



Pea protein based foods for the future: From chemical composition to statistical models to understand the mechanisms behind perceptions of beany, bitterness and astringency

Audrey Cosson

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Pea protein based foods for the future:
From chemical composition to statistical models
to understand the mechanisms behind perceptions
of beany, bitterness and astringency

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Invité

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PREFACE

Preface

This PhD is part of a CIFRE (*Conventions Industrielles de Formation par la Recherche*) program between the company ROQUETTE FRERE (Lestrem, France) AgroParisTech (Paris, France), the *Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement* (INRAE) and funded by the *Association Nationale de la Recherche et de la Technologie* (ANRT-Ministry of Higher Education and Research, ANRT-CIFRE 2017/0815). The AgroParisTech/INRAE partner laboratory of this project is the SayFood Joint Research Unit (Paris-Saclay Food and Bioproduct Engineering).

Founded in 1933, Roquette Frères is a French agro-industrial group with an international scope in the starch industry (roquette.com). It has 21 production sites in 13 countries and 8,000 employees. Roquette is a supplier to a large number of customers such as Nestlé, Danone and Wrigley. In 2016, its turnover amounted to 2 billion Euros. Roquette transforms 5 renewable resources (maize, wheat, potatoes, peas and microalgae) to develop a range of more than 700 derived products including polyols, sugars and proteins. These products are used in pharmacy, cosmetology, bio-industry, paper and cardboard industry, but also in animal nutrition and human nutrition. One of their challenges for the next few years is the development of a functional and sensory pleasant pea protein fraction for the development of a new range of products or the improvement of the existing range.

The SayFood Joint Research Unit (Grignon, France) is a joint research unit in co-supervision with AgroParisTech and INRAE. AgroParisTech, is a higher education teaching and research institute in the fields of agronomic sciences and techniques, the agri-food sector, forestry, management of spaces and natural resources, land management and development. Its mission is to train high level students and to advance scientific knowledge, in close association with public and private research centres (agroparistech.fr). INRAE is Europe's top agricultural research institute and the world's number two centre for the agricultural sciences (institut.inrae.fr). Its scientists are working towards solutions for society's major challenges linked to four main domains, food, nutrition, agriculture and the environment. The SayFood Unit is interested in the control of the physical and biological processes that govern food transformations with a view to delivering knowledge and tools to assist in the development of quality products. In particular, this unit is interested in the mechanisms of degradation of the food in the mouth (buccal and pharyngeal phases) and the modelling of the release kinetics of the associated target compounds (nutrients or stimuli responsible for perceptions). It combines an experimental multidisciplinary approach including modelling, in order to better understand the roles of the food (its composition and its structure) and of the individual (oral physiology) on the kinetics of release of the target molecules and perception. For several years now, it has been presenting an expertise on pea protein and its flavour thanks to its involvement in research projects around pulses and plant proteins.

In addition, research works were produced in collaboration with INRAE scientific platforms: the polyphenol platform (Montpellier, France) and the *Plateforme d'Analyse Protéomique de Paris Sud-Ouest* (PAPPSO, Jouy, France).

Because of the structural diversity, characterisation of phenolic compounds in plants and plant based food and non-food products, requires an integrated analytical approach. In this context, the polyphenol platform offers analytical, methodological and technical help, as well as expertise in mass spectrometry, nuclear magnetic resonance and chemometrics for structural characterisation, quantification, high throughput analysis of large series of samples. Within the framework of this PhD project, this platform has helped to set up a method for the characterization of phytochemical compounds in pea protein isolates and the identification of these compounds.

The *Plateforme d'Analyse Protéomique de Paris Sud-Ouest* (PAPPSO facility) provides research teams with equipment, skills and expertise in the field of proteomics, to allow them to respond to the most simple questions (identification of proteins from an organism the genome of which is entirely sequenced) as well as to complex questions (protein quantification in complex samples, dynamics of

post-translational modifications,...) in the framework of collaborative projects and contracts. PAPPISO is specialized in high throughput proteomics (quantitative analysis of large cohorts of samples) and in the analysis of highly complex samples (metaproteomics). The platform develops bioinformatics tools that allow the processing of these experiments and provides assistance to users for data interpretation. The PAPPISO platform (<http://pappiso.inra.fr>) is supported by INRAE (<http://www.inrae.fr>); the Ile-de-France regional council (<https://www.iledefrance.fr/education-recherche>); IBISA (<https://www.ibisa.net>); and CNRS (<http://www.cnrs.fr>). Within the framework of this PhD project, this platform has helped to identified peptides from pea protein isolate samples.

In addition, the following manuscript is built on "articles". Each of the result parts constitute either a published article or a draft article that will be submitted. These parts can therefore be read independently. They have a classical structure: introduction, materials and methods, results and discussion, conclusion and perspectives. Besides, an overall "materials and methods" part is presented before the results part. Its purpose is not to be redundant with the "materials and methods" of each part of the results, but to give some complements of the methods used during this PhD.

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List of abbreviations

Abbreviations of pea solutions:

Si = Pea protein solution from isolates i

Abbreviations of pea recombined solutions:

Refa = Reference pea solution from Pea Protein Isolate *a*

Refb = Reference pea solution from Pea Protein Isolate *b*

Pa = Permeate from Pea Protein Isolate *a*

Pb = Permeate from Pea Protein Isolate *b*

Ra = Retentate from Pea Protein Isolate *a*

Rb = Retentate from Pea Protein Isolate *b*

Ia = Pellet from Pea Protein Isolate *a*

Ib = Pellet from Pea Protein Isolate *b*

X Pb-Y Ra = Recombined product -X% of permeate *b*, Y% of retentate *a* and 20% of water

Abbreviations of pea beverages:

F- = 0% oil

F+ = 1.5% oil

G- = 0.12% gellan gum

G+ = 0.5% gellan gum

I = Isolate

P = Pellet

S- = 0.08% salt

S+ = 0.12% salt

Abbreviations for the sensory part:

X-M = X sensory attribute evaluated in mouth

X-N = X sensory attribute evaluated in nose

X-NC = X sensory attribute evaluate with nose-clip

BP = Block profiling protocol

CP = Profiling protocol

ASTM = American Society for Testing Materials

CATA = Check-All That-Apply

HPT = High PROP taster

ID = Product identity

LMS = Labelled Magnitude Scale

LPT = Low PROP taster

MPT = Medium PROP taster

PROP = 6-nPropylthiouracil

PSP = Polarized sensory positioning

TAS2R = Bitter Taste Receptors

TDS = Temporal Dominance of Sensations

TI = Time intensity

Abbreviations for the statistical part:

ANCOVA = Covariance analysis

ANN = Artificial Neural Network

ANOVA = Analysis of Variance

CCSWA = Common components and specific weights analysis

GPA = Generalized procrustean analysis

HCA = Hierarchical cluster analysis

LSD = Least Significant Difference

MANOVA = Multivariate analysis of variance

MCR-ALS = Multivariate curve resolution alternating least square

MDS = Multidimensional scaling method

MFA = Multiple factor analysis

MLR = Multiple Linear Regression

NA = Not available

NIPALS = Nonlinear Iterative Partial Least Squares

OLS = Ordinary Least Squares

PCA = Principal component analysis

PLS = Partial-Least-Square

PLS-MB = Partial-Least-Square multi-blocks

PLS-DA = PLS discriminant analysis

PLS-PM = PLS Path Modelling

RMSE = Root Mean Square Error

RMSEc = Root mean square error of calibration

RMSEv = Root mean square error of validation

VL = Latent variable

VM = Manifest variable

Abbreviations for the chemical part:

APCI = Atmospheric Pressure Chemical Ionization

API = Atmospheric pressure ionization

CZE = Capillary Zone Electrophoresis

DAD = Ultraviolet Diode Array Detector

DAG = Diglyceride

DDMP = 2,3-dihydro-2,5-dihydroxy-6-methyl-4Hpyran- 4-one

DL = Detection limit

DLS = Dynamic light scattering

DM = Dry matter

DSC = Micro Differential scanning calorimetry

E-noses = Electronic nose

ESI = Electrospray ionization

FAB = Fast atom bombardment

FID = Flame Ionization Detector

GC = Gas chromatography

GC-IMS = Ion mobility spectrometry

GC-O = GC olfactometry

GPC = Gel Permeation Chromatography

HESI = Heated-electrospray ionization probe

HPLC = High performance liquid chromatography

HR-CS = High-Resolution Continuum Source

HRGC = Headspace trap gas chromatography

HS-SPME = Headspace Solid Phase Micro Extraction

ICP-AES = Inductively Coupled Plasma - Atomic Emission Spectroscopy

ICP = Inductively Coupled Plasma

LAS = Laser absorption spectroscopy

LC = Liquid chromatography

LOD = Limit of Detection

MALDI = Matrix Assisted Laser Desorption Ionization

MIMS = Membrane introduction mass spectrometry

MLC = Micellar liquid chromatography

MRM = Multiple Reaction Monitoring

MS = Mass spectrometry

MW = Molecular weight

MWCO = Molecular weight cut-off

NMR = Nuclear magnetic resonance

OAV = Odour activity value

PTFE = Polytetrafluoroethylene

PTR = Proton Transfer Reaction Mass Spectrometry

QL = Quantification limit

RP-HPLC = Reverse phase HPLC

SAFE = Solvent Assisted Flavour Extraction

SESI = Secondary electrospray ionization

SIFT = Selected-ion flow-tube

SPE = Solid phase extraction

SPME = Solid Phase Micro Extraction

TDS = Thermodesorption

TGSC = thegoodscentcompany.com

TLC = Thin layer chromatography

TOF = Time-of-flight

UHPLC = Ultra high performance liquid chromatography

UHPLC-Q = UHPLC quadrupole

VOC = Volatile organic compounds

Scientific publications and communications **from this doctoral work**

Publications in scientific journals:

1. **Cosson, A.**, Delarue, J., Mabillet, A.-C., Druon, A., Descamps, N., Roturier, J.-M., Souchon, I., & Saint-Eve, A. (2020). Block protocol for conventional profiling to sensory characterize plant protein isolates. *Food Quality and Preference*, 83, 103927. <https://doi.org/10.1016/j.foodqual.2020.103927>
2. **Cosson, A.**, Souchon, I., Richard, J., Descamps, N., & Saint-Eve, A. (2020). Using multiple sensory profiling methods to gain insight into temporal perceptions of pea protein-based formulated foods. *Foods*, 9(8), 969. <https://doi.org/10.3390/foods9080969>
3. **Cosson A.**, Blumenthal D., Descamps N., Souchon I, Saint-Eve A. (2021). Using a mixture design and fraction-based formulation to better understand perceptions of plant-protein-based solutions. *Food Research International*, 110151. <https://doi.org/10.1016/j.foodres.2021.110151>
4. **Cosson A.**, Oliveira Correia L., Descamps N., Saint-Eve A., Souchon I (2022). Identification and characterization of the main peptides in pea protein isolates using ultra high-performance liquid chromatography coupled with mass spectrometry and bioinformatics tools. *Food Chemistry*, 367, 130747. <https://doi.org/10.1016/j.foodchem.2021.130747>.

Drafts of scientific publications:

5. **Cosson A.**, Meudec E., Ginies C., Danel A., Lieben P., Descamps N., Cheynier V., Saint-Eve A., Souchon I. Identification and quantification of main phytochemicals from pea protein isolates and first links with perception. (*To submit to Food Chemistry, September 2021*).
6. **Cosson A.**, Saint-Eve A., Peron A., Descamps N., Souchon I. Retention of volatile compounds in pea protein solutions and its impact on beany perceptions. *Discussion in progress for publication*.
7. **Cosson A.**, Saint-Eve A., Descamps N., Souchon I. Food-saliva interactions during the consumption of pea protein based foods and impact on aroma perceptions. *Discussion in progress for publication*.

Oral communications:

8. **Cosson A.**, Delarue J., Mabillet A.-C., Druon A., Descamps N., Souchon I., Roturier J.-M. & Saint-Eve A. (2018). Informed Profiling: a new and efficient sensory method to evaluate plant-protein isolates. **Eurosense conference**.
9. **Cosson A.** (2018). An efficient sensory method for understanding the perceptions of pea protein isolates in relation with their chemical composition. **European Sensory Science Society (E3S) Student and Early Career Researcher Group (SESERG) Knowledge exchange Webinar**.
10. **Cosson A.**, Blumenthal D., Druon A., Descamps N., Roturier J.-M., Souchon I. & Saint-Eve A. (2019). Proteins for the future: what are the link between composition and bitterness and beany perceptions for pea protein isolates? **Pangborn Sensory Science Symposium. Flash presentation poster**.
11. **Cosson A.** (2020). Using sensory profiling methods to gain insight into temporal perceptions of pea protein based formulated foods. **Roquette Frère International Scientific Review**.

12. Cosson A., Peron A.-C., Descamps N., Saint-Eve A., Souchon I. (2021). Food-saliva interactions during the consumption of pea protein based foods and impact on aroma perceptions. **Weurman Flavour Research Symposium.**

Posters:

13. Cosson A., Souchon I., Delarue J., Mabilhe A.-C., Druon A., Descamps N., Roturier J.-M. & Saint-Eve A. (2018). A new and efficient sensory method for understanding the perceptions of pea protein isolates in relation with their chemical composition. **EFFoST International Conference.**

14. Cosson, A., Souchon, I., Richard, J., Descamps, N., & Saint-Eve, A. (2020). Using multiple sensory profiling methods to gain insight into temporal perceptions of pea protein-based formulated foods. **NIZO Plant Protein Functionality.** Presentation of the poster on video: <https://youtu.be/geu-W20Cz4w>

15. Cosson A., Blumenthal D., Descamps N., Souchon I. & Saint-Eve A. (2020). Predictive statistical model explaining the perceptions of plant protein solutions according to protein fractions. **Eurosense conference.** Presentation of the poster on video: <https://youtu.be/shtR12JUM34>

16. Cosson A., Blumenthal D., Descamps N., Souchon I. & Saint-Eve A. (2020). Identification through statistical modelling of volatile and non-volatile compounds responsible for perceptions in pea protein isolates. **Eurosense conference.** Presentation of the poster on video: <https://youtu.be/PORsOL663qw>

17. Cosson A., Peron A.-C., Descamps N., Saint-Eve A., Souchon I. (2021). Retention of volatile compounds in pea protein solutions and its impact on beany perceptions. **Weurman Flavour Research Symposium.**

Awards and Grants:

18. Young Scientist Grants to attend Pangborn symposium. **Pangborn Sensory Science Symposium (2019).**

19. Young Scientist Award for best poster presented. **NIZO Plant Protein Functionality (2020).**

GENERAL INTRODUCTION

General introduction

Food is one of the main levers for optimizing human health and the sustainability of food systems (Willett *et al.*, 2019). Proteins are essential nutrients in our diet, with a role in protein synthesis for the body. On a global scale, an increase in protein demand caused by population growth (projected at 10 billion people by 2050) is observed. Today, the majority of proteins available for human consumption come from cereals (40%), meat (18%), milk (10%), plant and pulses (10%) and aquatic animals (7%) (FAOSTAT, 2019). In Western countries, animal protein consumption has not stopped increasing since the beginning of the 19th century and during the 20th, representing today about 60 to 70% of protein intake in France (Anses, 2017). In emerging countries (Asia, South America and Africa), a significant increase in demand for animal products is expected to possibly conduct to a doubling of meat consumption in the world within 15 years (Guillevic *et al.*, 2017).

However, there is a growing awareness of the environmental impacts of animal farming and the health consequences of high consumption of meat products. This awareness has led to a quest for more plant-based diets and more sustainable agriculture and processing. Many prospective studies, focusing on the challenge of demographic growth show that a transition towards food system by 2050 will require significant changes in sustainable diets. They highlight the need to change the relative part of animal and plant products in diets. For instance, the Agrimonde 2050 prospective proposes a "healthy" scenario including the partial substitution of meat by pulse proteins in diets, which would allow the improvement of nutritional quality (Le Mouël and Mora, 2019). In occidental countries, a decrease in meat consumption is already observed, for health as for ethical reasons. In France, it is recommended to have a diet rich in plant products and to limit animal food products (PNNS, 2019). This transition to healthy diets will have to be addressed by increasing and diversifying the food supply of plant-based foods, particularly pulse-based foods.

A direct consequence of these dietary trends is the increase of plant protein production with a global increase in the pulse harvest area (from 4.1 Mha in 2006 to 5.7 Mha in 2018, FAOSTAT, June 2020) and pulse production (from 3,200 kt in 2006 to 4,300 kt in 2018, FAOSTAT, June 2020). Pulses are protein-rich seeds with an indispensable amino acid profile complementary to cereals. Cereals are deficient in lysine and rich in sulphur amino acids, while pulses are deficient in sulphur amino acids and rich in lysine. Mixing the two raw materials within a diet can thus improve their protein quality for both animal and human consumption. Moreover, pulse crops have strong agronomic interest when they are inserted between other crops within crop rotations, or when they are grown in association with cereals. Indeed, they reduce the amount of nitrogen needed by crops by fixing atmospheric nitrogen, as well as the use of pesticides by creating a physical barrier to the spread of pests (Siddique *et al.*, 2012). Among pulses, yellow field pea (*Pisum Sativum* L.) has received much attention. In France, yellow field pea is the most widely produced grain pulse: 154,200 ha were cultivated in 2019, mainly in the northern part of France, compared to 137,200 ha in 2014 (CAP 2019).

Another consequence of these dietary trends is the modification of the plant industrial sector. At the end of 2016, the "Protéines France" collective have undertaken to implement a strategic development program in terms of research, investment and support for the industry in order to make France a world leader in proteins. The design of new foods enriched with plant proteins has also been targeted by companies. Many industrials are investing massively worldwide to develop sites capable of producing plant proteins in large quantities. This is notably the case of Roquette Frère, which opened in 2020 the world's largest pea protein plant in Manitoba, Canada; or of Cargill which, first implemented in the Americas, is now expanding its portfolio of pea protein ingredients in Europe and in Asia. But also Nestlé, which has invested massively in China in plant substitutes; or Unilever, which has built a large food innovation centre at the University of Wageningen in the Netherlands in particular to support research on plant ingredients and meat substitutes.

In France, peas are commonly used in animal feed, representing 10% of feed for poultry and 20% of feed for growing pigs, but they are also increasingly used for human food. The increase interest of pea

protein-based ingredients is also due to their low allergenicity, high nutritional value and restoration in the amino acid balance of cereal-based diets. Their proteins also have functional properties useful in food formulation: they promote emulsification, foaming, gelling and whipping properties (Adebiyi & Aluko, 2011; Gharsallaoui *et al.*, 2009). These valuable properties also explain why these products are used in the formulation of many foods. For example, they are used in sports nutrition and to replace casein and whey protein in fermented and unfermented dairy products (Akin & Ozcan, 2017). They can be used as substitutes for egg protein (Hoang, 2012); they can help to enrich protein levels in baked foods, cereals and snacks (Philipp *et al.*, 2017). They can also improve cooking efficiency, water/fat bonding and slicing ability of processed meat or fish (Baugreet *et al.*, 2016). They are also emerging as an alternative ingredient in specialty foods, such as gluten-free products (Mariotti *et al.*, 2009) and infant formula (Le Roux *et al.*, 2020). Within these products their concentrations vary widely (from 1% to 50%, with a median of 5%, Open Food Facts, 2019).

However, pulses are still struggling to expand in human diet due to lack of appeal and convenience for consumption. In particular, they are still strongly constrained by sensory off-notes (Roland *et al.*, 2017). Described as unpleasant or undesirable odours and tastes, they have often been reported in many plant products, particularly in pea proteins based-products (Trikusuma *et al.*, 2020; Bi *et al.*, 2020; Gläser *et al.*, 2020; Murat *et al.*, 2013; Humiski *et al.*, 2007). Three main types of off-notes are associated with plant proteins: beaniness, bitterness and astringency. They are either intrinsic to the seed, or generated during the various stages of fractionation of raw materials (Murat *et al.*, 2013) or during the final processing of the product. However, each of these notes has been studied separately leading clearly to a lack of knowledge to understand mechanisms at the origin of these perceptions in plant protein isolates. The main research works were focused mainly on the role of volatile aroma compounds in creating sensations of beaniness. Some phytochemical compounds have also been identified to contribute to astringency but literature concerns seeds or flour but not protein isolates. No studies used a more global approach to examine how the complex perception of pea protein isolates arises from both volatile and non-volatile compounds and their potential interactions. A lack of knowledge is clearly identified. In addition, if in the literature, works exist on the sensory and physico-chemical interactions that can modulate the perceptions of bitterness and astringency, the kinetics and dynamics of perceptions remain little studied in the case of products based on plant proteins, making it difficult to predict the dynamics of perceptions of pea proteins-based products. Thus, these off-notes constitute one of the main limits for the development of products enriched in plant proteins.

Therefore, the research question of this doctoral project falls within this context: **“What are the main physical, chemical and sensory mechanisms that explain the sensory notes of pea protein isolates?”** More precisely, the objective of the PhD is to acquire knowledge on perception of pea protein isolates (beany, bitter, astringent) in relationship with their composition as well as dynamic perceptions when consuming formulated products enriched with pea protein isolates. Pea protein isolates have been chosen as a "model" plant protein on the basis of its sensory off-notes very often highlighted in the literature, and for the growing interest of food industry in this protein. In order to meet these doctoral objectives, this work proposes to combine knowledge from three main disciplines that have been applied to this product: sensory analysis, chemical analysis and statistical analysis.

The first chapter of this manuscript is devoted to the state of the art of current knowledge on the mechanisms behind the sensory perceptions of pea protein isolates. These sensory perceptions resulting from consumption, as well as the methods used to study them, are detailed in the first part. Then, the main compounds at the origin of these perceptions are presented along with the methods used to study them. The third part of the state of the art deals with the experimental and statistical approaches used to study sensory mechanisms by linking sensory and instrumental data sets. Finally, the proposed approach to answering the research question is given in the final section.

The second chapter of the manuscript details the materials and methods used during the different phases of this PhD project. Although the result section of this manuscript summarizes the specific materials and methods used, this chapter outlines in particular the methodological choices. It is organized by themes, such as products, sensory methods, instrumental methods and statistical methods.

The third chapter of the manuscript presents the results. It is composed of four sections including results presented as papers (already published or to be submitted).

The distinct and persistent flavour of pea proteins calls into question their sensory evaluation and requires the adaptation of classical sensory methods. In addition, the apparent complexity of mechanisms at the origin of off-flavours in pea protein isolates calls for the establishment of an adapted strategy. Thus, the purpose of the first section of results is to present the adaptations of sensory profiling which have been made to describe the sensory perceptions of pea protein isolates; and the experimental strategy based on an experimental design which have been developed to create a “controlled diversity” of products.

The recent development of analytical technologies (modern mass spectrometry and bioinformatics) makes it possible a more precise identification of chemical compounds of pea protein isolates. Thus, the purpose of the second section of results is to determine the chemical characteristics of pea proteins solutions and to identify first links between these compounds and pea perceptions. Volatile compounds, peptides, and phytochemical compounds were studied.

The aim of the third section of results is to go further in understanding the complex and combined role of volatile and non-volatile compounds (peptides, phytochemicals) on overall perceptions of pea protein isolates. It concerns the construction of a statistical model taking into account all the different chemicals and the identification of their relative contribution to perceptions.

Beyond the chemical composition of the food, its structure and texture could also influence the dynamics of perception. Thus, as a final point, the purpose of the fourth section of results is to determine how the sensory perceptions of pea protein isolates are affected by the formulation as well as the consumption of a whole product. Among the different pea applications, pea beverages were studied, varying in their level of salt, fat, thickeners and type of proteins.

The fourth chapter provides a synthesis and discussion of the main findings of this PhD work, in particular on the knowledge sharing between solutions and beverages and the impact of inter-individual variability on perceptions. Finally this manuscript ends with general conclusions which bring recommendations to reduce pea protein-based products off-notes, and possible perspectives to deepen the knowledge acquired on the sensory mechanisms at the origin of these off-notes.

CHAPTER 1

STATE OF THE ART

State of the art

Introduction

Pea protein isolates are promising food ingredients for the development of new products, more sustainable and more appreciate than animal protein based ingredients. Pea protein isolates are a powder obtained from yellow field pea seed (*Pisum Sativum* L), after selective extraction of protein fraction. Pea seed are constituted of starch (30-50% w/w of dry matter), proteins (20-25%), fibres (10-15%) and lipids (2%). Proteins are found in the endosperm as well as in the cotyledons. These proteins have different function in the seed: structural proteins, biologically active proteins and reserve proteins. Pea proteins are characterized by a high lysine content and a relative deficiency of sulphur amino acids and threonine in relation to food requirements. Like all legume proteins, they are made up of three classes of proteins: globulins, albumins and glutelins, so-called 'insoluble' proteins (Creveieu-Gabriel, 1999). The first two fractions were initially characterized by their solubility in water and in saline medium respectively. Globulins represent 50 to 65% of total proteins. They are the main reserve proteins of the seed. Albumins represent 20 to 25% of total proteins. They are rich in lysine and sulphur amino acids, particularly methionine. Numerous factors as the pea variety, the farming practices, the soil, the climate or the dates of harvest can influence the protein content of the pea seed. For example, a study on 59 different varieties of peas showed that the protein composition on a dry basis ranged from 13.7 to 30.7% (Tzitzikas *et al.*, 2006).

Pea proteins can then be prepared in three forms: pea flour (about 20% of proteins), pea protein concentrate (38% - 65% of proteins), and pea protein isolate (80% - 90 % of proteins). Pea flour is produced by dry milling of dehulled peas. Pea protein concentrate can be produced through the acid leaching procedure traditionally used to produce soy protein concentrates, or through dry separation methods which are more economical (Owusu-Ansah & McCurdy, 1991). The dry process, or air-classification, consists in a grinding and then a turbo separation. It allows obtaining a protein extract composed of 38% to 65% of proteins and allows a good separation of the starches and the proteins (Pelgrom *et al.*, 2015). The wet process consist on two main steps (Schutyser *et al.*, 2015). The objective of the first step is to recover proteins. It consists in solubilizing the proteins and separating them by centrifugation from the carbohydrates. In the second step, the proteins are concentrated and purified from the other constituents, which can be done in two ways. Either the soluble non-protein molecules of low molecular weight are separated from the proteins by ultrafiltration, or the proteins are precipitated by lowering the pH of the mixture to their isoelectric point. Water washing, centrifugation, neutralization and drying steps are then carried out to purify the proteins of the last undesirable constituents. These steps make it possible to obtain isolates with 80% - 90 % of proteins. During these processes, glutelins are eliminated. It results a composition of pea protein isolates with mainly globulins and some albumins left. Pea protein isolates also still contains some lipids, carbohydrates, minerals, and other small compounds (e.g., phenolics), which are the products of seed metabolism. Figure 1. presents the pea protein isolates extraction process of Roquette commercial pea protein isolates (Murat *et al.*, 2013).

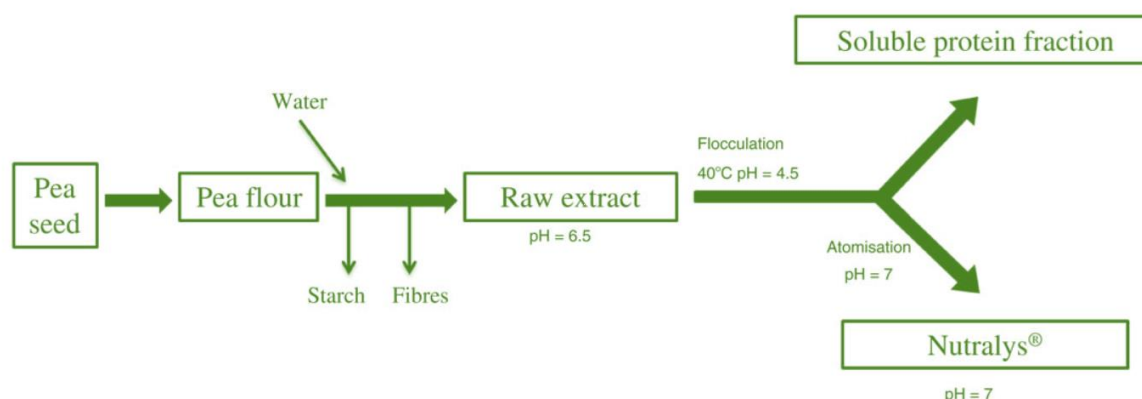


Figure 1.: Schematic description of the pea protein extraction process adapted from Murat *et al.*, 2013.

Pea protein isolates are used in numerous industrial recipes for human consumption. They offer high nutritional and technological functions for many types of foods, from dietary supplements, bakery to meat alternative. This wide variety of products leads to a wide range of sensory properties. The latter are known to be key factors in determining consumer acceptability and preferences. A challenge remains: pea-protein-based products are usually described as having strong sensory off-notes, which makes them less desirable to consumers and limit their use. Three main types of off-notes are associated with pea proteins: beaniness, bitterness and astringency.

Thus, the objective of the first part of this literature review is to identify the shared sensory perceptions of pea protein-based products. Pea off-notes being multimodal, we will first describe the physiological mechanisms at the origin of aromatic, sapid and somesthetic perceptions and describe these perceptions in pea protein-based products. Then the different sensory methods used in the literature for this type of product and their relevance will be discussed. Finally, the impact of the oral process and of the interactions on these perceptions will be presented.

The second objective of this state of the art is to identify the main chemical compounds present in pea protein isolates. We have chosen to focus on volatile compounds, protein and peptides, on phytochemical compounds. We will particularly detail their characteristics in pea protein isolates, the analytical methods and associated bioinformatics tools used to study them as well as their roles on pea protein perceptions. In addition, the role of other compounds (e.g. minerals and carbohydrates) on perception will be discussed.

The third objective of this state of the art is to understand the strategies used in the literature to lead to a better understanding of the mechanisms behind perceptions. An overview of the different strategic approaches used to link chemical and sensory datasets will be presented. Experimental and statistical approaches will be discussed.

Finally, the objectives of PhD project will be presented in a last part.

1.1. Perceptions generated by pea-based products

Human perceptions are the results of complex sensory and interpretation processes. When a food product is consumed, its properties of texture, aroma, taste and its trigeminal properties generate stimuli that will determine its perception, its acceptability to consumers and contribute to the development of their preferences.

Food consumption is also a multi-modal and dynamic experience for consumers. In the case of pea protein based-products, their perceptions are often described with "beany" notes, but also with bitter and astringent notes (Bott & Chambers, 2006; Humiski & Aluko, 2007). This three sensations are the subject of this first part of the state of art, which is followed by a presentation of the main sensory methods used in the literature to characterise this type of plant product and their relevance are discussed. Finally, a synthesis of the works about the role of the food oral processing on the dynamic of perceptions are presented.

1.1.1. Aroma, sapid and somesthesis perceptions of pea based-products

1.1.1.1. Physiology of aroma perceptions

Aroma perception results to the activation of olfactory receptors present in the olfactory epithelium in the nasal cavity of humans by volatile compounds. These last can generate olfactory nerve stimulus and signal transmission via the olfactory bulb to the brain (Figure 1.1.1.). Volatile compounds are either inhaled through the nose and interact with the olfactory system directly, then we are talking about the orthonasal way, or they initiate the sensory process of odour recognition in mouth, flowing from the mouth through the back of the throat reaching the nasal cavity through the pharynx, via the retronasal way (Bojanowski & Hummel, 2012). Olfactory perception originates from the interaction between the volatile chemical compounds and the olfactory neurons located in the olfactory epithelium. Odorant compounds generally have a fairly marked hydrophobic character and their transport to the olfactory receptors must take place through the olfactory mucus that covers the epithelium and in which odorants are very poorly soluble.

Olfactory receptors are carried in the cilia of dendritic end of each olfactory neuron. About 350 of receptor types (G-protein coupled receptors) are active in humans. They receive the chemical signal outside the cell and transfer it inside by activating the associated G protein. The latter then initiates the production of secondary chemical messengers, notably cyclic MPA, whose action triggers the opening of the cell's ion channels. The resulting flow of ions causes a change in the polarization state of the membrane. And so the transmission of nerve impulses. Olfactory neurons converge to the mitral cells. The clusters of these synaptic connections are the glomeruli. The axons of the mitral cells will then ensure the connections with the olfactory cortex (Buck et Axel, 1991).

It has been established that a given olfactory neuron expresses only one type of receptor, that each type of receptor has more or less strong affinity for several odorants and that, correlatively, an odorant compound can activate several receptors. Consequently, different odorants activate distinct sets of olfactory receptors more or less intensely, which may nevertheless partially overlap. Odour recognition is therefore similar to pattern recognition and we can speak of an olfactory image projected within the olfactory bulb. The high number of types of olfactory receptors, the combinatorial nature of the information and the "pattern recognition" aspect make it possible to understand that we are able to distinguish the smell from an incredibly high number of different compounds. It has been speculated that the humans can discriminate more than 1 trillion olfactory stimuli (Bushdid *et al.*, 2014). Natural olfactory stimuli are almost always mixtures of large numbers of diverse volatile compounds at different concentrations. The characteristic aroma of a wine, for example, is produced by a mixture of more than 100 compounds, although typically only a small number of compounds contribute to the perceived flavour (Francis & Newton 2005). In order to make these stimuli explicit, sensory attributes are commonly used by individuals.

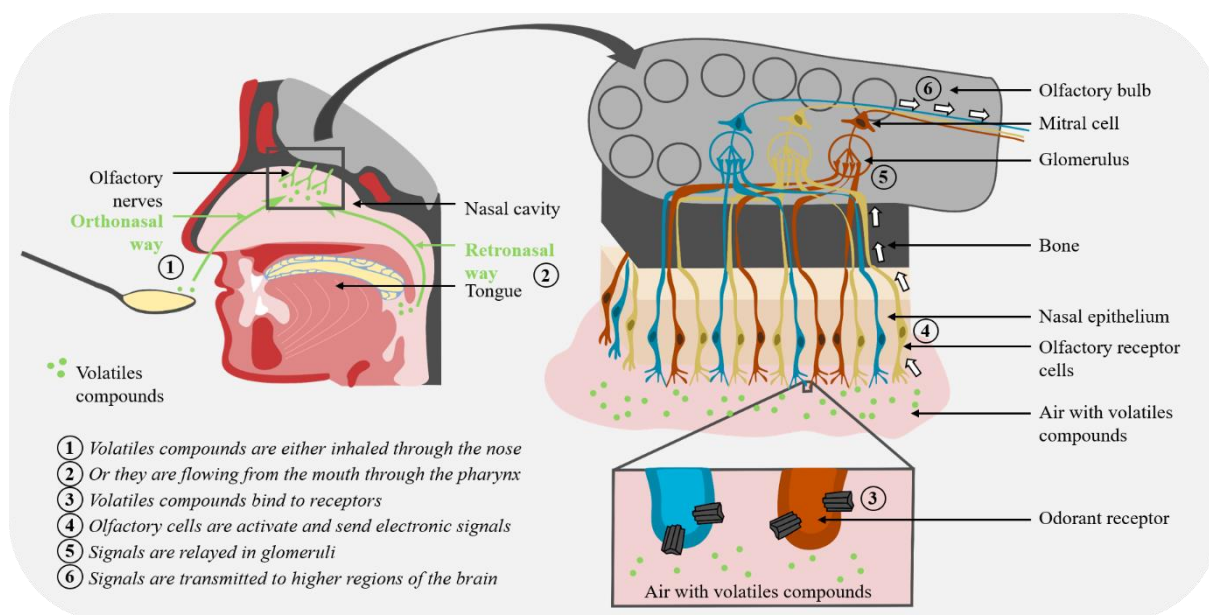


Figure 1.1.1.: Anatomy of the olfactory system (adapted from Buck et Axel, 1991).

In addition, the perception of volatile compounds varies between individuals. For example, in a study in Germany, among 1312 participants, 3.6 % were found functionally anosmic (decrease or complete loss of sense of smell) and 18% had olfactory dysfunction (Vennemann *et al.*, 2008). The most common anosmia is musky odour. It affects about 10% of the population (Triller *et al.*, 2008). These differences can be due to genetic factors or to smell dysfunction (aging, sinusal disease, head trauma, smoking...).

1.1.1.2. Physiology of bitter perceptions

Bitter perception can cause the rejection of certain foods and therefore has often been considered as a defence mechanism against the ingestion of toxic substances (Drewnowski & Gomez-Carneros, 2000). It is often considered to be the most complex taste due in particular to the multiplicity of receptors and compounds involved in this perception (Behrens *et al.*, 2004). Indeed, thousands of structurally diverse compounds with different chemical structures can activate the bitter receptors: amino acids, peptides, salts, organic ions, polyphenols, alkaloid compounds like caffeine or quinine, etc. Rodgers *et al.* (2005), identified 93 specific structures associated with the perception of bitterness. These structures include in particular amino acids, peptides and phenolics (Figure 1.1.2.).

Structural Class	Example Structure	Structural Class	Example Structure	Structural Class	Example Structure
Amino acid (Meiselman and Halpern, 1970; Suzuki <i>et al.</i> , 2002)	<chem>CC(N)C(=O)O</chem> Alanine	Quinolizidine alkaloid (Hatzold <i>et al.</i> , 1983; Ruiz and Sotelo, 2001)	<chem>C1CC2C(C1)N3CCCCC3C2=O</chem> 17-oxolupanine	Glucoside (Rouseff, 1980; Drewnowski and Gomez-Carneros, 2000)	<chem>O=C1C(OC2C(OC3C(OC4C(C3)O)O)O)O2)O1</chem> Prunin
Diketopiperazine (Ishibashi, <i>et al.</i> , 1988)	<chem>C1CC2C(C1)N3CCCCC3C2=O</chem> Cyclo(leucine-proline)	Sugar, sugar derivative (Raiz-Avalia <i>et al.</i> , 2000; Schiffman <i>et al.</i> , 1995)	<chem>OC[C@H]1O[C@H](O)[C@H](O)[C@@H](O)[C@H]1O</chem> Fructose	Lactone (Berry and Tatum, 1986; Price <i>et al.</i> , 1990)	<chem>O=C1C(OC2C(OC3C(OC4C(C3)O)O)O)O2)O1</chem> 7-hydroxycoumarin
Flavonoid (Robichaud and Noble, 1990; Lee <i>et al.</i> , 1999)	<chem>O=C1C(OC2C(OC3C(OC4C(C3)O)O)O)O2)O1</chem> Gallic acid	Terpene (Belitz and Wieser, 1985)	<chem>C1CC2C(C1)N3CCCCC3C2=O</chem> Quassin	Pyranoside (Bufe <i>et al.</i> , 2002)	<chem>O=C1C(OC2C(OC3C(OC4C(C3)O)O)O)O2)O1</chem> Salicin

Figure 1.1.2.: Main bitter structural groups identified by Rodgers *et al.*, 2005.

Bitterness is one of the key sensations perceived by the gustatory system. The gustation and its taste signals are originated from the taste buds that are stimulated by the soluble compounds when they come into contact with the tips of the apical cells of these taste buds as shown on the Figure 1.1.3. (Brondel *et al.*, 2013).

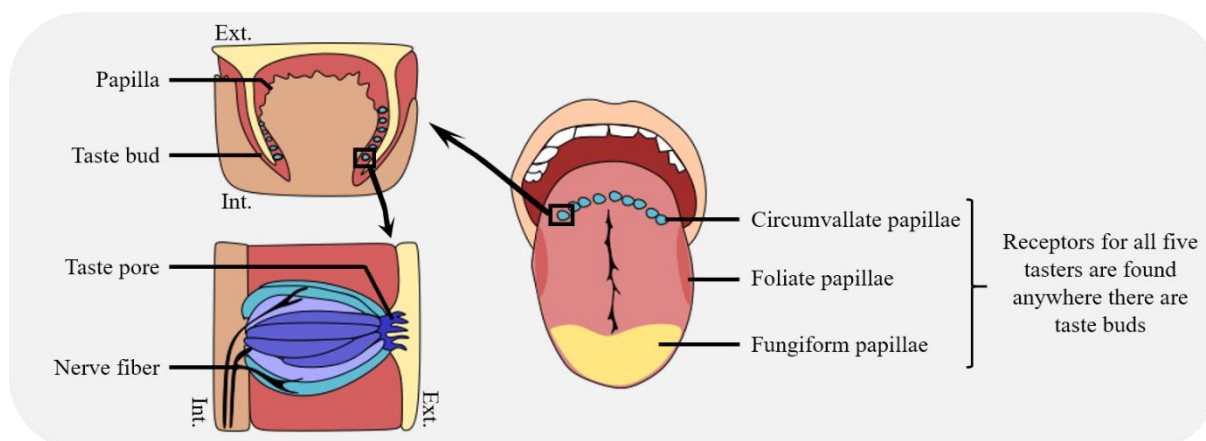


Figure 1.1.3.: Anatomy of the gustatory system (adapted from Muchlinski *et al.*, 2011).

Inside the taste buds, these bitter chemical compounds can activate the TAS2Rs receptors. These family receptors are constituted of 25 receptors positioned at the apical membranes of the receptor cells of taste (Briand & Salles, 2016; Maehashi *et al.*, 2009; Meyerhof *et al.*, 2010). Signal transduction of bitter stimuli is accomplished via a G protein. This protein may activate a taste phosphodiesterase and decreases cyclic nucleotide levels. Then it mediates taste by activating IP₃ (inositol triphosphate) and DAG (diglyceride). These second messengers may open gated ion channels and may cause release of internal calcium and then cause neurotransmitters release (Figure 1.1.4.) (Moyes & Schulte, 2008).

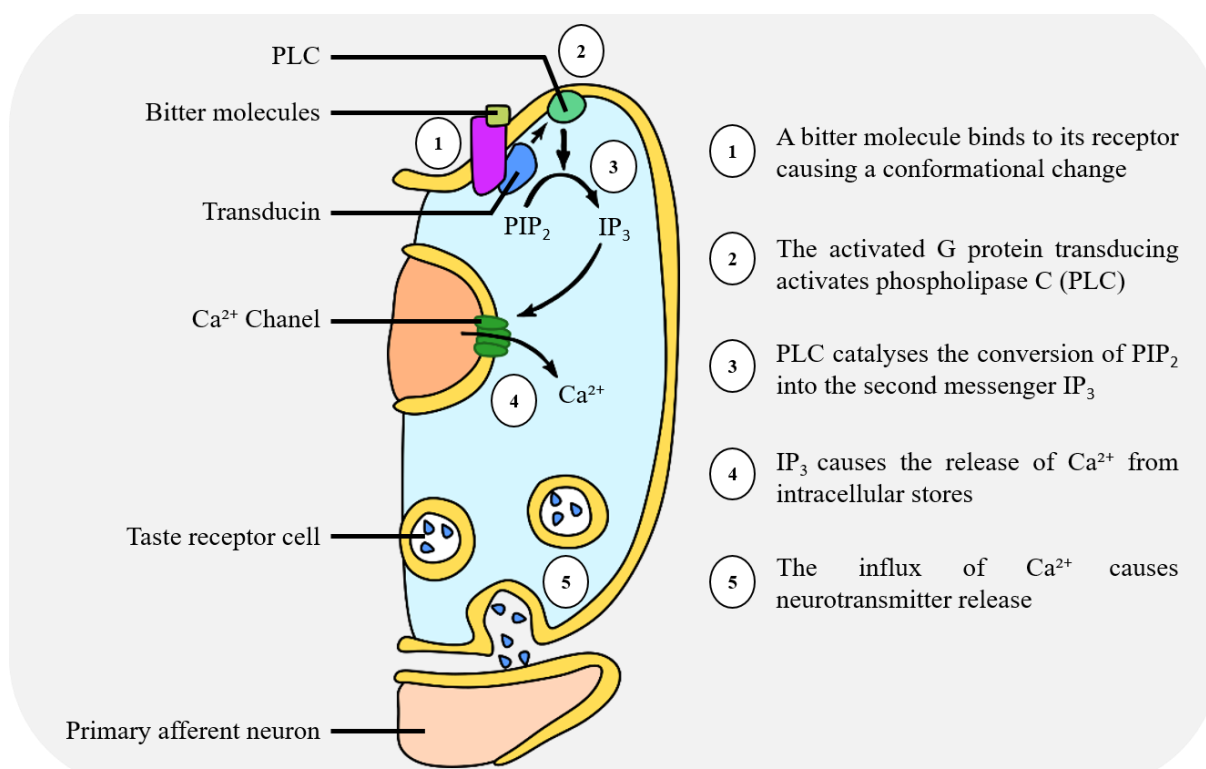


Figure 1.1.4.: Signal transduction in taste receptor cells (adapted from Moyes & Schulte, 2008).

Unlike receptors of other taste, TAS2Rs have a very strong ligand-receptor identity. Most TAS2Rs show an intermediate degree of specificity for bitter compounds that means they respond to several bitter compounds. But some TAS2Rs receptors show a strong specified and two receptors even seem to be specific to a single chemical compound (Meyerhof *et al.*, 2010). For example, at least 6 receptors respond to amino acids and peptides stimuli (Kohl *et al.*, 2013). The response of the receptors depends on the signal amplitude and the concentration of bitter compounds (Meyerhof *et al.*, 2010). Humans can

therefore detect many bitter compounds with a very small number of general receptors and a small number of compounds with specialized receptors (Bufe *et al.*, 2002). The sensitivity of TAS2R to different bitter compounds raises the question of how receptors interact with their ligands. Do they have multiple binding sites or do they have unique binding sites, each of which can accommodate several chemically different compounds? A study by Brockhoff *et al.* (2010) on the TAS2R43, TAS2R44 and TAS2R46 receptors strongly suggest that TAS2Rs possess only a single binding pocket, accommodating the different ligands via amino acid residues.

In addition, the perception of bitter compounds varies widely between individuals. Hansen *et al.* worked on the bitterness intensity for different bitter solutions (PROP, octa-acetate sucrose, quinine, caffeine). They showed that three sets of distinct genes were responsible for sensitivity to these compounds and that genetic factors may explain variations in perceptions of bitterness (Hansen *et al.*, 2006). Environmental factors (age, pathology, medication, etc.) can also affect the perception of bitterness (Mennella *et al.*, 2010) as well as eating habits and salivary composition (Dsamou, 2012).

1.1.1.3. Physiology of astringent perceptions

Astringency has been defined as “the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” by the American Society for Testing Materials. It may be a combination of taste and oral tactile mechanisms (Bajec & Pickering, 2008). For this reason, astringency has also been described as a tactile sensation in the mouth producing an impression of dryness and tightness of the mucous membranes. As the sense of touch, it is a somesthetic sensations which take place in the oral cavity of tasting and are activated by different stimuli (Brondel *et al.*, 2013). As bitterness, it could also be generally referred to a negative contributor to the liking of various foods (Breslin *et al.*, 1993).

Astringency is little studied in food product except in the case of wine and tea products. So the molecular and physiological mechanisms underlying astringency are still poorly understood. Gibbins and Carpenter (2013) as well as Ma *et al.* (2014) summarized in their respective reviews the different activation pathways of astringency as shown in Figure 1.1.5. A feeling of friction can occur by the presence of aggregates and the loss of the lubrication power of saliva. These aggregates can come from the binding and the precipitation of proteins of the saliva (salivary amylase, mucin, esterase...) with polyphenols. Charlton *et al.* (2002), pointed out that tannin-protein interactions are based on three phases. First, hydrophobic associations occur between the flat surfaces of the aromatic rings of tannins and the hydrophobic sites of proteins. At the same time, the hydrogen bonding effects stabilize the complexes formed. Then, tannin-protein complexes can self-associate via new hydrogen bonds thus generating larger soluble tannin-protein complexes and then aggregates. Finally, these aggregates have a sufficient size to form insoluble sediments. In parallel, the aggregates of tannin-proteins (possibly also free) polyphenols, can cause a breakage of the salivary layer leading to a loss of lubrication (Charlton *et al.*, 2002; Ma *et al.*, 2014). In addition, phenolics can be adsorbed at the surface of the oral mucosa : several studies showed that the mucin-rich mucous was also involved in the mechanism of perception of astringency. In addition, a direct activation pathway through chemical receptors were identified for galloylated polyphenols (Schobel *et al.*, 2014).

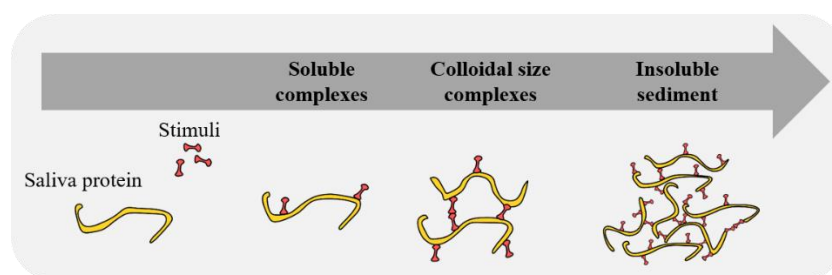


Figure 1.1.5.: Schematic representation of possible astringency mechanisms: A 3-stage model of the interaction between stimuli and proteins (adapted from Ma *et al.*, 2014).

1.1.1.4. Perceptions in the case of pea protein-based products

The beany aroma seems to be one of the key characteristics of pea protein perceptions. It is described as being a multidimensional and complex descriptor. According to Bott and Chambers, beany flavour is an aromatic sensation including musty/earthy, musty/dusty, sour aromatics, starchy, powdery feel, green pea, nutty, brown notes (Bott & Chambers, 2006). Different references can be used to explain the descriptors associated with beany flavour as shown on Table 1.1.1. (Cherdchu *et al.*, 2013; Vara-Ubol *et al.*, 2004). Nevertheless depending on the type of pulses (soybean, lupine, pea...), the type of studied food or depending to the research work, beany note definition may vary. For example, for lupin flour, beany/green, mushroom/soil, floral, meaty, nutty, woody/green, sweet, and baked attributes were used to describe beany sensation (Kaczmarek *et al.*, 2018), whereas beany, green, fresh, and grassy attributes are commonly used to describe the sensations in pea and soybean (Troszyńska *et al.*, 2007).

Table 1.1.1.: References used to explain the descriptors associated with beany flavour (adapted from Cherdchu *et al.*, 2013; Vara-Ubol *et al.*, 2004). Intensities were scores from 1 (just recognizable) to 15 (extremely intense).

Attribute	Descriptor	Reference and intensity (/15)
Green, pea pod	Aromatics notes associated with fresh, green pea pods	Food Club Lima Beans (frozen) = 8.0 (flavour)
Musty/dusty	Dry, dirt-like aromatics associated with dry, brown soil.	Potato peel (dry) = 5.0 (aroma) Kroger Pinto Beans (cooked dried) = 3.0 (flavour)
Musty/earthy	Humus-like aromatics that may or may not include damp soil, decaying vegetation, or cellar-like characteristics.	Raw white potato = 3.0 (aroma) Kroger Butter Beans (canned) = 5.5 (flavour)
Starchy	Aromatics associated with starch and starch based ingredients.	2% Argo corn starch in water = 7.5 (flavour) Kroger Pinto Beans (cooked dried) = 7.5 (flavour)
Nutty	A light, brown, slightly musty aromatic associated with nuts, wheat germ, and certain whole grains.	Arrowhead Mills Soy Beans (cooked dried) = 4.0 (flavour) Kretschner Wheat Germ = 10.0 (flavour)
Powdery feel	The feeling of undissolved starch in vegetable products such as potatoes	Kroger Northern Beans (canned) = 6.5
Sour aromatics	Sharp, pungent aromatics that suggest a product would taste sour.	Food Club Lima Beans (frozen) = 3.0 (flavour)
Brown	Full, round, aromatic impression always characterized as some degree of darkness, generally associated with other attributes (i.e. toasted, nutty, sweet, etc.).	Bush's Pinto Beans (canned) = 3.0 (flavour)

A synthesis issued from 23 different studies (Table 1.1.2.) permits to describe beany note in pea based products with a total of 79 different terms. Among them, 41 terms involved aromatic perceptions. The main ones were pea, beans/beany, nut/nutty, grass/grassy, green, yeast/yeasty, corn, flour, grain, off-flavour, rancid and wheaty. As a result, the use of beany as a descriptor is sometimes avoided (Lawrence *et al.*, 2016, Malcolmson *et al.*, 2014). In addition, recent publications propose also to consider beany flavour not only as an odour but also as a sapid perception (Meinlschmidt *et al.*, 2016). Bitter and astringent perception are also perceived in a consensual manner and described with significant persistence in the mouth with pea protein-based products (Table 1.1.2.). Bitterness and astringency constitute an inherent part of the global and complex perception of plant-based products. The hedonic tests presented were conducted in order to compare pea protein-based products with products based on other proteins: milk protein concentrate and pea protein isolates (Mazinani *et al.*, 2020); spaghetti with various concentration of pea flour (Padalino *et al.*, 2014); wheat flour, pea starch, pea flour and pea fiber (Pietrasik & Janz, 2010); pea protein isolate and rice protein isolate (Shoaib *et al.*, 2018); pea rice blend (Ravindran *et al.*, 2011); yellow pea flour and wheat flour (Dabija *et al.*, 2017); pea pastes samples (Xing *et al.*, 2018); yellow pea flour, wheat flour and flour blend (Bouasla *et al.*, 2016); maiza, pea and barley flour (Fikiru *et al.*, 2017); pea pod flour and broan bean pod flour (Belghith-Fendri *et al.*, 2016).

Table 1.1.2.: Sensory studies carried out on pea protein-based products with the characteristics of the panel, the type of product, the used palate-cleaning, the used sensory attributes and the references.

Products	Attributes	Palate-cleansing	Methods	Panel	References
Enzymatic pea protein hydrolysates	bitter	Water and unsalted cracker	Static descriptive profiling	Trained	Humiski & Aluko, 2007
Fermented pea protein-based product	global intensity, sour, bitter, astringent, tangy, sparkling, green flavour/vegetal, leguminous plant, citrus fruit, nut, beer/yeast, sourdough, cultured apple cider)	Water and crackers			El Youssef <i>et al.</i> , 2020
Pea flour	pea, bitter, astringent, metallic	Not specified			Price <i>et al.</i> , 1985
Pea milk	odour vinegar, odour earth, odour vegetable, Texture creamy, Sensation astringent, taste sweet, taste bitter, taste acid, aroma smoked, aroma dairy, aroma pea				Yousseef <i>et al.</i> , 2016
Pea noodle	firmness, chewiness, stickiness, springiness, surface roughness, ‘floury’ texture, and overall flavour				Water and one minute
Pea protein beverages	beany, cooked green beans, saw dust, pasta, potato, cardboard and oxidized	Not specified			Trikusuma <i>et al.</i> , 2020
Pea protein-fortified extruded rice snacks	appearance (lightness, surface uniformity, shape uniformity), flavour (rice flavour, pea flavour), texture (hardness, crispness, crunchiness, adhesiveness, moisture absorption)	Slice of carrot and water			Philipp <i>et al.</i> , 2017
Pea snacks	Odour (total odour intensity, corn, peas, grain/flour, old stuffy), taste (sour, sweet, salty, bitter, umami), flavour (corn, hay/grass, pea, grain/flour, roasted, nutty, old stuffy, cloying), texture (hardness, chewiness, crispness, juiciness, granularity, pungent)	Warm and cold water, unsalted crackers			Saldanha do Carmo <i>et al.</i> , 2019
Pea sprouts	Odour (beany, rancid, grassy), taste (beany, green, fresh, rancid, bitter, astringent, pungent, grassy), texture (juiciness, fibrousness), overall quality	Room temperature water			Troszyńska <i>et al.</i> , 2007
Pea-based bread	aroma (wheaty, sweet, nutty, yeasty, and pea), flavour/taste (wheaty, sweet, nutty, yeasty, and pea), texture (firmness, initial mouth dryness, cohesiveness of mass, and adhesiveness to teeth), appearance (yellow colour)	Water	Hedonic-scaling and Static descriptive profiling	Trained	Fahim <i>et al.</i> , 2019
Pea cheese	texture, flavour, colour, appearance, and overall acceptability	Not specified		Untrained	Omrani Khiabanian <i>et al.</i> , 2020
Pea pasta	No attribute used	Not specified		Trained	Padalino <i>et al.</i> , 2014
Pea protein-cheese	flavour, texture, appearance, overall acceptance		Mazinani <i>et al.</i> , 2020		
Bologna enriched with pea protein	appearance, flavour, moistness, firmness, and overall acceptability	Room temperature water and unsalted crackers	Hedonic scaling	Untrained	Pietrasik & Janz, 2010
Chicken nuggets enriched with pea protein	firmness, colour, flavour and overall acceptability	Not specified			Shoaib <i>et al.</i> , 2018
Extruded pea–rice blends	flavour, texture, colour and overall acceptability				Ravindran <i>et al.</i> , 2011

Products	Attributes	Palate-cleansing	Methods	Panel	References
Pea cake	No attribute used	Not specified	Hedonic scaling	Untrained	Belghith-Fendri <i>et al.</i> , 2016
Pea complementary food blended	colour, texture, flavour, odour, and overall acceptability				Fikiru <i>et al.</i> , 2017
Pea pasta	No attribute used				Bouasla <i>et al.</i> , 2016
Pea pastes	colour, texture, mouthfeel, smell, taste, and metallic off-flavour				Xing <i>et al.</i> , 2018
Pea-based bread	No attribute used				Dabija <i>et al.</i> , 2017
Protein-based systems flavoured with cocoa	chocolate flavour, sweetness, off-flavour and thickness	Water	Ranking	Trained	Tarrega <i>et al.</i> , 2012
Fermented pea protein-based gels	Long list of odour attributes (41)	Not specified	CATA	Untrained	Ben-Harb <i>et al.</i> , 2020

1.1.2. Methodologies used to characterize sensory properties of pea protein based-foods

1.1.2.1. Overall sensory methodology

The sensory analysis of food is an interdisciplinary scientific discipline used to establish lawful and specific relationships between product characteristics and human perception. More specifically, sensory tests may be divided into three groups based on the type of information that they provide. The three types are discrimination, descriptive, and affective. First, discrimination tests aim to determine if some products are different or not. Second, description tests permit to quantify the perceived intensities of the sensory characteristics of products (Murray *et al.*, 2001). Then, hedonic assessment emphasises the subjective impression, such as the preference for a product. It regroups different affective tests which explores consumer likings or preference levels of the products. In the case of pea protein-based products, the literature review shows that they are mainly characterized with hedonic assessment (12/23) and static descriptive profiling (11/23) (Table 1.1.2). Half of these studies used a trained panel (13/23). To our knowledge, no study takes the temporality of perceptions into account.

1.1.2.2. Key methodology difficulties related to the beany perceptions

The multidimensional nature of beaniness of plant based products and the persistence of beaniness can be a difficulty to be described and quantified by panellists. It is therefore difficult to find adequate vocabulary and then to quantify it. Moreover, in the case where attributes are selected, it is difficult to cross-check all the dimensions of the beany. As a result, there is an increased probability of omitting a sensory dimension (Torres-Penaranda & Reitmeier, 2001). Therefore, to describe the beany perception, a classic profile-type approach is suitable but difficult to implement because of persistence and attributes that are difficult for judges to learn. Faster sensory methods may also be of interest depending on the objective of the study, its context and on the type of product (see Delarue & Lawlor, 2014).

To cope with complex food and to limit the probability of omitting a dimension, some protocols allow each panellist to add descriptors to a pre-established list of attributes as with the Mixed profiling (Coulon-Leroy *et al.*, 2017). As food presentation schemes can impact how memory is activated, other researchers have recommended adapting how samples are presented (monadically, comparatively, or accompanied by a reference) (Mazzucchelli & Guinard, 1999; Saint-Eve *et al.*, 2006). Using a stable and known reference for product evaluation could also be an interesting proposition. For example, Polarized Sensory Positioning proposes to compare and indirectly describe a set of products to three known reference products (Teillet *et al.*, 2010). The use of references is also used by the method of Pivot Profile (Thuillier *et al.*, 2015), which is based on a free expression of the difference between a tested product and an identified pivot product. For both methods, the choice of the reference product is critical to produce meaningful descriptions. As a summary, the main methods used in sensory sciences with their advantages and their limits are presented on (Table 1.1.3.). These specific methods reflect various sensory philosophies and approaches. However, with the exception of CATA and classical profiling, these methods seem to be little used in the case of pea protein based-products. \$

Table 1.1.3.: Examples of different sensory methods with their advantages and limits.

Methods family	Methods	Principle	Advantages	Limits	References
Methods based on verbal description	Classical profiling	Rating of products on an intensity scale, based on common attributes.	Intensities. Common attributes.	Long and difficult method.	Varela & Ares, 2012
	Profil Flash	Rating of products on a scale of intensity, based on individual attributes.	Intensities. Allow to work with expert.	Complex data analysis. No consensus on vocabulary.	Dairou & Sieffermann, 2002
	Check-All-That-Apply (CATA)	Selection of the attributes that best describe the product from a list of terms.	Fast method. Easy to use.	Reductionist in term of descriptors. Dichotomous data (0/1): could mask differences between products (intensity).	Dooley <i>et al.</i> , 2010
Methods based on similarity and difference measurement	Free sorting	Classification of products according to their similarities.	Fast method. Holistic non-verbal approach.	No intensity data. Difficulty in interpreting vocabulary. Requires a specific number of products (often 6 to 12).	Delarue & Lawlor, 2014
	Projective mapping or Napping©	Positioning of products in a space defined (sheet of paper) according to their similarities.	Very easy to realize for the panellists.	No intensity data. Difficulty in interpreting vocabulary.	Risvik <i>et al.</i> , 2005
Methods based on reference	Profil Pivot©	Free description of the differences between a product and a reference (called pivot).	Useful for specific products that are difficult to test simultaneously	Choice of the pivot is decisive. Tasting over several sessions implies that the pivot is sensorially stable over time.	Thuillier <i>et al.</i> , 2015
	Polarized sensory positioning (PSP)	Positioning of products in relation to 3 poles or reference products.	Holistic non-verbal approach. No limit in terms of number of products.	Choice of the poles is decisive. Tasting over several sessions implies that the poles are sensorially stable over time.	Teillet <i>et al.</i> , 2010

1.1.2.3. Key methodology difficulties related to the bitter and astringent perceptions

If consumers are aware of the concept of astringency (Childs & Drake, 2010), they have often difficulties to identify and objectively quantify astringency or to differentiate astringency from other basic tastes such as sour or bitter (Carter *et al.*, 2020). In sensory evaluation, as bitterness and astringency are often confused they request a special training (Peleg *et al.*, 1999). In addition, the prolonged persistence of bitter and astringent notes can lead to physiological adaptation and sensory fatigue (Kallithraka *et al.*, 1997). These same difficulties occur when pea proteins are part of food matrices, where composition, texture, and structure play an essential role in shaping sensory properties (Guichard, 2002; Kühn *et al.*, 2009).

To limit persistence and potential bias in sensory profiling, several articles (9/23) propose to use a specific palate cleansing protocol: to rinse their mouth with water, or with the addition of a food product (either crackers, carrot) and/or with a waiting time between each sample. But it involved very long sensory tests which could induce possibly loss of stimulus memory for panellists (Colonna *et al.*, 2004; Lee & Vickers, 2010). Some articles proposed also to limit the number of samples to test in a session.

For this type of products, to evaluate persistence presents a real interest. For that, time intensity method was widely used (Colonna *et al.*, 2004; Courregelongue *et al.*, 1999). For example, time-intensity method was used to evaluate persistence of bitterness and astringency of Gallic acid, Tannic acid, Catechin and Epicatechin solution (Robichaud & Noble, 1990).

In addition, as the perception of bitter compounds varies widely between individuals, this attribute is often difficult to assess. To take into account the sensitivity of panellists, the 6-n-Propylthiouracil (PROP) test can be used (Figure 1.1.6.). Past work has found that PROP status could be a proxy for overall oral responsiveness, which means that high PROP tasters could potentially display greater overall sensitivity to sensory properties (Carney *et al.*, 2018). Dinehart and colleagues found that people with greater PROP sensitivity gave higher bitterness scores to vegetables (Dinehart *et al.*, 2006).

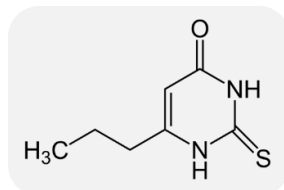


Figure 1.1.6.: Chemical structure of 6-n-propylthiouracil (PROP).

To conclude, it seems necessary to carry out a proper description and quantification to better understand the off-notes.

1.1.3. Consideration of the food oral processing and interactions for the understanding of these perceptions

1.1.3.1. Food oral processing: a dynamic process at the origin of perceptions

Food oral processing is a dynamic process involving in perception. In general, food product will go through several steps in order to be consumed. First, during the pre-oral phase, the product is cut or mixed and then taken by licking, drinking, crunching or grasping it to put it in the mouth. The second step is the oral process: food is progressively transformed into a bolus suitable for swallowing through different stages, occurring successively or simultaneously (Chen, 2009). The third and last step is the swallow, which allows the food to be transported into the stomach and digested.

Considering the second step of dynamic oral process, food and beverages can undergo many modifications (Figure 1.1.7.), depending of human physiology characteristics. They can be mechanically deformed by mastication and movements between the tongue and the palate (Stokes *et al.*, 2013). Their produced particle can be hydrated and lubricated by saliva to form a bolus (Mishellany *et al.*, 2006). They can be enzymatically degraded by salivary enzymes (Buettner, 2002; Pagès-Hélary *et al.*, 2014). These modifications depend of both the food structure and the individual physiology parameters. For example, while solids need to be fragmented and mixed with saliva to form a cohesive bolus, in the case of beverages, products can be swallowed in a shorter period of time after being diluted by saliva and warmed to body temperature (Engelen, 2003). As regards individual physiology parameters, food processing depend in particular on masticatory capacity, volume of the mouth and saliva flow.

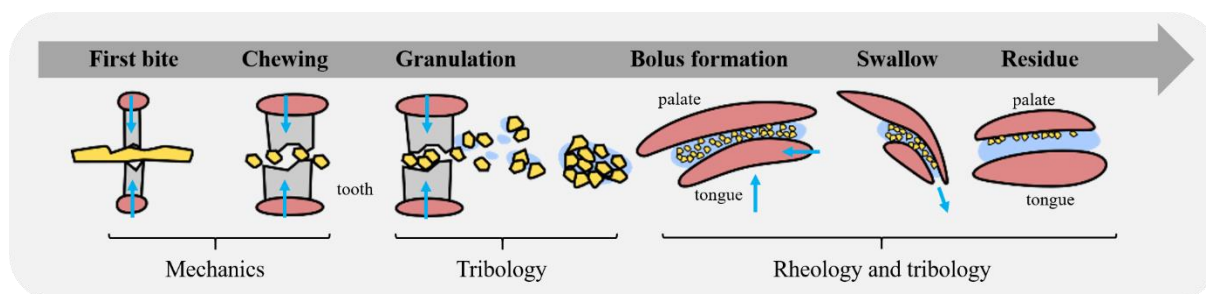


Figure 1.1.7.: Depiction of 6 key stages of the oral processing (adapted from Stokes *et al.*, 2013).

To capture the temporal dimension of the perceptions that arise during food intake, temporal sensory profiling methods are usually used. They allowed to obtain a complementary picture of the sensory experiences elicited by food products. Among the diversity of temporal methodologies, one widely adopted approach is the temporal dominance of sensations (TDS) method. It yields information on the sequence and duration of dominant sensations (Pineau *et al.*, 2009), which could be described as the sensations which attract the most attention from consumers (Schlich, 2017). The TDS method has been used in tandem with static sensory profiling to evaluate different types of products, making it possible to identify sensory characteristics that are not picked up by one method alone (Figure 1.1.8.). For example, this combined approach has proven to be useful for studying interactions between texture and aroma in model candies (Saint-Eve *et al.*, 2011); solid foods with contrasting textural layers (i.e., fish sticks (Albert *et al.*, 2012); interactions between olive oil composition and pureed beans and tomatoes (Dinnella *et al.*, 2012); the influence of aroma on taste and texture in an apple matrix (Charles *et al.*, 2017); and the key flavours perceived in strawberries (Oliver *et al.*, 2018).

Another widely used method is the TCATA. In this method, panelists are presented with a list of terms and are asked to select all the terms they consider applicable to describe the sensations they perceive at each moment of the product evaluation (Castura *et al.*, 2016). A key difference between the two methods is the concept of dominance, which is fundamental to the TDS. By focusing exclusively on the dominant attribute, the other sensory characteristics that are perceived simultaneously during the consumption of a product are not captured by the TDS. TCATA may be a better methodological choice when a detailed description of the evolution of product sensory characteristics over time is required. And TDS may be the most appropriate choice when the research question requires the identification of attributes that attract the attention of panelists at each point in the evaluation.

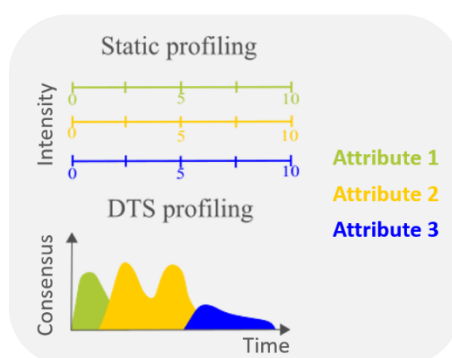


Figure 1.1.8.: Schematic representation of static profiling and temporal dominance of sensations profiling (TDS).

Another point to be noted is that static or TDS profiling method are usually applied to a single instance of food intake (i.e., one bite of a solid food or one sip of a beverage). However, in real life, food intake involves a series of instances. Several studies have shown that repeated intake of a product can change the perception of product attributes due to sensory adaptation and/or perception persistence (Lawless & Heymann, 2010). The multi-intake TDS method can provide a sensory profile for a full portion of food. It has recently proven its utility in studies evaluating the influence of wine on cheese perception (Galmarini *et al.*, 2017) and in studies characterizing the sensory properties of an oral nutritional supplement (Thomas *et al.*, 2016), fat-free strawberry yogurts (Lesme *et al.*, 2020), and yogurts with granola (van Bommel *et al.*, 2019). But to our knowledge, no TDS study was performed on plant based products.

1.1.3.2. The specific role of saliva on perceptions

Many physiological parameters are involved in the dynamics of perception (saliva, air flow, volume, masticatory capacity). In this state of the art we focused on the impact of saliva as it may have a strong impact on the bitter and astringent perceptions of plant proteins. The incorporation of saliva into the food bolus contributes greatly to the modification of the food and so of the perceptions. In addition to

dilute and hydrate food products, saliva also occurs in the perception of taste by acting either directly on the sapid compounds or on the taste receptors (Mese & Matsuo, 2007). More precisely, saliva provides protection and maturation of taste buds (Henkin *et al.*, 1999) and plays a protective role against apoptosis (Leinonen *et al.*, 2001). Saliva compounds can also stimulate taste receptors and thus change their sensitivity to sapid compounds. For example, the detection threshold of NaCl or glutamate depends on basal concentrations in saliva (Mese & Matsuo, 2007). Saliva also ensures the dilution and transport of compounds to their receptors (Spielman, 1990). Knowing that this dilution depends on the physical properties of the food in which they are present, it will be even faster than the medium is liquid. Table 1.1.4. summarizes the different impacts of saliva on sensory perceptions. The production of saliva by the different salivary glands changes during the day and can be stimulated by different factors such as chewing or acidity. Salivary flow and saliva composition varies significantly between individuals. Each individual is therefore characterized by a unique oral physiology.

Table 1.1.4.: Participation of saliva in food oral processing (adapted from Mosca & Chen, 2017).

Level	Saliva modifications	Impact on sensory perceptions	References
Structural	Surface coating, wetting and particle clustering	- Facilitate deformation and breakdown of food structures, bolus formation and swallowing - Dissolution and transport of taste compounds	Chen <i>et al.</i> 2009
Molecular	Colloidal destabilization	- Impact on rheological properties and mouthfeel sensations	Silletti <i>et al.</i> , 2008
	Enzymatic breakdown	- (Partial) hydrolysis of macromolecules - Conversion of flavour compounds - Impact on texture and flavour perception - Possible contribution to oil/fat sensations	Engelen <i>et al.</i> , 2003
	Complexation	- Impact on lubrication and friction of saliva - Rise of astringency and friction-related sensations	Canon <i>et al.</i> , 2018
	Binding of aroma compounds	- Impact on aroma release and flavour perception	Pagès-Hélary <i>et al.</i> , 2014

Saliva, in addition to participate in the mechanisms that cause astringency, can also impact other pea off-notes. It can modify the dissolution and transport of the compounds responsible for bitterness. It can also modify the release of the volatile compounds responsible for beany perceptions or converts these compounds.

1.1.3.3. Modulation of perceptions through interactions between perceptions

To understand the mechanism of perceptions, it is necessary to include two main types of interactions : cognitive interactions and physiological interactions (Figure 1.1.9.) (Keast & Breslin, 2003). Physiological interactions are due to a compound that interferes with another at the receptor cells (Keast & Breslin, 2003). Cognitive interactions are neurophysiological interactions that occur at the level of the transduction mechanism and at the convergence of afferent signals to the nucleus located in the central nervous system (Frijters, 1982). It is due to the fact that the perception of the flavour is not equivalent to a sum of individual perceptions generated by different stimuli. The cognitive interactions can be modulated by attention, habit, fatigue or emotional state but also knowledge, culture and language (Lawrence *et al.*, 2009). The classic example is the sweet characterization of a sucrose solution which can increase with the addition of a raspberry odour when only the sweet intensity is evaluated and disappears when subjects evaluate sweetness intensity and fruit intensity (Frank & Byram, 1988). These interactions occur when people eat products in natural context, however if we are interested in understanding the sensory mechanisms and to relate them to specific compounds in the food, it may be interesting to limit these cognitive interactions. To limit interactions between sapid and flavour

perceptions, some researchers have suggested that nose clips could be employed to allow panellists to focus on sapid perceptions (Abegaz *et al.*, 2004; Schoumacker *et al.*, 2017). These interactions are important in the case of products based on pea protein isolates because of the complexity of their perceptions. It is highly likely that there are strong sensory interactions between the perceptions of bitterness, astringency and beaniness. Disassociating these perceptions and understanding these interactions is so an essential step in order to reduce pea off-notes.

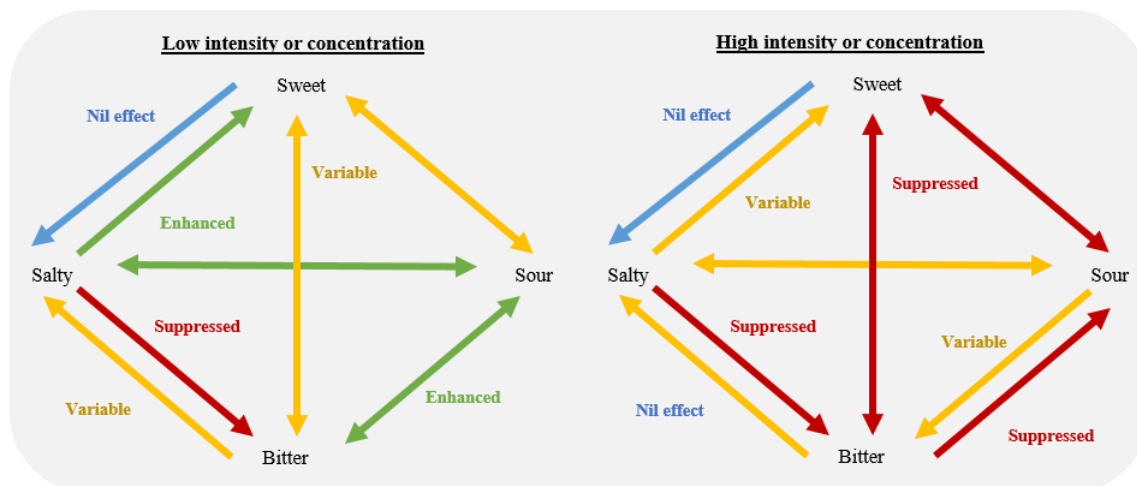


Figure 1.1.9: Schematic review of binary taste interactions. Research investigating taste–taste interactions is variable for a number of reasons and the schematic reviews are merely indications of what happens to taste qualities when two are mixed together (adapted from Keast & Breslin, 2003).

Main conclusions

- Pea protein-based products are studied most of the time in their final application matrices with hedonic tests or classical profiling.
- They are sensory described as beany, bitter or astringent with strong persistence of these notes.
- Their distinct and persistent flavour challenges their sensory evaluation and calls for the adaptation of classical methods.
- The dynamics of pea perceptions and in particular their persistence has been very little studied.
- Food formulation and oral processing (especially saliva step) may strongly affected the perceptions of pea protein-based products.
- A large number of compounds are likely to participate in the mechanisms at the origin of their perceptions either directly or via interactions of various kinds.
- Thus, to better understand the mechanisms at the origin of pea protein-based perceptions we need to look at their chemical composition.

1.2. Composition of pea protein isolates and their potential role on perceptions

Sensory properties of pea protein isolates came from various chemical compounds which act directly or via interactions on the olfactory and gustatory systems. Thus, to better understand mechanisms at the origin of perceptions of pea protein isolates, it is necessary to study their chemical composition. Pea protein isolates have a high protein content but also contain various peptides, volatile compounds, phytochemicals, carbohydrates, lipids, and minerals. All these constituents could participate to the perception of pea-based ingredients. Previous research works on the perception of pea-based products have mainly focused on the role of volatile compounds in creating sensations of beaniness. And only few studies identified amino acids and peptides that could be related to bitterness and others taste. Phytochemical compounds, as minerals and carbohydrates can also highly impacted plant product perception but their role were only few studied in literature. Thus we chose to focus this part of the state of the art on the composition of volatile, protein and phytochemical fractions to better understand possible mechanisms underlying beany, bitterness and astringency sensations. In addition, the role of other compounds (e.g. minerals and carbohydrates) on perception will be briefly discussed at the end.

1.2.1. Volatiles composition and their contribution to beany aroma

To understand the mechanisms at the origin of aroma perceptions, most of the article studied the composition on volatile compounds. A synthesis issued from 32 different studies were used to better understand volatile composition of pea protein isolates and their links to beany perceptions.

1.2.1.1. Analytical methods to study volatile compounds

To study volatile compounds composition, headspace analysis are widely used. However, a large number of extraction, concentration and analysis methods are used in the literature. The choice of the method depends on the objective of the study, its context and on the type of products and volatile compounds.

For example, to extract volatile compounds, steam distillation/extraction, supercritical CO₂ extraction, solvent assisted flavour evaporation (SAFE) (Engel *et al.*, 1999), static headspace methods such as solid-phase microextraction (SPME) (Yang & Peppard, 1994) and dynamic headspace methods such as Purge and Trap (Pinnel & Vandegans, 1996) could be used. Murat *et al.* (2013), compared three extraction methods (SPME, Purge and Trap extraction SAFE) on pea flour. Their results showed that the extracts of the volatile fraction are qualitatively and quantitatively different. The Purge and Trap extract was the richest in volatile compounds. The SPME extract was the poorest. They concluded that SAFE extraction was the most suitable method because of its good extraction capacities and its high sensory representativity of the global odour of pea flour.

As regards analysis methods, the gas chromatograph coupled with a mass spectrometer is the most widely used method. The gas chromatograph is a temperature-controlled oven that acts as a separation device. It allows sample compounds to travel through a mobile gas phase over a stationary phase and to be separated according to their retention time prior to the mass-spectrometer introduction. The mass-spectrometer allow to characterize each of the compounds individually thanks an analyser. Single, dual or triple quadrupole mass analyser, time-of-flight or ion trap analyser can be used (Giannoukos *et al.*, 2019) (Table 1.2.1.).

Table 1.2.1.: Advantages and limits of the existing molecular technologies for volatile compounds analysis (adapted from Giannoukos *et al.*, 2019).

Technics	Principle	Main field of application	Advantages	Limitations	References
GC-MS	Combination of separate of volatile compounds by gas chromatography and detection of compounds by mass spectrometry.	Food and aroma compounds detection	1. Pre-concentration 2. Low LOD (ppt-ppb) 3. High sensitivity 4. High specificity 5. High selectivity 6. Accuracy 7. Qualitative & quantitative analysis 8. Commercial libraries	1. High purchase and maintenance costs 2. Require specialised personnel for operation 3. Bulky size and high weight 4. Long analysis times SIFT-MS	Adebo <i>et al.</i> , 2020
SIFT-MS	Chemical ionization of trace volatile compounds by selected positive precursor ions during a well-defined time period along a flow tube	Disease detection	1. Real-time analysis 2. Low LOD (sub-ppb) 3. High sensitivity 4. High specificity 5. Self-calibration	1. Uncertain identification of ions 2. Limit of quantification 3. Lack of commercial libraries	Langford <i>et al.</i> , 2019
PTR-MS	On-line mass spectrometry measurements allowing the monitoring of the release of aroma compounds in the air exhaled by a subject consuming food	Food and aroma compounds detection, environment VOC detection	1. Real-time analysis 2. Low LOD (sub-ppt) 3. High sensitivity 4. High specificity 5. No sample preparation 6. Soft ionization – small number of fragments 7. No sample pre-concentration	1. Maximum measurable concentration 2. Lack of commercial libraries	Lindinger <i>et al.</i> , 1998
MIMS	Directly sampling in gaseous, liquid and solid samples through a semi-permeable membrane coupled to a mass spectrometer	Environment VOC detection	1. Near-real time analysis 2. Low LOD (ppt) 3. High selectivity 4. No sample preparation 5. Gas, liquid and soil analysis 6. Low cost 7. Low power consumption 8. Integrates with portable systems	1. Requires stable membrane temperature for reliable quantitative analysis 2. Requires temperature-programmed desorption for resolving complex mixtures	Davey <i>et al.</i> , 2011
High performance MS with ambient ionization (e.g. APCI, SESI)	ionization method which utilizes gas-phase ion-molecule reactions at atmospheric pressure commonly coupled with high-performance liquid chromatography	Food and aroma compounds detection	1. Real-time analysis 2. No sample preparations 3. MS/MS capabilities 4. Low-invasive	1. Possible contamination of new samples	Viry <i>et al.</i> , 2018
gas chromatography-ion mobility spectrometry (GC-IMS)	short multi-capillary separation column that allows separation according to gas chromatography principle	Food and aroma compounds detection	1. Real-time analysis 2. Low LOD (ppb) 3. High sensitivity 4. High specificity 5. Fast response times 6. Portability (size & weight) 7. Robustness	1. False-positive alarms 2. Lack of performance in highly contaminated chemical environments 3. High bureaucracy for the systems with radioactive sources	Wang <i>et al.</i> , 2020
E-noses	Pattern recognition of volatile compounds in idea to mimic the human olfactory receptors and their communication with the human brain	Food and aroma compounds detection	1. Fast response times 2. Low cost 3. Portability	1. Unstable results 2. Saturation effects 3. Low specificity 4. Relatively poor sensitivity	Hou <i>et al.</i> , 2020
Laser absorption spectroscopy (LAS)	Use of lasers to assess the concentration or amount of a species in gas phase in breath by absorption spectrometry	Disease detection	1. Qualitative information 2. Near real-time measurements 3. Low instrument costs 4. Sensitivity 5. Selectivity	1. Cannot resolve complicated mixtures – identification of single (or a small combination) of compounds	Nidheesh <i>et al.</i> , 2020
Nanotechnology	Specialized nano-sensors capable of detecting volatile molecules in the breath	Disease detection	1. High sensitivity 2. Fast response times 3. High selectivity	1. Cannot resolve complicated mixtures 2. Need for results cross-validation	Broza & Haick, 2013

Derived from this method, the GC-MS olfactometer allows a panelist to sniff the volatile compounds after the gas chromatograph in the same time that they are analysed by the mass spectrometer (Figure 1.2.1). This method allows to detect volatile compound that actually have an odour and thus may be important in the sensory aroma of the product (Chambers & Koppel, 2013). To take into account the dynamic of volatile compounds release during the food oral processing, Atmospheric Pressure Chemical Ionization mass spectrometry (APCI-MS) (Viry *et al.*, 2018) and Proton Transfer Reaction Mass Spectrometry (PTR-MS) allow on line measurement of volatiles release (Lindinger *et al.*, 1998).

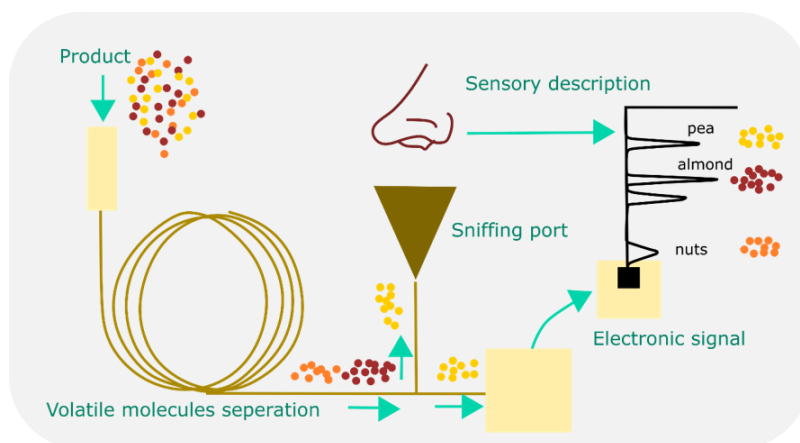


Figure 1.2.1.: General workflow of the volatile analytical pipeline by GC-MS Olfactometry (GC-O-MS): on-line mass spectrometry measurements in parallel to human perception allow the association between chemical compounds and odour and the identification of key odorous compounds (Jourden *et al.*, 2017).

1.2.1.2. Volatile compounds composition of pea protein isolates

In the literature, a large number of volatile compounds were identified on pea protein-based products or on other pulses protein-based products. Among 32 papers, which studied beany flavour, more than 200 compounds were identified (Table 1.2.2.). These compounds were extracted mainly by SPME or HS-SPME (15/32) and identified by GC-MS (25/32) with polar columns (22/32). Nine papers have studied volatile compounds by GC-O. Overall studies worked on an average of 40 volatile compounds (between 2 and 124 compounds according to the papers). These compounds belonged to many chemical families: aldehydes, ketones, alcohols, esters, carbonyl compounds, pyrazines, cyclic amines, furans and of sulphides.

In pea protein-based products, alcohols and aldehydes were often the most prominent family in terms of the number of compounds and the total quantity, followed by ketones and esters. Hexanal, 1-hexanol, and 1-octen-3-ol are often the most abundant compounds (Bi *et al.*, 2020; Murat *et al.*, 2013; Trikusuma *et al.*, 2020; Zhang *et al.*, 2020). In the literature, the main origin of these compounds is indicating coming from endogen origin of pea seed, from microbial spoilage, from thermic degradation or from oxidation of the lipids/peptides/amino acids in the seed or during the storage (Fischer *et al.*, 2020; Murat *et al.*, 2013; Sethi *et al.*, 2016; Trikusuma *et al.*, 2020). For example, they could come from the oxidation of unsaturated lipids and phospholipids (Cowan *et al.*, 1973) or the oxidation because of the lipoxygenase (Baysal & Demirdöven, 2007) which could be accelerated by oxidation and auto-oxidation (Damodaran & Arora, 2013) (Figure 1.2.2.).

Some of these volatile compounds were highlighted as key volatile beany compounds. For example Bi *et al.* identified six volatile compounds which significantly contributed to the characteristic aroma of peas. Among these, 3-methylbutanoic acid (odour activity value = 382, ratio of the concentration of an odorant to its odour threshold) and hexanal (odour activity value = 280) significantly contributed to the

aroma of peas (Bi *et al.*, 2020). Zhang *et al.* identified 6 compounds which contributed most to the characteristic beany of pea milk, among which 2-methoxy-3-isopropyl-(5 or 6)-methyl pyrazine, hexanal, (E,E)-2,4-nonadienal, and (E,E)-2,4-decadienal contributed more than others (Zhang *et al.*, 2020). Murat *et al.* (2013) identified 15 volatile compounds as described on the Table 1.2.3.

Table 1.2.2.: Volatiles studies carried out on beany aroma on pulse.

Product	Chemical methods	Extraction methods	Columns	Identified compounds	Objective of the study	References
Raw and roasted peas	GC-MS, GC-O	SPME	DB-5MS and HP-WAX	30	Identify compound at the origin of beany aroma	Bi <i>et al.</i> , 2020
Pea yogurt	GC-MS	Dynamic Headspace	DB-5MS	87	Impact of fermentation on beany aroma	El Youssef <i>et al.</i> , 2020
Yellow pea protein isolate	GC-MS	HS-SPME	ZB-WAX	6	Study beany aroma related compounds obtained at different alkaline extraction pHs	Gao <i>et al.</i> , 2020
Pea beverages	GC-MS/MS, GC-MS-O	SAFE	HP-5 column	21	Study volatile composition according the process	Trikusuma <i>et al.</i> , 2020
Pea protein enriched flour	GC-MS	HS-SPME	DB-5MS	28	Study volatile removal with aqueous solvents	Wang <i>et al.</i> , 2020
Fermented soybean curd	GC-MS	HS-SPME	DB-WAX, TR-WAX	>60	Study volatile composition according the process	Wei <i>et al.</i> , 2020
Pea and soy milk	GC-O-MS	HS-SPME	DB-WAX	80	Identify compound at the origin of beany aroma	Zhang <i>et al.</i> , 2020
Pea-protein-enriched emulsions fermented	GC-MS	Purge and trap	DB-5	74	Impact of fermentation on beany aroma	Ben-Harb <i>et al.</i> , 2020
Red kidney beans	GC-MS, GC-O	Simultaneous distillation extraction	DB-5	14	Identify compound at the origin of beany aroma	Mishra <i>et al.</i> , 2019
Chickpea, lentil, and yellow pea flours	GC-MS/O	HS-SPME	ZB-Wax	124	Study volatile composition according the germination process	Xu <i>et al.</i> , 2019, 2020
Lupin and soybean flour	GC-O	Dynamic Headspace	VF-WAX	35	Study the effect of germination on beany aroma	Kaczmarek <i>et al.</i> , 2018
Cucumbers, pepper, carrot, and peas	GC-MS	SPME	ZB-WAX	40	Study of methoxypyrazine	Mutarutwa <i>et al.</i> , 2018
Yellow pea flour	GC	HS-SPME	ZB Wax	13	Study volatile composition according the process	Vatansever & Hall, 2020
Pea pastes	GC-MS	SPME	InnoWax	36	Study volatile composition according the process	Xing <i>et al.</i> , 2018
Soybean and soymilk	GC-MS	HS-SPME	DB-WAX	10	Study the volatile composition of soymilk relatively to the volatile composition of soybean	Shi <i>et al.</i> , 2015
Pea proteins	GCMS	HS-SPME	DB-Wax	41	Study a pea proteins–maltodextrin aqueous two phases system	Nguyen <i>et al.</i> , 2014
Canola, pea and wheat proteins	GC-MS	Dynamic headspace	A VF-5 ms	6	Study protein-flavour binding	Wang & Arntfield, 2016

Product	Chemical methods	Extraction methods	Columns	Identified compounds	Objective of the study	References
Pea flour and pea isolates	GC-MS, GC-O	SAFE, SPME, Purge and Trap	Non polar ZB1.MS	87	Identify compound at the origin of beany aroma and follow their concentration along the process	Murat <i>et al.</i> , 2012;, 2013
Lupin and pea protein extract	GC-FID, GC-MS, TDS-GC-O	Dynamic headspace	Optima Wax, DB-Wax and a HP-5	56	Impact of fermentation on beany aroma	Schindler <i>et al.</i> , 2012
Soymilk	GC-FID	SPME	DB-23	8	Study effect of process on volatile composition	Zhang <i>et al.</i> , 2012
Yellow and green pea cultivars, raw flour, whole cooked peas	GC/MS	HS-SPME	VF-5MS	33	Compare the volatile profile of products and evaluate the effect of processing and storage	Azarnia <i>et al.</i> , 2011
Lupin flour	HRGC-O, HRGC-MS	SAFE	DB-FFAP	50	Identify compound at the origin of beany aroma	Bader <i>et al.</i> , 2009
Soymilk	GC-MS	headspace autosampler	CP-Wax	4	Reduce beany aroma	Blagden & Gilliland, 2005
Soymilk	GC-MS	SPME	HP-5	8	Study effect of cyclodextrin to reduce headspace concentrations beany flavour compounds	Suratman <i>et al.</i> , 2006
Soy protein isolates	FID, GC-MS, GC-MS-O	Chloroform recovered headspace	EC-Wax	14	Better understand beany aroma	Boatright & Lei, 1999
Blanched Green Peas	GC-MS, GC-O	Dynamic Headspace	WCOT fused silica	47	Study volatile composition according to storage and genotype and identify compound responsible of beany aroma	Jakobsen <i>et al.</i> , 1998
Soybean	GC	SPE	DB-624	2	Study volatile composition	Wang <i>et al.</i> , 1997
Unblanched Frozen Peas	GC-MS	Vacuum sublimation	Carbowax	117	Identify compound at the origin of beany aroma and follow their concentration along the process	Murray <i>et al.</i> , 1976
Green Peas	GC	Vacuum distillation	Carbowax	24	Compare different method to extract volatile compounds	Shipton <i>et al.</i> , 1969
Unblanched Frozen Peas	Thin-layer partition chromatogram	Distillation	NA	12	Study volatile composition	Whitfield & Shipton, 1966
Pea Blancher	GC-MS	Charcoal extraction	Carbowax	41	Study volatile composition	Ralls <i>et al.</i> , 1965

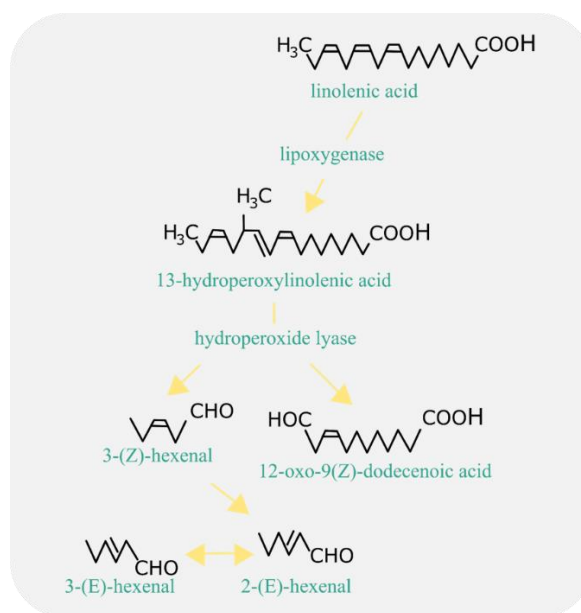


Figure 1.2.2.: Parts of the pathway of plant oxylipin metabolism leading from linolenic acid to volatile aldehydes (Baysal & Demirdöven, 2007).

Table 1.2.3.: Odorant compounds identified in the commercial pea protein extract (Murat *et al.*, 2013).

Molecules	CAS	Experimental descriptors	Theoretical descriptors
Hexanal	66-25-1	Fresh grass	Green, vegetable
3-methylthiopropional	3268-49-3	Potato	Potato, french fries
Heptanal	111-71-7	Green vegetable	Fresh, green
Hexanoic acid	142-62-1	Faeces, meat broth, sewer	Sour, fat, sweat
or dimethyl trisulfide	3658-80-8		Sulphurous, cooked, meaty, egg
1-octen-3-ol	3391-86-4	Mushroom	Mushroom, earthy, green
or 2,3-octanedione	585-25-1		Mushroom, dill, broccoli
Benzeneacetaldehyde	122-78-1	Floral	Floral
or 2-ethyl-1-hexanol	104-76-7		Citrus, fresh, floral
(E)-2-octenal	2548-87-0	Pea, dry vegetable	Green, cucumber
Heptanoic acid	111-14-8	Animal, vomit	Rancid, sour, cheese
(E,E)-3,5-octadien-2-one	30086-02-3	Vegetable, hay, earth	Woody, mushroom, green
4-ethyl-benzaldehyde and 2-methoxy-3-isopropyl-(5 or 6)-methyl pyrazine	53951-50-1	Sweet, honey, hay, dry vegetable	Sweet
Benzothiazole.	32021-41-3		Vegetable
5-butylidihydro-2(3H)-furanone	95-16-9	Grilled meat	Sauce, grilled, sulphurous, meaty
2-undecanone	104-50-7	Floral, anise, mint	Sweet, fruity, leafy
(E,E)-2,4-decadienal	112-12-9	Floral, fruity	Fruity
5-pentyl-5(H)-furan-2-one	25152-84-5	Peanut, grilled meat	Oily, nut, meat
4-hydroxy-3-methoxy-benzaldehyde	21963-26-8	Mint, chlorophyll	Mint, fruity
	121-33-5	Vanilla, caramel	Vanilla

1.2.1.3. Contribution of volatile compounds for pea protein isolates perceptions

Aromatic perceptions can be explained through the kinetics of volatile compounds from the product (transport and transfer mechanisms of the volatile compounds from the product to the air phase) to the olfactory receptors in nasal cavity. The release of volatile compounds from pea protein isolates can be due to different mechanisms. It can depend on the mobility (diffusion and convective transport) and on the partition properties of volatile compounds within each phase and between them (Poling *et al.*, 2001). Thus, the release of the volatile compounds depends on the matrix and on environmental conditions. For example, volatile compounds may more or less diffuse within the food or bolus according to the food

texture and structure of gels or macromolecules (Gostan *et al.*, 2004). Volatile compounds release depend also on their interactions with hydrophobic constituents of the matrix, such as lipids or proteins. These interactions can influence their release, in proportion relative to their hydrophobicity value (Druaux *et al.*, 1998; Relkin *et al.*, 2004).

In the case of protein based systems, interactions between volatile compounds and proteins were highlighted in the literature (Heng *et al.*, 2006). Evidence for protein/volatile compounds interactions have been found for numerous compounds as for example vanillin or 2-octanone, thanks to gas chromatography methods coupled with headspace extraction. These studies have been conducted mainly with soy proteins (Damodaran & Arora, 2013; Damodaran & Kinsella, 1981). In the case of pea, pea vicilin and pea legumin were shown also to be able to bind with volatile compounds (Heng *et al.*, 2006; Wang & Arntfield, 2016). Physico-chemical interactions between volatile compounds and non-volatile compounds of a food matrix are mainly non-covalent bonds as Van der Waals type (hydrophobic interactions, hydrogen bonds, Debye or London forces) or ionic bonds. The nature of these interactions will depend on physicochemical characteristics of the volatile compounds and matrix components considered. In the case of proteins, most volatile compounds show hydrophobic and reversible binding to proteins (Damodaran & Kinsella, 1981). These interactions can be modified by different factors: the protein conformation (denaturation level), composition, concentration...), the volatile compounds (chemical function, chain length, composition, concentration...) and the environment (pH, ionic strength, temperature...) (Andriot *et al.*, 2000; Damodaran & Kinsella, 1981; Druaux *et al.*, 1998; Wang & Arntfield, 2016). Especially, volatile compounds binding by proteins is very dependent on the conformational state of the proteins which affect both the binding affinity and the number of binding sites on proteins for volatile compounds (Damodaran & Kinsella, 1981) (Figure 1.2.3.). Protein-volatile interaction mechanisms is usually expressed as a function of the volatile headspace concentration compared to a reference (i.e. solution without proteins) as (Wang & Arntfield, 2016):

$$(a) \quad \text{Binding coefficient (\%)} = (1 - P/C) \times 100\%$$

where P (mol/L) is the headspace concentration for the protein buffer solution and C (mol/L) is the headspace concentration in the control buffer.

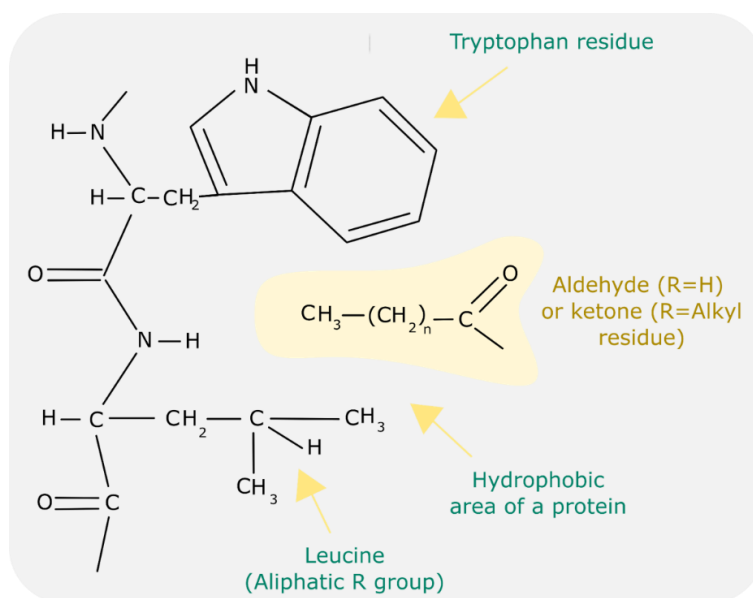


Figure 1.2.3.: Illustration of protein hydrophobic interactions with aldehydes and ketones (Wang & Arntfield 2016).

In addition, during the dynamic oral process, food and beverages can undergo many modifications. The mechanical deformation by mastication and movement between the tongue and the palate may modify the release and diffusion of volatile compounds (Stokes *et al.*, 2013). As regards the saliva, its role on volatile compounds release is described as complex and explained by several effects. Previous research have reported direct mechanisms (molecular interactions, enzymatic conversion, salting-out effect,

dilution) involving salivary components (minerals, proteins including enzymes, microbiota) and indirect mechanisms (changes of volatile compound diffusion through modification of the physico-chemical properties of the food matrix) (Buettner, 2002; Muñoz-González *et al.*, 2018; Pagès-Hélary *et al.*, 2014; Ployon *et al.*, 2017).

Main conclusions: Therefore, several studies, have already highlighted that beany notes are rooted in the complex composition of the volatile compounds found in pulses and belonging to many chemical families (mainly aldehydes, ketones and alcohols compounds). Several studies highlighted key beany-compounds such as hexanal and 1-octen-3-ol. However, this knowledge does not allow yet to control beany aroma to formulate food products with high sensory desirability. It is necessary to integrate the complexity of involved mechanisms to understand perception: complexity of volatile composition, chemical interactions with matrices and impact of the food oral processing.

1.2.2. Peptides composition and their contribution to bitterness

The high content of protein in pea protein isolates may surely participate to pea perceptions. In particular, amino acids and peptides could be related to bitterness and others taste.

1.2.2.1. Analytical methods to study peptides

More studies have been performed over the past decades to identify and quantify the taste-active peptides using sensoproteomics. The development of peptidomic technologies was allowed by modern mass spectrometry and bioinformatics. These tools are ideally suited for comprehensive peptide analysis, especially combined with the massive information available in today's genomic and transcriptomic databases. Different solvents and technologies can be used for the fractionation, separation and analysis of peptides (Table 1.2.4.). The liquid chromatograph-mass spectrometer is the most widely used method. Experimental fragmentation spectra are compared with those theoretically expected from the sequences stored in databases to identified peptides. Then, identified peptides are assigned to the proteins that contain their sequences (Langella *et al.*, 2017). Several bioinformatics tool have been developed to perform these operations: X!Tandem (Craig & Beavis, 2004), OMSSA (Geer *et al.*, 2004), Andromeda (Cox *et al.*, 2011), COMET (Eng *et al.*, 2013), Morpheus (Wenger & Coon, 2013), MS-GF+ (Kim & Pevzner, 2014).

Table 1.2.4.: Peptides studies carried out on different food products in relation with perceptions (*except Liu et al., 2015 that does not link to perceptions*).

Product	Analytical method	Reference
Brown rice protein	LC-ESI-MS/MS	Selamassakul <i>et al.</i> , 2020
Soy sauce	RP-HPLC, UPLC-Q-TOF-MS/MS	Zhu <i>et al.</i> , 2020
Bovine milk proteins	RP-HPLC, RP-HPLC-MS/MS	Iwaniak <i>et al.</i> , 2020
Stir-fried beef	LC-Q-TOF/MS	Huang <i>et al.</i> , 2019
Yeast extract	RP-HPLC, UP-HPLC-QTOL	Alim <i>et al.</i> , 2019
Bamboo shoots	UHPLC-MS	Gao <i>et al.</i> , 2019
Cocoa Beans	UPLC-TOF-MS, LC-MS/MS	Salger <i>et al.</i> , 2019
UHT milk	MALDI-TOF-MS	Dalabasmaz <i>et al.</i> , 2019
Whey protein hydrolysates	RP-HPLC, MALDI-MS	Carvalho <i>et al.</i> , 2019
Cheese	UPLC-MS/MS	Sebald <i>et al.</i> , 2018
Casein hydrolysate	UPLC-Q-TOF-MS	Murray <i>et al.</i> , 2018
Bovine muscle and porcine plasma	LC-MS/MS	Fu <i>et al.</i> , 2018
Cheese	MALDI-MS	Baptista <i>et al.</i> , 2017
Douchi	RP-HPLC-MS	Ding <i>et al.</i> , 2017
Cheese	MALDI-ToF-Pro	Silva <i>et al.</i> , 2016
Cheese	LC-MS/MS	Hillmann & Hofmann, 2016
Whey protein hydrolysates	LC-Q-TOF/MS	Liu <i>et al.</i> , 2015
Pea protein hydrolysate	UPLC-API MS/MS	Li & Aluko, 2010
Cheese	GPC, HPLC-MS/MS	Toelstede & Hofmann, 2008
Chicken broth	GPC, PFPP-HPLC, HILIC	Dunkel & Hofmann, 2009
Cheese	RP-HPLC-MS/MS	Gómez-Ruiz <i>et al.</i> , 2007
Beans	LC-MS/MS	Dunkel <i>et al.</i> , 2007

However, because of sequence redundancy (a same peptide can be included in several proteins), this raw list may contain irrelevant proteins with respect to the actual composition of the biological sample. To deal with this problem, softwares (e.g. X!TandemPipeline) have been designed to perform protein inference and to manage redundancy in the results of phosphosite identification by database search (Figure 1.2.4.). It provides a minimal list of proteins or phosphosites that are present in a set of samples using grouping algorithms based on the principle of parsimony (Langella *et al.*, 2017). However, to our knowledge, no study have analyses peptides from pea protein isolates in relation with their perceptions.

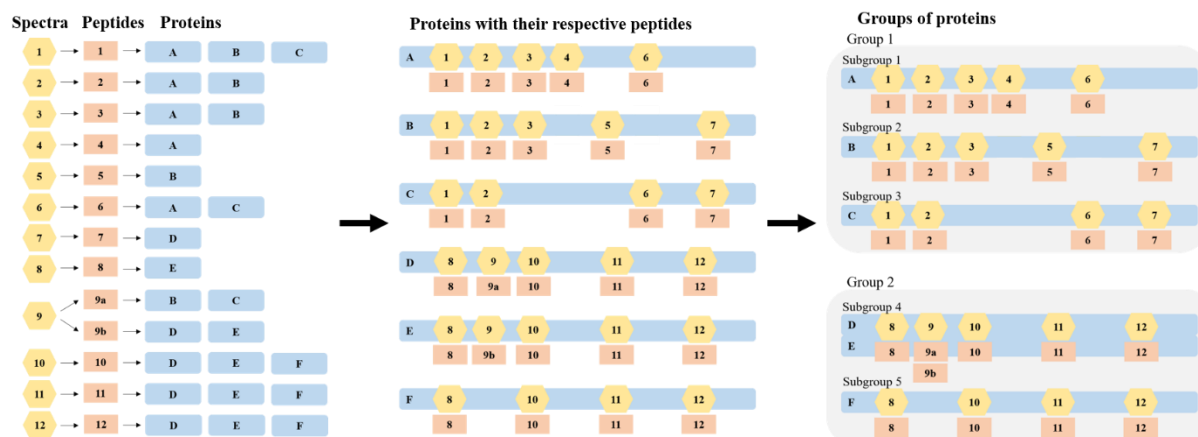


Figure 1.2.4.: Schematic representation of X!TandemPipeline postprocessing (Langella *et al.*, 2017).

1.2.2.2. Peptides composition of commercial pea protein isolates

In term of composition, pea proteins are characterized by a complete composition on essential amino acids with especially high lysine content, low tryptophan content and low sulphur-containing amino acids content (Pownall *et al.*, 2010). Composition of amino acid is detailed in Table 1.2.5. They are mainly composed of three classes of proteins: globulins (50-65%), albumins (20-40%) and so-called 'insoluble' proteins: glutelins (15-20%) (Creveu-Gabriel, 1999). The first two fractions were initially characterized by their solubility in water and in saline respectively. During the process to extract and concentrate proteins from seeds, glutelins are eliminated. It results a composition of pea protein isolates with mainly globulin and some albumins left. In addition, globulins are the main proteins reserve of the seed. They consist of two fractions characterized by their sedimentation coefficient in ultracentrifugation, 7S (20%-40%) and 11S (20-30%).

Table 1.2.5.: Composition on amino acids (Gwiazda *et al.*, 1980).

Amino acid [g/16g N]	Pea flour	Pea albumin	Pea globulin	11S	7S
Lys	6.92	9.34	6.41	5.02	7.64
His	2.30	2.63	2.55	2.65	1.95
Arg	8.28	5.67	8.00	9.18	6.75
Asp	12.25	11.9	12.99	12.19	12.31
Thr	3.65	5.66	3.34	3.41	2.54
Ser	4.79	5.03	5.30	4.89	5.43
Glu	17.41	14.95	18.66	19.66	17.32
Pro	3.90	4.46	4.36	4.89	4.01
Gly	4.29	5.97	3.89	5.14	2.78
Ala	4.06	5.85	3.97	5.34	3.07
Cys	1.39	3.15	0.80	1.14	—
Val	4.69	4.41	4.73	4.53	4.43
Met	39.00	1.34	0.70	0.73	0.28
Ile	4.23	3.86	4.39	4.25	4.83
Leu	7.20	4.87	8.23	7.78	8.79
Tyr	3.19	4.71	3.37	3.15	3.01
Phe	4.75	4.52	5.40	4.58	5.86
Try	0.95	1.47	0.64	0.70	—

The fraction 7S is constituted of vicilin and convicilin. The pea vicilin (Figure 1.2.5.) is a glycoprotein (Crevieu-Gabriel, 1999). According to Lawrence *et al.* (1994), this protein would have a structure trimerically organized along an axis of symmetry of type three. It is a trimer of 50 kDa subunits that can be identical or not, more or less modified by post-translational proteolysis and/or glycosylations. This leads to polypeptides of molecular weights from 12.5 to 35 kDa. The structure of this protein is stabilized by non-covalent forces. The amino acid composition of convicilin is close to vicilin composition, but unlike vicilin, it contains sulphur amino acids and is not glycosylated. It is a trimeric structure protein like vicilin, but whose subunits have a molecular mass of 68.2 kDa (Crevieu-Gabriel, 1999). Globally, the 7S fraction contains more lysine, leucine, isoleucine and phenylalanine.

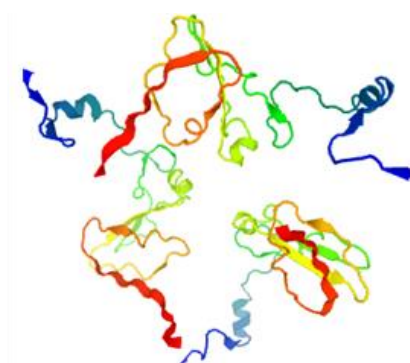


Figure 1.2.5.: Vicilin, 14 kDa modelling (swiss model, 2017).

The fraction 11S is constituted of legumins. They are hexamers of 350-400 kDa. They are not glycosylated. Each of its six subunits is consisting of an α -acid polypeptide of about 40 kDa and a basic β polypeptide of approximately 20 kDa linked by a disulfide bridge. The subunits are gathered in the hexamer in a compact structure. Polypeptides α , much less hydrophobic than β polypeptides, are located outside the molecule. The secondary structure of legumins is rich in β -sheets (Subirade *et al.*, 1994). According to Lawrence *et al.*, monomers of the 11S protein ($\alpha\beta$ groups) would have a similar structure to the monomers of the 7S protein (Lawrence *et al.*, 1994). The hexamer of the protein 11S would consist of the superposition two trimers of the type of the 7S protein. Globally, the 11 S protein contains more methionine, cystine, tryptophan and threonine (Crevieu-Gabriel, 1999).

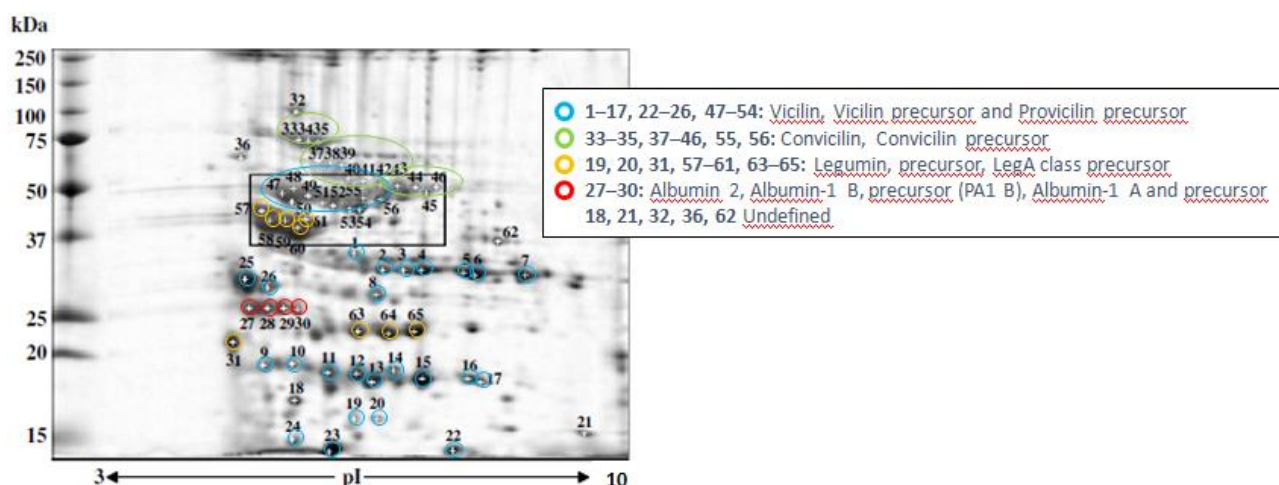


Figure 1.2.6.: Pea storage proteins (vicilins, convicilins, legumins, and albumins) in the proteome reference maps of *Pisum sativum* proteins (Pisane): 2D-electrophoresis, denaturing condition (Sirtori *et al.*, 2012).

In commercial pea protein isolates, proteins are highly denatured. This is largely due to the extraction process of pea protein isolates (pH change, high temperature). Because of this denaturation, the proteins form aggregates. In the isolates, with the protein aggregates, there are also numerous peptides of different sizes as well as free amino acids. Most vicilin are denatured at temperatures around 65°C, whereas higher temperatures are generally acceptable for legumin. The average pH of the proteins is 4.5 and the pH of the proteins suspended in water around 7. Figure 1.2.6 extracted from Sirtori et al, presents the proteomic map of a commercial pea protein isolate for the higher protein compounds at 15kDa (Sirtori *et al.*, 2012).

1.2.2.3. Contribution of peptides for pea perceptions

As we just described below, pea proteins isolates are characterized by a complex protein composition, with different proteins, numerous peptides and free amino acids. As regards native proteins, if some are known to have a sweet taste, majority of proteins does not have flavour. However, proteins can interact with many compounds, which can give them taste. In addition, although the mechanisms of receptor activation are not well known, C or N-terminus of some proteins may active the bitter receptors and so participate to the bitterness perception (Brondel *et al.*, 2013). As regards peptides and amino acids, it has been shown that they could activate the receptors of bitterness. Matoba and Hata explained the formation of bitterness in protein hydrolysates by the following mechanism. In a native globular protein, the majority of its hydrophobic side-chains may be concealed inside and cannot interact with the taste receptor cells. When the protein is hydrolysed (by enzyme actions or process actions) peptide moieties containing many hydrophobic amino acids would be exposed to the solvent and can interact with the taste receptor cells (Matoba & Hata, 1972).

However, to perceive bitterness, it would be necessary for amino acids and peptides to be on a large concentration. For example, for the bitter peptide VVYPWTQRF from Bovine hemoglobin, a 0.25 mM of peptide solution was equivalent in bitterness to 0.073mMof quinine sulphate or 21mMof caffeine (Aubes-Dufau *et al.*, 1995). Moreover all the peptides are not bitter: peptides having a size greater than 10 residues would not be able to activate the bitter receptors because of their large size. Bitter peptides would have rather a size between 5 and 6 residues (Aubes-Dufau *et al.*, 1995; Kanehisa & Okai, 1984). In addition, bitter peptides would be mainly hydrophobic peptides at their side chain, rich in proline and in leucine, especially at their C-terminal end at their C-terminal end (Kanehisa & Okai, 1984; Kim *et al.*, 2008; Shinoda *et al.*, 1987). N-terminal Arg would be also indispensable for bitterness. Kanehisa et al, studied the bitter character of peptides for hydrolysate casein. They found that the structure of the peptides would influence more the hydrophobic character than the position of basic / hydrophobic moieties. And a basic amino acid at the N-terminal position and a hydrophobic amino acid at the C-terminal position significantly enhance bitterness (Kanehisa & Okai, 1984). The review of Maehashi and Huang proposed a model for binding of bitter peptides to bitter receptors (Figure 1.2.7.). The intensity of bitterness is hypothesized to depend on two binding sites called binding unit and stimulating unit. The size of the pocket of the proposed bitter receptor would be around 15 Å, which could allow contact with a peptide of eight amino acids maximum (Maehashi & Huang 2009; Tamura *et al.*, 1990, Ishibashi *et al.*, 1988).

In addition Henriksen showed that the broth note of dried sausage could be related to a mixture of different amino acids and peptides and that the intensity of the potato note could be positively correlated with levels of tyrosine (in both its free and peptide residue forms) (Henriksen, 1997). Taste information about peptide properties can then find in databases of low-molecular-weight compounds, including BitterDB (Wiener *et al.*, 2012), and in databases of peptides such as EROP-Moscow (Zamyatnin, 2006) or BIOPEP (Iwaniak *et al.*, 2016).

Main conclusions: Therefore, pea protein-based products perceptions (especially bitterness) may be rooted in the complex composition of the proteins and peptides found in pulses. The characteristics of bitter peptides are still poorly understood. However, the development of peptidomic technologies (modern mass spectrometry and bioinformatics) in relation with sensory analysis could allow the identification of key bitter peptides.

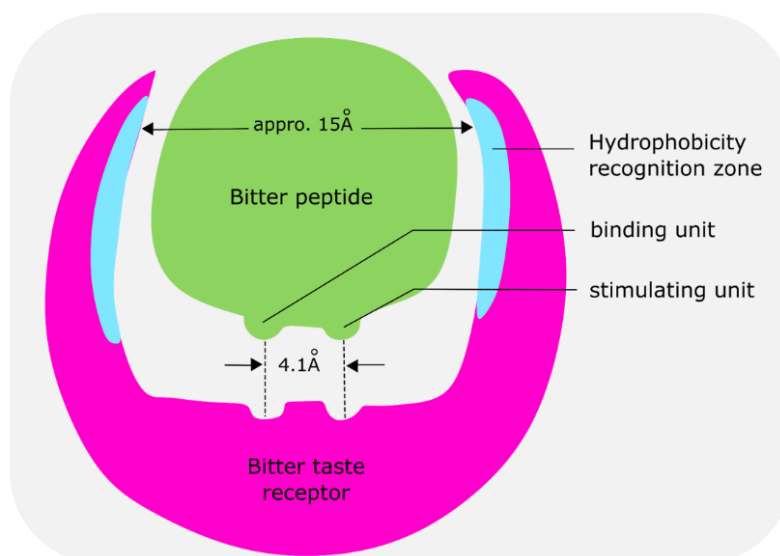


Figure 1.2.7.: Model for binding of bitter peptides to bitter receptors (Maehashi & Huang 2009).

1.2.3. Phytochemicals composition and their contribution to bitterness and astringency

Pea protein isolates contain some phytochemical compounds such as phenolic acids, flavonoids, and saponins. These compounds are being increasingly studied because of their health benefits (antioxidants, enzyme detoxification agents, immune system stimulants, lipid, hormone metabolism regulators and anti-angiogenic agents) (Singh *et al.*, 2017). However, these compounds could also contribute to bitterness and astringency of pea protein-based products.

1.2.3.1. Analytical methods to study phytochemical compounds

The identification and characterization of phytochemicals were increased by the development of modern mass spectrometry and bioinformatics. Different solvents and technologies can be used for the fractionation, separation and analysis of phytochemical compounds (Table 1.2.6.). The liquid chromatograph-mass spectrometer is the most widely used method in tandem with NMR Spectroscopy.

However, comprehensive identification or annotation of phytochemicals remains a real challenge because of 1) the high diversity of compounds; 2) the limited availability of authentic standards for peak identification in tandem mass spectrometry; 3) the great number of modifications of the structure that can occur in the seed and then during food processes; and 4) the highly complex fragmentation during mass spectrometry analysis of these compounds which is largely influenced by the aglycon but also of substituents (Akimoto *et al.*, 2017).

Nevertheless recent data bases are developing. For example, Akimoto *et al.* (2017) developed a system for annotating flavonoids (FlavonoidSearch), which consists of a database of virtual mass fragments (FsDatabase) predicted from 6,867 known flavonoid structures in one of the largest flavonoid databases (metabolomics.jp) and a computational tool (FsTool) to search the database. The objective of this data base is to allow a comprehensive annotation of flavonoids and so to give lists of MSMS-aglycones and substituents for the identification of novel flavonoids (Akimoto *et al.*, 2017).

Others free data bases are also available online:

- <http://webs2.kazusa.or.jp/msmsfragmentviewer;>
- <http://spectra.psc.riken.jp;>
- http://www.knapsackfamily.com/KNAPSAcK_Family;
- [http://metabolomics.jp/wiki/Category:FL.](http://metabolomics.jp/wiki/Category:FL)


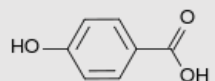
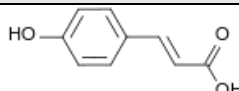
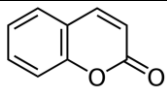
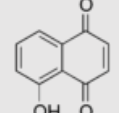
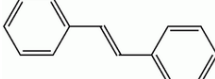
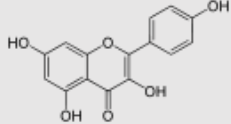
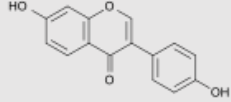
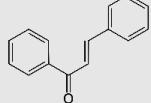
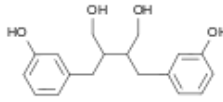
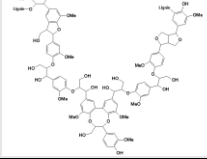
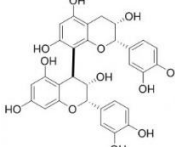
Table 1.2.6.: Studies carried out on phytochemical compounds on various plant proteins through different methods.

	Product	Chemical technics	References
Total phenolics and/or total flavonoids	Pea	Colorimetric method and/or enzymatic method	Zhao <i>et al.</i> , 2020
	Fava-bean, green-pea, yellow-pea flour		Millar <i>et al.</i> , 2019
	Field pea, lentil, bean and chickpea		Padhi <i>et al.</i> , 2017
	Pea peel		Hadrich <i>et al.</i> , 2014
	Chick pea, green pea		Nithiyanantham <i>et al.</i> , 2012
	Lentil, pea hulls		Oomah <i>et al.</i> , 2011
	Pea protein isolates		Agboola <i>et al.</i> , 2010
	Lentils, chickpeas, peas, soybeans		Han & Baik, 2008
	Field pea, grass pea		Wang <i>et al.</i> , 1997
	Pea seed		Bishnoi <i>et al.</i> , 1994
Overall phenolics	Pea	HPLC–UV	Nazir <i>et al.</i> , 2020
	Pea hulls	UHPLC-LTQ-OrbiTrap-MS	Guo <i>et al.</i> , 2019
	Oat	HPLC-DAD	Soycan <i>et al.</i> , 2019
	31 Pulses	HPLC-MS/MS, dynamic-MRM triple quadrupole	Caprioli <i>et al.</i> , 2018
	36 Pulses	HPLC-DAD	Giusti <i>et al.</i> , 2018
	Pea	UHPLC-LTQ OrbiTrap MS	Stanisavljevic <i>et al.</i> , 2016
	Grass pea, lentils, chickpea	UHPLC-MS	Fратиanni <i>et al.</i> , 2014
	Pea shoots, ready-to-eat baby-leaf	HPLC-DAD-ESI-MSn, HR-CS-AAS	Santos, <i>et al.</i> , 2014
	Pea seed	HPLC	Dvořák <i>et al.</i> , 2011
	Pea	HPLC-DAD, HPLC-(ESI)MS	Duenas <i>et al.</i> , 2004
Flavonoids	Canola, rapeseed protein isolates	LC–MS/MS	Hald <i>et al.</i> , 2019
	Pea, fava bean	HPLC-DAD-ESI-MSn	Neugart <i>et al.</i> , 2015
	Kale	HPLC-DAD/ESI-MSn	Schmidt <i>et al.</i> , 2010
	Spinach	ESI-LC-MS, NMR	Brock & Hofmann, 2008
	Soy and plant extract	HPLC/ESI-MS	Klejdus <i>et al.</i> , 2007
	Soy flakes, soy protein isolate, soy germ	HPLC	Aldin <i>et al.</i> , 2006
	Pea shoot	NMR, HPLC, FAB-MS	Ferrerres <i>et al.</i> , 1995
	Soybeans	HPLC, TLC, UV, MS, IR, NMR	Kudou <i>et al.</i> , 1991
Saponins	Pea	1D/2D-NMR, (LC-)MS/MS, LC-TOF-MS	Gläser <i>et al.</i> , 2020
	Oat	LC-TOF-MS, LCMS/MS, 1D/2D-NMR	Günther-Jordanland <i>et al.</i> , 2016
	Pea	HPTLC-MS	Reim & Rohn, 2015
	Asparagus	HPLC–MS/MS, LC-MS/MS, LC-TOF-MS, 1D/2D-NMR spectroscopy	Dawid & Hofmann, 2014
	Soy flakes, soy protein isolate, soy germ	HPLC	Aldin <i>et al.</i> , 2006
	Pea	RP-HPLC	Heng <i>et al.</i> , 2006
	Soybeans	HPLC, LCQ-MS	Decroos <i>et al.</i> , 2005
	Soybeans	HPLC, LCQ-MS, NMR	Berhow <i>et al.</i> , 2002
	Pea	HPLC, LCMS	Daveby <i>et al.</i> , 1998
	Mung bean, sorghum, barley, peas	GC-MS, FAB-MS, 2D-NMR	Ohana <i>et al.</i> , 1998
	Soybeans	HPLC, NMR	Kudou <i>et al.</i> , 1992
	Soybean seed	HPC, TLC, IR, MS, NMR	Shiraiwa <i>et al.</i> , 1991
	Chick Pea, Haricot Bean, Red Kidney Bean	HPLC	Ireland, 1987
	Pea and soya	LC-MS	Curl <i>et al.</i> , 1985
	Pea flour, soybean flour	RP-HPLC, TLC-MS	Price <i>et al.</i> , 1985
	Food plant	TLC	Fenwick & Oakenfull, 1983

1.2.3.2. Phenolics composition of pea protein isolates

Phenolic compounds constitute one of the most numerous and ubiquitous groups of plant metabolites. Ranging from simple phenolic compounds to highly polymerized compounds with molecular weights greater than 30,000 Da, the occurrence of this complex group of substances in plant foods is extremely variable (Bravo, 1998). Table 1.2.7. presents the main classes of phenolic compounds based on carbon structure.

Table 1.2.7.: Main classes of phenolic compounds based on carbon structure (adapted from Cheynier *et al.*, 2013)

Formula	Family	Example	Structure
C ₆	Phenols	Hydroquinone	
C ₆ -C ₁	Hydroxybenzoic acid	Parahydroxybenzoic acid	
C ₆ -C ₃	Hydroxycinnamic acid	Paracoumaric acid	
	Coumarin	1-benzopyrane-2-one	
C ₆ -C ₄	Naphtoquinone	Juglon	
C ₆ -C ₂ -C ₆	Stilbenoid	Stilbene	
C ₆ -C ₃ -C ₆	Flavonoid	Kaempferol	
	Isoflavonoid	Daidzein	
	Anthocyane	Chalcone	
(C ₆ -C ₃) ₂	Lignan	Enterodiol	
(C ₆ -C ₃) _n	Lignin		
(C ₆ -C ₃ -C ₆) _n	Condensed tannin	Procyanidine	

In pulse, different polyphenols have been identified. For example Fratianni *et al.* (2014) identified gallic acid, chlorogenic acid, catechin, caffeic acid, epicatechin, coumaric acid, rutin, ferulic acid in grass pea and in chickpea. Kaempferol and quercetin glycosides, sophotriosides were also identified in leaves of *V. faba* and *P. sativum* (Neugart *et al.*, 2015), and phenolic acids were found in soy (Arai *et al.*, 1966; Damodaran & Arora, 2013). Among the phenolic compounds, flavonoids and especially isoflavones (Figure 1.2.8) have been studied in pulse. Isoflavones are substituted of 3-phenyl-chromen-4-ones and their conjugates. They are phytochemicals that are found in many plants and plant-derived foods in both native (“aglycon”) form and as acetyl-, or malonyl-, etc., β -glucosides. For example they are present in soymilk, tofu, miso, and tempeh with average total isoflavone contents of 0.37, 1.00, 0.84, and 2.00/moles isoflavones/g product, respectively (Aldin *et al.*, 2006). Specific isoflavone like Genistein, daidzein, and glycitein have especially been identified in soy (Aldin *et al.*, 2006; Klejdus *et al.*, 2007).

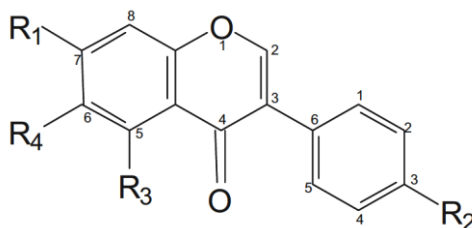


Figure 1.2.8.: Isoflavone representation_(Klejdus *et al.*, 2007)

In *Pisum sativum*, more than 110 structurally different phenolic compounds have been described in the literature with the majority being glycosylated flavonols, along with their related counterparts (e.g. flavones, flavanols, flavanones, anthocyanins and isoflavonoids). A great number of sophorotriosides quercetin and 3-O-glycosides of kaempferol were identified (Fahim *et al.*, 2019). In pea seed, according to Padhi and al, the polyphenols are concentrated in the cotyledon. The total polyphenol content was 1-4 mg gallic acid equivalent/ g dry weight and the total carotenoid content 20-40 micro g/g dry weight (Padhi *et al.*, 2017). In protein isolates, according to Damodaran et Aora, in soy more than 90% of phenolic acids are removed from soybean during extraction of proteins (Damodaran & Arora, 2013) so we can make the hypothesis that pea protein isolates contains less polyphenols than pea seed. The presence of phenolic compounds within the pea can also be reduced through additional steps during processing, such as the use of charcoal filters and alcohol washes (Lam *et al.*, 2018).

1.2.3.3. Saponins composition of pea protein isolates

Saponins are non-volatile, amphiphilic, surface-active sterol or triterpene glycosides present in a wide variety of plants (Fenwick & Oakenfull, 1983). They consist in non-polar aglycones coupled with one or more sugar chains (Heng *et al.*, 2006). In pulse, triterpene glycosides have been commonly identified (Barakat *et al.*, 2015). They are categorized as monodesmosides which have a single sugar chain linked to the aglycone and as bidesmosides which have two sugar chains (Lásztity *et al.*, 1998). According to the plant species, they present variation in their structure (type and composition of the aglycone and the sugar chains). They are classically categorized into A, B & E group saponins according to their aglycone structures (Singh *et al.*, 2017). In pea cultivar, saponin content was found in the range of 0,8-2,5 g/kg depending of the varieties (Daveby *et al.*, 1998; Heng *et al.*, 2006). In pea flour, Fenwick and oakenfull reported approximately 2.5 g/kg of saponins (Fenwick & Oakenfull, 1983) and Curl *et al.* (Curl *et al.*, 1985) around 1,8% (w/w). In soy protein isolates, studies reported that most of the saponins are recovered because, unlike isoflavones, they are heat stable and resist losses during processing. Nevertheless, in pea protein isolates, it appears that concentration of saponins is very low compared to soy protein (Heng *et al.*, 2006).

Peas contain two types of saponins: saponin B and DDMP saponin (Figure 1.2.9.). They are monodesmoside saponins with a sugar chain linked to the C-3 position of the aglycones. DDMP saponin has in addition a 2,3-dihydro-2,5-dihydroxy-6-methyl-4Hpyran- 4-one (DDMP) group at the C-22 position (Heng *et al.*, 2006). Some studies report that DDMP saponin could be converted on saponin B, corresponding to the release of a maltol, during extraction and processing and especially in acidic or

basic solutions (Berhow *et al.*, 2002; Heng *et al.*, 2006). So varieties, harvesting, process condition and storage conditions could influenced the ratio of saponin B/DDMP saponin. Heng *et al* investigated saponin content and composition of 16 dry pea varieties. They found that in two varieties, DDMP saponin was the only saponin present whereas in the rest of the varieties, DDMP saponin was more abundant than saponin B. The amounts of DDMP saponin ranged from 0.7 to 1.5 g/kg (dry matter), whereas those of saponin B ranged from 0 to 0.4 g/kg (Heng *et al.*, 2006).

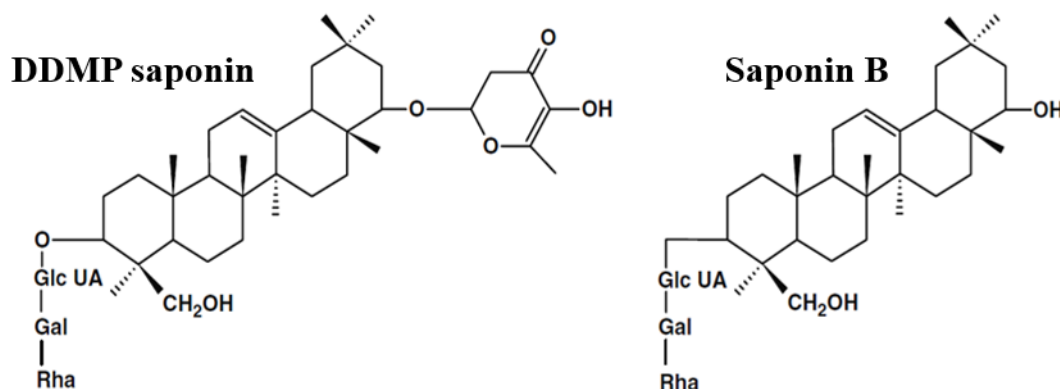


Figure 1.2.9.: Structures of saponin B and DDMP saponins (Heng *et al.*, 2006).

1.2.3.4. Contribution of phytochemical compounds for pea perceptions

Polyphenols and especially flavonoids are described in the literature as contributing to bitterness and astringency. However, the majority of these studies deal with polyphenols of red wine and are concerned by tannins, tannic acid polymer fractions and flavan-3-ol monomers / dimer / trimers (Drewnowski & Gomez-Carneros, 2000; Hufnagel & Hofmann, 2008; Symoneaux, 2015). So there are not much studies on the bitter taste of polyphenols that do not belong to tannin classes, such as kaempferol derivatives. Regarding their structure, it seems that the conformation of polyphenol compounds plays a role in the activation of bitterness receptors. (+) - catechin would activate the TAS2R14 & TAS2R39 receptors (Roland *et al.*, 2013) while one of these isomers (-) - epicatechine would activate the TAS2R4, TAS2R5, TAS2R14 & TAS2R39 receptors (Roland *et al.*, 2013) (Figure 1.2.10). Moreover, the size of the polyphenol compounds would also play a role in the activation of bitterness receptors. Polymer with a high degree of polymerization would tend to be less bitter and more astringent whereas polymers with a low degree of polymerization would tend to be more bitter and less astringent (Brossaud *et al.*, 2001; Peleg *et al.*, 1999; Robichaud & Noble, 1990). In addition, the presence of galloyl groups on epicatechin could modify receptor activation (Yamazaki *et al.*, 2013). The aglycone isomers of isoflavones, being more hydrophobic, tend to be more adapted to receptors than their glucoside counterparts (Okubo *et al.*, 1992).

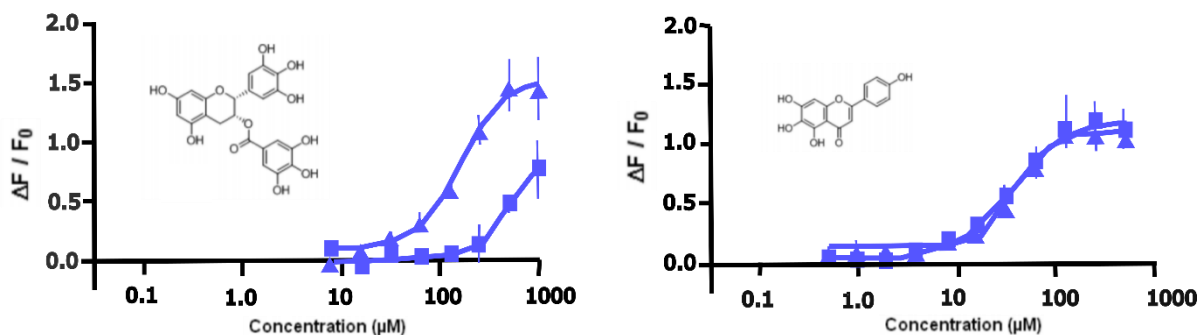


Figure 1.2.10.: Dose-response curves of epigallocatechin gallate (left), and scutellarein (right) on bitter receptors hTAS2R14 (■) and hTAS2R39 (▲) (Roland *et al.*, 2013).

As regards saponins, like tannins, they are also generally referred to bitter and astringent perceptions (Heng *et al.*, 2006). Price *et al.* (1985) estimated that the concentration of soyasaponin I for which the undesirable bitter and astringent tastes were rated as moderate (score 6-0) by a panel, was approximately 0.08% in pea flavour. In Heng *et al.* study, bitterness of saponins was perceived by the sensory assessors at very low concentrations around 2 mg L⁻¹ for saponins mixture (Saponin B and DDMP saponin in a ratio of 1:4) and around 8 mg L⁻¹ for saponins B. According to Heng *et al.*, both saponins have a bitter taste, in which intensity increases with concentration, but the DDMP saponin is significantly bitterer than the saponin B. Therefore, pea varieties having a high DDMP saponin content or a high total saponin content can likely taste more bitter than those with a low content in saponins (Heng *et al.*, 2006). Gläser *et al.* (2020) studied the bitterness of saponins in pea protein isolates, and their results highlighted that not saponins but 14 bitter lipids and lipid oxidation products such as 9,10,13-trihydroxyoctadec-12-enoic acid were implied in this perception.

In addition several studies showed that these non-volatile compounds could interact with proteins. Phenolics could interact with pea protein and thus impact the flavour. According to Damodaran and Arora (2013), phenolics are mostly present in the protein-bound state and remain associated with proteins during the manufacture of soy isolates protein. Recent studies showed that tannins were likely to interact with lipids with consequences on the perception of bitterness. Studying at the molecular level in wine, tannin-lipid interactions, by disrupting the lipid environment of taste receptors embedded in the oral membranes, could affect the functionality of the receptor and its interaction with tannins, and therefore bitterness. Furthermore, a possible competition between the tannin-lipid and tannin-saliva protein interactions could reduce the astringency during the tasting of a wine (Saad *et al.*, 2016).

Saponins could also interact with protein. For example they can interact with casein and soy proteins (Potter *et al.*, 1993) to form complexes with proteins at low pH in Beet (Morton & Murray, 2001). These interactions of saponins with proteins may affect the interactions of flavour compounds with the proteins. Saponins may occupy the available binding sites on proteins and lead to less flavour compounds that can be bound to the proteins. On the contrary, it may induce more binding of flavour compounds by creating hydrophobic pockets when it itself is bound to the proteins (Heng *et al.*, 2006).

Main conclusions: Therefore, pea protein-based products bitterness and astringency may be root in the complex composition of the phytochemicals present in pulses. The characteristics of these compounds are still poorly understood. However, the development of peptidomic technologies (modern mass spectrometry and bioinformatics) in relation with sensory analysis could allow the identification of key bitter and astringent compounds.

1.2.4. Other pea compounds and their contribution to perceptions

Pea protein isolates also contain various lipids, carbohydrates, or minerals. The most important lipids in pea are linoleic acid, oleic acid, palmitic acid, and linolenic acid (Fischer *et al.*, 2020). Lipids have been particularly studied in peas, especially the unsaturated ones, because they are the substrates of several volatile compounds known to be beany. For example, linoleic acid leads to the formation of hexanal, and oleic acid to the formation of octanal (Murray *et al.*, 1976; Fischer *et al.*, 2020). They can also be responsible for sapid perceptions. For example Gläser *et al.*, (2020) identified 14 lipids and lipid oxidation products related to bitter perceptions. In addition, pea seed and pea flour are a good source of carbohydrates (Millar *et al.*, 2019). However the main carbohydrates are washed during the wet process of pea protein isolates (Schutyser *et al.*, 2015). And there is little data in the literature on the impact of the left carbohydrates on perceptions for pea protein isolates. Pea seed and pea flour are also a good source of minerals such as calcium, iron, potassium, manganese, phosphorus and zinc (Millar *et al.*, 2019; Wang *et al.*, 2008). However, variety and processing (soaking, cooking and dehulling) affected the composition in mineral. Soaking and cooking increased calcium, copper, magnesium and

phosphorus content in peas and reduced iron, potassium magnesium and zinc content in peas. Dehulled seeds had higher potassium and phosphorus content and lower calcium, copper, iron, magnesium and manganese than raw seeds (Wang *et al.*, 2008). The mineral concentrations in the protein isolates are therefore likely to be modified compared to the seed and the flour. As regards minerals perceptions, most mineral have taste properties. Bitter and astringent tastes are usually used to describe aluminium, calcium, copper, iron, magnesium, potassium, sodium or zinc solutions. In addition some minerals (e.g. zinc and copper), are necessary to maintain normal chemoreception (Delompré *et al.*, 2019). Minerals can also have an indirect role by modifying pH and ionic strength. The modification of these chemical parameters can impact the interactions with proteins and therefore the perceptions of bitterness and astringency.

Main conclusions

- A synthesis issued from 32 different studies shown that pea isolates are composed to more than 100 volatile compounds belong to many chemical families.
- A synthesis issued from 45 different studies shown that pea seed have a complex and not fully known composition on phytochemical compounds.
- Pea isolates have also a complex composition on proteins, peptides, lipids, minerals and carbohydrates and few studies have studied it.
- From a chemical point of view, the development of technologies (modern mass spectrometry and bioinformatics) could allow to go further in identification of these compounds.
- However, if several studies highlight key beany-compounds (such as hexanal and 1-octen-3-ol), the mechanisms at the origin of the taste of pea protein are still not fully known (especially bitter and astringent perceptions).
- Thus, to better understand the mechanisms at the origin of pea protein-based perceptions, it is necessary to examine how perceptions of pea protein isolates arises from both volatile and non-volatile compounds and their potential interactions.

1.3. Implemented strategies for a better understanding of mechanisms behind perceptions

As highlighted in the present state of art, the mechanisms at the origin of perceptions of food are multimodal and complex. In this last bibliographic section, the strategies used in the literature to lead to a better understanding of the mechanisms behind perceptions will be presented and discussed. To link sensory properties to food product composition, several types of strategies were identified in literature and will be presented in this section: strategies based on product deconstruction, chemical strategy, receptor strategy and statistical modelling. These methods are briefly exposed in the Figure 1.3.1. and will be presented in more details on the following sub-sections.

Thus, this last bibliographic section deals with research strategies in sensory perception. Beyond the theme addressed, it leads to question the acceptability of plant proteins in relation to societal expectations, or even the link with nutritional properties. To go further, it would be interesting to question the impact of these sociological and/or physiological factors on sensory perception. In particular, it would be interesting to consider whether these factors may not constitute a limit for the models that are developed to understand perceptions.

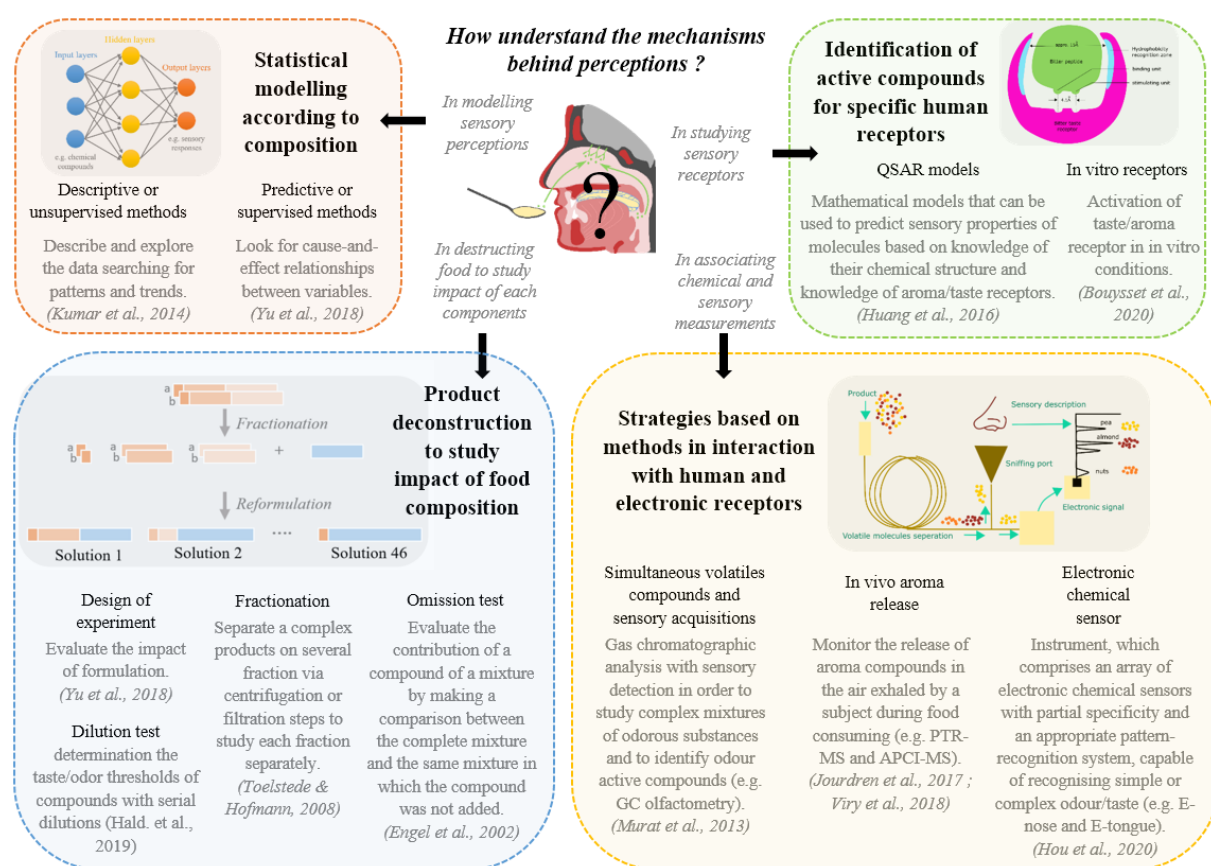


Figure 1.3.1.: Examples of strategies used in the literature in order to lead to a better understanding of the mechanisms behind perceptions doing links between sensory and chemical data.

1.3.1. Strategies based on product deconstruction to study impact of food composition on human perceptions

Deconstruction strategies focus on the evaluation of the contribution of an ingredient or a factor (for example temperature of a process), a molecule or a group of molecules to the food perceptions. In these

strategies, the product is modified according to a fractionation process, an addition or omission of molecules. Different methods can be used according to the objectives and studied object (investigation of ingredient or chemical composition).

1.3.1.1. Design of experiment strategies

At ingredient level, combination of experimental design and predictive models are commonly used. The data collection are often planned following an experimental design (Vatansever *et al.*, 2020; Fikiru *et al.*, 2017; Symoneaux *et al.*, 2015). The design of experiments allows to study input parameters (predictor variables) in generating output variables (response variables), taking interactions into account that may exist between the variables. They are usually represented by a matrix with columns representing the independent variables and rows representing samples or experimental runs. They are built up of different steps as explained in the Figure 1.3.2. Several methods of experiment designs are used to link sensory properties and composition of food products. Their choice depends on the research question, type and number of variables, and number and type of food products. The most widely used methods are factorial designs. They are based on a combination of factors. They are orthogonal and balanced designs and allow the estimation of main factors and interaction factors. However, they often involve a large number of experimental runs or samples. To reduce the number of runs/samples while minimising the degree of aliasing, optimal designs are sometime used (Yu *et al.*, 2018).

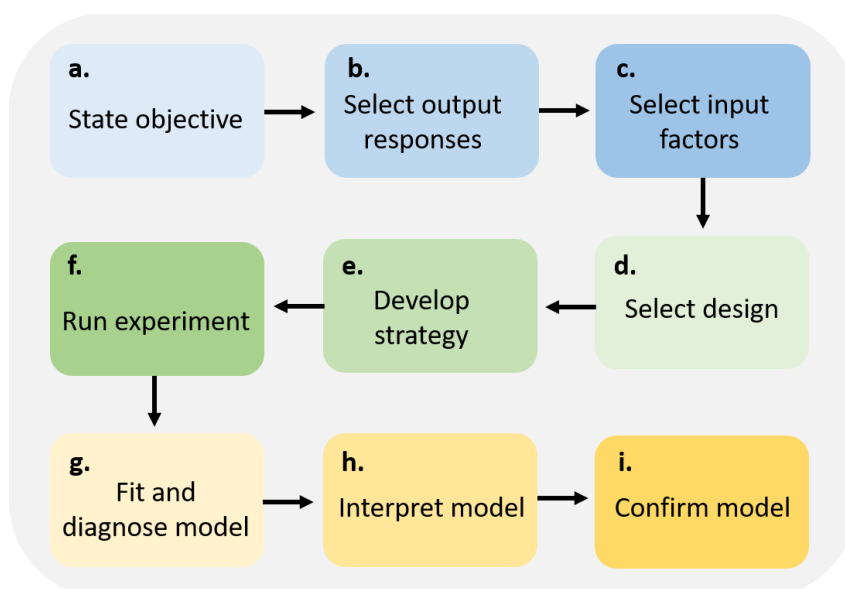


Figure 1.3.2.: Flowchart of an experimental design combined with a model.

1.3.1.2. Strategies of fractionation, omission or dilution test strategies

At molecular level (or group of molecules), several strategies are used either separately or in combination: fractionation, omission or dilution test. The aim of fractionation strategy is to separate a complex products into several fractions via mechanical processes (as centrifugation, filtration steps), then to evaluate each obtained fraction with sensory and instrumental measurements. The links between the chemical data and the perceptions of these different fractions are thus studied, in order to identify the key molecules at the origin of perception (Engel *et al.*, 2002). As an example, Sebald *et al.* used a fractionation strategy for the identification of bitter peptides in fresh cheese. This study allowed the identification of 17 peptides as bitter peptide candidate. Among them, 15 of the 17 target peptides showed bitter taste thresholds ranging from 30 to 690 $\mu\text{mol/L}$. Finally, the evolution of the bitter peptides throughout two different manufacturing processes of fresh cheese was quantitatively recorded (Sebald *et al.*, 2018).

Omission tests are often associated to reconstitution tests (Hillmann & Hofmann, 2016; Ferreira *et al.*, 2002). Reconstitution consists in constructing a synthetic model mixture with composition and perceptions as close as possible to the raw product. Omission test consists in evaluating the contribution of one compound or several compounds in a synthetic mixture to perceptions by making a sensory comparison between the perceptions of the complete mixture and the perceptions of a mixture in which one compound or several compounds were omitted (Engel *et al.*, 2002). Several studies have shown that taste or aroma perceptions may be similar for a raw product and a product obtained with a fraction-based reconstruction.

Table 1.3.1.: Examples of fractionation, omissions, and reconstitution or dilution tests carried out on food products to link chemical composition and perceptions.

Main chemical molecules identified	Product	Main strategy	Reference
Alapyridaine	Beef broth	Fractionation, reconstitution	Ottinger & Hofmann, 2003
Amino acids	Dairy protein hydrolysates	Fractionation	Murray <i>et al.</i> , 2017
Amino acids, minerals, lipids, organic acid, volatiles	Cheese	Omission	Engel <i>et al.</i> , 2002
Amino acids, organic acids		Fractionation and omission	Andersen <i>et al.</i> , 2010
Amino acids, organic acids, fatty acids, biogenic amines, minerals		Reconstitution, dilution and omission	Hillmann & Hofmann, 2016
Avenanthramides and saponins	Oat	Fractionation and dilution	Günther-Jordanland <i>et al.</i> , 2016
Peptides	Beans	Fractionation and dilution	Dunkel <i>et al.</i> , 2007
	Beef stir-fried	Fractionation	Huang <i>et al.</i> , 2019
	Brown rice protein	Fractionation	Selamassakul <i>et al.</i> , 2020
	Casein hydrolysate fractions	Fractionation	Murray <i>et al.</i> , 2018
	Cheese	Fractionation and dilution	Toelstede <i>et al.</i> , 2009
	Cheese	Fractionation	Gómez-Ruiz <i>et al.</i> , 2007
	Cocoa beans	Fractionation and dilution	Salger <i>et al.</i> , 2019
	Cooked Crustaceans	Fractionation and dilution	Meyer <i>et al.</i> , 2016
	Fermented foods	Fractionation and dilution	Sebald <i>et al.</i> , 2018
	Soy sauce	Fractionation	Zhu <i>et al.</i> , 2020
Phenolics	Yeast extract	Fractionation and dilution	Alim <i>et al.</i> , 2019
	Canola/Rapeseed Protein Isolates	Fractionation and dilution	Hald <i>et al.</i> , 2019
	Cheese	Fractionation and dilution	Toelstede & Hofmann, 2008
Saponins	Hops	Fractionation, reconstitution and dilution	Dresel <i>et al.</i> , 2015
	Asparagus	Fractionation and dilution	Dawid & Hofmann, 2014
	Pea protein isolates	Fractionation and dilution	Gläser <i>et al.</i> , 2020
Volatiles	Cheese	Reconstitution	Thomsen <i>et al.</i> , 2017
	Pellets of hop varieties	Fractionation and dilution	Brendel <i>et al.</i> , 2019
	Raw and Roasted Peas	Omission	Bi <i>et al.</i> , 2020
	Soy sauce	Omission	Feng <i>et al.</i> , 2015
	Wine	Dilution analysis, omission, reconstitution	Ferreira <i>et al.</i> , 2002
Volatiles, fatty acids	Cheese	Omission	House & Acree, 2002

For example, artificial ikura (Japanese salmon caviar) was prepared using vegetable oil and a low-calorie natural gel (e.g., one made with alginic acid) (Hayashi *et al.*, 1990); each component of the food was then analysed using chemical and sensory methods. Based on the analytical data, a synthetic ikura was reconstituted using pure reagents. There were very few sensory differences in the taste profiles between the reference food and the reconstructed food (Hayashi *et al.*, 1990). In another study (Niimi *et al.*,

2014), a cheese solution was reconstituted using a mixture of sucrose, NaCl, monosodium glutamate, lactic acid, and caffeine that was then adjusted using a fractional factorial design. From sensory profiling data, the reconstructed products did not significantly differ from the cheddar cheese reference in overall intensity, as in saltiness, sourness, umami, and bitterness perceptions (Niimi *et al.*, 2014).

Dilution tests are based on the determination of the odour thresholds of compounds using gas chromatography-olfactometry and performed with serial dilutions of an aroma extract or of the taste/aroma threshold of compounds with serial dilutions of the compound extract. These tests allow to identify compounds detection thresholds, but also compounds psychophysical slopes. They allow to understand the impact of a compound according to its concentration. For example, the study of Hald *et al.* (2019) with activity-guided fractionation and dilution tests led to the identification of kaempferol 3-O-(2''-O-sinapoyl- β -sophoroside) which exhibited a bitter taste above its threshold concentration of 3.4 $\mu\text{mol/L}$. This compound contributes to an unpleasant bitter taste of rapeseed protein isolates. However, dilution test cannot accurately predict the perceptions of these compounds in mixture.

Combining instrumental analysis and human perception, on fractions or compounds isolated allow to identify tasteless and taste enhancing compounds in foods. Thanks to these methods, aroma models have been built in order to reconstitute complex odours—such as different types of wine, olive oil, cheese, boiled beef, coffee, and whey protein—using only a small fraction of the great number of volatiles occurring in these foods (Dinnella *et al.*, 2012; Ferreira *et al.*, 2002; Whitson *et al.*, 2010). However, in addition to be very time consuming, these experiments are less effective when volatile compounds are included in mixtures because they can interact with other ingredients. Indeed, the ability of volatile compounds to modify product odour depends on both their relative concentrations in the products and their interactions within the food matrix (Guichard, 2002). Moreover, mixing congruous volatiles and taste stimuli can enhance taste intensity, while mixing incongruous stimuli can suppress taste intensity (Caporale *et al.*, 2004). These methods were also used to study peptides, phenolic or lipid compounds as presented on the Table 1.3.1. In particular, Thomas Hofmann has worked on many different food products to identify key sensory chemical compounds with these methods. One of his latest studies based on a fractionation strategy is on pea protein isolates and led to the identification of 14 key bitter lipids and lipid oxidation products (Gläser *et al.*, 2020).

Concerning the perceptions of pea protein isolates, the identification of the main compounds (e.g., peptides, phenolics, minerals) potentially responsible for aroma and taste notes remains a real challenge for scientists and industrials, due to the complexity of composition of materials. Moreover, most analytical techniques require the use of non-food-grade solvents or buffers that are difficult to handle and that can pose problems if the extracts have to be used in sensory evaluations.

1.3.2. Strategies based on methods in interaction with human and electronic receptors

Different strategies focused on chemical measurements in interaction with human or mimicking human receptors. The main used methods are GC-olfactometry, on line mass spectrometry measurements (PTR-MS and APCI-MS), as well as electronic measurement (E-nose and E-tongue).

GC-olfactometry allows a panelist to sniff the volatiles molecules at the output of the gas chromatograph column and in the same time that they are analysed by the GC detector (for instance mass spectrometer or FID). For example, Murat *et al.*, studied odour active compounds and followed their evolution during four steps of the process (from pea flour to pea protein) using Gas Chromatography coupled with Mass Spectrometry and Olfactometry (Murat *et al.*, 2013). Results showed that in pea protein isolates, 16 key compounds could be linked to volatile compounds. The most perceived compounds in term of frequency were linked to (E,E)-3,5-octadien-2-one (panel detection 100%); 3- methylthiopropional (panel detection 94%); (E)-2-octenal (panel detection 94%); and 5-butylidihydro-2(3H)-furanone (panel detection 94%). However, this method does not take into account physico-chemical interactions as well as matrix effect or sensory interactions.

On-line mass spectrometry measurements (PTR-MS or APCI-MS) allow the monitoring of the release of aroma compounds in the air exhaled by a subject consuming food. A lot of applications are described

in literature. For example, Jourdren *et al.*, studied the influence of bread structure, volatile compounds, and oral processing on aroma perception using proton transfer reaction-mass spectrometry (PTR-MS). The release of aroma compounds of three types of bread were characterized by the study of thirty-nine ion fragments from m/z 45 to 139. In addition, volatile compounds of boli from panelists were studied using PTR-MS headspace analysis at 3 stages of oral processing (10, 40, and 100% of individual swallowing time). This study demonstrated for the first time that the perceived aroma of crumb with crust was influenced more by volatile profiles than by crumb texture (Jourdren *et al.*, 2017). However, this method focused only on the monitoring of the release of a selected and low number of ion fragments and links with dynamic perceptions were not easy to establish.

Finally, electronic measurements through E-nose or E-mouth systems are based on pattern recognition of flavour and taste compounds based on principle to mimic the human taste and olfactory receptors and their communication with the human brain. However, these methods do not generate information on sample composition but provide only a digital fingerprint through pattern recognition. For example, Hou *et al.*, studied colour values, amino acid, and sugar composition of fresh and roasted sweet potatoes from 11 cultivars. They used a combination of sensory evaluation, GC-MS, electronic nose, and electronic tongue. Results showed that the electronic nose could distinguish the differences in flavour among the different roasted sweet potatoes (Hou *et al.*, 2020).

1.3.3. Strategies based on identification of active compounds for human receptors

When receptors to specific perception are known, it makes possible to study the specific interaction between receptors and some active compounds. These experimental strategies aim to predict how a molecule could be perceived based on its chemical structure and knowledge of aroma/taste receptors. Prediction can be done by mathematical model (Quantitative Structure–Activity Relationship models). For example Huang *et al.*, developed a web server tool that can be used to predict the human bitter taste receptors used for certain small molecules (BitterX, Huang *et al.*, 2016). This tool first identifies a compound as bitter and then predicts its candidate TAS2Rs (Huang *et al.*, 2016). Prediction can be also validated by cell-based expression system for human receptors (Bouysset *et al.*, 2020). These methods are interesting to confirm the taste or aroma of specific compound. However, they require to work on a small number of known compounds and do not allow to take into account the interactions. For example, Bouysset *et al.*, used machine learning to predict novel agonists of the sweet taste receptor. A was setup based on open-source chemical features optimized on a curated database of 316 known sweet agents (SweetenersDB, Bouysset *et al.*, 2020). The virtual screening of a large database of natural compounds identified thousands of putative sweeteners, of which three were selected for in vitro functional assays of the human sweet taste receptor and dose–response analyses. Among them, they identified arctiin as a novel agonist of the T1R2/T1R3 sweet taste receptor (Bouysset *et al.*, 2020).

1.3.4. Statistical modelling of perceptions according to chemical composition

To model perceptions in function of the chemical composition, two kinds of statistical approach are commonly used. The first approach aims to describe and explore the data in an unsupervised manner searching for patterns and trends in the data or at investigating the effect of specific treatments on the samples. The second approach aims to predict in a supervised manner interesting characteristics about the samples by using the data together (regression or classification models). Predictor variables (independent variables, chemical compounds) and response variables (dependent variable, sensory intensities) are usually referred to as X and Y respectively.

1.3.4.1. Extracting all information from chemical and sensory datasets

The first step before modelling is the pre-processing of data in order to extract all information from the chemical and sensory data (Figure 1.3.3.). As regards chemical data, it can be separated into two main directions: removing data artefacts and transforming or rescaling the data by using a function (auto-scaling, mean-centring, log-transformation). For example, in mass spectrometry analysis it is necessary to remove variations attributed to experimental sources (e.g. analytical noise or experimental bias) in chemical data intensities among measurements while preserving the relevant variation (due to biological source) (Gorrochategui *et al.*, 2016). Another problem is the general presence and/or intensity of the peaks in the data (e.g. ion suppression, adduct formation, fragmentation) (Proust *et al.*, 2019).

In addition, the pre-processing step depends on the approach of the study (targeted or untargeted). In targeted analysis, such as usually in GC-MS, a predefined group of compounds are characterized and annotated according to referential databases. In this type of analysis, there is less artefacts and less compounds to study (Johnson *et al.*, 2015). However process becomes quite time consuming and complex if there is a large number of compounds to identify such as for peptides identifications (Daher *et al.*, 2020). In untargeted analysis, all measurable compounds in a sample, including uncharacterized metabolites, are considered (e.g. consideration of each measured ions). In this type of analysis, no previous knowledge of the sample is required and no referential database is necessary. However, it requires the analysis of whole data sets which include gigabytes of information. Thus it requires previous reduction of their dimensions into more computationally manageable formats (Gorrochategui *et al.*, 2016). Data preprocessing may occur also in descriptive sensory analysis. However, in most publications, it is not mentioned or specified. For example, the data may be screened for panellists who are outliers or for mistakes (Seisonen *et al.*, 2016).

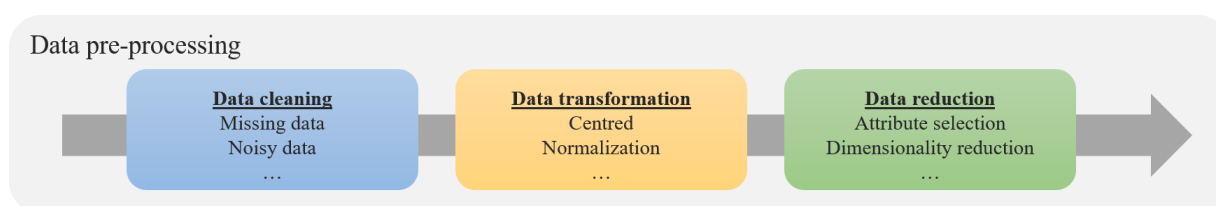


Figure 1.3.3.: Data pre-processing in data mining.

1.3.4.2. Statistic descriptive or unsupervised approaches

To link sensory and chemical datasets, some studies used descriptive or unsupervised approaches. The aim of these approaches is to describe and explore the data searching for patterns and trends in the data or at investigating the effect of specific treatments on the samples. Two main types of methods are used: multivariate decomposition methods and correlations analysis. Correlation coefficients measure linear correlations between two variables X and Y (Ludbrook, 2002).

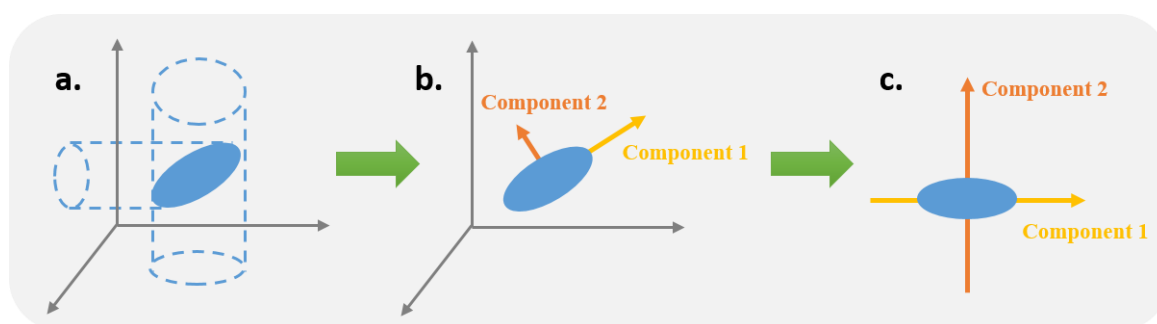


Figure 1.3.4.: Schematic description of PCA construction: a. object described by a complex set of variables (or coordinate system); b. new variables orthogonal to each other and pointing to the direction of largest variances; c. new variables to describe object in a more concise way.

For example, Moon & Li-Chan (2007) studied the links between volatile compounds and 12 soy protein isolates. Another example, Bi *et al.*, studied volatile compounds of raw and cooked adzuki beans under three cooking methods. The results of correlations between volatile compounds and sensory attributes showed that pyrazines and maltol might be responsible for “caramel-like” and “cream-like” aromas (Bi *et al.*, 2021). Correlation tests may provide trends and patterns within a set of data, but provide limited

information relating multiple datasets (Zielinski *et al.*, 2014). In addition, the presence of different sensory interactions give a highly complex relationship, which cannot be easily analysed using correlations. Thus, correlation tests are also often used in conjunction with multivariate statistical methods (Yu *et al.*, 2018).

Multivariate decomposition methods are used to explore and visualize datasets. Principal component analysis (PCA), for example, is used to explore multidimensional data sets made up of quantitative variables (Kumar *et al.*, 2014). It is a projection method that allows observations to be projected from the p-dimensional space of the p variables to a k-dimensional space ($k < p$) such that a maximum of information is conserved (the information is here measured through the total variance of the point cloud) on the first dimensions (Figure 1.3.4.).

Table 1.3.2.: Example of regression studies carried out on food products to link chemical composition and perceptions.

Main instrumental parameters	Product	Number of products	Main statistical methods	Reference
Nuclear Magnetic Resonance and infrared spectroscopy parameters	Tomato	112	ComDim, PLS-DA	Monakhova <i>et al.</i> , 2016
Infrared spectroscopy parameters	Oil	32	ComDim	Rosa <i>et al.</i> , 2017
Volatiles	Beers	24	MLR, ANN	Gonzalez Viejo <i>et al.</i> , 2019
Fatty acids, volatile	Chicken	8	PLS-R	Niu <i>et al.</i> , 2016
Organic acid, amino acids	Cheese	459		Kraggerud <i>et al.</i> , 2014
Peptides	Bovine muscle and porcine plasma protein hydrolysates	20		Fu <i>et al.</i> , 2018
	Beef seasoning	7		Tian <i>et al.</i> , 2015
	Bitter cocktail	16		Johnson <i>et al.</i> , 2015
	Liquor	16		Xiao <i>et al.</i> , 2016
	Cheese	16		Carpino <i>et al.</i> , 2002
	Commercial egg flavour Sachimas	4		Yang <i>et al.</i> , 2019
	Garlic Oil	8		Sun <i>et al.</i> , 2019
	Honey	6		Tahir <i>et al.</i> , 2016
	Tea	27		Qin <i>et al.</i> , 2013
	Wine	21		Schmidtke <i>et al.</i> , 2013
	Yogurt	10		Sfakianakis & Tzia, 2017
	Bread	10		Heenan <i>et al.</i> , 2009
Volatiles and peptides	Hydrolysates derived from porcine blood	8		Fu <i>et al.</i> , 2019
427 varied compounds	Soy sauce	25		Shiga <i>et al.</i> , 2014
Peptides	Bamboo shoots	16	PLS-DA	Gao <i>et al.</i> , 2019
Volatiles	Chocolate	206		Deuscher <i>et al.</i> , 2019
	Cheese	7	PLS-DA, PLS	Shiota <i>et al.</i> , 2015
Physical properties	Bread	3	PLS-MB	Jourdren <i>et al.</i> , 2016
Nutritional content, instrumental texture, structure, properties and colour	Yogurt	8	PLS-PM	Masson <i>et al.</i> , 2016

For example Casassa *et al.* (2015), linked phenolics concentrations of 6 wines to sensory properties via PCA. The multidimensional scaling method (MDS) is also a method used to reconstitute a map of individuals from a matrix of proximities (highlighting similarities or dissimilarities) between individuals. In ögütçü and Yilmaz (2009), physicochemical and sensory properties of 5 olive oil samples were compared with MDS. Other methods can be used such as multiple factor analysis (AFM) (Bernhardt *et al.*, 2015), or generalized procrustean analysis (GPA) (Guàrdia *et al.*, 2010). For example, Giacalone *et al.* (2018) have studied sensory and chemical properties of common roasting off-note in

coffee. In this study, instrumental-sensory correlations were modelled by MFA, leading to different results such as sensory attributes “Burnt”, “Astringent” and “Burnt (Aftertaste)” which could be due to high concentrations of pyridine and furfuryl alcohol. Another example, Mitchell *et al.* (2011), have studied the impact of salt reduction on flavour profile of vegetable soup with GPA. The results revealed that reducing the salt content of the low salt soup had a significant effect on the attributes “green colour”, “sweet flavour” and “pepper flavour”.

1.3.4.3. Predictive or supervised approaches

In order to obtain cause and effect links, it is necessary to use regression models. Multivariate regression methods are prediction methods that look for cause-and-effect relationships between variables. Once again, different methods of analysis can be distinguished. Table 1.3.2. presents some examples of regression studies carried out on food products to link chemical composition and perceptions.

Multiple Linear Regression (MLR) are usually described in the literature as models with good predictive ability to link texture attributes in relation to sensory attributes (Krzeminski *et al.*, 2013). However, these models do not work when the number of individual is greater than the number of variables X . Partial Least Square Regressions (PLS-R) are used when there is a large number of explanatory variables or when there are strong collinearities between the variables (Höskuldsson, 1988). It allows the comparison of two blocks of variables taking into account the correlations between the variables (Wold, Sjöström, *et al.*, 2001) (Figure 1.3.5.). PLS methods are widely used to correlate consumer sensory descriptive attributes with instrumental or chemicals parameters (Sfakianakis & Tzia, 2017; Fu *et al.*, 2019; Shiga *et al.*, 2014; Heenan *et al.*, 2009). PLS-DA (discriminant analysis) is a special case of PLS where the qualitative q variable with q classes is replaced by q indicator variables of these classes.

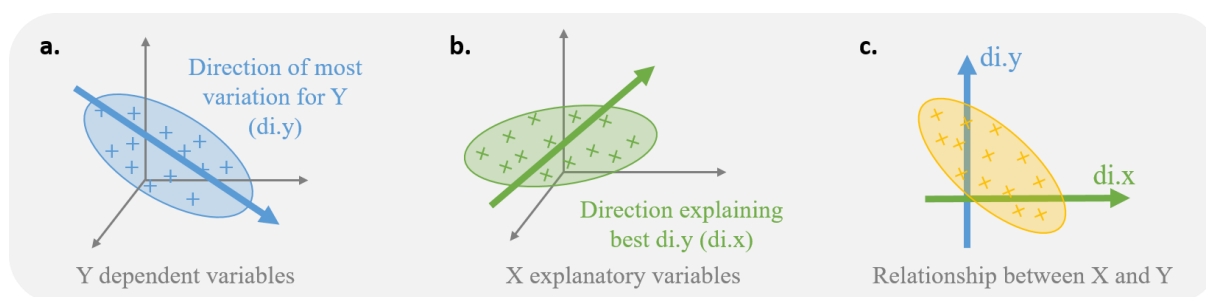


Figure 1.3.5.: Schematic description of PLS-R construction: matrices of Y independent variables (a.) and then matrices of X explanatory variables (b.) are decomposed into latent structure in an iterative process. The latent structure corresponding to the most variation of Y is extracted and explained by a latent structure of X that explains it the best (c.).

However, simple PLS-R can present some limits. They have been developed for data sets comprising about twenty variables, but today the work includes hundreds, or thousands of variables. This increase in the number of variables poses a lot of limits in the modelling and interpretation of the results. Besides it deals with difficulty for Ys which measure different concepts that are quite independent (Wold *et al.*, 2001). Several solutions exist in the literature. For example, the ComDim method is an analysis in common components and specific weights (CCSWA) (Hanafi *et al.*, 2006). It is used in the literature to study food products analysed using Nuclear Magnetic Resonance and infrared spectroscopy (Monakhova *et al.*, 2016; Rosa *et al.*, 2017). Another example is the PLS Multi-Block (PLS-MB), a PLS variant, which preserve the original structure of the data and thus allows to analyse the different tables simultaneously. In particular, the usefulness of PLS-MB lies on the ease of visualization of importance of each block of variables. In addition, PLS-MB is low sensitive to multicollinearity within explanatory blocks (Bougeard & Dray, 2018). Another solution is to use the PLS-Path Modelling method, which permits to divide the X and Y variables into sub-blocks, to create a PC or PLS model of each sub-block, and then to use the resulting scores as a new set of variables in a new PLS model (Wold, *et al.*, 2001).

It is very useful in calculating global indices, such as the consumer satisfaction index, but it is little used today in food applications (Tenenhaus *et al.*, 2005).

Another type of method used in the literature are non-linear models such as artificial neural networks (ANN) (Gonzalez Viejo *et al.*, 2019). ANN are a type of machine-learning prediction method with the ability to self-learn relationships from labelled experimental data and generalize to unlabelled situations. They were designed to mimic pattern recognition and information storage processes performed by the brain and biological nervous system. An ANN consists of a network of connected nodes which are divided into the input, output, and hidden layers (Figure 1.3.6.). The input layer receive the independent variables. The output layer generate the predicted values. The hidden layer is a layer of nodes between the input and output layers where multiple connection patterns are possible (Yu *et al.*, 2018).

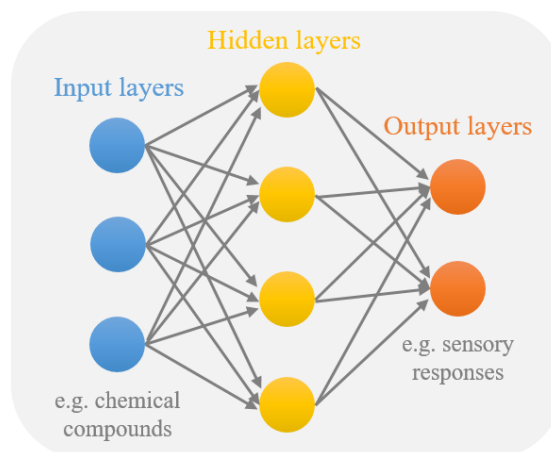


Figure 1.3.6.: Simplified view of a feedforward artificial neural network.

Main conclusions

In food science, two main strategies are used to link sensory and chemical datasets:

- The first strategy combines fractionation and test of omissions or dilution, but:
 - Compounds in pea protein isolates that are potentially responsible for sensory attributes are very complex and challenging to purify and identify;
 - Most analytical techniques require the use of non-food-grade solvents or buffers that are difficult to handle with sensory evaluations.
- The second strategy combines design of experiments and predictive models:
 - Data pre-processing is a complex task in order to obtain quality data;
 - PLS methods are widely used;
 - However, links between chemical and sensory data are often nonlinear;
 - For that, artificial neural networks are sometimes used.

1.4. Objectives of the PhD work and experimental approach

As highlighted in the literature review, the perception of pea proteins is complex and could limit their use as food ingredients. A large number of molecules (volatile compounds, peptides, phytochemicals, lipids, minerals and carbohydrates) are likely to participate in the perception mechanisms either directly or via interactions between molecules, with food ingredients in matrix and with individual physiology parameters. These last ones play a role in food oral processing with consequences on the dynamics of perceptions, which also directly participate at the global image of perception. Understanding the physicochemical and sensory mechanisms associated with pea protein isolates may subsequently enable us to formulate pea based-products that meet consumer expectations.

In this context, the work carried out in this PhD project aims to answer the following research question: “What are the main physical, chemical and sensory mechanisms that explain the sensory notes of pea protein isolates?” Consequently, the overall objective of this PhD project was to evaluate i) the role of the main groups of chemical compounds (volatile compounds, peptides, phenolics/saponins) on sensory perceptions, and ii) the role of food formulation, sensory interactions (chemical, physiological and cognitive interactions) and oral process on the release of these compounds during food intake and on temporal perceptions. In order to meet these objectives, some hypotheses have been formulated:

- The overall aromatic composition of pea protein isolates, the protein-flavour interactions, the formulated matrix (fat, salt, texture, etc.) and the dilution with saliva during the oral process impact the release of volatile compounds, which can interact with olfactory receptors and lead to the dynamics of aromatic perceptions.
- Perceptions of bitterness root in the complex composition of pea protein isolates into soluble molecules (peptides, phenolics, saponins). They are impacted by the protein interactions, the formulated matrix and the physiological characteristics of individuals involved during consumption act.
- The phenolic composition of pea protein isolates and the formulated matrix impact the formation of saliva protein aggregates which lead to the astringent perceptions.

In addition, as the literature review points out, the study of pea proteins requires adapting the used physico-chemical and sensory methodologies. The distinct and persistent flavour of pea proteins calls into question their sensory evaluation and requires the adaptation of classical methods. To understand the origin of perception, scientific studies usually use the combination of molecular fractionation and omission tests. However, these methods need to be adapted to simultaneously study different groups of molecules. It is therefore necessary to also develop a strategy adapted to these complex ingredients. The development of technologies (modern mass spectrometry and bioinformatics) could allow a more precise identification of peptides and phytochemical compounds, but still requires further development. Finally, to link chemical and sensory data, scientific studies have generally focused on aromatic or sapid perceptions related to volatile or non-volatile compounds with linear regressions. However, in order to apprehend a global picture of perception, it is also necessary to propose a more global approach on all properties and composition.

Thus, to test these hypotheses, a four-step experimental approach was undertaken for this PhD. The following diagram summarizes the different sections of the PhD work and their relationships with pea protein isolates (Figure 1.4.1.).

In order to investigate the mechanisms that lead to sensory properties of pea-protein based products, it is necessary to describe and quantify their perception, and by consequences a relevant sensory profiling protocol is required, adapted to this type of products. In addition, the apparent complexity of mechanisms at the origin of off-flavours in pea protein isolates calls for the establishment of an adapted strategy taking into account volatiles and non-volatile compounds. Thus, the first step of the project aims to propose i) a relevant sensory protocol for the identification and quantification of pea protein-based samples perception (Section 3.1.1.); and ii) a relevant strategy to develop products enabling to

study the relative role of volatile and non-volatile fractions of pea protein isolates on these perceptions (Section 3.1.2.). For this last point, an original approach based on the recombination of different fractions (blocks of molecules) were considered. In order to have a larger number of products with an interesting variability, the idea was to artificially create different protein isolates from pea protein isolates. The objectives of these artificial products are to create variability based on the three main block compositions: the volatile part, the protein part and the other components.

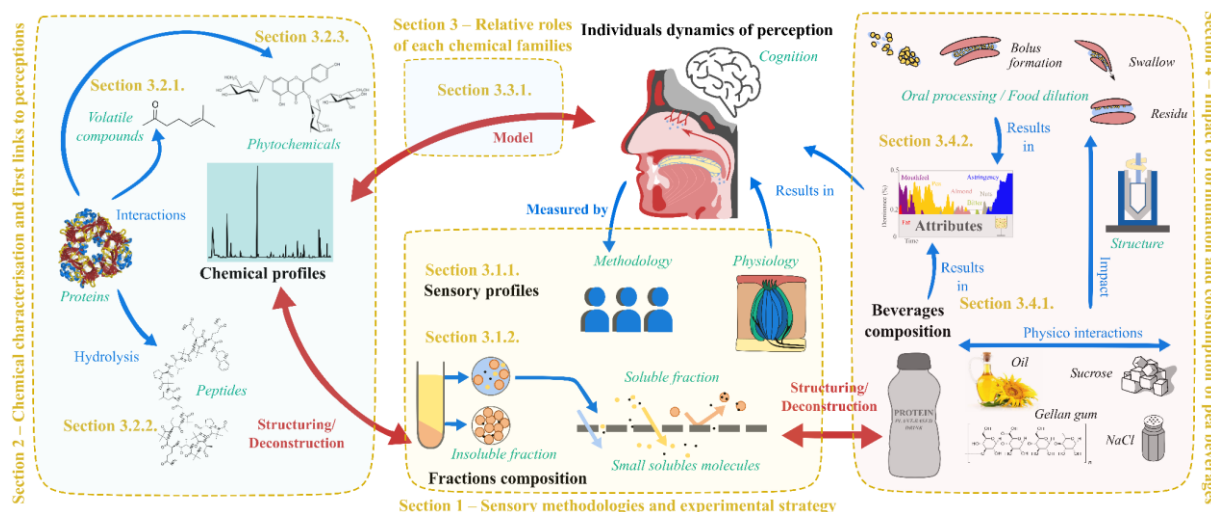


Figure 1.4.1.: Schematic representation of the experimental approach of the PhD work. The sections labels correspond to the Chapter 3 - Results.

As highlighted in the literature, the chemical composition of pea protein isolates (with the exception of volatile compounds) is still poorly known. However, the recent development of analytical technologies could make possible a more precise identification of chemical compounds of pea protein isolates. Thus, the second step of the project aims to characterize the chemical composition of pea proteins, in particular i) the main volatile compounds (Section 3.2.1.); ii) the main oligopeptides and polypeptides (Section 3.2.2.); and iii) the main phytochemicals (phenolic acids, flavonoids and saponins – Section 3.2.3.). For each chemical profile, the links with sensory properties were considered.

The third step of the project aims to go further in understanding the complex and combined role of volatile and non-volatile compounds on overall perceptions of pea protein isolates and to build a statistical model taking into account key compounds coming from different chemical families to explain sensory perceptions (Section 3.3.1.).

Beyond the chemical composition of the food, its structure and texture could also influence the dynamics of perception. To better understand sensory off-notes and to be able to provide advice to improve them, it is therefore necessary to consider a formulated product. In addition, as shown in the literature review, sensory interactions and dynamic of perceptions seem very important for pea protein-based products and need to be studied further. Thus, the purpose of this fourth step of the project is to determine how the sensory perceptions of pea protein isolates are affected by the formulation as well as the consumption of a whole product. The temporal sensory properties were considered when consuming a spoonful, but also a whole portion of food (Section 3.4.1.). The rheological properties and volatile compound profiles were also considered (Section 3.4.2.). Among the different pea applications, pea beverages were considered.

The following sections detail the materials and methods used in these four experimental phases.

CHAPTER 2

MATERIALS AND METHODS

Chapter 2 – Materials and methods

This part presents the products and protocols implemented during the different phases of the PhD project to meet their different objectives. The pea based products (protein isolates, solutions and food products) used during the different phases of the project are presented in the first section. Then, the description of the sensory methods used are presented, followed by the physicochemical methods. Finally, the last part focuses the statistical analyses carried out between the different sensory and instrumental data sets. Since some techniques and methods are common to different phases of the project, a thematic organization was preferred to a chronological one. In addition, this materials and methods section has been written to be complementary to the information given in the results sections and to give a synthetic vision of all the experiments carried out.

2.1. Pea protein based products used in the project

To conduct this research work, three types of pea protein-based products were produced from commercial pea protein isolates: 6 pea protein-based solutions, 46 pea protein fraction-based products and 12 pea protein-based beverages (Figure 2.1.1.).

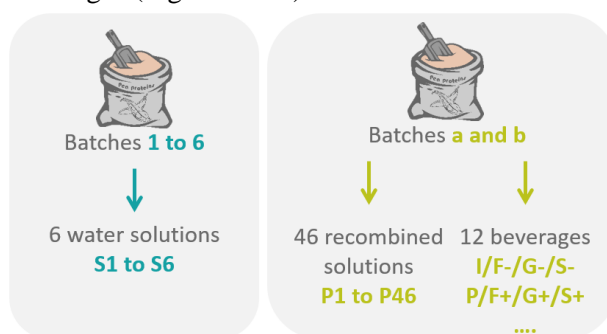


Figure 2.1.1.: Schematic representation of the pea-protein products (solutions S, products P and beverages) studied during PhD work.

2.1.1. Choice of pea proteins isolates

For the first phase of the project, 6 quite different sensory products were first selected (S1 to S6) among the diversity of commercial products. No further information on the isolates and their characterizations can be given for reasons of confidentiality. However, the overall composition of S1 is given on the Figure 2.1.2.

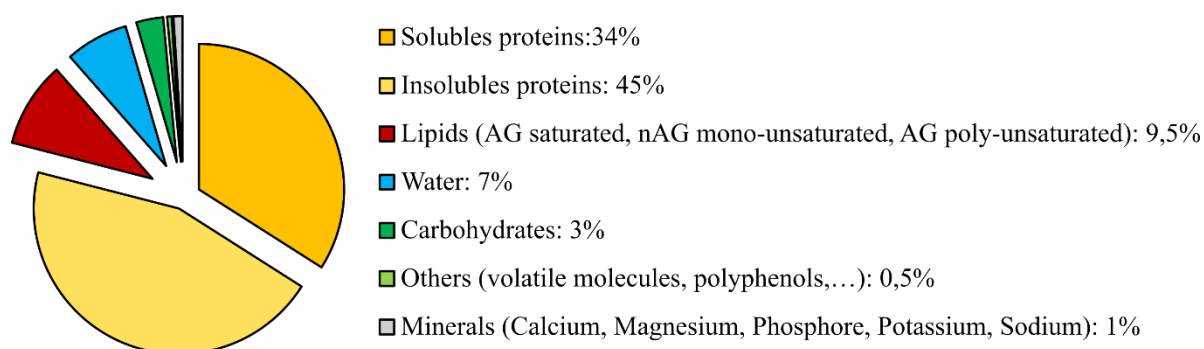


Figure 2.1.2.: Overall composition of commercial pea protein isolates S1.

Then, for the fraction-recombination methodology, we wanted to work on products belonging to a range of similar products and not to work on different commercial products. Among batches and range of commercial products from a single manufacturer, we had to select the most different products possible on sensory and physicochemical criteria. For that, in order to select different pea protein isolates, fast

sensory analysis and physicochemical characterizations were performed on 10 pre-selected pea protein isolates (called *a* to *h*).

First a free sorting test on the 10 batches was carried out. However the results did not yield any significant results. The 10 batches were very close in term of perceptions, so it was very difficult for panellists to sort them. Thus, volatiles composition (GC-MS) were analysed as well as physical characteristics: pH, residing at calcination, protein content, colorimetry index (L, A, B and ratio A/B), bulk density and granulometry ($> 250\mu\text{m}$, $> 100\mu\text{m}$ and $> 80\mu\text{m}$). The figure 2.1.3., presents the Multiple Factor Analysis between the volatile molecule data and the physical data. Among the 10 products the two most different products were selected (Batch *a* and *b*). For confidentiality reasons, the choice of these isolates *a* and *b* will not be discussed further. In the same way, in the results, the differences between *a* and *b* will not be discussed because of confidentiality issues.

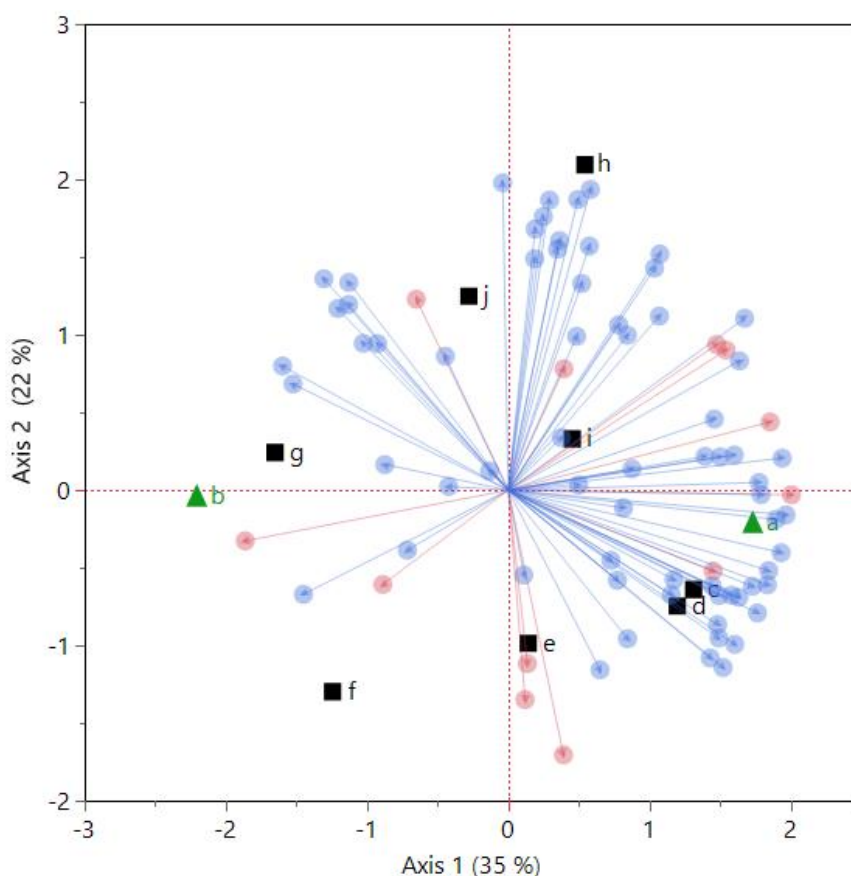


Figure 2.1.3.: Representation of 10 pea protein isolates pre-selected in the first and second dimensions of the multiple factor analysis performed on physico-chemical datasets. Rv coefficient = 0.64 between physical parameters and volatile molecules areas. In green are the pea protein isolates *a* and *b* selected for PhD work. In red are the physical parameters: pH, residing at calcination, protein content, colorimetry index (L, A, B and ratio A/B), bulk density and granulometry ($> 250\mu\text{m}$, $> 100\mu\text{m}$ and $> 80\mu\text{m}$). In blue are the volatile molecules areas.

Finally, the sensory differences were confirmed by triangular tests. Table 2.1. presents the results of the triangular tests between products *a* and *b*. Significant differences were perceived by panellists between these two products, confirming the interest to work with them. No further information on the isolates and their characterizations can be given for reasons of confidentiality.

Table 2.1.: Results of the triangular tests between pea protein isolates *a* and pea protein isolates *b* performed by 19 non trained judges with 2 repetitions.

Repetition	Corrected responses	Pvalue
1st repetition	12 /19	< 0.01
2nd repetition	14 /19	< 0.01

2.1.2. Pea protein-based solutions

For the implementation of a sensory method, it was chosen to work on the isolates present in water solution, without any other addition of ingredients (e.g. sugars) in order to not decrease or mask the potential off-notes. They were presented in the form of solutions with 4% (w/w) pea protein (S1 to S6): the isolates were mixed in water (Evian, France) for 1 min and then allowed to soak for 30 min.

2.1.3. Pea protein fraction-based products

To establish a predictive model of perceptions of pea protein isolate as a function of their physicochemical compositions and properties, it was necessary to study a larger number of products (about twenty). Thus, a market study was carried out and different samples were requested from both Roquette and other companies. The variability of the batches obtained was statistically studied (standard deviation, box plot) on sensory profile data, general composition data, total amino acids and free amino acids. The results showed that there was a variability within the Roquette products that could be interesting to exploit. On the other hand, the variability within the products from different companies was sometimes very high. In addition, it appeared that there were not so many pea protein isolates on the market and that working on these products could raise confidentiality issues. The choice was therefore made to work only on Nutralys products.

In order to have a larger number of products with an interesting variability, the idea was to artificially create different protein isolates from pea protein isolates. The objectives of these artificial products are to create variability based on the three main block compositions: the volatile part, the protein part and the other components. The analysis of the literature has led us to imagine to separate a soluble fraction (mainly proteins, called pellet), an insoluble fraction (mainly proteins, called retentate) and a soluble fraction with molecular weight lower than 10 kDa (mainly "non-protein" molecules, called permeate).

2.1.3.1. Preliminary experiments to define conditions of fractionation

The aim of the study was to separate a soluble fraction with molecular higher molecular weight (mainly composed of proteins), an insoluble fraction (mainly composed of proteins) and a soluble fraction with lower molecular weight (mainly composed of peptides and "non-protein" molecules) from two protein isolates *a* and *b*. The six fractions obtained were then re-associated to formulate the 46 samples of the mixing plan. For this purpose, it was necessary to obtain fractions under strictly similar conditions for both isolates, in large quantities and with a process that minimizes product modifications (modification of protein structure, loss of compounds...). Storage conditions were also adapted in order to optimize the long-term storage of the samples (about 12 months) and to be able to defrost them gradually according to the needs of the analyses. In addition, the products were aimed to be consumed, some specific handling precautions were also applied to produce safely the products.

First, several experiments were carried out in the SayFood laboratory on a laboratory membrane filtration system (Figure 2.1.4.). Different centrifugation conditions and different membranes and filtration conditions (salt, diafiltration) were tested. These various conditions made it possible to determine the optimal parameters for these processes at pilot scale. These studies showed the feasibility of fractionation and interesting sensory differences between products. Results showed that the addition of the centrifugation stage was determinant to limit fouling during the filtration step. However, the use of a NaCl solution to extract proteins was not retained, in particular because it greatly alters the taste of the products obtained. However, the non-controlled temperature during filtration and the high fouling of the membrane have limited the fractionation but help in the establishment of further specifications.

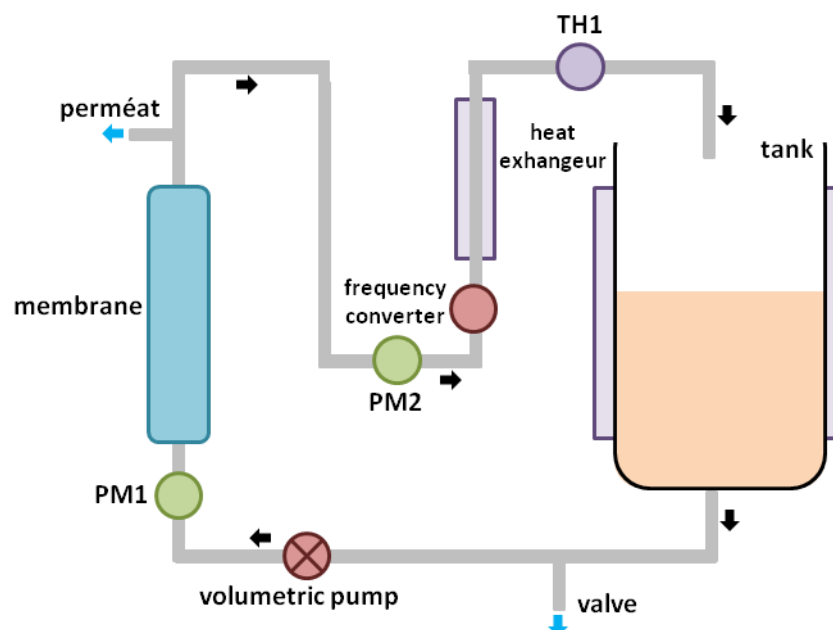


Figure 2.1.4.: Microfiltration system used at SayFood laboratory.

Thus the tests were quickly limited by the pilot's capacity (filtration rate, filtration temperature...). They were then carried out within the Improve platform. Improve (Amiens, France) is a European platform for research and development fully dedicated to the valorisation of proteins (<http://www.improve-innov.com/>). A first experiment was carried out to choose the method for the preparation of the pellets: a decanter Lemitec was used. The pea protein isolate *a* was used in this study and prepared on water solution: 4% (w/w) of pea protein isolates were mixed during on water (Tap water) and hydrated during 12h in a 200L double-walled tank with stirring. It was refrigerated to +4°C by a cooling unit to limit the proliferation of microorganisms. The isolate was added to the water previously cooled to 10°C. The powder was added with care to avoid excessive foam production. The pH and the solubility of the solution were measured after 30min and after 12h. After hydration, the settling tests with the Lemitec were carried out in two stages: a series of pre-tests to determine the settling parameters allowing to reach the target and a repetition of the 3 best conditions to validate the process (Figure 2.1.5.).

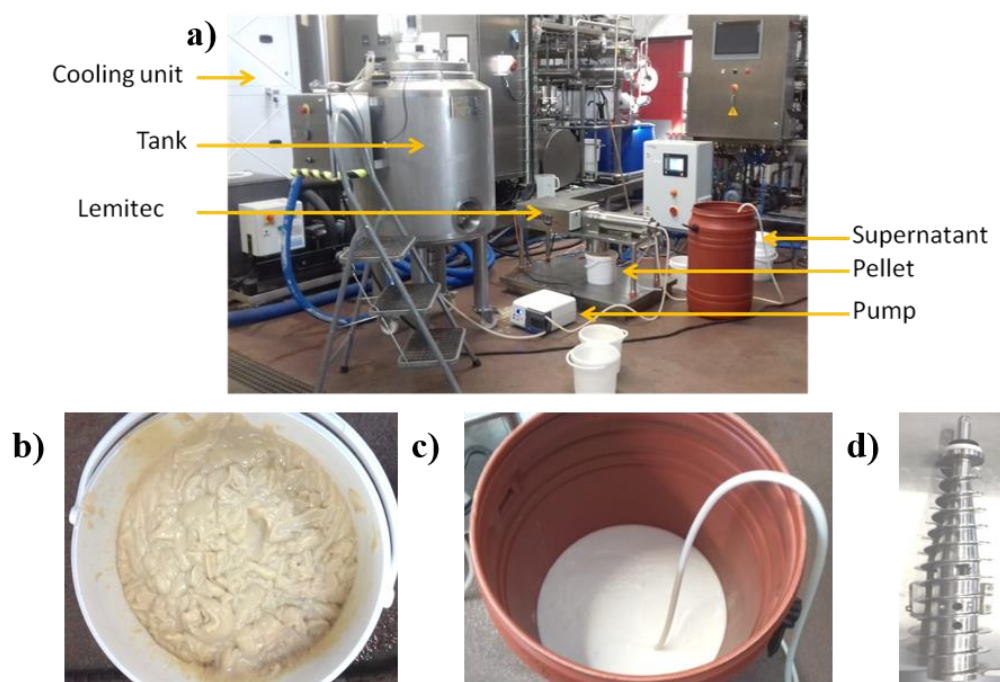


Figure 2.1.5.: a. Photographs of the device (continuous decantation with the Limitec system), b. of the pellet, c. of the supernatant and d. of the screw used for separation.

Different parameters were modified as listed on Table 2.2.: modification of the screw rotation speed, modification of the bowl rotation speed, modification in feed rate. A spin test of the initial suspension is performed 4 hours after the start of the settling tests to check the stability of the product as well as a pH measurement. Results are compared to results obtained with centrifugation on a lab scale (12 000g during 15min). After this test phase, the three best tests (n°3, 4 and 7) were reproduced with an analysis of the dry matter, the pellet and the supernatant.

Table 2.2.: Conditions used for decantation tests with Limitec.

Tests n°	1	2	3	4	5	6	7	8	9
Flow rate (L.h-1)	40	40	40	40	40	40	30	20	10
Bowl acceleration (g)	4000	4000	4000	3500	3000	3000	3500	3500	3500
Screw differential speed (rpm)	100	50	40	40	40	40	40	40	40
Screw motor torque (%)	14	34	40	32	28	33	28	22	18
Pellet volume (%)	18	4,5	4	4	4,5	15	3	4	7

Results indicated that the settling was not sufficient for optimal product separation. The pellet was not sufficiently clarified for the following planned steps. Two possible alternatives to settling (on a small scale) were therefore considered. First, centrifugation of the suspension by batch (on rotor bucket) was considered: it allows an optimal separation of the supernatant and the sludge, but the limit is an important number of handling of the product which could be a source of microbiological contamination. The second option considered was a plate centrifugation: it allows a very good clarification of the supernatant with a treatment rate of 200 to 400 L.h-1. The limit of this process is a lower concentration of the solid phase. Finally, the choice of batch centrifugation was made.

2.1.3.2. Production of pea protein isolate fractions

Then samples were produced in four days as described on Figure 2.1.6.

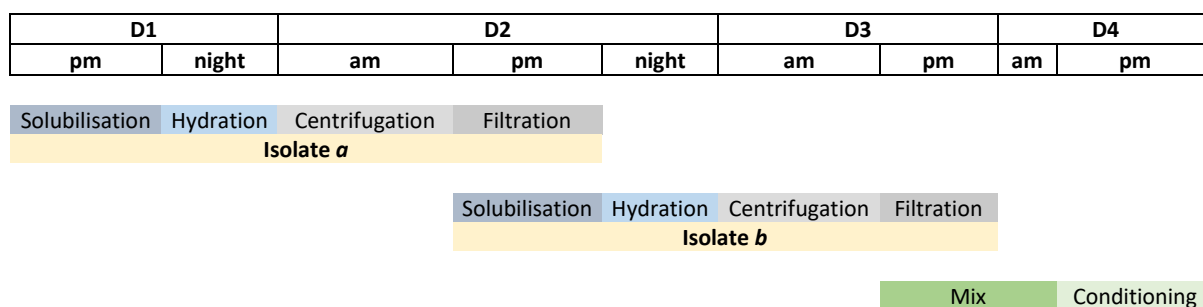


Figure 2.1.6.: Calendar of the production of isolate fractions.

The isolates were dispersed in tap water in a tank to obtain a final suspension containing 4% (w/w) dry matter content (Figure 2.1.7.). This suspension was maintained under agitation for 12 h at 3°C with an external agitator (U-shaped stirrer shafts).

a) Weight of the powder



b) Protein solution



c) Mixed of the solution



d) Hydration of the protein solution



Figure 2.1.7.: Photographs of the a. weight of the powder, b. of the protein solution, c. of the mixed of the solution and d. of the hydration of the protein.

It was then centrifuged with two centrifuges (Jouan Kr4i and Sorvall Lynx 4000, Thermo Scientific, US) at 6000 g, 10 min, 4°C (Figure 2.1.8.). The supernatant was manually separated from the pellet. The pellet was subsequently diluted with tap water to arrive at a dry matter content of 12.35%, which facilitated solution creation.

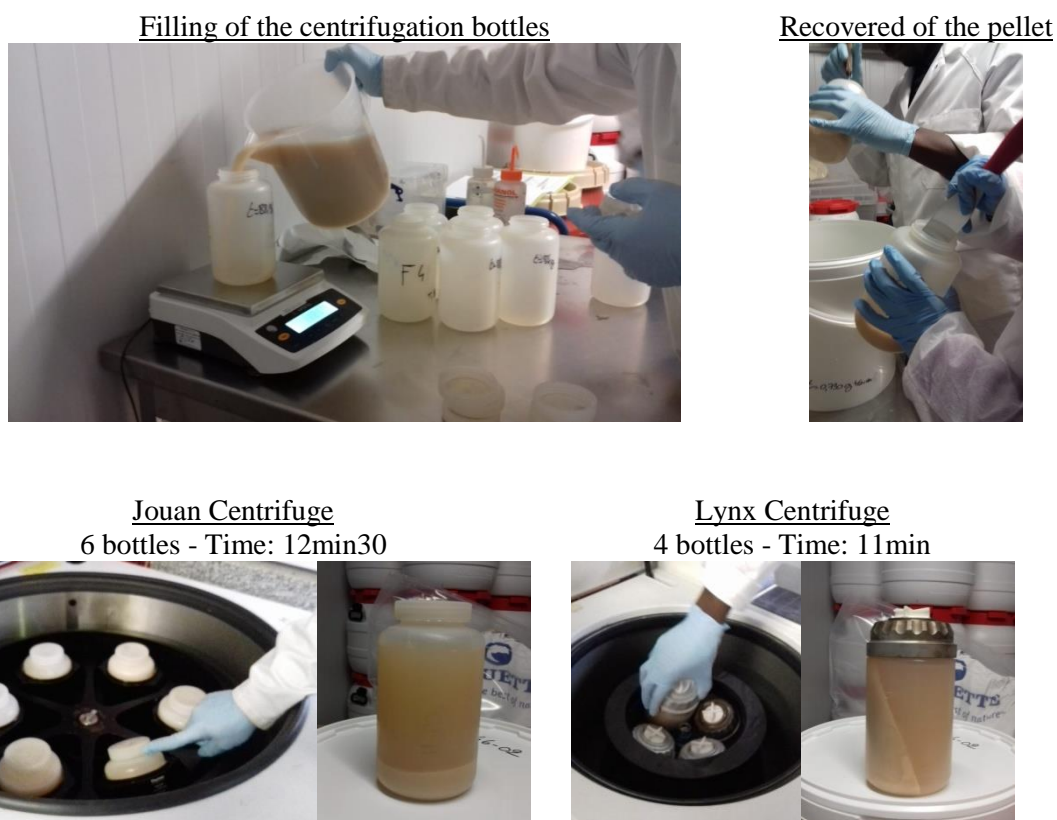


Figure 2.1.8.: Photographs of the Jouan and Lynx centrifuge, the filling of the bottles and the recovered of the pellet.

A tangential filtration module (TIA, Bollene, France) was used for the ultrafiltration process. The module employed two ST-3B-1812 PES Synder membranes (46-mil spacer; 10-kDa MWCO). Total membrane surface was 0.67 m². The filtration pilot was equipped with a high-pressure diaphragm pump (Wanner Hydra-Cell G10, Wanner International Ltd, UK)). The retentate was maintained at 13°C throughout filtration. The inlet pressure (P1) was 1.5 bar, the outlet retentate pressure (P2) was 1 bar, and the mean transmembrane pressure ($[P1 + P2]/2$) was 1.25 bar. First, ultrafiltration was used to obtain around 10 L of permeate; then, diafiltration was performed employing the same parameters to partially wash the retentate (one diavolume was used). Six fractions were obtained: permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b* (Figure 2.1.9. and 2.1.10). Complementary details on protocol can be found in the section 3.1.2.

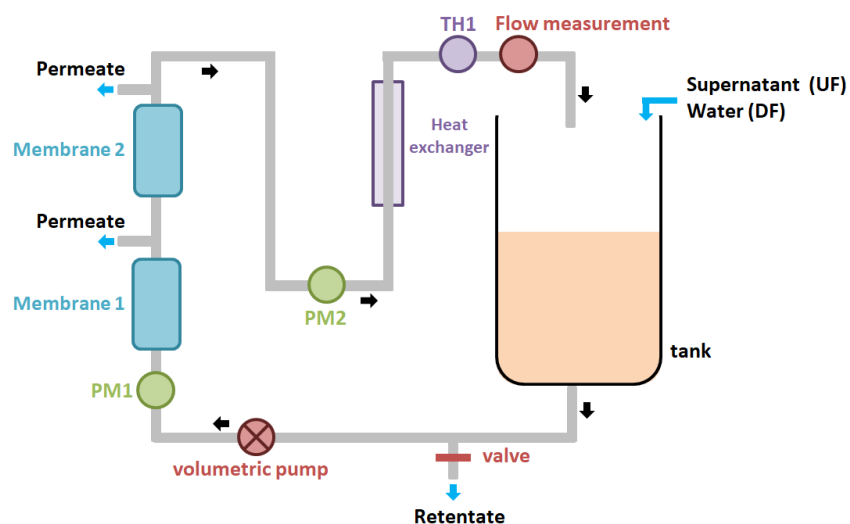
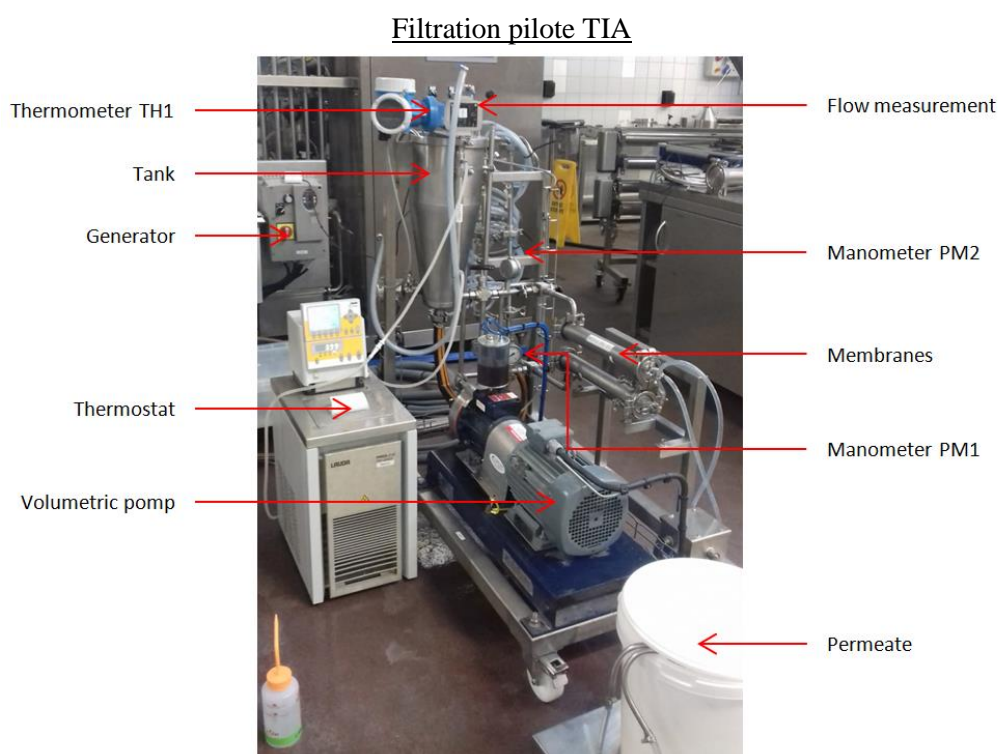


Figure 2.1.9.: Schematic representation of the filtration pilot.



Recovered of permeate



Recovered of retentate



Figure 2.1.10.: Photographs of the filtration pilot, the recovered of the permeate and the retentate.

2.1.3.3. Mixture design

An optimal mixture design was developed to have a wide range of reference and experimental solutions from the fractions (permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b*). Response surface models were performed and included quadratic terms and first-order interactions. The experimental design was such that there was orthogonality among all the terms, which allowed variable effects to be differentiated from one another. A blocking factor was used to control for the effect of the day on which sensory evaluation took place. Variable levels were chosen so as to represent a wide range of variation while remaining realistic in terms of the protein concentrations (similar to real food products). To validate the model's predictive capacity, six solutions that were not initially included in the design were added to the sensory evaluations (in italics in the Table 2.3.—sensory session ID 9). Complementary details on protocol can be found in Section 3.1.2.

Table 2.3.: Composition of the different solutions used in this study by mixture design model. The 46 solutions were produced by mixing different levels of permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b*. In bold are the solutions that were replicated. In italics are the supplementary solutions used for validation purposes.

Solution ID	Permeate <i>a</i> (%)	Permeate <i>b</i> (%)	Retentate <i>a</i> (%)	Retentate <i>b</i> (%)	Pellet <i>a</i> (%)	Pellet <i>b</i> (%)	Water (%)	MS (%)	Sensory session ID
P12	100	0	0	0	0	0	0	0.20	3
P29	100	0	0	0	0	0	0	0.20	6
P9	0	100	0	0	0	0	0	0.20	2
P38	0	100	0	0	0	0	0	0.20	8
P4	0	0	100	0	0	0	0	1.70	1
P8	0	0	100	0	0	0	0	1.70	2
P13	0	0	100	0	0	0	0	1.70	3
P14	0	0	0	100	0	0	0	1.70	3
P31	0	0	0	100	0	0	0	1.70	7
P40	0	0	0	100	0	0	0	1.70	8
P3	0	0	0	0	0	0	100	0.00	1
P19	0	0	0	0	0	0	100	0.00	4
P25	0	0	0	0	0	0	100	0.00	5
P34	0	0	0	0	0	0	100	0.00	7
P6	0	0	0	0	0	25	75	3.09	2
P24	0	0	0	0	0	25	75	3.09	5
P11	25	0	25	0	12.5	0	37.5	2.02	3
P28	0	0	0	0	30	0	70	3.71	6
P1	40	0	0	0	0	0	60	0.08	1
P35	40	0	0	0	0	0	60	0.08	7
P18	0	40	0	0	0	0	60	0.08	4
P17	0	0	40	0	0	30	30	4.39	4
P37	0	0	0	40	30	0	30	4.39	8
P7	0	0	0	40	0	30	30	4.39	2
P2	0	0	0	0	50	0	50	6.00	1
P20	0	0	0	0	50	0	50	6.00	4
P30	0	0	0	0	0	50	50	6.00	6
P39	0	0	0	0	0	50	50	6.00	8
P33	50	0	0	0	0	25	25	3.19	7
P36	50	0	50	0	0	0	0	0.95	8
P10	50	0	0	0	25	0	25	3.19	2
P5	0	50	0	0	0	25	25	3.19	1
P23	0	50	0	50	0	0	0	0.95	5
P26	0	50	0	0	0	0	50	0.10	6
P22	0	0	50	0	25	0	25	3.94	5
P16	0	0	0	50	0	0	50	0.85	4
P21	0	0	0	50	0	0	50	0.85	5
P32	0	0	60	0	0	0	40	1.02	7
P15	0	70	30	0	0	0	0	0.65	3
P27	40	0	0	60	0	0	0	1.10	6
<i>P41</i>	0	0	0	0	67	0	33	8.27	9
<i>P42</i>	0	0	0	0	0	50	50	6.00	9
<i>P43</i>	0	0	0	0	8	0	92	0.99	9
<i>P44</i>	0	0	0	0	0	8	92	0.99	9
<i>P45 (Refa-R)</i>	38	0	34	0	28	0	0	4.10	9
<i>P46 (Refb-R)</i>	0	40	0	36	0	24	0	3.70	9

Note on the construction of this mixture design:

- The choices of construction of this mixture design led to be very strongly constrained to dilution effects (dry matter effect). These effects are clearly visible in the results. An approach with an equivalent dry matter rate had previously been considered. However, in view of the fractionation results, it was difficult to separate the dry matter rate from the protein rate. In addition, the permeate fractions present dry matter and protein rates too low to be able to work at constant dry matter or protein rate. Dry matter variations were therefore chosen between 0 and 6%. These values are relatively low and in the order of magnitude of the formulated products on the market.
- In addition, we chose in this design to add a 100% water sample. This product was necessary for the chemical analyses. This sample is also interesting because it allows to study the threshold effects. In addition, the permeates were much diluted and therefore very close to water. Thus, the water sample was not inconsistent in terms of composition.
- Moreover, it was initially planned to study the quadratic effects. However, due to the complexity of the results, these effects could not be studied. Therefore, afterwards, the experimental design could have been simplified.
- Finally, we added to this design, 6 specific sample. These points allowed us to validate the model's predictive capacity. They have been chosen because in view of the first sensory results, it seemed that the products with high concentrations of dry matter were very close sensorially to each other. We therefore wanted to add products with high concentrations of dry matter to study how the panelists would describe them.

2.1.3.4. Solutions creations

The six different fractions were combined in various ways to formulate the 46 solutions of the mixture design. This process was carried out at 4°C in glass flasks, which were stored at -20°C. During fractionation and recombination, good hygiene practices were used to limit microbial contamination (usage of coat, gloves, and hygienic cap; cleaning and disinfection of hands and all equipment with pure ethanol, followed by air drying; work carried out in a 4°C chamber). In addition, the microbial safety of the solutions was tested by a certified external laboratory (Eurofins Scientific, France):

- Mesophilic aerobic flora (30°C) (NF EN ISO 4833-1)
- Yeasts (NF V 08-059)
- Mould (NF V 08-059)
- *Escherichia coli* β -glucuronidase positive (NF ISO 16649-2)
- *Escherichia coli* β -glucuronidase positive (NF ISO 16649-2)
- Presumptive *Bacillus cereus* (30°C) (NF EN ISO 7932)
- *Clostridium perfringens* (NF EN ISO 7937)
- *Listeria monocytogenes* (AES 10/03-09/00)
- *Salmonella* (BRD 07/11-12/05)
- Coagulase-positive *staphylococci* (37°C) (NF EN ISO 6888-2)

However, the pellet *b* presented a high level of Mesophilic aerobic flora and presumptive *Bacillus cereus*. To face this problem, our first proposal was to autoclave the samples. This solution would allow us to have safe products, but possible evolution of flavour properties. In order to validate this process, sensory triangle tests were performed between non-autoclaved products and autoclaved products with two conditions (Pea protein isolates *a*, 4%, 110°C-10min and 100°C-5min). Results showed that the heat treatment considerably modified the perceptions for both heat treatments (significant triangular test). This solution was therefore not satisfactory.

Thus, we decided for the project to evaluate the samples on the aromatic notes with odour (orthonasal perceptions) in addition to an in-mouth evaluation (retronasal perceptions) and to add the descriptor sweet, i. e.:

- For samples without microbiological problems (32/40 samples): in-nose evaluation on aromatic descriptors, in-mouth evaluation on sapid descriptors and in-mouth evaluation on aromatic descriptors.
- For samples with microbiological problems (8/40 products): in-nose evaluation on aromatic descriptors on samples initially made, in-mouth evaluation on sapid descriptors on autoclaved samples (110°C-10 min) and in-mouth evaluation on aromatic descriptors on autoclaved samples (110°C-10 min).

In parallel, we proposed to added products during the sensory profiling to study the autoclave effect (110°C, 10 min) on the initial products (Refa and Refb) as well as on the pellet *b* fraction (duplicate evaluation by the panel). Table 2.4. presents the results of the two-way analysis of variance performed on the intensities of all the attributes (Autoclaved, Product, Repetition and Product*Autoclaved). The Autoclaved effects were significant for almond, cereals and granularity. The Product*Autoclaved effects were significant for nuts and granularity. Thus for the others attributes (8/12) there is no impact of the autoclave on their intensities on products.

Table 2.4.: Effect of the autoclaved for the evaluation of the intensities of the 12/12 attributes evaluated by mouth for Pellet *b* (6% of dry matter) and Refb (4% of dry matter): (a) F value: Fisher statistic for fixed factors. Pvalue for the Fisher test. In bold p-values significate at the threshold of 0.05.

	Autoclaved		Product		Product*Autoclaved		Repetition	
	F value	Pvalue	F value	Pvalue	F value	Pvalue	Rapport F	Pvalue
Almond-M	13.67	0.00	0.09	0.76	0.43	0.51	0.53	0.47
Astringent-NC	0.50	0.48	1.84	0.18	0.18	0.67	0.36	0.55
Bitter-NC	2.98	0.09	0.13	0.71	0.85	0.36	0.11	0.74
Broth-M	0.86	0.36	9.80	0.00	1.66	0.20	0.06	0.81
Cereals-M	6.88	0.01	0.21	0.65	1.22	0.27	0.23	0.64
Granularity-NC	418.49	0.00	9.36	0.00	6.41	0.01	0.75	0.39
Mouthfeel-NC	0.82	0.37	9.11	0.00	2.56	0.11	0.03	0.86
Nuts-M	1.18	0.28	0.55	0.46	5.81	0.02	2.45	0.12
Pea-M	1.84	0.18	0.49	0.48	0.25	0.62	0.08	0.78
Potato-M	0.82	0.37	0.94	0.33	0.24	0.62	0.12	0.73
Salty-NC	0.17	0.68	10.87	0.00	0.04	0.84	1.24	0.27
Sugar-NC	3.00	0.09	0.39	0.53	0.09	0.77	0.00	0.94

Considering average perceived intensities and Newman-Keuls groups, the difference of intensities between autoclaved and not autoclaved was low for nuts, cereals and almonds (Table 2.5.). Nevertheless, the difference of intensity was very high for granularity (difference of 6/10). Autoclaved products were less granular than the not autoclaved products. We can assume that the autoclaved process induce important change on the structure of the pea protein isolates solutions.

Table 2.5.: Averaged perceived intensity (across subjects) and Newman-Keuls group associated for products autoclaved and no autoclaved for the significant attributes: (1) Pellet b (6% of dry matter) and (2) Refb (4% of dry matter). Differences of average intensities between autoclaved and not autoclaved were calculated. Significant differences between groups are indicated by differences in letters.

	Nuts-M	Cereals-M	Almond-M	Granularity-NC
(1)				
Refb	4.25 a	3.02 a	3.22 b	5.37 b
Autoclaved Refb	3.76 a	3.56 a	4.41 ab	0.57 c
Differences	0.49	-0.54	-1.19	4.81
(2)				
Pellet b	3.09 a	2.79 a	3.09 b	6.87 a
Autoclaved Pellet b	4.38 a	4.11 a	4.79 a	0.71 c
Differences	-1.29	-1.33	-1.70	6.17

2.1.4. Pea protein-based beverages

In addition to the study on pea protein solutions, some beverages were developed in order to understand the role of this ingredient in real products. For that, we studied the impact of composition of beverages on perception. The pea protein isolates *a* was used to study formulated products. Among the different applications using pea proteins (dietary supplements, bakery and meat products...), we chose to study beverages because they allow to vary the concentrations of various ingredients (salt, sugar, fat, texturizing agent) while being easy to produce in a repeatable way over a long period of time (6 months). Two mixture designs were used to produce a wide range of plant-beverages from different ingredients while being realistic in term of ingredient concentrations. The first mixture design was formulated with pea protein isolates and had three independent variables with two levels: sunflower oil concentration (0% or 1.5% w/w), gellan gum concentration (0.12% or 0.5% w/w) and salt concentration (0.08% or 0.12% w/w). The second mixture design was formulated with pea pellets and had also two independent variables: the protein, sunflower oil concentration (0% or 1.5% w/w) and two levels of gellan gum concentration (0.12% or 0.5% w/w). Thus, the total number of trials was 12 (composition and ingredient concentrations are presented in Table 2.6. Complementary details on protocol can be found in the Section 3.4.1.

Table 2.6.: Composition (ingredient concentrations [w/w %]) of the pea protein-based beverages used in this study. Abbreviations: I = isolate, P = pellet, F+ = 1.5% oil, F- = 0% oil, G+ = 0.5% gellan gum, G- = 0.12% gellan gum, S+ = 0.12% salt, and S- = 0.08% salt.

Product name	Protein type	Sunflower Oil (%)	Soy lecithin (%)	Gellan gum (%)	Salt (%)	Sugar (%)	Pea protein (%)	Water (%)
	(P or I)	(F+ or F-)		(G+ or G-)	(S+ or S-)			
I/F-/G-/S-	Isolate	0.00	0.00	0.12	0.08	1.00	7.00	91.80
I/F-/G-/S+	Isolate	0.00	0.00	0.12	0.12	1.00	7.00	91.76
I/F-/G+/S-	Isolate	0.00	0.00	0.50	0.08	1.00	7.00	91.42
I/F-/G+/S+	Isolate	0.00	0.00	0.50	0.12	1.00	7.00	91.38
I/F+/G-/S-	Isolate	1.50	0.10	0.12	0.08	1.00	7.00	90.20
I/F+/G-/S+	Isolate	1.50	0.10	0.12	0.12	1.00	7.00	90.16
I/F+/G+/S-	Isolate	1.50	0.10	0.50	0.08	1.00	7.00	89.82
I/F+/G+/S+	Isolate	1.50	0.10	0.50	0.12	1.00	7.00	89.78
P/F-/G-/S-	Pellet	0.00	0.00	0.12	0.08	1.00	7.00	91.80
P/F-/G+/S-	Pellet	0.00	0.00	0.50	0.08	1.00	7.00	91.42
P/F+/G-/S-	Pellet	1.50	0.10	0.12	0.08	1.00	7.00	90.20
P/F+/G+/S-	Pellet	1.50	0.10	0.50	0.08	1.00	7.00	89.82

2.2. Sensory characterizations of pea protein-based solutions and beverages

To sensory characterize pea protein-based products (solutions and beverages), two different panels were used as well as four types of sensory methods: a classical profile, a static block profile, a mono-intake TDS method, and a multi-intake TDS (multi-TDS) method (Figure 2.2.2.).

Table 2.7.: List of sensory methods carried out with used products and panels and section references.

Product	Sensory method	Panel	Section references
S1 to S6	Classical profiling Block profiling	2018-2019	Section 3.1.1.
46 Pea solutions	Block profiling	2018-2019	Section 3.1.2.
12 Pea Beverages	Block profiling Mono and multi-intake TDS	2019-2020	Section 3.4.1.
NA	Prop Test	2018-2019 and 2019-2020	Section 3.1.1, and 3.4.1.

2.2.1. Experimental conditions and panels composition

2.2.1.1. Experimental condition

Panellists performed the sensory evaluations in individual booths under white light in an air-conditioned room (20°C) (Figure 2.2.1.). Sensory analysis was managed using Fizz Acquisition software (v. 2.51, Biosystemes, France). As some beverages were viscous, participants were instructed to intake beverages and solutions with spoons, instead of sipping. To reduce sensation build-up, the following palate-cleansing protocol was used between samples during our formal experiments: panellists had to consume an apple slice, drink water, and wait 40 seconds.



Figure 2.2.1.: Photography of the individual booths.

2.2.1.2. Panel composition

Two different panels were used during this PhD project. Panellists were recruited based on their will to participate and availability to participate in a long-term study (Table 2.7.). The first panel was trained and used from September 2018 to February 2019 (Panel 2018-2019) and included 17 panellists (13 women and 4 men). They had initially no prior experience with pea products or sensory evaluation methods. This panel evaluated pea protein-based solutions and pea protein fraction-based products. The second panel was trained and used from September 2019 to February 2020 (Panel 2019-2020) and included sixteen panellists (15 women and 1 man, 18–39 years in age). Two of the panellists had participated in the first panel. The other panellists had no prior experience with pea protein-based products. This panel evaluated exclusively pea protein-based beverages. Figure 2.2.2. is a schematic representation of the PhD sensory experimental set up.

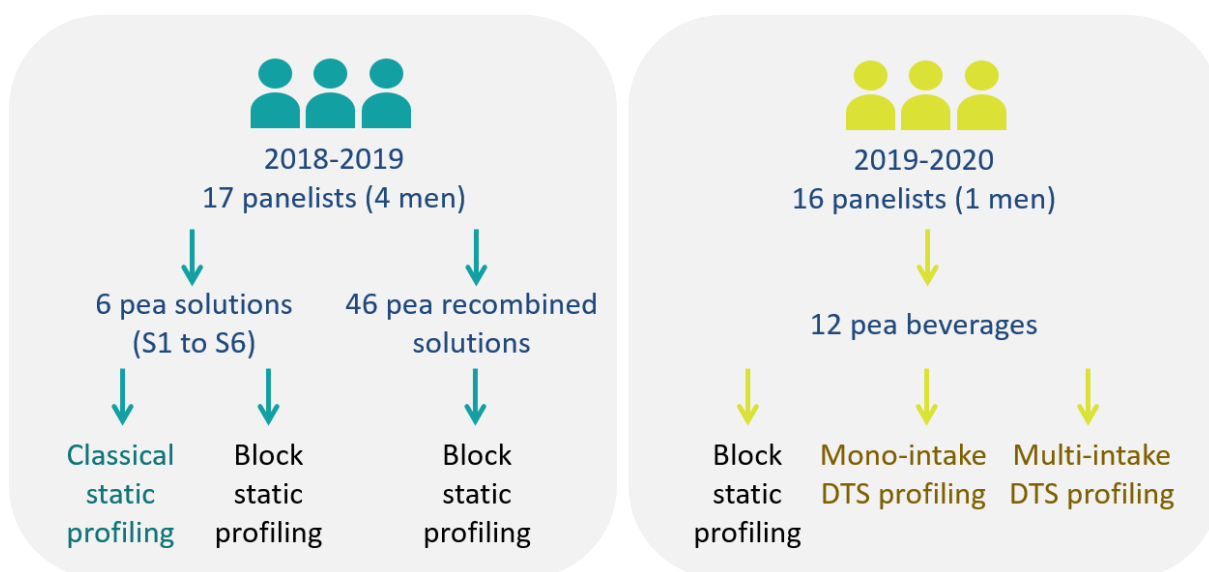


Figure 2.2.2.: Schematic representation of the two panels used for PhD sensory experimental set up.

2.2.1.3. Characterization of panellist PROP status

We assessed the PROP taster status of all the panellists using the methodology of the three-solution test, with sodium chloride as the standard (Tepper *et al.*, 2001) (Figure 2.2.3.). Taste intensity ratings were collected for three solutions of suprathreshold 6-n-Propylthiouracil (Sigma-Aldrich, USA) (0.032, 0.32, and 3.2 mM) and of sodium chloride (NaCl, Sigma-Aldrich, USA) (0.01, 0.1, 1.0 M); the solvent was water (Evian, France). The Labelled Magnitude Scale (LMS) was used for evaluating the samples.

Based on the results of the PROP test, we defined three groups of panellists. In the low PROP taster (LPT) group were panellists who had rated sodium chloride as having a more intense taste than PROP. Panellists who gave similar ratings to sodium chloride and PROP were in the medium PROP taster (MPT) group. Those who had rated PROP as having a more intense taste than sodium chloride were in the high PROP taster (HPT) group. Differences among groups were analysed using repeated-measures analysis of variance (ANOVA) and post-hoc comparisons (Newman-Keuls method).

Among the panel 2018, 7/17 panellists were assigned to the HPT group (PROP intensity > sodium chloride intensity) and 5/17 panellists were assigned to the LPT group (PROP intensity < sodium chloride intensity). The 5/17 panellists who did not perceive a difference between PROP and sodium chloride were assigned to MPT group. Among the panel 2019, 6/16 panellists were assigned to the HPT group, 8/16 were assigned to the LPT group and 2/16 panellists were assigned to MPT group.

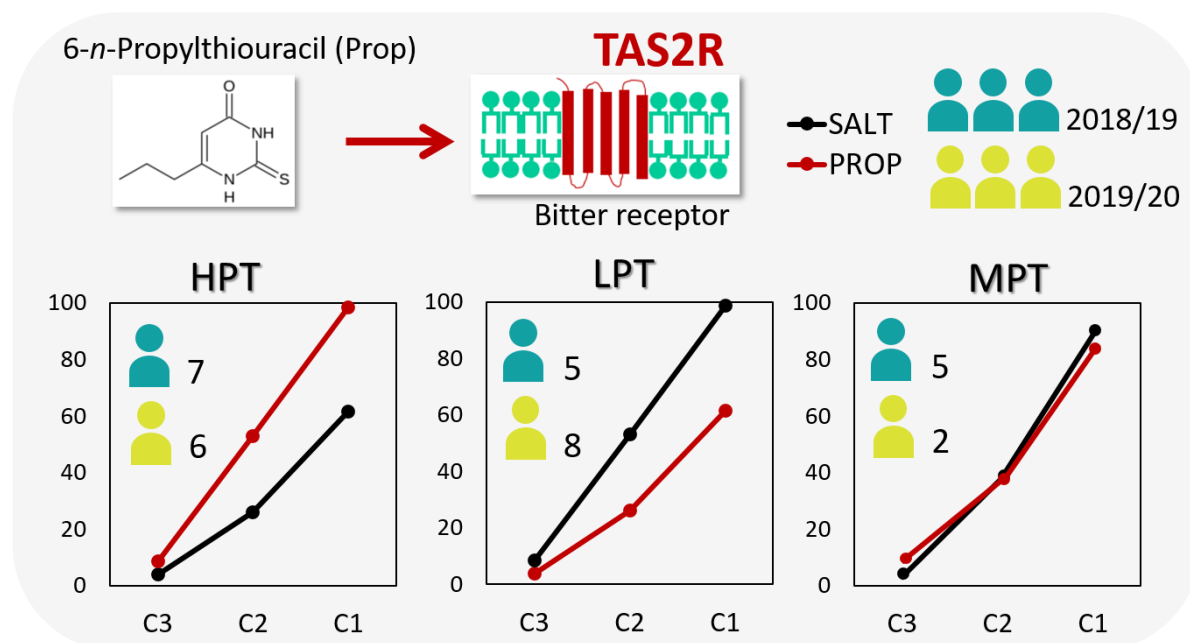


Figure 2.2.3.: Schematic representation of characterization of panellist PROP status.

2.2.2. Static profiling

2.2.2.1. Attribute generation, selection and training

For attribute generation step, panellists were asked to complete a check-all-that-apply (CATA) questionnaire. It listed 30 attributes, and panellists could add more. After discussion with the panel, a final list of 17 attributes was selected (Table 2.8.). As the study was conducted in French, the terms used in French as well as their translation into English are presented in Table 2.8. This list of attributes was then adapted to each sensory study in the PhD.

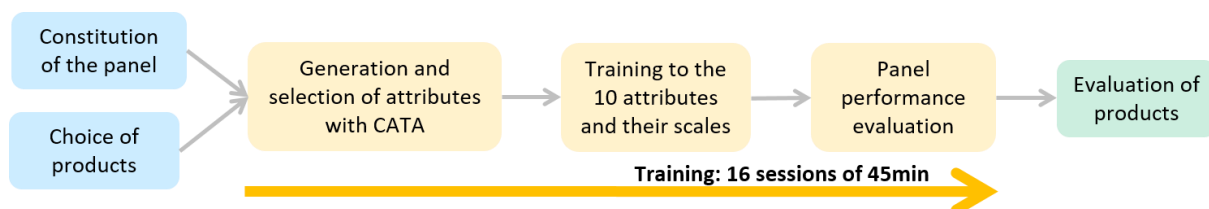


Figure 2.2.4.: General workflow of attribute generation, selection and training.

The panellists were trained to evaluate the intensity of these 17 attributes along an unstructured scale (range: 0–10) using external references. Training took place over 8 sessions (for Panel 2018-2019) and 10 sessions (for panel 2019-2020) that each lasted 45 min (Figure 2.2.4.). Afterward, panellist performance was evaluated and controlled. Overall performance (repetition, discrimination and homogeneity) was assessed using ANOVA with three independent variables (product type, panellist ID, and replicate) and their first-order interactions. We used fixed-effect ANOVAs here because we considered that the panellists were well trained. Another possibility would have been to use mixed-effects ANOVAs that attribute a random effect to the panellists. There was a product effect, indicating that panellists distinguished among the different beverages ($p < 0.05$). The significance of various interactions revealed whether the panellists consistently scored attributes across replicates (panellist*replicate), whether there was consistency in scoring among panellists (product type*panellist ID), and whether panellists scored products consistently across replicates (product type*replicate). The performance of

individual panellists was also evaluated based on their ability to discriminate among products and on repeatability criteria.

Table 2.8.: Definition of the different attributes evaluated by the panellists for the different sensory studies.

Attributes	Attributes in French	Definition	Blocks profiling (Section 3.1.1.)	Experimental design profiling (Section 3.1.2.)	Beverages profiling (Section 3.4.1.)	Beverages DTS (Section 3.4.1.)
Salty	<i>Salé</i>	A fundamental taste – sodium chloride is a typical example	X	X	X	X
Bitter	<i>Amer</i>	The fundamental taste associated with a caffeine solution	X	X	X	X
Astringent	<i>Astringent</i>	A sensation of drying out, roughening, and/or puckering that is felt in the mouth, like when consuming red wine or unripe fruit	X	X	X	X
Sweet	<i>Sucré</i>	A fundamental taste – sucrose is a typical example		X	X	X
Fat	<i>Gras</i>	Property relative to the perception of the quantity of fat in the product			X	X
Watery	<i>Aqueux</i>	Opposite to fat				X
Granularity	<i>Granulosité</i>	The presence of grains or granules in a food	X	X		
Mouthfeel	<i>Epais</i>	The way a food feels in the mouth in relation to its viscosity	X	X	X	X
Liquid	<i>Liquide</i>	Opposite to mouthfeel				X
Overall aromatic intensity	<i>Intensité aromatique globale</i>	Total aroma impressions created by the product in the mouth			X	
Pea	<i>Pois</i>	The flavour characteristic of beans and bean-based foods	X	X	X	X
Almond	<i>Amande</i>	The flavour associated with almonds	X	X	X	X
Nuts	<i>Noix</i>	The flavour associated with nuts, like walnuts or hazelnuts	X	X	X	X
Broth	<i>Bouillon</i>	The flavour associated with boiled vegetables, soup, or stock	X	X	X	X
Potato	<i>Pomme de terre</i>	The flavour associated with cooked potato	X	X		
Grains	<i>Céréales</i>	The flavour associated with grains	X	X		

2.2.2.2. Classical and block profiling

Two static protocols were used in sensory profile: classical protocol and block protocol (Figure 2.2.5.). The block protocol was the static profiling specifically developed for pea based-products during this PhD. In both cases, panellists were asked to evaluate the intensity of a sample's sensory attributes using an unstructured scale ranging from 0 to 10. To account for order and carry-over effects, sample order was balanced across panellists using a Latin square (Williams design). When panellists employed the classical protocol, samples were presented monadically in sequence: panellists evaluated all attributes (printed on the same survey page) at once for each sample.

When panellists employed the block protocol, attributes were evaluated in three blocks. Prior to the experiment, the attribute blocks were defined and discussed with the panellists. For each block, samples were presented monadically in sequence: panellists evaluated all the attributes in the block (printed on the same survey page) for each sample. Blocks were similar for all panellists. The samples order was the same for the three blocks for one subject (but different between subjects). The blocks were built according to the type of perception (sapid / aromatic). To create them, we discussed with the panelists to get their feedback. We also thought about the potential dumping or halo effects and the order of the attributes within the blocks, especially those of the most important attributes (e.g. bitterness). In analysing the data, we kept these potential effects in mind, especially for the last attributes evaluated (potato and grains). In addition, the first sample in each session, for the three blocks, was also the reference used to limit drift between sessions. This reference was available in large quantities and was stored under highly stable conditions.

Note: we have proposed during these PhD a new methodology for several reasons. First of all, it came from an internal need of the company who had difficulties to characterize the pea based products with their trained panel. Moreover, according to the interlocutors, there was no homogeneity in the definitions of beany. In addition, beany perception is complex and multidimensional. It is therefore difficult to describe it and it is possible to omit sub-dimensions of this perception. The training of the panelists and the choice of references is therefore important. Finally, the pea based products are also very persistent and there are therefore potential risks of sensory fatigue or sensory adaptation.

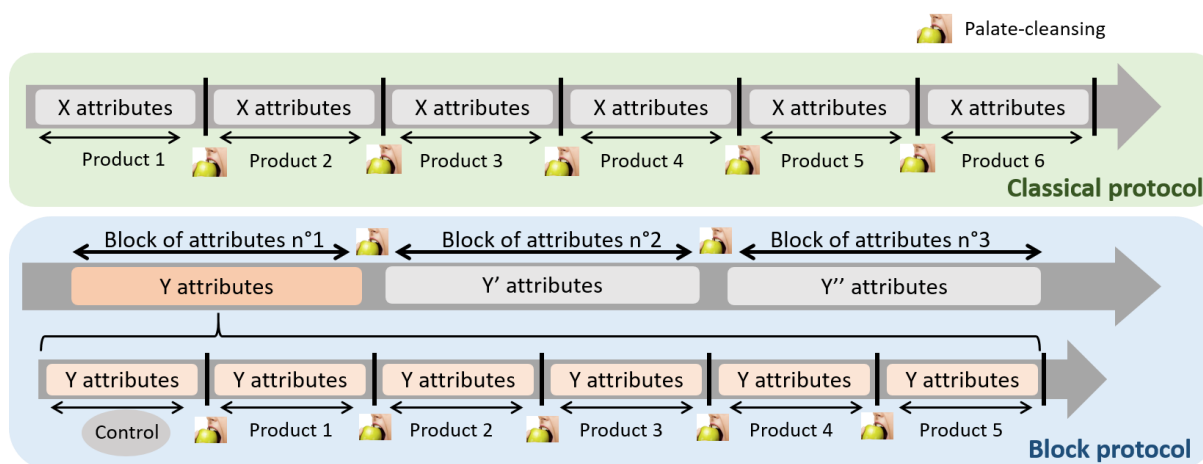


Figure 2.2.5.: Schematic representation of the profiling protocols used in this study: classical profiling protocol in green and block profiling protocol in blue.

2.2.3. Temporal dominance of sensations profiling

2.2.3.1. Mono-intake temporal dominance of sensations profiling

To evaluate the temporal evaluation of perceptions, a mono-intake TDS method was used (Figure 2.2.6.). Panellists evaluated the sequence in attribute over a period of 120s after taking a sip of a given beverage. They evaluated six beverages per session. All the attributes were simultaneously presented on the computer screen. Attribute list was the same for each panellist for all the mono-intake TDS sessions but the attributes order in the list was randomly assigned and balanced among panellists. The evaluation process started as soon as the panellists took a sip of the beverage. The panellists then had to click on the attribute that they perceived as dominant, which was defined for them as “the attribute that draws the most attention.” When this dominant attribute changed, the subject had to click on the new dominant attribute. The panellist was free to choose the same dominant attribute several times or, conversely, to never select a dominant attribute. The panellists also had to click on the button “I swallowed” each time they swallowed the beverage or their saliva. For each panellist and each beverage, the following data were collected: the time at which an attribute was selected as dominant, the specific attribute, the time that had elapsed before the panellist clicked on “I swallowed” for the first time (i.e., the panellist had largely consumed the product), and the number of times that the panellist clicked on the button “I swallowed”.

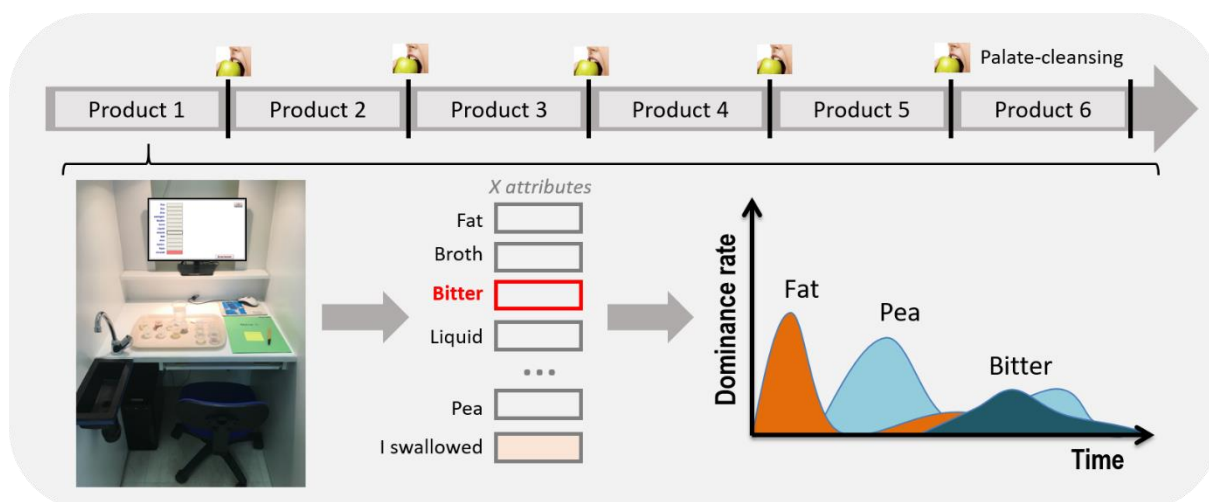


Figure 2.2.6.: Schematic representation of the TDS profiling protocol used in this study.

2.2.3.2. Multi-intake temporal dominance of sensations profiling

To evaluate the temporal evaluation of perceptions, a multi-intake TDS method was used. The multi-intake TDS profiling method can be used to evaluate changes in attribute perceptions as people consumed a full portion of a product (Figure 2.2.7.). Here, a portion was defined as 120 mL, which is equivalent to an entire ready-to-drink beverage or a cup of yogurt. First, the panellists had to cleanse their palates. Over the course of the session, they were not allowed to consume anything except the product to allow for the possible cumulative effects of persistent sensations. Second, the panellists evaluated the products using the same general approach as in the mono-intake TDS profiling method, except that a given product was evaluated at three time points. The first evaluation took place after the first spoonful of the product was consumed (hereafter, first spoonful). The second evaluation took place after panellists had consumed 60 mL of the product (~ half the portion); they then had to evaluate a second spoonful of the product (hereafter, second spoonful). The third evaluation took place after panellists had consumed the remaining 60 mL of the product; they then had to evaluate a final spoonful of the product (hereafter, third spoonful). We thus obtained three sets of data reflecting the shift in sensations from the beginning to the end of product consumption. No time limits were placed on this process.

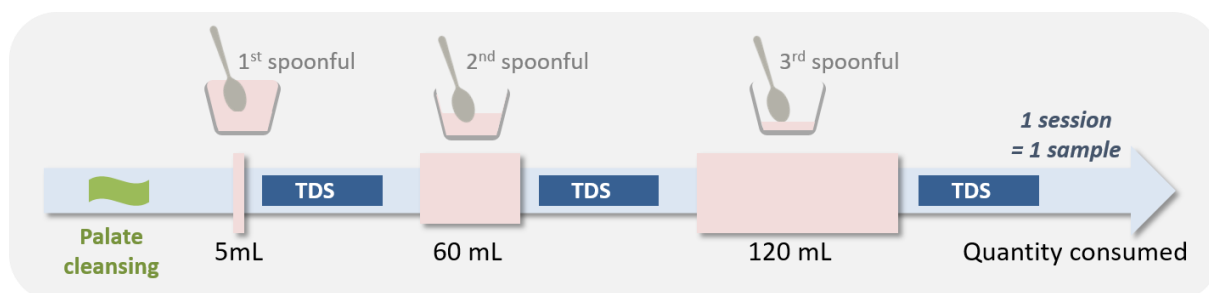


Figure 2.2.7.: Schematic representation of the multi-intake temporal dominance of sensations (TDS) method used in this study.

2.2.4. Statistical analysis

The data were automatically acquired using Fizz Acquisition software (v. 2.51; Biosystemes, 1990). Data analysis was performed using XLSTAT (Addinsoft, 2017, Paris, France), R (R Core Team, 2019) and JMP software version 13.1.0 (SAS Institute Inc., Cary, SC, USA). The threshold for statistical significance was $\alpha = 0.05$.

The static profiling data were analysed using ANOVA. To assess panellist performance, ANOVA were carried out in which product type, panellist ID, and replicate were fixed effects and there were first-order interactions. Post-hoc comparisons were then performed to interpret the specific effect of product type (Newman-Keuls method). To visually explore differences in the results obtained, we carried out principal component analysis (PCA) on a correlation matrix; the data were averaged across replicates and panellists. To study the possible drift between sessions, we carried out a two-way ANOVA on the data for the reference solution. To visually explore differences in the results obtained using classical versus block protocol, we carried out multiple factor analysis (MFA) (centred by group; Pearson type); the data from both protocols were averaged across replicates and panellists.

Note: For all of the PCAs in the PhD manuscript, the Cos2 values that were considered acceptable for PCA interpretation are values greater than 0.7. But in each case, the PCAs were used for visualization purposes. The results were confirmed by regressions and analysis of variances. In the same way, only the first two axes will generally be presented but all the axes have been studied each time.

In the case of TDS profiling analyses, the time to the first instance of swallowing and the total duration of the evaluation period were extracted from the data collected during the sessions. ANOVA were performed in which product type, panellist ID, and replicate were fixed effects and there were first-order interactions. For the multi-intake TDS profiling data, the ANOVA had product type, panellist ID, replicate, and spoonful ID as fixed effects and included first-order interactions. Relative attribute dominance (i.e., the percentage of panellists who perceived a given attribute as dominant) was determined for each beverage at each time point, and the TDS curves were graphed. As suggested by Pineau *et al.* (Pineau *et al.*, 2009), two lines were drawn on the TDS graph: one line representing the relative dominance an attribute could achieve by chance alone when considering all the attributes evaluated and one line representing the minimum relative dominance an attribute must obtain for the result to be significantly different from that expected by chance alone (binomial distribution, $\alpha = 0.05$).

2.3. Physico-chemical characterizations of pea protein-based products

2.3.1. Overall characterisation of composition of pea protein-based products

A large number of characterization methods were used during the PhD work in order to select the best to answer to the objectives and along the decided strategy (Table 2.9.). So, the volatile molecules of some products have been also characterised by GC-MS with Olfactrometry (GC-MS-O) and Proton-transfer-reaction mass spectrometry (PTR-MS). However, GC-MS-O does not take into account the physico-chemical interactions and the matrix. For PTR-MS analysis, whatever the conditions (pea protein concentrations and quantity consumed), monitor ions presented areas under the curve at the limit of detection of the PTR-MS. Results presented also a high variability between panellists (5) complicating the analysis of results. We thus preferred to avoid these types of measurements.

In addition, the protein fraction of some isolates was characterised by 1D and 2D electrophoresis. However, the 1D results showed only little difference between the samples. The 2D results showed more difference between the samples. The intensity of the spots could be measured and correlated with sensory perceptions. However, no relevant results could be drawn. Moreover, this method was difficult to apply to fractions.

The free amino acids were also characterised in few samples. However, the differences between the samples were not correlated with sensory differences. Finally, the structure of some isolates was characterised by micro DSC and DLS. The DSC analyses did not lead to any relevant results. Indeed, it is highly probable that the proteins of the commercial isolates are highly denatured due to their process. The DLS results showed few relevant results between the different isolates. Moreover, these two methods were also difficult to apply to fractions. Therefore, the choice was made not to further study these methods and not to present the results in more detail in this PhD manuscript.

Table 2.9.: List of characterizations carried out, location of the information and principle of the characterization methods.

Products	Compounds/ characteristics	Methods	Abbreviation	Laboratories	References if used for the models if not explanation of why it was not continued the characterization with this method
Fractions	Phytochemicals	Ultra-high performance liquid chromatography mass spectrometry	UHPLC-MS	Sayfood	Section 3.2.3.
Solutions	Free amino acids	Ultra-high performance liquid chromatography mass spectrometry	UHPLC-MS	Sayfood	No relevant differences between products
Solutions, Beverages	Volatiles	Proton-transfer-reaction mass spectrometry	PTR-MS	Sayfood	Monitor ions presented areas under the curve at the limit of detection
Beverages	Texture	Rheometer	NA	Sayfood	Section 3.4.2.
Solutions	Hydrophobicity index	Spectrofluorometry	Na	Sayfood	Section 3.2.2.
Solutions	pH and conductivity	Conductivity probe	Na	Sayfood	Section 3.1.2.
Isolates	Proteins	Electrophoresis 1D	NA	Sayfood	No relevant differences between products. Not applicable for fractions
Isolates	Proteins	Electrophoresis 2D	NA	Sayfood	Difficult to achieve. Not applicable for fractions
Solutions, Beverages	Volatiles	Gas Chromatography-Mass Spectrometry	GC-MS	Sayfood	Section 3.2.1. and 3.4.2.
Solutions	Volatiles	Gas Chromatography-Mass Spectrometry coupled to olfactometry	GC-MS-O	Roquette	Do not take into account physico-chemical interactions
Solutions	Peptides	Ultra-high performance liquid chromatography mass spectrometry	UHPLC-MS	PAPPSO	Section 3.2.2.
Solutions	Protein content	Kjeldahl method	Na	External	Section 3.1.2.
Isolates	Heat capacity	Micro Differential scanning calorimetry	micro DSC	External	No relevant differences between products. Not applicable for fractions
Isolates	Size distribution particle	Dynamic light scattering	DLS	External	No relevant differences between products. Not applicable for fractions

The following sections provide the description of relevant methods to better understand the perceptions of pea protein isolates. In order not to multiply the information presented in this manuscript, only a brief description of each used method will be detailed in the following sections. In order to obtain more information, the reader could refer to the Material and Methods sections of the Results following the correspondence presented in the Table 2.9.

2.3.2. Characterisation of volatile composition of pea protein-based products

To analyse the aroma compounds present in the pea protein beverages, GC-MS analysis was performed. The Figure 2.3.1. describe the general workflow of the volatile analytical pipeline and characteristics of the different samples analysed.

A Dynamic Headspace Gas Chromatography coupled with a Mass Spectrometer (MPS: Gerstel, GC: Agilent 7890B, MS: Agilent 5977B MSD) was used to quantify aroma compounds released from the different beverages. Protocol was carried out as follows. After thawing during one night at 4°C, 5g of each product was stored at 10°C on the GC-MS sample holder. Each sample was then incubated at 40°C for 3min. The headspace was then purged with a constant flow of helium for 10 min for pea solutions and 20 min for beverages at 30°C and aroma compounds were trapped on an adsorption unit (tenax polymer). The trap was dried under a stream of helium to remove traces of water. Then the trap unit was desorbed from 30°C to 270°C. The GC was equipped with polar or an apolar column (respectively HP-INNOWax Agilent, 60m x 320µm x 0.25µm and DB-5, 60m x 320µm x 1µm) with a helium flow of 1.6 mL/min.

The two types of chromatography column were used to identify a larger number of volatile compounds. A mass spectrometer was used to characterize aroma compounds. The compounds were identified by comparison of their mass spectra with those of the NIST 2017 Mass Spectral Library. The retention times and the Kovats retention index were also used for the tentative identification. The data were reported as peak area for each compounds detected. Quantification data were obtained from the integration of the areas from the total ion current (TIC). MassHunter software was used for instrument control, data acquisition and data analysis.

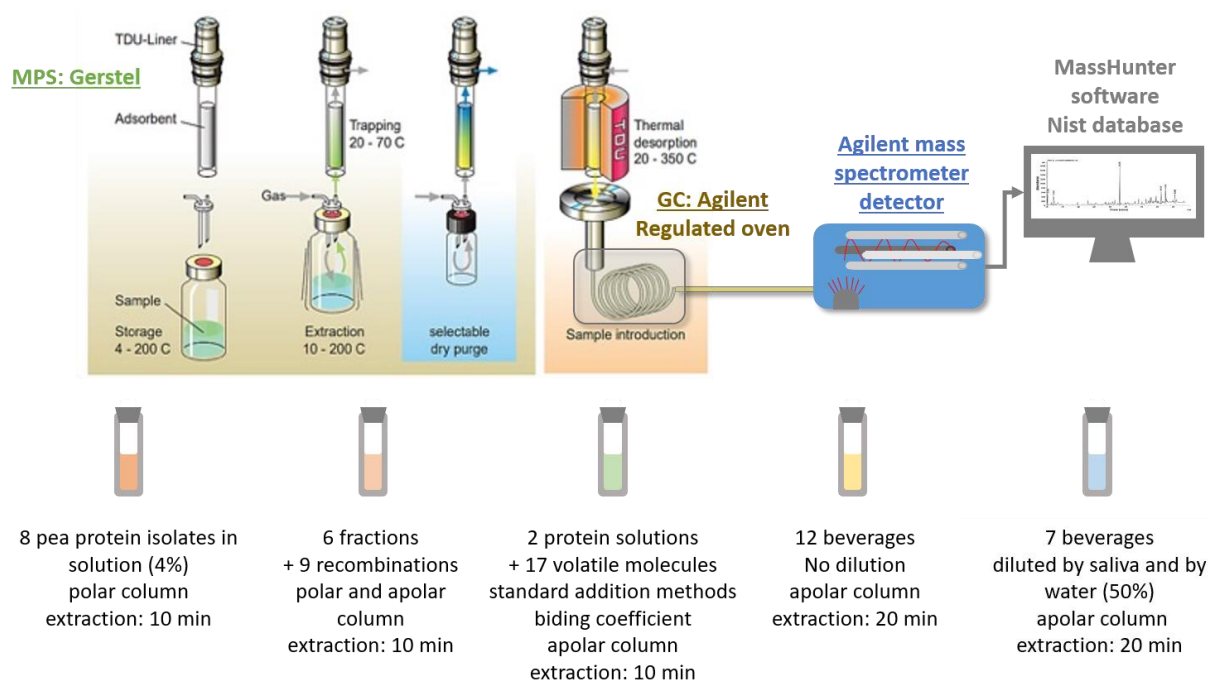


Figure 2.3.1.: General workflow of the volatile analytical pipeline and characteristics of the different samples analysed.

2.3.3. Characterisation of peptides composition of pea protein-based products

The Figure 2.3.2. describe the general workflow of the peptidomics analytical pipeline, starting from the experimental part to the bioinformatic analyses. Before LC-MS analysis, pea solutions were centrifuged (15 000g, 4°C, 15min). Supernatants were filtrated via Vivaspın centrifugal concentrator (20mL, 10 kDa, sigma Aldrich) at 8000g (30min, at 4°C). Filtrate solution were stored in the dark at -80°C previous to analysis. Mass spectrometry was performed on the PAPPSO platform (MICALIS, INRAE, Jouy-en-Josas, France). An Orbitrap Fusion™ Lumos™ Tribrid™ (Thermo Fisher Scientific) coupled to an UltiMate™ 3000 RSLC nano System (Thermo Fisher Scientific) was used. Eluted peptides were analysed on line on the Orbitrap mass analyser. Peptide identification was performed with X!Tandem version 2017.2.14 (Alanine) and X!Tandem Pipeline (C++) version 0.2.40 (Langella *et al.*, 2017) on the protein sequence of *L. Pisum Sativum*. MassChroQ version 2.2.17 (mass Chromatogram Quantification) was used to perform alignment, XIC extraction, peak detection and quantification (Valot *et al.*, 2011).

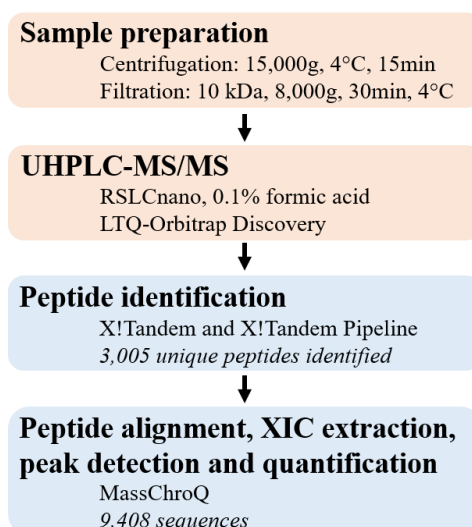


Figure 2.3.2.: General workflow of the peptidomics analytical pipeline, starting from the experimental part (in orange) to the bioinformatic analyses (in blue).

2.3.4. Characterisation of phytochemicals composition of pea protein-based products

The Figure 2.3.3. describe the general workflow of the phytochemicals analytical pipeline, starting from the experimental part to the bioinformatic analyses.

Samples with a high dry matter content (flour, isolates *a* and *b*, pellets *a* and *b*) were extracted three times with methanol/formic acid mixture (99/1, v/v) for 2h with a magnetic stirrer at room temperature, protected from light. After extraction, supernatants were kept for 10 min at -20°C then separated by centrifugation. The supernatant was evaporated under vacuum at 40°C and in the dark to protect sample from light. In order to remove some precipitates occurring during the concentration step, a centrifugation was performed before total evaporation of solvent. After that, supernatants were evaporated under vacuum at 40°C protected from light until the solvent has evaporated completely. After resolubilization, in methanol/water mixture (80/20, v/v), samples were kept for 10 min at -20°C to promote precipitation. At that point, samples were separated by centrifugation. Supernatants were filtered through a micrometer PTFE filter and stored in the dark at -80°C previous to analysis. All these steps of filtration and centrifugation were added to ensure obtaining a clear extract without any precipitate. Samples with a low dry matter content (retentate *a* and *b*, permeate *a* and *b*) were directly mixed to methanol/formic acid mixture (99/1, v/v) for 10 min at room temperature, protected from light. Then, solvents were prepared as previously explained.

Chromatographic separations were performed using UHPLC system (Ultimate 3000 Thermo Scientific, USA). The analytical column used for separations was a Hypersil GOLD (length 100mm, internal diameter 2.1mm, granulometry 1.9µm, Thermo Scientific). The mobile phase consisted of (A) water + 0.1% formic acid and (B) acetonitrile + 0.1% formic acid (Optima, Thermo Fisher Scientific). The UHPLC system was coupled to a Q Exactive Orbitrap high resolution mass spectrometer (Thermo Scientific, USA) equipped with heated-electrospray ionization probe (HESI II, Thermo Scientific, USA). The mass spectrometer was operated in negative and in positive ion modes. MS spectra (MS and MS²) were acquired by full MS and Full MS/ddMS² range (m/z). The system was also coupled to a diode array detector covering full range acquisition between 190 and 600 nm.

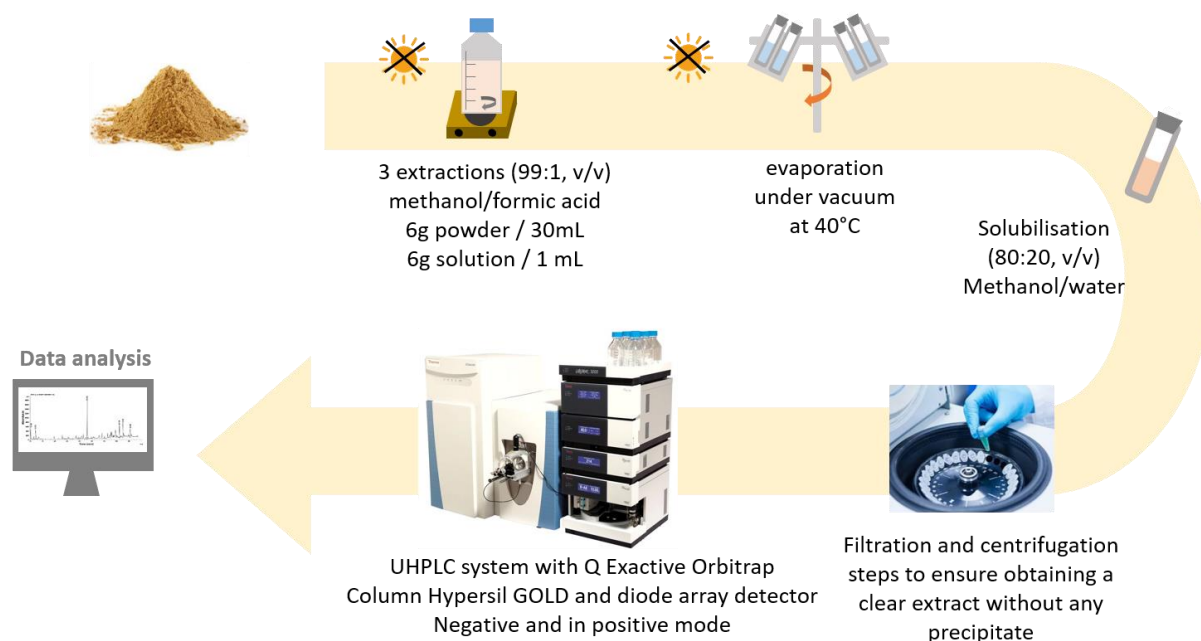


Figure 2.3.3.: General workflow of the phytochemicals analytical pipeline, starting from the experimental part to the bioinformatic analyses.

Phytochemicals were identified according to the corresponding spectral characteristics: mass spectra, accurate mass, characteristic fragmentation, UV spectrum and characteristic retention time. Xcalibur (Thermo Scientific, USA) was used for instrument control, data acquisition, and data analysis. Phytochemical quantification was obtained by integration of specific reconstructing ion current in comparison with the calibration curves with TraceFinder software (Thermo Scientific, USA). Compound discoverer (Thermo Scientific, USA) with several databases (PubChem, Phenol explorer, Flavonoid database, Arita database, NPASS database) were used to propose formula and identification of peaks.

2.3.5. Characterisation of mechanical properties of pea protein beverages

A rheometer (MCR301, Anton Paar, Graz, Austria) equipped with sanded coaxial cylinders (diameter of 27 mm) was used to test the gels' rheological properties. The beverages were carefully poured into the cylinder, which was kept at 20 °C. Different tests were subsequently carried out on the samples (Figure 2.3.4.):

- Steady shear rate (1 to 1000 1/s, 15 measurement points) were performed at 20 °C using rotation mode. From the shear rate and the shear stress data, the flow curves were plot using logarithmic scales and modelled with a power law ($\tau = \gamma^n$ such as τ = shear stress, γ = shear rate, n = power law index and k = consistency).
- Strain sweeps (0.001–100%, 1 Hz) were performed at 20 °C. From the shear stress data, the loss modulus [Pa] and the storage modulus [Pa] were calculated.
- Frequency sweeps (10–0.01 Hz, 1%, 11 measurement points) were performed at 20 °C. From the shear stress data, the loss modulus [Pa] and the storage modulus [Pa] were calculated.

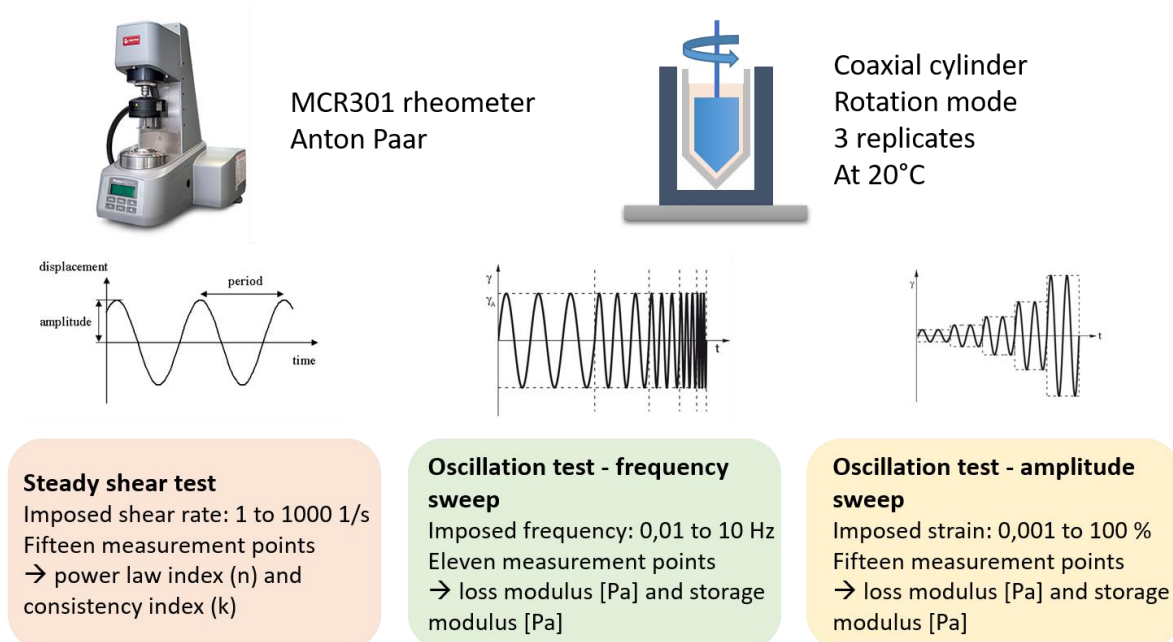


Figure 2.3.4.: Characterisation of mechanical properties of pea protein beverages.

2.4. Statistical approaches used to link different instrumental and sensory data sets

Several statistical methods were used between the different sets of data obtained experimentally. They have been chosen from a large panorama of multidimensional statistical methods in order to best answer to the different objectives of the PhD project. The aim of these models was to model sensory attributes as a function of composition. In a first step, the sensory attributes were modelled from the composition in fractions (Optimal mixture models).

Then the relevant sensory attributes were correlated to the different chemical data sets separately. Volatile compounds were correlated to sensory attributes with Pearson correlation coefficients, Partial Least Square (PLS) regressions and Artificial Neural Network (ANN) regressions. Phytochemical compounds and peptides were correlated to sensory attributes with Pearson correlation coefficients. This step allowed the selection of key compounds and the reduction of the number of variables.

Finally, to establish the relative contribution of each group of chemical compounds present on pea protein isolates on sensory perceptions, a multi-block PLS model was used. Figure 2.4.1. summarizes all the physic-chemical and sensory variables as well as the main models developed.

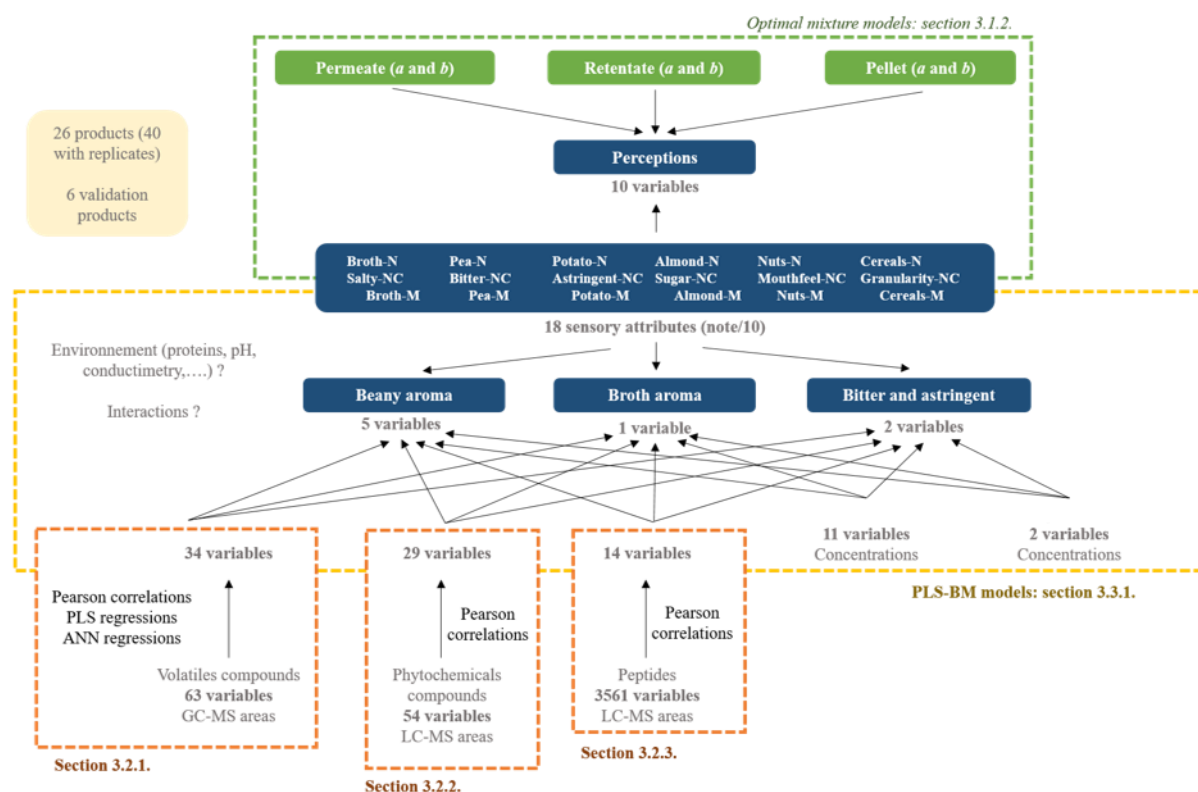


Figure 2.4.1.: Generals workflow of the statistical analysis done during this PhD.

2.4.1. Variables clustering

This dataset allowed us to compare different clustering methods used on sensory data but also chemical data: hierarchical clustering, k-means, latent class and VARCLUS®. Clustering methods allow products to be grouped according to similarity or difference. In particular, they have been used in the construction of latent variables. These methods yielded similar results as shown on the Figure 2.4.2. for sensory attributes. So finally, in a classical way, we have always used hierarchical clustering.

A) Blocks as evaluated by panellists: 4 groups

1. Salty-NC, Bitter-NC, Astringent-NC, Sugar-NC
2. Mouthfeel-NC, Granularity-NC
3. Broth-N, Pea-N, Potato-N, Almond-N, Nuts-N, Cereals-N
4. Broth-M, Pea-M, Potato-M, Almond-M, Nuts-M, Cereals-M

B) Varclus (6 groups)

1. Sugar-NC
2. Mouthfeel-NC, Granularity-NC
3. Broth-M, broth-N, Salty
4. Pea-N, Pea-M
5. Nuts-N, Almond-N, Cereals-N, Potato-N, Nuts-M, Almond-M, Cereals-M, Potato-M
6. Bitter-NC, Astringent-NC

C) Kmeans (9 groups)

1. Sugar-NC
2. Mouthfeel-NC
3. Granularity-NC
4. Broth-N, Broth-M
5. Salty-NC, Pea-M
6. Pea-N, Almond-N, Nuts-N, Cereals-N
7. Almond-M, Cereals-M
8. Potato-N, Astringent-NC, Potato-M, Nuts-M
9. Bitter-NC

D) Hierarchical clustering (9 groups)

1. Sugar-NC
2. Mouthfeel-NC
3. Granularity-NC
4. Broth-N, Broth-M
5. Salty-NC, Pea-M
6. Almond-M, Cereals-M
7. Potato-N, Almond-N, Nuts-N, Cereals-N
8. Pea-N, Astringent-NC, Potato-M, Nuts-M
9. Bitter-NC

E) Latent class (9 groups)

1. Sugar-NC
2. Mouthfeel-NC
3. Granularity-NC
4. Broth-M, Broth-N
5. Salty-NC
6. Pea-M, Pea-N
7. Almond-N, Cereals-N, Nuts-N, Potato-M, Potato-N
8. Almond-M, Cereals-M, Nuts-M
9. Astringent-NC, Bitter-NC

Figure 2.4.2.: Clustering of sensory attributes (scores/10) evaluated for the 46 pea solutions in duplicate by 17 trained panellists by block profiling with different clustering methods.

2.4.2. Optimal mixture models (fractions)

The objective of the mixing design is to model the effects of composition on fraction (X effect) on the sensory perceptions of the products (Y responses) and to find the levels of X factors to optimize the Y responses (predictive capability of the model) (Figure 2.4.3.). JMP (v. 13.1.0; SAS Institute Inc., Cary, SC, USA) was used to generate and analyse the optimal mixture design.

	Pellet <i>a</i>	Permeate <i>b</i>	...	Water	Salty	Bitter	...	Broth	
P1	0%	0%		60%	1.73	1.94		1.07	} 40 Products
P2	50%	0%		50%	2.90	3.34		1.73	
...									
P40	0%	0%		0%	3.43	4.66		5.65	

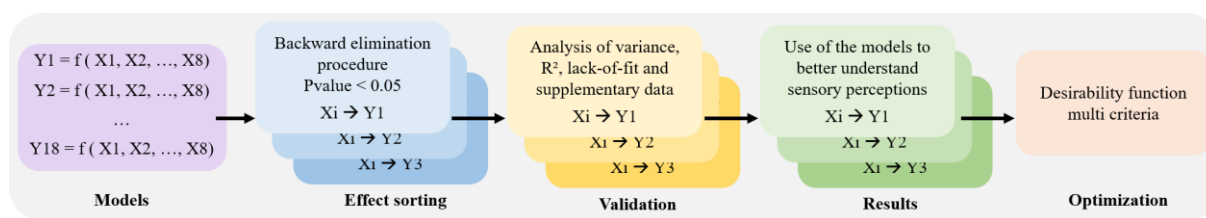


Figure 2.4.4.: Schematization of the analysis process of the mixing design models.

2.4.3. Pearson correlations (phytochemicals, volatiles and peptides datasets)

Due to the complexity of the data sets, it was sometimes difficult to make prediction models. In this case, only Pearson's correlation coefficients were calculated. Pearson correlation coefficient is a statistic method that measures linear correlation between two variables X and Y. They have been used to determine correlations between different sensory attributes as well as to correlate sensory attributes with volatile compounds, peptides and phytochemical compounds.

2.4.4. Partial Least square regressions (volatiles datasets)

These datasets allowed us also to compare different multivariate regression methods. Multivariate regression methods are prediction methods that look for cause-and-effect relationships between variables. Principal component regressions (PCR) were first used. There are regression analysis techniques based on principal component analysis (PCA): the principal components of the explanatory variables are used as regressors (Figure 2.4.5.). PCR are efficient to result in dimension reduction through substantially lowering the effective number of parameters characterizing the underlying model. They are efficient to deal with multicollinearity problem which arises when several explanatory variables are close to being collinear. However, with PCR model, the first components, associated with the largest eigenvalues, are not necessarily correlated with Y and are therefore not necessarily the best candidates for modelling Y (Jackson & Mudholkar, 1979).

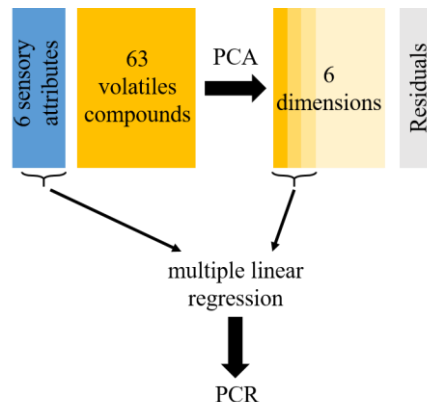


Figure 2.4.5.: Schematization of the Principal Component Regression as applied to the volatile compounds datasets (data not presented).

Thus, Partial Least Square Regressions (PLS) were then used. PLS are also useful when there is a large number of explanatory variables and when there are strong collinearities between the variables (Höskuldsson, 1988). It allows the comparison of two blocks of variables taking into account the correlations between the variables (Wold, Sjöström, *et al.*, 2001). One of the advantage of this model is to obtain the variable importance projection scores which could be easily calculated. They are a measure of a variable's importance relative to model both X and Y; 0.8 being the commonly used criterion to say that the variable is significant (Eriksson *et al.* 2006). This method has been used to predict aromatic perceptions as a function of volatile compounds (Figure 2.4.6.). In order to obtain more information, the reader could refer to the Material and Methods section 3.2.1.

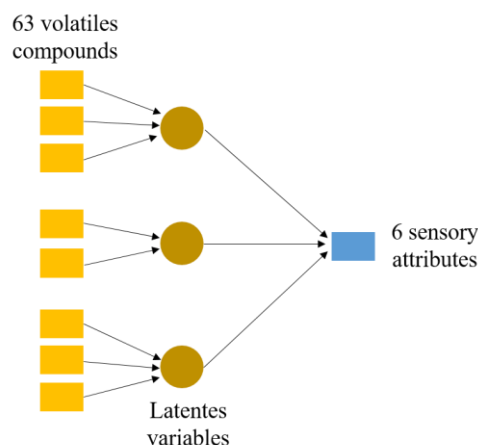


Figure 2.4.6.: Schematization of the Partial-Least-Square regression as applied for the volatile compounds dataset on section 3.2.1.

2.4.5. ANN model (volatiles datasets)

We tested also artificial neural networks (ANN) to model pea perceptions in function of composition. If ANN model allows to make more complex models taking into account interactions and threshold, they are however black box prediction machine with no insight into relationships between predictors and variables and which do not allow formal hypothesis tests. In addition, with ANN model, it is difficult to identify the most important variables in predicting the response (Seisonen *et al.*, 2016). This method has been used to predict aromatic perceptions as a function of volatile compounds (Figure 2.4.7.). In order to obtain more information, the reader could refer to the Material and Methods section 3.2.1.

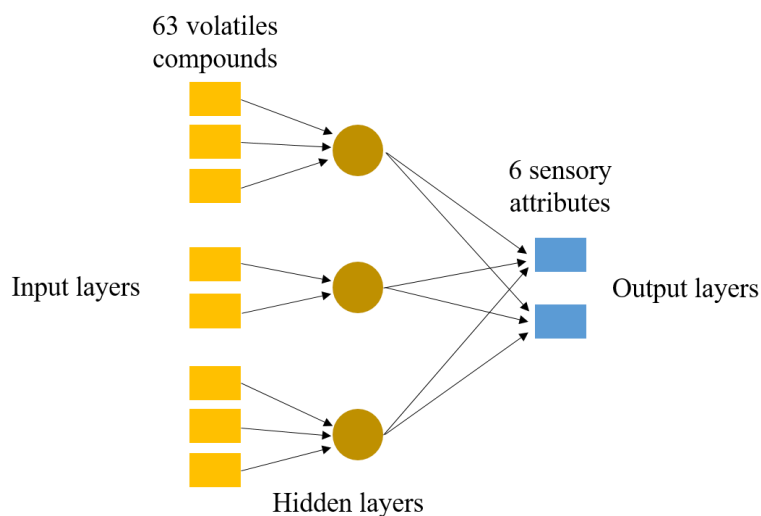


Figure 2.4.7.: Schematization of artificial neural networks as applied for the volatile compounds dataset on section 3.2.1.

2.4.6. Partial Least square Multi-Block regressions

In order to consider the block structure of the datasets, more complex models were also used. First, PLS-Path Modelling (PLS-PM) method was tested to model pea perceptions in function of composition model. In PLS-PM method, X and Y variables are divided into sub-blocks, to create a PLS model of each sub-block, and then to use the resulting scores as a new set of variables in a new PLS model (Figure 2.4.8.). It allows to study in the same model different variables to explain using latent variables (Wold, Sjöström, *et al.*, 2001). However, within our dataset, it was difficult to group the manifest variables (VM) of the external model and thus create the latent variables (VL) of the internal model. These difficulties were particularly important for the phytochemicals and peptides datasets. In addition, it was

difficult to give meaning to the results obtained. And the resulting models were not very efficient. We therefore decided to work with another regression method.

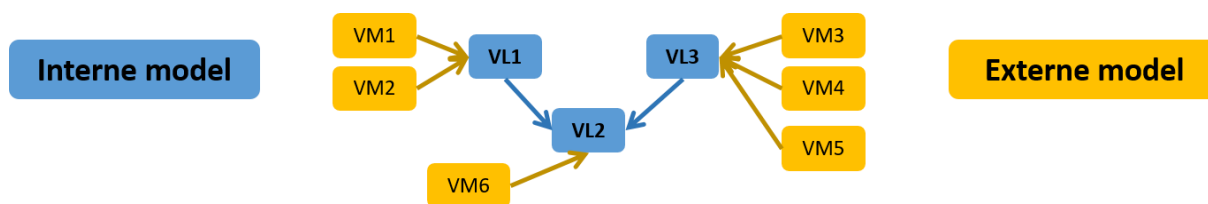


Figure 2.4.8.: Schematization of Partial-Least-Square Path-Modelling regressions.

Subsequently, we then built a PLS Multi-Block (PLS-MB) model. Different algorithms have been proposed for PLS-MB (Biancollilo *et al.*, 2019). The one adopted in the present work is the one proposed by the mbpls function of the R package “ade4” version 1.7-16 (Bougeard and Dray 2018; Dray and Dufour 2007). This method is based on a classical PLS algorithm. However, it makes it possible to group the variables by blocks of the same concept and to calculate the respective contributions of each of these blocks (Figure 2.4.9). To build these models, the 90 chemical compounds were grouped by chemical family (peptides, volatile compounds, phytochemical compounds). In addition, sensory attributes were also grouped together. Bitter and astringent attributes were clustered together. Broth attribute, very different from the other sensory attributes was clustered separately. The other aromatic attributes pea, potato, almond, nuts and cereals were clustered together. Thus for each of these 3 groups of sensory attributes an optimal model was selected with a two-fold cross-validation. In order to obtain more information, the reader could refer to the Material and Methods section 3.3.1.

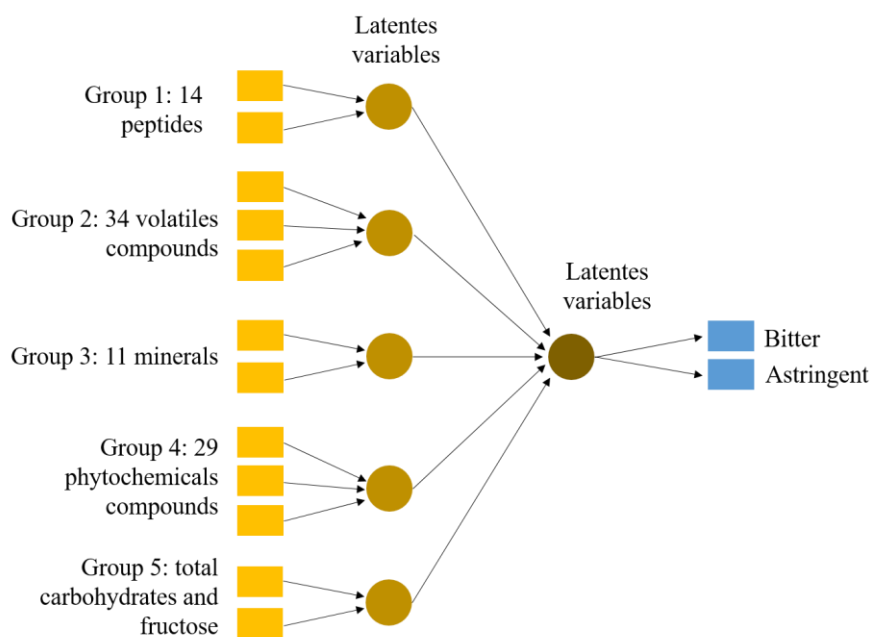


Figure 2.4.9.: Schematization of Partial-Least-Square Multi-Blocks regressions as applied on the bitter and astringent attributes on section 3.3.1.

RESULTS

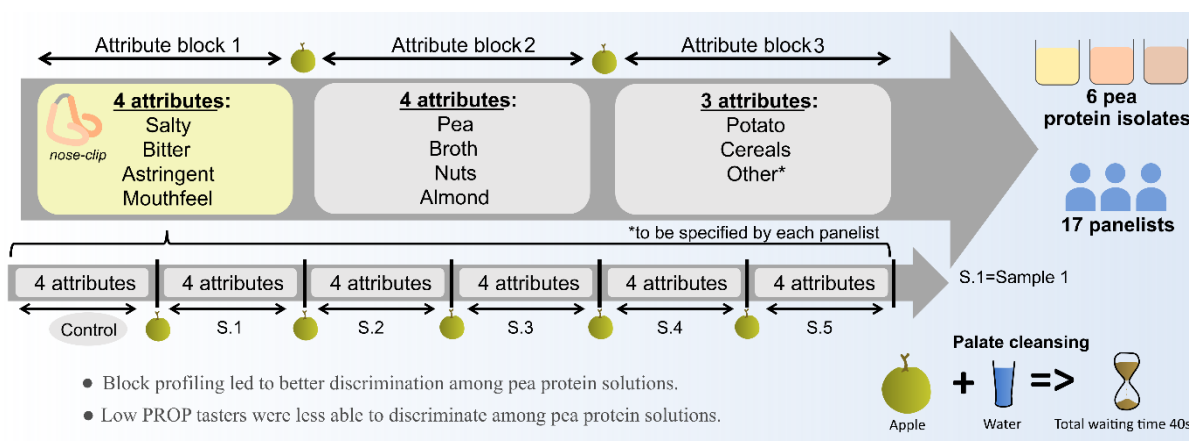
Section 1 - Sensory methodologies and experimental strategy to study pea protein isolates perceptions

Section 3.1. – Sensory methodologies and experimental strategy to study pea protein isolates perceptions

Context, objectives and presentation of section 3.1.

In order to investigate the mechanism that lead to sensory properties of pea-protein based products, it is necessary to describe and quantify their perception, and by consequences a relevant sensory profiling protocol is required, adapted to the type of products. The literature review shows that pea protein isolates are usually sensory described as beany, bitter and astringent with strong persistence. However, 1) there is no consensus definition of beany's perceptions; 2) high differences among panellists in bitterness sensitivity could greatly influence sensory perceptions of foods containing pea proteins; 3) during a sensory evaluation using conventional methods, some bias can strongly impact the sensory assessment of studied products (carry over, sensory fatigue, etc.). Thus, the aim of the work of this section was to propose a relevant protocol with relevant attributes for the evaluation and the characterization of pea protein-based samples.

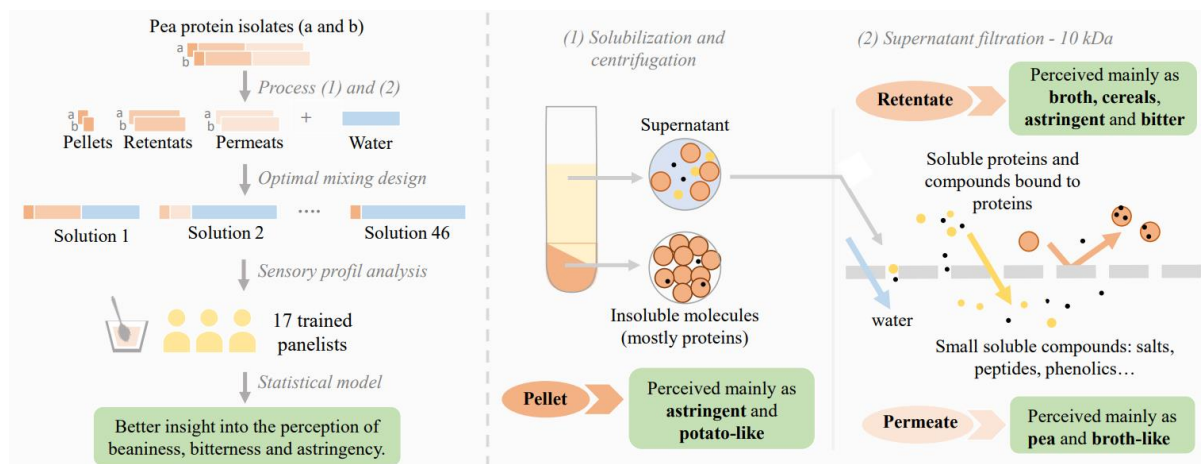
Here, we developed a sensory profiling protocol (block profiling) that could be used by trained panellists to effectively evaluate the perception of pea-protein-based foods (Section 3.1.1.). In short, as explained on the graphical abstract (Graphical abstract 3.1.1.), we adapted a classical profiling protocol —we incorporated relative-to-reference evaluation, and panellists assessed attributes in blocks rather than all at once. A focus on palate-cleansing was performed, to optimise the conditions of evaluations. In addition, sensitivity to bitterness of panellists (PROP test) was evaluated to better characterize the used panel. Results were published and discussed on *Food Quality and Preference* as presented below (Cosson, A., Delarue, J., Mabil, A.-C., Druon, A., Descamps, N., Roturier, J.-M., Souchon, I., & Saint-Eve, A. (2020). Block protocol for conventional profiling to sensory characterize plant protein isolates. *Food Quality and Preference*, 83, 103927. <https://doi.org/10.1016/j.foodqual.2020.103927>).



Graphical abstract 3.1.1.: Block protocol for conventional profiling to sensory characterize plant protein isolates

In a second step, the apparent complexity of mechanisms at the origin of off-flavours in pea protein isolates calls for the establishment of an adapted strategy. As highlighted in the state of art, different strategies could be used to focus on the impact of composition of perception. In this study, we chose to use a mixture design based on fractions from protein isolates (Section 3.1.2.). Thus, the aim of the work of this second part was to propose a relevant strategies to link composition to perceptions. In short, as explained on the graphical abstract (Graphical abstract 3.1.2), a mixture design was used. First, we separated the pea protein isolates into three fractions (the pellet, permeate, and retentate) from two pea

protein isolates, resulting in a total of six fractions. Second, we used various combinations of the six fractions to create a set of 46 pea-protein-based solutions via various processes (solubilisation, centrifugation, filtration, and mixing). Third, trained panellists were asked to score the attributes of the solutions using sensory profiling to build optimal mixture models. Results were published and discussed on *Food Research International* as presented below (Cosson, A., Blumenthal, D., Descamps, N., Souchon, I., & Saint-Eve, A. (2021). Using a mixture design and fraction-based formulation to better understand perceptions of plant-protein-based solutions. *Food Research International*, 110151. <https://doi.org/10.1016/j.foodres.2021.110151>).



Graphical abstract 3.1.2.: Using a mixture design and fraction-based formulation to better understand perceptions of plant-protein-based solutions

Section 3.1.1. – Block protocol for conventional profiling to sensory characterize plant protein isolates

3.1.1.1. Introduction

Over the last decade, the food industry has been searching for alternatives to animal proteins as more consumers are making dietary choices based on health, ethical, or environmental criteria. In this context, pea (*Pisum sativum* L.) protein isolates are becoming increasingly common in new plant-based foods, enhancing the sustainability of food systems (Siddique *et al.*, 2012). Pea proteins have desirable functional properties (e.g., emulsification, foaming, and whipping) (Gharsallaoui *et al.*, 2009); they also have low allergenicity and high nutritional value (Gharsallaoui *et al.*, 2009). If many articles deal about their physico-chemical characteristics, little deal about their sensory characteristics reflecting difficulties to sensory describe them (Roland *et al.* 2017).

Like other pulse plant proteins, pea proteins have a distinct flavour. Sensory characterizations have highlighted the existence of three highly persistent notes: bitterness, astringency, and beaniness (Bott and Chambers, 2006; Humiski and Aluko, 2007). Beaniness corresponds to a complex flavour perception that is associated with bean products: consumers perceive such diverse notes as musty/earthy, musty/dusty, sour, starchy, powdery, green pea, nutty, and brown (Bott and Chambers, 2006). Such notes are rooted in the complex composition of the aroma compounds found in pulses (Murat *et al.*, 2013). In mouth, pea proteins are also often described as astringent, which has been defined as “the complex of sensations due to shrinking, drawing, or puckering of the epithelium as a result of exposure to substances such as alums or tannins” by the American Society for Testing Materials (ASTM, 1991). Finally, pea proteins are often perceived as bitter, which could result from the interaction of bitter compounds (e.g., amino acids, phenolics) with the TAS2R family of receptors, which are found on the apical membranes of taste receptor cells (Maehashi *et al.*, 2009; Meyerhof *et al.*, 2010).

Consequently, the distinct and persistent flavour of pea proteins limits their use as food ingredients (Lam *et al.*, 2018) and challenges their sensory evaluation. In sensory evaluation of foods, descriptive analysis such as classical profiling is conventionally employed (Lawless and Heymann, 2010). However, in classical profiling, the multidimensional nature of beaniness can be difficult for panellists to precisely describe and quantify. As a result, there is an increased probability of omitting a sensory dimension (Torres-Penaranda *et al.*, 2001). In addition, the prolonged persistence of bitter and astringent notes can lead to physiological adaptation and sensory fatigue (Kallithraka *et al.*, 1997). These same difficulties occur when pea proteins are part of food matrices, where composition, texture, and structure play an essential role in shaping sensory properties (Kühn *et al.* 2009; Guichard *et al.*, 2002). Perception could also be affected by cognitive interactions (i.e., neurophysiological interactions) between taste qualities (e.g., bitterness, saltiness, sweetness; Keast and Breslin, 2003). These cognitive interactions may act differently for individuals depending on their cultural background, food use, food consumption habits, and physiology (Lawrence *et al.*, 2009). These interactions occur when people eat products in natural context, however if we are interested in understanding the sensory mechanisms and to relate them to specific compounds in the food, it may be interesting to limit these cognitive interactions.

Several alternatives to the classical sensory profiling protocol have already been described in literature to deal with these issues. To limit interactions between sapid and flavour perceptions, some researchers have suggested that nose clips could be employed to allow panellists to focus on sapid perceptions (Abegaz *et al.*, 2004; Schoumacker *et al.*, 2017). In descriptive analysis, to cope with complex food and to limit the probability of omitting a dimension, some protocols allow each panellist to add descriptors to a pre-established list of attributes as with the Mixed profiling (Coulon-Leroy *et al.*, 2017). As food presentation schemes can impact how memory is activated, other researchers have recommended adapting how samples are presented (monadically, comparatively, or accompanied by a reference) (Mazzucchelli and Guinard, 1999; Saint-Eve *et al.*, 2006). Using a stable and known reference for

product evaluation could be interesting. For example, Polarized Sensory Positioning proposes to compare and indirectly describe a set of products to three known reference products (Teillet, *et al.*, 2010). The use of references is also used by the method of Pivot Profile (Thuillier *et al.*, 2005), which is based on a free expression of the difference between a tested product and an identified pivot product. For both methods, the choice of the reference product is critical to produce meaningful descriptions.

In addition, pronounced variability in sensitivity to bitterness has been observed in the general population and among panellists (Hansen *et al.*, 2006); furthermore, past research has shown that panellists who are less sensitive to bitterness are less able to discriminate among foods (Dinehart *et al.*, 2006). Consequently, when carrying out sensory assessments, it is necessary to characterize panellists' sensitivity to bitterness. For example, the 6-n-Propylthiouracil (PROP) test can be used. Indeed, past work has found that PROP status could be a proxy for overall oral responsiveness, which means that high PROP tasters could potentially display greater overall sensitivity to sensory properties (Carney *et al.*, 2018). This hypothesis is based on research that has found links between sensitivity to PROP bitterness and sensitivity to i) other basic taste sensations (Fischer *et al.*, 2015; Hayes *et al.*, 2008; Prescott and Swain-Campbell, 2000; Webb *et al.*, 2015); ii) chemesthetic sensations (pungency associated with capsaicin and other oral irritants) (Prescott and Swain-Campbell, 2000; Yang *et al.*, 2014); and iii) tactile sensations (astringency associated with alum) (Bajec and Pickering, 2008). Dinehart and colleagues found that people with greater PROP sensitivity gave higher bitterness scores to vegetables (Dinehart *et al.*, 2006). As a result, differences among panellists in bitterness sensitivity could greatly influence sensory perceptions of foods containing pea proteins. In summary, the complex sensory properties of pea proteins require an improved sensory profiling protocol if we wish to better and more extensively characterize food products containing pea proteins and understand the mechanisms that lead to sensory perceptions.

The aim of this study was to propose a relevant protocol for the evaluation and the characterization of pea protein-based samples. Based on this past research, we adapted a classical protocol to evaluate pea-protein-based foods. To decrease the effects of sensation saturation and sensory fatigue, we limited sample number and employed a strict palate-cleansing protocol. To deal with the complex and multidimensional perception of beaniness, we asked panellists to evaluate attributes by blocks. In addition, panellists were extensively trained to recognize different attributes, and we employed a reference sample to evoke these sensory memories during the experiment. Finally, to limit cognitive interactions between taste and flavour, and to dissociate cognitive interactions from chemical interactions, panellists wore nose clips during the evaluation of sapid perceptions. In this study, we compared how panellists evaluated solutions of pea protein isolates using both the classical protocol and the adapted protocol called here block protocol.

3.1.1.2. Materials and methods

Samples preparation

Six commercial pea protein isolates (representing different batches and suppliers) were used in this study. They were presented in the form of solutions (S1 to S6). No further information on the isolates will be given for reasons of confidentiality. We prepared 4% (w/w) pea protein solutions: the isolates were mixed in water (Evian, France) for 1 min and then allowed to soak for 30 min. The solutions were then mixed by hand before being served to the panellists. The samples were stored at 4°C before serving. They were served at 20°C (room temperature) in transparent cups (29.5 mL) bearing three-digit codes.

Experimental conditions

We recruited 17 panellists (13 women and 4 men) based on their interest in participating in a long-term study. They were grouped on two sub-groups: 9 panellists (8 women and 1 man, mean age = 20 years) and 8 panellists (5 women and 3 men, mean age = 26 years). These groups were formed according to the availability of panellists. They had no prior experience with pea products or sensory evaluation methods. They were not informed of the precise aim of the experiment. They gave their free and informed consent to participate in the study and received compensation for their participation. They

were asked not to eat, drink, or smoke for at least 1 h prior to any experiments or training sessions. All sensory profiling was carried out in individual booths under white light (samples had similar colour) in an air-conditioned room (20°C). To reduce sensation build-up, three main strategies have been used: limiting sample number, increasing waiting time between samples, and more effectively cleansing the oral mucosa between samples (Courregelongue *et al.*, 1999). Among these possibilities, the following palate-cleansing protocol was used between samples during our formal experiments: panellists had to consume an apple slice, drink water, and wait 40 seconds.

Characterization of panellist PROP status

We assessed the PROP taster status of the panellists using the three-solution test, with sodium chloride as the standard (Tepper *et al.*, 2001). Taste intensity ratings were collected for three solutions of suprathreshold 6-n-Propylthiouracil (Sigma-Aldrich, USA) (0.032, 0.32, and 3.2 mM) and of sodium chloride (NaCl, Sigma-Aldrich, USA) (0.01, 0.1, 1.0 M); the solvent was water (Evian, France). The panellists first assessed the three sodium chloride solutions and then the three PROP solutions; the order of presentation in three-solution tests does not produce systematic differences in taste ratings. The Labelled Magnitude Scale (LMS) was used for evaluating the samples (Tepper *et al.*, 2001). For each solution type, the order in which different solutions were presented was balanced across panellists. Between solutions, panellists rinsed their mouths with the palate-cleansing. The panellists assessed each solution twice.

Attribute selection and panellist training

Panellists were asked to fill out a check-all-that-apply (CATA) survey. It contained 30 attributes, and it was possible for panellists to add more. For our final list, we selected attributes that were cited more than 20% of the time and that allowed significant discrimination among sample types. We also wished to limit attribute number to avoid panellist fatigue. The eleven attributes that we retained were salty, bitter, astringent, mouthfeel, granularity, pea, broth, nuts, almond, potato, and grains. For the evaluation of textured food matrices, the granularity attribute was not relevant to be used.

Table 3.1.1.1.: Definition of the sensory attributes evaluated by the panellists for the pea-protein-based sample types.

Attributes	Attributes in French	Definition
Salty	<i>Salé</i>	A fundamental taste - sodium chloride is a typical example
Bitter	<i>Amer</i>	The fundamental taste associated with a caffeine solution
Astringent	<i>Astringent</i>	A sensation of drying out, roughening, and/or puckering that is felt in the mouth, like when consuming red wine or unripe fruit
Mouthfeel	<i>Epaisseur</i>	The way a food feels in the mouth in relation to its viscosity
Granularity	<i>Granulosité</i>	The presence of grains or granules in a food
Pea	<i>Pois</i>	The flavour characteristic of beans and bean-based foods
Broth	<i>Bouillon</i>	The flavour associated with boiled meat, soup, or stock
Nuts	<i>Noix</i>	The flavour associated with nuts, like walnuts or hazelnuts
Almond	<i>Amande</i>	The flavour associated with almonds
Potato	<i>Pomme de terre</i>	The flavour associated with cooked potato
Grains	<i>Céréales</i>	The flavour associated with grains

We then trained all the panellists to assess these attributes using external references and an unstructured scale ranging from 0 and 10 (Table 3.1.1.1.). Training took place over 8 sessions that lasted 45 min. Afterward, panellist performance was evaluated and confirmed. The panel performance was assessed for the entire panel (subgroup 1 and subgroup 2) using an ANOVA model with three factors (sample, panellist, repetition) and their interactions. The sample effect indicates the discrimination of products by panellists ($p < 0.05$). Interactions were also used to determine whether panellists used the intensity

scale between repetitions in a similar way (panellist * repetition), whether there was agreement among panellists (product * panellist) and whether panellists generated similar values between repetitions (product * repetition). Panellist performance was also evaluated for panellist discrimination, agreement and repeatability.

General experimental set-up

Nine panellists (9) were taught to use classical protocol, and eight others (8) were taught to use block protocol. They were then asked to evaluate the solutions S1 to S6. The panellists were unaware of sample identity and they evaluate the samples twice during two different sessions. After this first round of evaluation, the panellists were taught the profiling protocol they had not yet used, and they were asked to repeat the evaluation process. So in total 17 panellists evaluated products with both block and classical protocol. This repeated measures approach to sensory profiling allowed us to account for potential order and learning effects.

Sensory profiling methods

Two protocols were used: classical protocol and block protocol. In both cases, panellists were asked to evaluate the intensity of a sample's sensory attributes using an unstructured scale ranging from 0 to 10. To account for order and carry-over effects, sample order was balanced across panellists using a Latin square (Williams design). Panellists were given two replicates of each sample. The palate-cleansing protocol described above was used between samples.

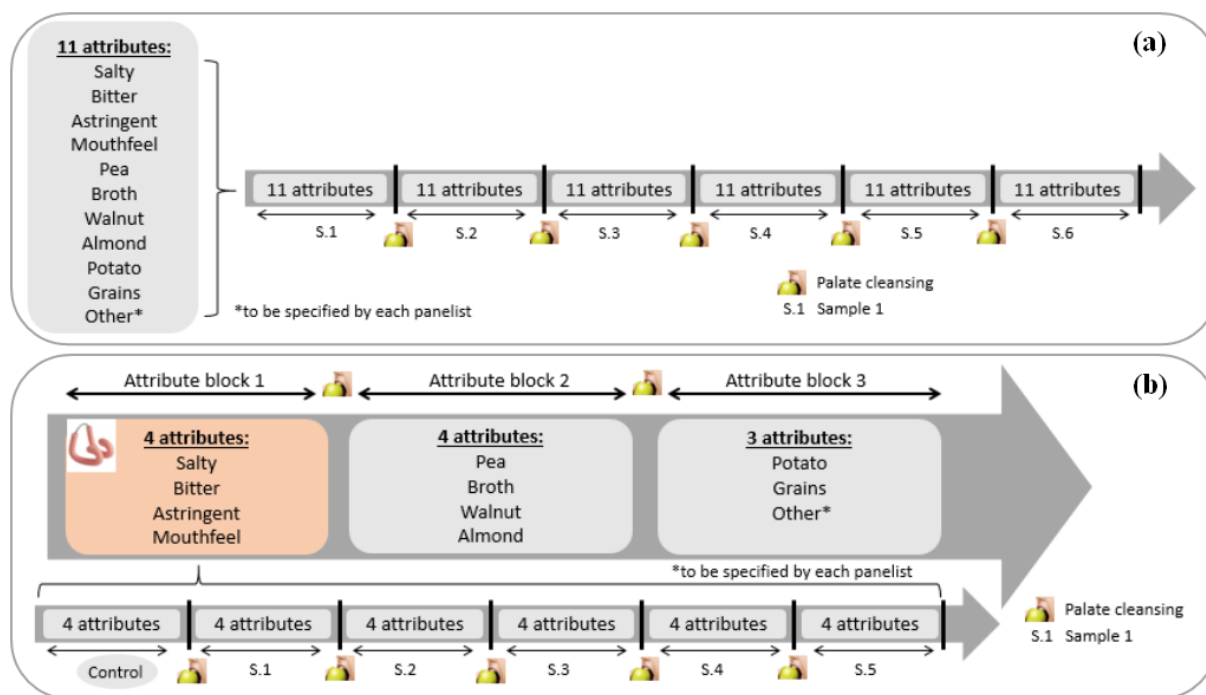


Figure 3.1.1.1.: Schematic representation of the profiling protocols used in this study. (a) Classical profiling protocol. (b) Block profiling protocol.

When panellists employed the classical protocol, samples were presented monadically in sequence (Figure 3.1.1.1.): panellists evaluated all eleven attributes (printed on the same survey page) at once for each sample.

When panellists employed the block protocol, attributes were evaluated in blocks (Figure 3.1.1.1.). Prior to the experiment, the attribute blocks were defined and discussed with the panellists. The first attribute block focused on taste perception and mouthfeel (attributes of block 1: salty, bitter, astringent,

mouthfeel, and granularity), for which panellists wore nose clips. The next two attribute blocks focused on olfactory perception (attributes of block 2: pea, broth, nuts, almond; attributes of block 3: potato, grains and other), and panellists did not wear nose clips during the evaluation. For each block, samples were presented monadically in sequence: panellists evaluated all the attributes in the block (printed on the same survey page) for each sample. The samples order was the same for the three blocks for one subject (but different between subjects). In addition, the first sample (S1) in each session, for the three blocks, was also the reference used to limit drift between sessions. This reference was available in large quantities and was stored under highly stable conditions.

Data analysis

All the analyses were performed using XLStat (Addinsoft, 2017, Paris, France) and R (R Core Team, 2017). For analyses of an inferential nature, we used $\alpha = 0.05$ to determine statistical significance.

Based on the results of the PROP test, we defined three groups of panellists. In the low PROP taster (LPT) group were panellists who had rated sodium chloride as having a more intense taste than PROP. Panellists who gave similar ratings to sodium chloride and PROP were in the medium PROP taster (MPT) group. Those who had rated PROP as having a more intense taste than sodium chloride were in the high PROP taster (HPT) group. Differences among groups were analysed using repeated-measures analysis of variance (ANOVA) and post-hoc comparisons (Newman-Keuls method).

To analyse the sensory profiling results, in a classical way, we carried out three-way ANOVA. There were two sets of models: models using the classical protocol data and models using the block protocol data. Within each set, there was one model focused on the results for a single attribute. For all the models, sample type, replicate, and panellist identity were the fixed effects, and all first-order interactions were included. When the fixed effects were significant, we carried out post-hoc comparisons (Newman-Keuls method). We also performed additional three-way ANOVA and Newman-Keuls tests to assess the effect of PROP status on the intensity scores for the 11 attributes: sample type, replicate, PROP status and panellist identity (nested factor with PROP status) were the fixed effects, and all first-order interactions were included. To visually explore differences in the results obtained using classical versus block protocol, we carried out multiple factor analysis (MFA) (centred by group; Pearson type); the data from both protocols were averaged across replicates and panellists.

3.1.1.3. Results

Assessing panellist performance

We examined how well panellists performed (reproducibility and homogeneity) when using classical versus block protocol to evaluate attribute intensity. Table 3.1.1.2. presents the results from the three-way ANOVA that used the scoring data for each attribute from each of the protocols. The interaction between replicate and sample type were not significant (except in the case of granularity as evaluated with classical protocol). Replicate was significant for 6 attributes for the block protocol and for 2 attributes for the classical protocol. The interaction between panellist and replicate was not significant for potato, nuts, broth, grains and bitter for the classical protocol and, granularity for the block protocol. But the F values for these factors are low compared to the F values for the effects produced. Nevertheless, these effects indicated a low level of repeatability of the panel and ask to be circumspect for the following analysis. The interaction between panellist and sample type was significant for seven attributes and one attribute for the block and the classical protocols, respectively. Such interactions are common when sensory attributes are evaluated using unstructural scales and are difficult to control even when panellists have undergone extensive training (Jourjon *et al.*, 2005). Nevertheless, the block protocol seems to enhance differences between products and panellists. These results nonetheless suggest that the panellists generally came up with repeatable and homogeneous scores, even if there was some disagreement for some attributes.

Table 3.1.1.2.: Results of the three-way ANOVA examining panellist performance and comparing the two protocols during the experiment where attribute intensity for pea protein solutions was evaluated using the classical profiling protocol (CP) and the block profiling protocol (BP) (degrees of freedom: 118; residual degrees of freedom: 85). Significant p-values are in bold ($\alpha = 0.05$).

Sensory attributes	Sample type		Replicate		Panellist ID		Sample*Panellist		Sample*Replicate		Panellist*Replicate	
	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue
Almond-BP	8.26	<0.01	0.76	0.39	15.31	<0.01	1.86	<0.01	0.68	0.64	3.17	<0.01
Almond-CP	4.35	<0.01	1.03	0.31	13.84	<0.01	1.27	0.15	1.01	0.42	2.42	0.01
Astringent-BP	4.10	<0.01	6.54	0.01	6.86	<0.01	1.13	0.29	1.64	0.16	2.46	<0.01
Astringent-CP	2.16	0.07	2.77	0.10	9.73	<0.01	1.12	0.30	2.33	0.05	3.41	<0.01
Bitter-BP	13.29	<0.01	15.19	<0.01	14.34	<0.01	1.34	0.10	1.15	0.34	2.91	<0.01
Bitter-CP	5.62	<0.01	1.06	0.31	11.50	<0.01	0.79	0.85	0.87	0.50	1.22	0.27
Broth-BP	12.44	<0.01	1.83	0.18	13.56	<0.01	1.41	0.06	1.46	0.21	3.65	<0.01
Broth-CP	6.98	<0.01	7.26	0.01	4.70	<0.01	0.93	0.64	1.76	0.13	1.71	0.06
Grains-BP	4.54	<0.01	11.96	<0.01	16.14	<0.01	1.57	0.02	1.62	0.16	3.14	<0.01
Grains-CP	1.84	0.11	1.05	0.31	7.02	<0.01	0.98	0.53	1.35	0.25	1.29	0.22
Granularity-BP	8.67	<0.01	0.13	0.72	8.81	<0.01	1.39	0.07	0.62	0.69	1.38	0.17
Granularity-CP	10.04	<0.01	3.77	0.06	6.97	<0.01	1.69	0.01	2.85	0.02	3.81	<0.01
Mouthfeel-BP	21.14	<0.01	22.52	<0.01	10.72	<0.01	1.68	0.01	0.52	0.76	2.19	0.01
Mouthfeel-CP	5.37	<0.01	4.34	0.04	13.81	<0.01	1.29	0.13	1.88	0.11	5.11	<0.01
Nuts-BP	21.61	<0.01	21.64	<0.01	5.35	<0.01	2.50	<0.01	0.93	0.47	3.08	<0.01
Nuts-CP	7.69	<0.01	0.70	0.41	6.23	<0.01	1.26	0.15	2.34	0.05	1.38	0.17
Pea-BP	9.19	<0.01	5.15	0.03	8.11	<0.01	2.76	<0.01	2.38	0.05	1.93	0.03
Pea-CP	1.62	0.17	1.60	0.21	6.16	<0.01	1.30	0.12	1.91	0.10	1.97	0.03
Potato-BP	11.89	<0.01	0.05	0.83	13.16	<0.01	2.19	<0.01	0.99	0.43	5.22	<0.01
Potato-CP	6.71	<0.01	1.12	0.29	8.37	<0.01	1.29	0.13	2.29	0.05	1.66	0.07
Salty-BP	16.69	<0.01	0.38	0.54	14.22	<0.01	2.24	<0.01	1.69	0.15	3.39	<0.01
Salty-CP	7.49	<0.01	0.00	0.95	15.99	<0.01	1.11	0.32	0.72	0.61	4.14	<0.01

Comparison of the classical protocol and block protocol results

In the three-way ANOVA, sample type was significant, revealing that there were differences in how the different pea protein solutions were perceived (Table 3.1.1.3.). When the block protocol was used, panellists discriminated among the six sample types for all the attributes; for the classical protocol, the same was true for eight of the attributes (except pea, grains and astringent). For the block protocol, the greatest degree of discrimination was observed with the attributes nuts and mouthfeel ($F = 21.61$ and $F = 21.14$); for the classical protocol, it was with granularity ($F = 10.04$). For the block protocol, there was some degree of discrimination displayed in granularity, pea, and almond (resp. $F = 8.67$, $F = 9.19$, $F = 8.26$) as well with grains and astringent (resp. $F = 4.54$, $F = 4.10$), although F-values were weaker in the latter case. In contrast, with classical protocol, panellists did not discriminate among the sample types for the attributes astringent, grains, and pea. Consequently, the block protocol allowed a greater degree of discrimination among a larger number of attributes. Table 3.1.1.3. presents the results of the Newman-Keuls groups associated for the evaluation of pea protein isolates for significant attributes with the classical and the block protocols. Overall products are ranked in the same way. Nevertheless there is a higher number of different groups with the block protocol for the attributes bitter, astringent, mouthfeel, pea, broth and grains (respectively from 2 to 4, and 1 to 3 groups).

Table 3.1.1.3.: Results from the Newman-Keuls post-hoc comparisons for the evaluation of pea protein isolates in water solutions by different protocol. (CP) Classical profiling protocol. (BP) Block profiling protocol. Nb_group number of Newman-Keuls group. Significant differences between groups are indicated by differences in letters.

Solutions	Salty	Bitter	Astringent	Mouthfeel	Granularity	Pea	Broth	Nuts	Almond	Potato	Grains
S1-BP	a	a	a	a	ab	a	b	a	a	a	a
S1-CP	abc	a	a	a	c	a	b	a	ab	b	a
S2-BP	b	ab	ab	b	bc	bc	c	a	a	b	ab
S2-CP	bc	ab	a	a	c	a	b	ab	a	b	a
S3-BP	a	c	ab	cd	a	b	a	b	b	a	b
S3-CP	a	b	a	c	ab	a	a	c	b	a	a
S4-BP	b	c	b	d	ab	bc	bc	b	a	b	ab
S4-CP	c	b	a	bc	a	a	b	bc	ab	ab	a
S5-BP	b	bc	b	d	a	c	bc	b	b	b	b
S5-CP	c	ab	a	abc	bc	a	ab	ab	b	b	a
S6-BP	b	c	b	bc	c	ab	bc	a	a	b	ab
S6-CP	ab	ab	a	ab	c	a	b	bc	a	b	a
Nb_group -BP	2	3	2	4	3	3	3	2	2	2	2
Nb_group -CP	3	2	1	3	3	1	2	3	2	2	1

The results of the MFA are shown in Figure 3.1.1.2. There was a good correlation between the results obtained with the two protocols (RV coefficient = 0.778) (Josse *et al.*, 2008). The first two axes accounted for a large proportion of the variance (81.01%) in the sensory profiles. The loadings of the attributes showed that evaluations of granularity were well correlated between the two protocols, as were evaluations of broth, salty, mouthfeel, bitter, nuts, and almond. The only attribute for which this was not the case, was potato. This is because of higher potato intensity scores when panellists used block protocol, especially for S1 (5.31 for block profiling vs. 2.68 for classical profiling). It may be that, with the classical protocol, it was easier to confuse the potato attribute with other attributes; in contrast, block protocol might have limited such confusion. Along the first axis (52.95% of variance explained), sample types were mainly differentiated based on the attributes nuts, mouthfeel, almond, and granularity. Along the second axis (28.05% of variance explained), sample types were mainly differentiated based on the attributes salty and potato. The sample types evaluated with classical protocol are mostly distributed along the first axis, which means that panellists mainly used a single dimension when differentiating among sample types. In contrast, the sample types evaluated with block protocol are distributed along axes 1 and 2, which means that panellists used two dimensions in the discrimination process. Overall, this finding indicates that there was a greater degree of discrimination among sample types when block protocol was employed.

For both protocols, panellists had the possibility to complete their descriptions with another attribute in ‘Other’. However, with both protocols, this attribute has been used only rarely and never in a consensual way. These results do not allow us to conclude on the usefulness of such an attribute.

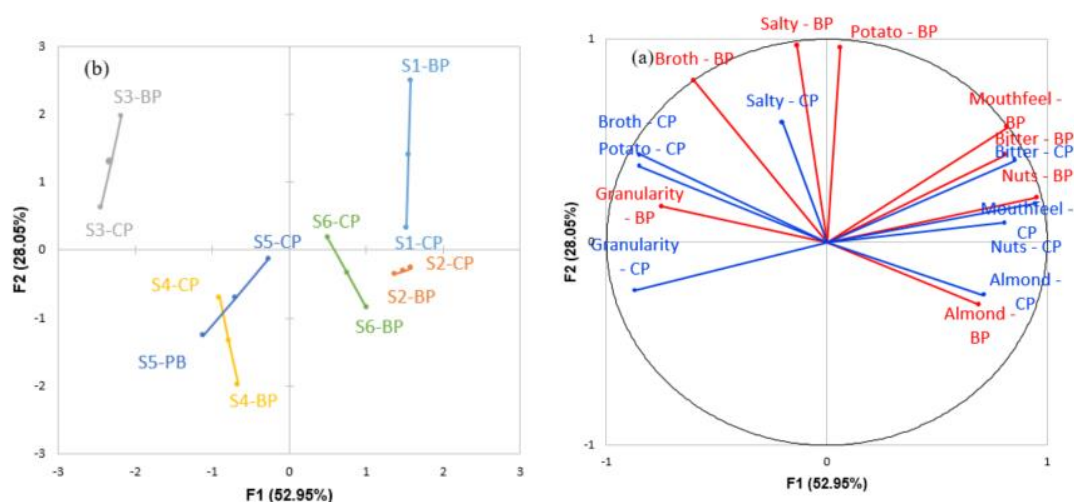


Figure 3.1.1.2.: Results of the multiple factor analysis comparing the sensory evaluations of pea protein solutions by classical (CP) versus block profiling protocol (BP) (RV coefficient: 0.778) (8/11 attributes which are significant for both methods). (a) Correlation map of the variables along the first two axes. (b) Observations and projected points along the first two axes.

PROP status of panellists and its relationship with perceptions

Among the 17 individuals who evaluated pea protein isolates in solution with both protocols, 7 were assigned to the HPT group (PROP intensity > sodium chloride intensity) and 5 were assigned to the LPT group (PROP intensity < sodium chloride intensity). The 5 panellists who did not perceive a difference between PROP and sodium chloride were assigned to MPT group.

Table 3.1.1.4.: Effects of panellist PROP status on the evaluation of the attribute intensities of pea protein solutions from the results from the ANOVA (degrees of freedom: 34; residual degrees of freedom: 169). Significant p-values are in bold ($\alpha = 0.05$). (CP) Classical profiling protocol. (BP) Block profiling protocol.

Sensory attributes	Panellist [PROP]		Sample type		PROP status		Replicate		Replicate* PROP		PROP* Sample	
	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue
Almond-BP	9.50	<0.01	5.37	<0.01	12.45	<0.01	0.39	0.53	1.55	0.21	1.03	0.42
Almond-CP	12.21	<0.01	4.08	<0.01	12.32	<0.01	0.16	0.69	6.06	<0.01	1.77	0.07
Astringent-BP	3.74	<0.01	3.24	0.01	19.51	<0.01	4.83	0.03	2.27	0.11	0.78	0.65
Astringent-CP	5.20	<0.01	1.52	0.19	21.66	<0.01	1.66	0.20	1.47	0.23	0.42	0.93
Bitter-BP	11.99	<0.01	10.74	<0.01	8.79	<0.01	12.46	<0.01	5.31	0.01	1.41	0.18
Bitter-CP	13.16	<0.01	5.75	<0.01	6.91	<0.01	1.09	0.30	0.19	0.83	1.08	0.38
Broth-BP	5.08	<0.01	8.21	<0.01	39.35	<0.01	0.63	0.43	3.06	0.05	0.35	0.96
Broth-CP	4.46	<0.01	7.00	<0.01	5.32	0.01	8.11	<0.01	1.10	0.34	1.34	0.21
Grains-BP	11.61	<0.01	3.09	0.01	14.29	<0.01	11.61	<0.01	4.57	0.01	1.74	0.08
Grains-CP	7.03	<0.01	1.63	0.16	4.35	0.01	0.98	0.32	1.19	0.31	0.61	0.80
Granularity-BP	8.01	<0.01	7.07	<0.01	3.04	0.05	0.04	0.84	0.32	0.73	1.18	0.30
Granularity-CP	4.28	<0.01	6.23	<0.01	8.60	<0.01	0.75	0.39	10.88	<0.01	0.99	0.46
Mouthfeel-BP	9.16	<0.01	14.79	<0.01	0.52	0.59	17.48	<0.01	0.84	0.43	1.96	0.04
Mouthfeel-CP	11.23	<0.01	3.60	<0.01	2.76	0.07	2.13	0.15	10.00	<0.01	1.29	0.24
Nuts-BP	2.42	<0.01	11.54	<0.01	8.18	<0.01	13.97	<0.01	1.28	0.28	2.42	0.01
Nuts-CP	4.54	<0.01	6.98	<0.01	10.73	<0.01	0.39	0.53	0.79	0.45	1.28	0.24
Pea-BP	2.80	<0.01	4.64	<0.01	15.97	<0.01	2.80	0.10	0.01	0.99	1.97	0.04
Pea-CP	5.57	<0.01	0.89	0.49	2.21	0.11	1.63	0.20	1.05	0.35	1.74	0.08
Potato-BP	3.90	<0.01	6.02	<0.01	28.99	<0.01	0.08	0.78	3.92	0.02	0.70	0.72
Potato-CP	6.27	<0.01	5.06	<0.01	11.99	<0.01	1.71	0.19	2.77	0.07	1.04	0.42
Salty-BP	8.12	<0.01	9.88	<0.01	10.06	<0.01	0.37	0.55	0.97	0.38	1.82	0.06
Salty-CP	13.26	<0.01	5.60	<0.01	4.56	0.01	0.18	0.67	2.86	0.06	0.71	0.71

We used the block protocol data and the classical protocol data for the S1-S6 solutions to examine whether panellist PROP status influenced attribute evaluation (Table 3.1.1.4.). The ANOVA results show that PROP status affected the panellists' evaluation of 9 of the 11 attributes (all except mouthfeel

and granularity) for block protocol and 9 of the 11 attributes (all except mouthfeel and pea) for classical. Table 3.1.1.5. presents the results from the Newman-Keuls post-hoc comparisons. With block protocol, in contrast to the HPT and MPT groups, the LPT group perceived all the attributes as less intense (all except mouthfeel and granularity which are not significant). With classical protocol, LPT group perceived the products as less intense in astringent, granularity and aroma attributes (except pea attribute). The differences between the two protocols therefore depend on the attributes. Nevertheless, for bitter attribute, block protocol allow the expression of a greater difference between PROP status populations.

Table 3.1.1.5.: Results from the Newman-Keuls post-hoc comparisons, where HPT = high PROP taster group, MPT = medium PROP taster group, and LPT = low Prop taster group. (CP) Classical profiling protocol. (BP) Block profiling protocol. Nb_group number of Newman-Keuls group. Significant differences between groups are indicated by differences in letters.

Prop taster	Salty	Bitter	Astringent	Mouthfeel	Granularity	Pea	Broth	Nuts	Almond	Potato	Grains
HPT - BP	a	a	a	a	a	a	b	a	b	a	a
HPT - CP	a	a	a	a	a	a	a	a	a	a	a
MPT - BP	a	a	a	a	a	a	a	a	a	a	a
MPT - CP	a	a	b	a	a	a	ab	ab	ab	a	ab
LPT - BP	b	b	b	a	a	b	c	b	b	b	b
LPT - CP	a	a	c	a	b	a	b	b	b	b	b
Nb_group-BP	2	2	2	1	1	2	3	2	2	2	2
Nb_group-CP	1	1	3	1	2	1	2	2	2	2	2

3.1.1.4. Discussion

We found that classical and block protocols yielded correlated results. However, the block protocol allowed better discrimination among sample types. These differences were likely due to the differences in the presentation schemes, which activated different cognitive processes. In past research, monadic presentation has been found to be faster and less tiring than comparative presentation (Mazzucchelli and Guinard, 1999); however, comparative presentation allows panellists to detect smaller differences among foods and to make more accurate decisions about these relative differences (Saint-Eve *et al.*, 2006). Here, our classical protocol employed a monadic presentation scheme, which means that panellists based their attribute evaluations on their memory of the references from the training period. They thus had to activate both their long-term and short-term memory. In contrast, in our block protocol, the presentation scheme combined both monadic and comparative elements. Consequently, the panellists could base their attribute evaluations on both their memories from the training period as well as on the reference sample, which was the first sample in the sequence (Hastie and Park, 1986). As a result, they might have needed to rely less on their long term-memory than when using the classical protocol.

The differences in the results obtained with the two protocols could also be rooted in bias. For example, during sensory evaluation, one of a food's attributes can strongly influence or bias the perception of the food's other attributes (halo effect) (Thorndike, 1920), or, in contrast, the absence of a potentially salient attribute from a food can affect how the food's other attributes are perceived (dumping effect) (Clark and Lawless, 1994). By having panellists evaluate attributes in groups, block protocol may reduce correlations among attributes and thus limit halo and dumping effects. Such factors could explain the distribution patterns along the first and second axes in the MFA (Figure 3.1.1.2.).

Here, we asked panellists to evaluate sapid and texture attributes with nose clips. If nose-clip evaluation does not mimic what happens when people taste the products in real life, it allows to dissociate chemical interactions from cognitive interactions in order to study the relationships between sensory data and instrumental data to move towards an understanding of the mechanisms of sensory perceptions (Abegaz *et al.*, 2004).

In term of practicality, the block protocol takes more time than the classical protocol for products evaluation (about 20 min versus 15 min). Although using the nose clip for only one of the attributes blocks requires to panellists to put on and remove it during the evaluation, panellists found it easier to evaluate perceptions than classical protocol in particular due to the focus on a smaller number of attributes. In addition, the use of the reference, which makes sample preparation more cumbersome, was greatly appreciated by the panellists to help them to rate the products.

In summary, with this explorative study, block protocol is more efficient (better repeatability and discrimination) than classical protocol. Nevertheless, due to the high presence of the replicate effect, particularly in the classical protocol, and to the low number of evaluated products, as well as the limited number of subjects used in the panels, results have yet to be confirmed by other studies.

This research has also improved our knowledge of how pea proteins are perceived. Plant proteins are often described as “beany,” a multidimensional and ambiguous descriptor. In our study, we decided against using the term beany. Instead, we parsed out its multiple components and expressed them via other terms. Indeed, in the literature, the definition of beany varies across consumers and food types. For example, lupin flour has been described as having beany/green, mushroom/soil, floral, meaty, nutty, woody/green, sweet, and baked attributes (Kaczmarek *et al.*, 2018), and pea and soybean have been described as having beany, green, fresh, and grassy attributes (Troszyńska *et al.*, 2007). As a result, the use of beany as a descriptor is sometimes avoided (Lawrence *et al.*, 2016). For example, one study allowed cooked peas to only be described with the attributes pea, cooked vegetable, earthy, brothy, grainy, and hay like (Malcolmson *et al.*, 2014).

In our study, pea protein solutions could be described using attributes such as potato, pea, grains, broth, almond, and nuts. This definition of pea flavour (in particular the following attributes: green/pea, grain/starchy, nuts, and broth) is consistent with the definition developed by Vara-Ubol, Bott, and Chambers (Bott and Chambers, 2006; Vara-Ubol *et al.*, 2004), where beany is a combination of musty/earthy, musty/dusty, and one or more of the characteristic attributes green pea pod, nutty, brown, sour, starchy, and powdery. However, there are some differences in the beaniness of pea proteins (as illustrated in our results) and the beaniness of proteins in soybeans, green peas, or lupin flour. More specifically, the attributes musty/dusty and earthy/mushroomy are not associated with pea proteins.

Another aspect of our study is that we found a relationship between panellist PROP status and evaluations of pea protein solutions. The percentage of HPTs and LPTs that we identified among our panellists generally concurs with what has been reported in the literature. However, it is important to note that there is pronounced variability among populations worldwide (India: 26% HPT and 42% LPT; West Africa: 3% LPT; China: 6%–23% LPT; North America [Caucasians]: 30% LPT (Deshaware and Singhal, 2017)). Here, we found that PROP status affected evaluations of bitterness (as also shown by Dinehart *et al.*, 2006) as well as evaluations of other sapid and flavour attributes. In particular, LPTs seemed to have a harder time discriminating among sample types for a number of attributes; the only attribute for which there was evidence of discrimination was a texture attribute, which suggests that mechanisms underlying texture perception are very different from those underlying chemical perception. However, some researchers have been questioning whether PROP status is a global indicator of taste function given that the PROP test only measures sensitivity to one bitter compound (Webb *et al.*, 2015). That said, certain studies have shown that, compared to LPTs, MPTs and HPTs do perceive a variety of compounds with greater intensity (Fischer *et al.*, 2015). Therefore, to improve the quality of food descriptions and discrimination, sensory studies might want to strictly employ panellists who are HPT or MPT. Alternatively, researchers could control for panellist PROP status when carrying out the analyses.

3.1.1.5. Conclusions

In this study, we drew upon classical sensory protocol to develop a block protocol in which attributes are evaluated in groups. Panellists evaluated samples that were presented monadically and sequentially;

for each evaluation sequence, the first sample in the series was the reference. This protocol made it easier to evaluate samples with strong persistent attributes, such as bitterness or astringency, and multidimensional attributes, such as beaniness. The use of a reference sample may help limit drift between sessions. The block protocol made it possible for panellists to provide a more complete sensory characterization of the samples, which included a large number of attributes. In this study, the use of nose clips limited interactions between taste and flavour with a view to dissociate cognitive interactions from chemical interactions. We also assessed the PROP status of our panellists, which allowed us to better understand each panellist's evaluations. Furthermore, our results suggest that PROP status could help researchers select panellists based on sensory sensitivity. In closing, our overall findings should improve the characterization and formulation of innovative products based on pea proteins. Nevertheless, these results have yet to be confirmed for its use when evaluating other types of products. In particular, foods with complex, multidimensional sensory properties, such as foods incorporating pea protein isolates would be interesting to consider.

Main results

- Here, we proposed an explorative study for the use of an adapted sensory evaluation protocol, that could be used by trained panellists to effectively evaluate the intensities of sensory properties of pea-protein-based foods. In short, we adapted a classical protocol: we incorporated relative-to-reference evaluation, and panellists assessed attributes in blocks rather than all at once.
- We conducted an experiment in which both classical and block protocols were used to evaluate pea protein solutions made with one set of isolates. Similar results were obtained with classical versus block protocol, although panellist performance (reproducibility and homogeneity) was better with block protocol.
- Sensitivity to bitterness was also investigated for this limited number of panellists (PROP test), and it was found that high and medium PROP tasters could better discriminate among pea protein solutions but this observation could be confirmed on a greater number of individuals.

Section 3.1.2. – Using a mixture design and fraction-based formulation to better understand perceptions of plant-protein-based solutions

3.1.2.1. Introduction

Over the last few years, plant-based protein ingredients have received much attention from the food industry and consumers because of their environmental sustainability, attractive prices, nutritional values, and protein content (Davis *et al.*, 2010). In particular, yellow field pea (*Pisum sativum* L.) is an increasingly common ingredient in plant-based foods (Siddique *et al.*, 2012). Its proteins exhibit low allergenicity; have a high nutritional value; and can restore the amino acid balance of grain-based diets. They also display functional properties that are useful in food formulation: they promote emulsification, foaming, gelation, and whipping (Adebisi & Aluko, 2011; Gharsallaoui *et al.*, 2009).

Industrial pea-protein ingredients are traditionally generated via a several-step wet process. Pea seeds are solubilized in an alkaline solution, which is then centrifuged to remove insoluble compounds; the precipitate is obtained at the isoelectric point using acidification and centrifugation. The resulting isolate has a protein content of 80–90% (mainly globulins), but also contains lipids, carbohydrates, minerals, and other small compounds (e.g., phenolics), which are the products of seed metabolism (Schutyser *et al.*, 2015). The isolate can also serve as an ingredient in the formulation of many food products, including dietary supplements, bakery and confectionery products, beverages, yogurts, ice creams, meat products, and meat and dairy alternatives.

However, a challenge remains: pea-protein-based products are usually described as having strong beany, bitter, and astringent notes, which makes them less desirable to consumers. The mechanisms and chemical compounds underlying the perception of food are partly understood and may be multifarious (Owusu-Ansah & McCurdy, 1991). Indeed, the composition of pea protein isolates is complex: they have a high protein content but also contain various peptides, volatile compounds, phenolics, complex heterosides, carbohydrates, fibres, and minerals. All these constituents could influence the perception of pea-based ingredients.

Research in this area has often focused on the perception of beaniness, which is a complex flavour associated with bean products (Bott & Chambers, 2006). It results from the intricate composition of the volatile aroma compounds found in pulses; present at the highest concentrations is hexanal, whose occurrence is linked to the green notes of peas (Murat *et al.*, 2013). Bitterness arises from the interaction of bitter compounds (e.g., amino acids, peptides, phenolics, and complex heterosides) with the TAS2R family of receptors, which are found on the apical membranes of taste receptor cells (Maehashi *et al.*, 2009; Meyerhof *et al.*, 2010). For example, the caffeic acid in coffee and other plant products generates an intense sensation of bitterness (Frank *et al.*, 2007; Streit *et al.*, 2007). Astringency is produced by “the complex sensations due to shrinking, drawing, or puckering of the epithelium” and results from interactions between phenolics and saliva proteins (ASTM, 1991; Gibbins & Carpenter, 2013). From an industrial and scientific point of view, it has proven extremely challenging to clarify how pea-based ingredients give rise to these sensory attributes.

Research on the perception of pea-based products has largely focused on the role of volatile aroma compounds in creating sensations of beaniness (Azarnia *et al.*, 2011; Ben-Harb *et al.*, 2020; Bi *et al.*, 2020; Bott & Chambers, 2006; El Youssef *et al.*, 2020; Murat *et al.*, 2013; Mutarutwa *et al.*, 2018; Schindler *et al.*, 2012; Trikusuma *et al.*, 2020; Wang & Arntfield, 2016; Xu *et al.*, 2019; Xu *et al.*, 2020). A few studies have exclusively examined the peptides that could be related to bitterness (Akin & Ozcan, 2017; Pownall *et al.*, 2010; Sirtori *et al.*, 2012); the phenolics related to bitterness and astringency (Guo *et al.*, 2019; Padhi *et al.*, 2017); and the saponins related to bitterness (Daveby *et al.*, 1998; Heng *et al.*, 2006; Price *et al.*, 1985). However, to our knowledge, no study to date has used a more global approach

to examine how the complex perception of pea protein isolates arises from both volatile and non-volatile compounds and their potential interactions.

Several research strategies have been used to understand how complex products are perceived and to account for the interactions between matrix molecules. Omission testing is commonly used to estimate the effect of specific compounds on the sensory characteristics of products (Engel *et al.*, 2002). Thanks to this technique, aroma models have been built that reconstitute complex foods—such as those of different types of wine, olive oil, cheese, boiled beef, coffee, and whey protein—using only a small fraction of the great number of volatiles occurring in these foods (Dinnella *et al.*, 2012; Ferreira *et al.*, 2002; Whitson *et al.*, 2010). However, in addition to being very time consuming, these experiments are less effective when volatile compounds are included in mixtures because the volatiles interact with other ingredients. Indeed, the ability of volatile compounds to modify how something tastes depends on both their relative concentrations and their interactions within the food matrix (Guichard, 2002). Mixing congruous volatiles and taste stimuli can enhance taste intensity, while mixing incongruous stimuli can suppress taste intensity (Caporale *et al.*, 2004). Omission testing has also been used in tandem with gel permeation chromatography to study the water-soluble fraction of peptides found in cheese (Andersen *et al.*, 2010; Engel *et al.*, 2002; Gómez-Ruiz *et al.*, 2007; Toelstede & Hofmann, 2008). The compounds in pea protein isolates that are potentially responsible for sensory attributes (e.g., peptides, phenolics, minerals) are very complex and challenging to purify and identify. Moreover, most analytical techniques require the use of non-food-grade solvents or buffers that are difficult to handle and that can pose problems if the extracts are to be used in sensory evaluations.

Studies have shown that attribute perception may be similar for a complex product and a fraction-based reconstruction of the product. For example, artificial ikura (Japanese salmon caviar) was prepared using vegetable oil and a low-calorie natural gel (e.g., one made with alginic acid) (Hayashi *et al.*, 1990); each component of the food was then analysed using chemical and sensory methods. Based on the analytical data, a synthetic ikura was reconstituted using pure reagents. There were very few sensory differences in the taste profiles between the reference food and the reconstructed food (Hayashi *et al.*, 1990). In another study (Niimi *et al.*, 2014), a cheese solution was reconstituted using a mixture of sucrose, NaCl, monosodium glutamate, lactic acid, and caffeine that was then adjusted using a fractional factorial design. The reconstructed products did not significantly differ from the cheddar cheese reference in overall intensity, saltiness, sourness, umami, and bitterness (Niimi *et al.*, 2014).

Thus, the aim of this study was to examine how the main fractions of pea protein isolates individually affected the perception of sensory attributes, namely undesirable attributes such as beaniness, bitterness, and astringency. To this end, an original approach was employed, in which different fractions were combined in various ways to create a range of pea-protein-based solutions. These fractions were obtained from commercial pea protein isolates and were an insoluble fraction (called the pellet), a soluble fraction (called the retentate), and a soluble fraction with a molecular weight of less than 10 kDa (called the permeate). Through this approach, the focus was thus on different groups of compounds instead of on a single compound type.

3.1.2.2. Materials and methods

Production of pea protein isolate fractions

Two pea protein isolates (protein content Nx6.25, 83% dry matter) were used; they were called isolate *a* and *b*, respectively. The isolates were dispersed in tap water in a tank to obtain a final suspension containing 4% (w/w) dry matter content. This suspension was maintained under agitation for 12 h at 3°C with an external agitator (U-shaped stirrer shafts); it was then centrifuged with two centrifuges (Jouan Kr4i and a Sorvall Lynx 4000 [Thermo Scientific, Waltham, US]; 6000 g, 10 min, 4°C). The supernatant was manually separated from the pellet. The pellet was subsequently diluted with tap water to arrive at a dry matter content of 12.35%, which facilitated solution creation. A tangential filtration module (TIA, Bollene, France) was used for the ultrafiltration process. The module employed two ST-3B-1812 PES Synder membranes (46-mil spacer; 10-kDa MWCO). Total membrane surface was 0.67

m². The filtration pilot was equipped with a high-pressure diaphragm pump (Wanner Hydra-Cell G10, Wanner International Ltd, Church Crookham, UK)). The retentate was maintained at 13°C throughout filtration. The inlet pressure (P1) was 1.5 bar, the outlet retentate pressure (P2) was 1 bar, and the mean transmembrane pressure ($[P1 + P2]/2$) was 1.25 bar. First, ultrafiltration was used to obtain around 10 L of permeate; then, diafiltration was performed employing the same parameters to partially wash the retentate (one diavolume was used). Six fractions were obtained: permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b*.

Characterization of the pea protein isolate fractions

Each fraction was characterized to determine the key pea protein compounds it contained (Figure 3.1.2.1.). Nitrogen content was determined via the Kjeldahl method (nitrogen content $\times 6.25$), and dry matter content was determined by a certified external laboratory (SAS IMPROVE, Amien, France) via drying (prepASH®219 analysis system). Sodium content was also determined by a certified external laboratory (SAS QUALTECH, Vandoeuvre-les-Nancy, France) using inductively coupled plasma mass spectrometry. Caffeic acid content was determined using gas chromatography–mass spectrometry (GC-MS) and comparison with an external standard (CAS 331-39-5, grade $\geq 98.0\%$ HPLC, MW 180.16, Sigma Aldrich, Saint-Louis, US). Hexanal levels were determined using GC-MS as per El Youssef *et al.* (2020).

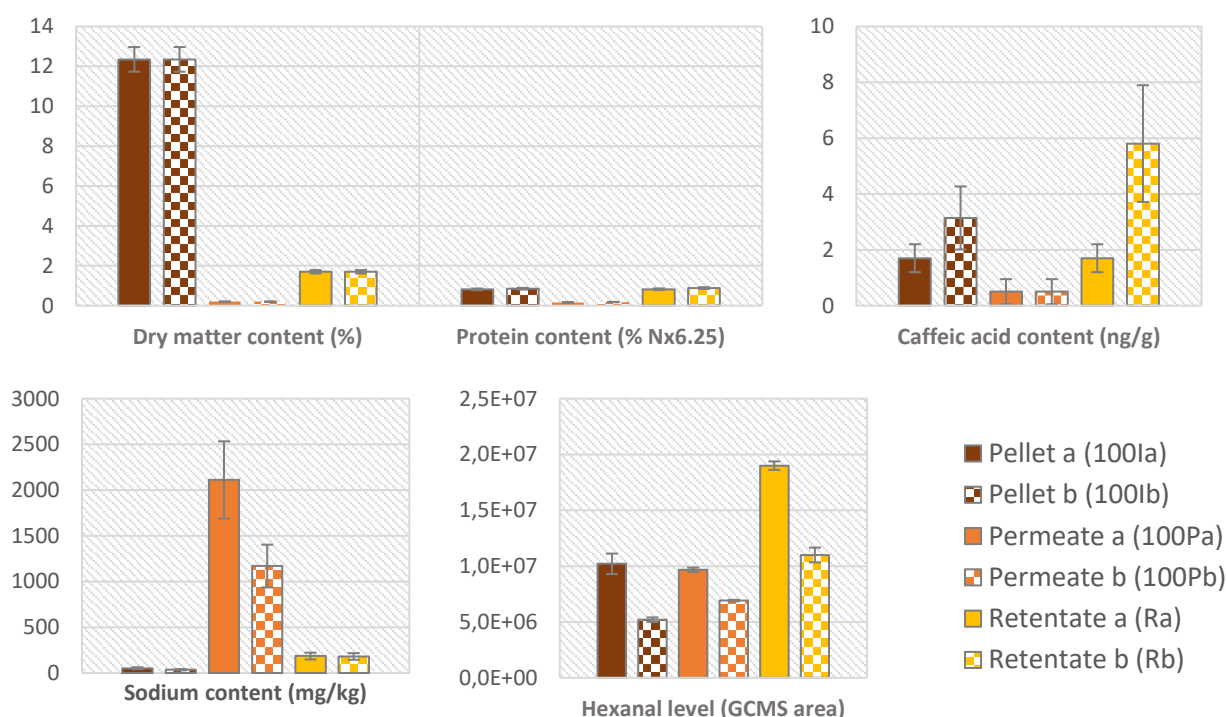


Figure 3.1.2.1.: Key characteristics of the six pea protein isolate fractions used in the study (pellet *a* and *b*, permeate *a* and *b*, retentate *a* and *b*): dry matter content (%), protein content (% Nx6.25), caffeic acid content (ng/g), sodium content (mg/kg), and hexanal levels (GCMS area).

Mixture design

An optimal mixture design was used to create a wide range of reference and experimental solutions from the fractions (permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b*). Response surface models were created and included quadratic terms and first-order interactions. The experimental design was such that there was orthogonality among all the terms, which allowed variable effects to be differentiated from one another. A blocking factor was used to control for the effect of the day on which sensory evaluation took place. The order of solution evaluation within the blocks was fully balanced. Overall, the mixture design had eight independent variables (see Table 3.1.2.1. for the levels). Ten repetitions were performed to estimate experimental error. The total number of trials was 40. Variable levels were chosen

so as to represent a wide range of variation while remaining realistic in terms of the protein concentrations actually experienced when pea protein isolates are used to create foods.

Table 3.1.2.1.: Composition of the different solutions used in this study, which were created by mixing permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b*. In bold are the solutions that were replicated. In italics are the supplementary solutions used for validation purposes.

Solution ID	Permeate <i>a</i> (%)	Permeate <i>b</i> (%)	Retentate <i>a</i> (%)	Retentate <i>b</i> (%)	Pellet <i>a</i> (%)	Pellet <i>b</i> (%)	Water (%)	MS (%)	Sensory session ID
P12	100	0	0	0	0	0	0	0.20	3
P29	100	0	0	0	0	0	0	0.20	6
P9	0	100	0	0	0	0	0	0.20	2
P38	0	100	0	0	0	0	0	0.20	8
P4	0	0	100	0	0	0	0	1.70	1
P8	0	0	100	0	0	0	0	1.70	2
P13	0	0	100	0	0	0	0	1.70	3
P14	0	0	0	100	0	0	0	1.70	3
P31	0	0	0	100	0	0	0	1.70	7
P40	0	0	0	100	0	0	0	1.70	8
P3	0	0	0	0	0	0	100	0.00	1
P19	0	0	0	0	0	0	100	0.00	4
P25	0	0	0	0	0	0	100	0.00	5
P34	0	0	0	0	0	0	100	0.00	7
P6	0	0	0	0	0	25	75	3.09	2
P24	0	0	0	0	0	25	75	3.09	5
P11	25	0	25	0	12.5	0	37.5	2.02	3
P28	0	0	0	0	30	0	70	3.71	6
P1	40	0	0	0	0	0	60	0.08	1
P35	40	0	0	0	0	0	60	0.08	7
P18	0	40	0	0	0	0	60	0.08	4
P17	0	0	40	0	0	30	30	4.39	4
P37	0	0	0	40	30	0	30	4.39	8
P7	0	0	0	40	0	30	30	4.39	2
P2	0	0	0	0	50	0	50	6.00	1
P20	0	0	0	0	50	0	50	6.00	4
P30	0	0	0	0	0	50	50	6.00	6
P39	0	0	0	0	0	50	50	6.00	8
P33	50	0	0	0	0	25	25	3.19	7
P36	50	0	50	0	0	0	0	0.95	8
P10	50	0	0	0	25	0	25	3.19	2
P5	0	50	0	0	0	25	25	3.19	1
P23	0	50	0	50	0	0	0	0.95	5
P26	0	50	0	0	0	0	50	0.10	6
P22	0	0	50	0	25	0	25	3.94	5
P16	0	0	0	50	0	0	50	0.85	4
P21	0	0	0	50	0	0	50	0.85	5
P32	0	0	60	0	0	0	40	1.02	7
P15	0	70	30	0	0	0	0	0.65	3
P27	40	0	0	60	0	0	0	1.10	6
<i>P41</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>67</i>	<i>0</i>	<i>33</i>	<i>8.27</i>	<i>9</i>
<i>P42</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>50</i>	<i>50</i>	<i>6.00</i>	<i>9</i>
<i>P43</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>8</i>	<i>0</i>	<i>92</i>	<i>0.99</i>	<i>9</i>
<i>P44</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>8</i>	<i>92</i>	<i>0.99</i>	<i>9</i>
<i>P45 (Ref_a-R)</i>	<i>38</i>	<i>0</i>	<i>34</i>	<i>0</i>	<i>28</i>	<i>0</i>	<i>0</i>	<i>4.10</i>	<i>9</i>
<i>P46 (Ref_b-R)</i>	<i>0</i>	<i>40</i>	<i>0</i>	<i>36</i>	<i>0</i>	<i>24</i>	<i>0</i>	<i>3.70</i>	<i>9</i>

This experiment was designed with a view to minimizing solution number (final solution count: 40), which facilitated solution evaluation. In contrast, a central composite design or a Box-Behnken design would have required ~60 and ~80 solutions, respectively. Furthermore, we used an optimal design because it is the only design that allows the addition of a blocking factor. This experiment displayed better or equivalent efficiency—with a D-optimal value of 13.25% and a G-optimal value of 50.85%—compared to experiments based on other designs. These metrics reflect goodness of fit relative to a hypothetical orthogonal design: the D-optimal value indicates whether the design minimizes the volume of the joint confidence region for the vector of regression coefficients, and the G-optimal value indicates whether the design minimizes the maximum prediction variance over the design region.

To validate the model's predictive capacity, six solutions that were not initially included in the design were added to the sensory evaluations (for more details, see Table 3.1.2.1—sensory session ID 9).

Solution creation

The six different fractions were combined in various ways to formulate the 46 solutions of the mixture design. This process was carried out at 4°C in 500 mL and 100 mL glass flasks, which were stored at -20°C. During fractionation and recombination, good hygiene practices were used to limit microbial contamination (usage of coat, gloves, and hygienic cap; cleaning and disinfection of hands and all equipment with pure ethanol, followed by air drying; work carried out in a 4°C chamber). In addition, the microbial safety of the solutions was tested by a certified external laboratory (Eurofins Scientific, France). However, for microbiological reasons, the solutions containing pellet *b* had to be heat treated (autoclaved at 110°C for 10 min) before oral sensory evaluation, so the supplemental effect of the autoclave procedure on perception was also evaluated. It was slightly significant for the attributes nuts, cereals, and almond and strongly significant for the attribute granularity (mean difference between autoclaved and unautoclaved solutions: 0.89/10 for nuts; 0.94/10 for cereals; 1.44/10 for almond, and 5.49/10 for granularity). Because this effect was minor (except in the case of granularity) and collinear with pellet *b*, it will not be discussed further.

Sensory evaluation conditions

We recruited 17 panellists (13 women and 4 men; mean age = 23 years old) based on their interest in participating in a long-term study that required their presence at two evaluation sessions per week for three months. They had already been trained to carry out sensory evaluations of pea products or to use sensory evaluation methods, but they all received additional training for this study. They were not informed of the precise aim of the experiment. They gave their free and informed consent to participate and received compensation for their participation. They were asked to not eat, drink, or smoke for at least 1 h prior to any of the sessions (training or experimental). Sensory profiling was carried out in individual booths under white light (the solutions were similar in colour) in an air-conditioned room (20°C). To reduce sensation build-up, the following palate-cleansing protocol was used between solutions during the experimental sessions: panellists had to consume an apple slice, drink water, and wait 40 seconds before consuming the subsequent solution (as described in Cosson *et al.*, 2020 – Section 3.1.1.).

Sensory profiling method

The process to lexicon development was as followed. Panellists were asked to fill out a check-all-that-apply (CATA) survey. It contained 30 attributes, and it was possible for panellists to add more. For our final list, we selected attributes that were cited more than 20% of the time and that allowed significant discrimination among solution types. To select the list of attributes, we also wished to limit total attribute number to avoid panellist fatigue. Panellists were then asked to assess solutions using the sensory profiling method (with a block protocol) described by Cosson *et al.* (2020 – Section 3.1.1.). The objective was to score the intensity of a solution's sensory attributes along an unstructured scale ranging from 0 to 10. Panellists were trained to assess the attributes along the unstructured scale using external references (Table 3.1.2.2). Training took place over 8 sessions that each lasted 45 min. Afterward, panellist performance was evaluated.

Attributes were evaluated in blocks. The first attribute block (pea, broth, nuts, almond, potato, and cereals) focused on aroma perception (i.e., evaluated by nose). The second attribute block (salty, sugar, bitter, astringent, mouthfeel, and granularity) focused on taste perception and mouthfeel, and the panellists wore nose clips. The third attribute block (pea, broth, nuts, almond, potato, and cereals) again focused on aroma perception, but the solutions were evaluated in mouth; the panellists did not wear nose clips. For each block, the solutions were presented on a sequential monadic presentation: for each solution, the panellists evaluated all the attributes within the block, which were printed on the same survey page. Solution order was the same for all three blocks for a given panellist; however, it differed among panellists. In addition, for the three blocks, the first solution in each session was always the reference solution (Refa), which limited and controlled drift between sessions. This reference was available in large quantities and was stored -20°C in 500 mL glass flasks to ensure sensory stable

conditions for the entire study period. To account for order and carry-over effects, solution order was balanced across panellists using a Latin square (Williams design). Each solution was evaluated in duplicate by the 17 panellists.

Table 3.1.2.2.: Definitions and references of the sensory attributes evaluated by the panellists to describe the perception of the pea-protein-based sample types by profiling method.

Descriptors	Attributes in French	Definition	Reference
Salty	Salé	A fundamental taste of which sodium chloride is typical.	2 g/L of sodium chloride solution
Sugar	Sucré	A fundamental taste of which saccharose is typical.	12 g/L of saccharose solution
Bitter	Amer	The fundamental taste associated with a caffeine solution.	0,8 g/L of caffeine
Astringency	Astringent	A drying-out, roughening, and puckery sensation felt in the mouth like with red wine or unripe fruit.	1,7g/L of sulphate aluminium potassium
Mouthfeel	Epaisseur	The way the product feels in the mouth in relation with his viscosity.	4% of pea protein isolates
Granularity	Granulosité	The presence of grains or granules in the products.	4 % of semolina
Pea	Pois	Aromatics characteristic of beans and bean products.	Split peas cooked during 5 minutes in boiling water
Broth	Bouillon	The aromatic associated with boiled meat, soup, or stock.	1 stock cube in 2L of bolt water
Nuts	Noix	The aromatics associated with nuts like walnuts or hazelnuts.	Walnut cooked during 5 minutes in boiling water
Almond	Amande	The aromatics associated with almonds.	Almond powder
Potato	Pomme de terre	The aromatics associated with cooked potato.	Potato cooked during 5 minutes in boiling water
Cereals	Céréales	The aromatics associated with cereals.	Wheat cooked during 5 minutes in boiling water

Statistical analysis of the sensory data

Analyses were performed using XLStat (Addinsoft, 2017, Paris, France) and R (R Core Team, 2017). For analyses of an inferential nature, $\alpha = 0.05$ was the threshold for statistical significance. To analyse the sensory profiling results, we carried out a three-way ANOVA. Solution identity (ID), replicate ID, and panellist ID were the fixed factors, and all the first-order interactions were included. To visually explore differences in the results obtained using the classical versus block profiling protocol, we carried out principal component analysis (PCA) on a correlation matrix; the data were averaged across replicates and panellists. To study the possible drift between sessions, we carried out a two-way ANOVA on the data for the reference solution. Panellist ID, sensory session ID, and their interaction were the fixed factors.

Statistical analysis of the mixture design

JMP (v. 13.1.0; SAS Institute Inc., Cary, SC, USA) was used to generate and analyse the optimal mixture design. Multiple regression analysis was performed to evaluate the effects of all the independent variables on each response variable (i.e., via the regression coefficients). The most influential independent variables ($p \leq 0.05$) were identified using backward elimination. The regression coefficients were calculated for each final model. Model performance was assessed via ANOVA (F-test for significance), lack-of-fit tests, and coefficients of determination (R^2). For the six validation solutions, the predicted and observed responses (with 95% confidence intervals) were calculated.

3.1.2.2. Results

The aim of this study was to understand how the sensory perception of pea protein isolates is affected by the isolates' main fractions. To this end, we used a mixture design. The first part of the results/discussion section examines how the design model was built: it provides an assessment of panellist performance over the 3-month experiment, an explanation of how attributes were chosen, a validation of the study methodology (i.e., creating solutions by combining isolate fractions), and a

statistical representation of the model. The second part of the results/discussion section focuses on how different sensory attributes (primarily beaniness, bitterness, and astringency) are affected by pea protein isolate composition (i.e., the main constituents—insoluble proteins, volatiles, and soluble compounds [proteins, peptides, phenolics, and minerals]).

Construction of surface response models from the sensory data

Assessment of panellist performance over the 3-month experiment

Panellists used sensory profiling to assess the 46 solutions (reference and experimental; in duplicate) during two weekly sessions over the course of three months. Because solution number was high and study duration was long, it was important to examine panellist performance over time (i.e., reproducibility, homogeneity, and between-session drift). To do so, a three-way ANOVA was used to analyse the attribute scoring data (Table 3.1.2.3.).

Table 3.1.2.3.: Assessment of panellist performance in scoring the intensities of the six aroma attributes evaluated by nose (N), the six taste attributes evaluated in mouth with the nose clip (NC), and the six aroma attributes evaluated in mouth (M) for the range of solutions used in the study; solution evaluation employed a block protocol. In the three-way ANOVA, the fixed factors were solution ID, panellist ID, replicate ID, and their first-order interactions. F: Fisher statistic for the fixed effects. Pvalue: p-value for the Fisher test. Significant p-values (threshold of 0.05) are in bold. Model degrees of freedom (DF): 735; residual DF: 624.

Sensory attributes	Solution ID		Panellist ID		Replicate ID		Panellist* Solution		Replicate* Panellist		Replicate* Solution	
	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue	F	Pvalue
Almond-M	45.78	<0.01	86.43	<0.01	19.15	<0.01	2.17	<0.01	3.02	<0.01	1.22	0.18
Almond-N	22.20	<0.01	100.15	<0.01	0.22	0.64	2.03	<0.01	4.62	<0.01	0.99	0.49
Astringent-NC	33.94	<0.01	58.61	<0.01	0.17	0.68	1.75	<0.01	4.85	<0.01	1.42	0.05
Bitter-NC	14.83	<0.01	44.76	<0.01	11.48	<0.01	1.23	<0.01	2.72	<0.01	1.20	0.19
Broth-M	54.09	<0.01	49.60	<0.01	5.39	0.02	1.98	<0.01	3.08	<0.01	1.43	0.04
Broth-N	27.26	<0.01	48.37	<0.01	10.46	<0.01	1.24	<0.01	2.30	<0.01	1.66	0.01
Cereals-M	47.01	<0.01	48.42	<0.01	1.58	0.21	2.34	<0.01	2.72	<0.01	1.86	<0.01
Cereals-N	23.43	<0.01	53.96	<0.01	0.91	0.34	1.46	<0.01	3.43	<0.01	0.82	0.78
Granularity-NC	261.12	<0.01	24.39	<0.01	0.05	0.82	2.29	<0.01	1.77	0.03	1.11	0.30
Mouthfeel-NC	116.94	<0.01	42.39	<0.01	35.61	<0.01	2.07	<0.01	3.53	<0.01	2.16	<0.01
Nuts-M	29.50	<0.01	89.91	<0.01	0.93	0.34	1.80	<0.01	3.62	<0.01	1.10	0.31
Nuts-N	25.95	<0.01	99.72	<0.01	2.13	0.15	1.60	<0.01	3.39	<0.01	1.62	0.01
Pea-M	27.57	<0.01	41.31	<0.01	55.09	<0.01	1.88	<0.01	6.76	<0.01	1.54	0.02
Pea-N	28.12	<0.01	45.61	<0.01	28.95	<0.01	1.52	<0.01	2.92	<0.01	1.94	<0.01
Potato-M	23.79	<0.01	61.59	<0.01	1.52	0.22	1.91	<0.01	3.83	<0.01	1.34	0.08
Potato-N	19.63	<0.01	40.01	<0.01	2.18	0.14	1.16	0.03	3.18	<0.01	0.94	0.57
Salty-NC	15.74	<0.01	70.85	<0.01	0.51	0.48	1.33	<0.01	4.24	<0.01	0.83	0.76
Sugar-NC	9.20	<0.01	94.31	<0.01	17.01	<0.01	1.96	<0.01	5.53	<0.01	1.37	0.07

Reproducibility and homogeneity were examined first. Solution ID was significant for all 18 attributes, which indicates that panellists distinguished among solutions. Panellist ID and the interaction between panellist ID and solution ID were also significant for all the attributes. Such interactions are common when sensory attributes are evaluated using unstructured scales and are difficult to control even when panellists have undergone extensive training (Jourjon *et al.*, 2005). The interaction between replicate ID and solution ID was not significant for 10/18 attributes. Replicate ID was not significant for 11/18 attributes, but the interaction between panellist ID and replicate ID was significant for all 18 attributes. However, the F-values for these interactions were low compared to the F-values for the main effect of solution ID. For example, for the broth-M attribute, $F(39,624) = 54.09$ for solution ID; $F(1,624) = 5.39$ for replicate ID; $F(16,624) = 3.08$ for the panellist-by-replicate interaction; and $F(16,39) = 1.43$ for the solution-by-replicate interaction (model degrees of freedom [DF] = 735, residual DF = 624).

The presence of between-session drift was examined by looking at the scores for the reference solution across the entire experiment. To this end, a two-way ANOVA (fixed factors: panellist ID and sensory session ID) was performed using scores for each attribute given to the reference solution (Table 3.1.2.4.). Sensory session ID was not significant for any of the attributes except broth-M and granularity-NC:

these attributes were assigned slightly higher and slightly lower scores, respectively, during a single session. Although using the reference can make solution preparation more cumbersome, it was important in helping to validate panellist performance. In addition, panellists found the reference useful as they scored the other solutions. In past research, monadic presentation has been found to be faster and less tiring than comparative presentation (Mazzucchelli & Guinard, 1999). However, comparative presentation allows panellists to detect smaller differences among food products and to make more accurate decisions about these relative differences (Saint-Eve *et al.*, 2006). Here, via its use of blocks, the presentation method combined monadic and comparative elements. Consequently, the panellists could base their attribute scoring on both their memories from the training period as well as on the reference, which was always the first solution in the sequence (Hastie & Park, 1986).

Table 3.1.2.4: Assessment of panellist performance in scoring the intensities of the six aroma attributes evaluated by nose (N), the six taste attributes evaluated in mouth with the nose clip (NC), and the six aroma attributes evaluated in mouth (M) for the main reference solution. In the two-way ANOVA, the fixed factors were panellist ID, sensory session ID, and their interaction. F value: Fisher statistic for the fixed effects. Pvalue: p-value for the Fisher test. Significant p-values (threshold of 0.05) are in bold. Model DF: 35; residual DF: 304.

Sensory attributes	Sensory session ID		Panellist ID	
	F	Pvalue	F	Pvalue
Almond-M	1.17	0.28	89.99	<0.01
Astringent-NC	0.50	0.96	30.02	<0.01
Bitter-NC	1.49	0.09	32.90	<0.01
Broth-M	2.48	<.01	30.36	<0.01
Cereals-M	0.88	0.61	40.19	<0.01
Granularity-NC	2.88	<.01	19.64	<0.01
Mouthfeel-NC	1.51	0.08	24.90	<0.01
Nuts-M	0.69	0.83	35.17	<0.01
Pea-M	1.37	0.14	24.88	<0.01
Potato-M	0.99	0.47	36.41	<0.01
Salty-NC	1.29	0.18	22.97	<0.01
Sugar-NC	1.08	0.37	63.31	<0.01

Taken together, these results suggest that the panellists generally came up with repeatable and homogeneous scores and that there was no between-session drift in scoring. There was some disagreement in the case of certain attributes (e.g., sugar-NC), which was taken into account when the results were analysed.

Attribute choice

Plant-protein-based ingredients are often said to be “beany,” a multidimensional and complex descriptor (Bott & Chambers, 2006). Here, the decision was made not to use the term “beany.” Instead, its multiple components were parsed out and expressed via other terms (see Cosson *et al.*, 2020 – Section 3.1.1.). Thus, six aroma attributes were selected: potato, pea, cereals, broth, almond, and nuts. Plant-protein-based ingredients are also often described as being persistently bitter and astringent (Roland *et al.*, 2017); consequently, bitterness and astringency were included as well. Finally, two taste attributes—salty and sugar—and two texture attributes—mouthfeel and granularity—were also chosen because they have been found to be important in descriptions of food quality and preference (van Vliet *et al.*, 2009).

Attribute intensities for the different solutions were investigated using a three-way ANOVA (Table 3.1.2.3.). Solution ID was significant for the 18 attributes (model DF: 735; residual DF: 624), which means the solutions had distinct sensory profiles. There were pronounced differences in perceived texture ($F = 261.12$ for granularity and $F = 116.94$ for mouthfeel) and smaller differences in perceived sweetness ($F = 9.20$ for sugar). These results are not surprising. It is easier to describe food products based on texture and taste than on aroma (Saint-Eve *et al.*, 2011). Furthermore, temporally, they are the first attributes to become dominant in the mouth (Le Calvé *et al.*, 2019; Pineau *et al.*, 2009; Saint-Eve

et al., 2011). Additionally, when describing overall preferences and sensory satisfaction, consumers appear to primarily focus on taste and then on texture, paying the least attention to aroma (van Vliet *et al.*, 2009). Finally, since the solutions had very low levels of natural sugar content (and no sugar was added), it was not surprising that sweetness did not greatly contribute to the perceived differences among the solutions. Consequently, this attribute was not included in the statistical model.

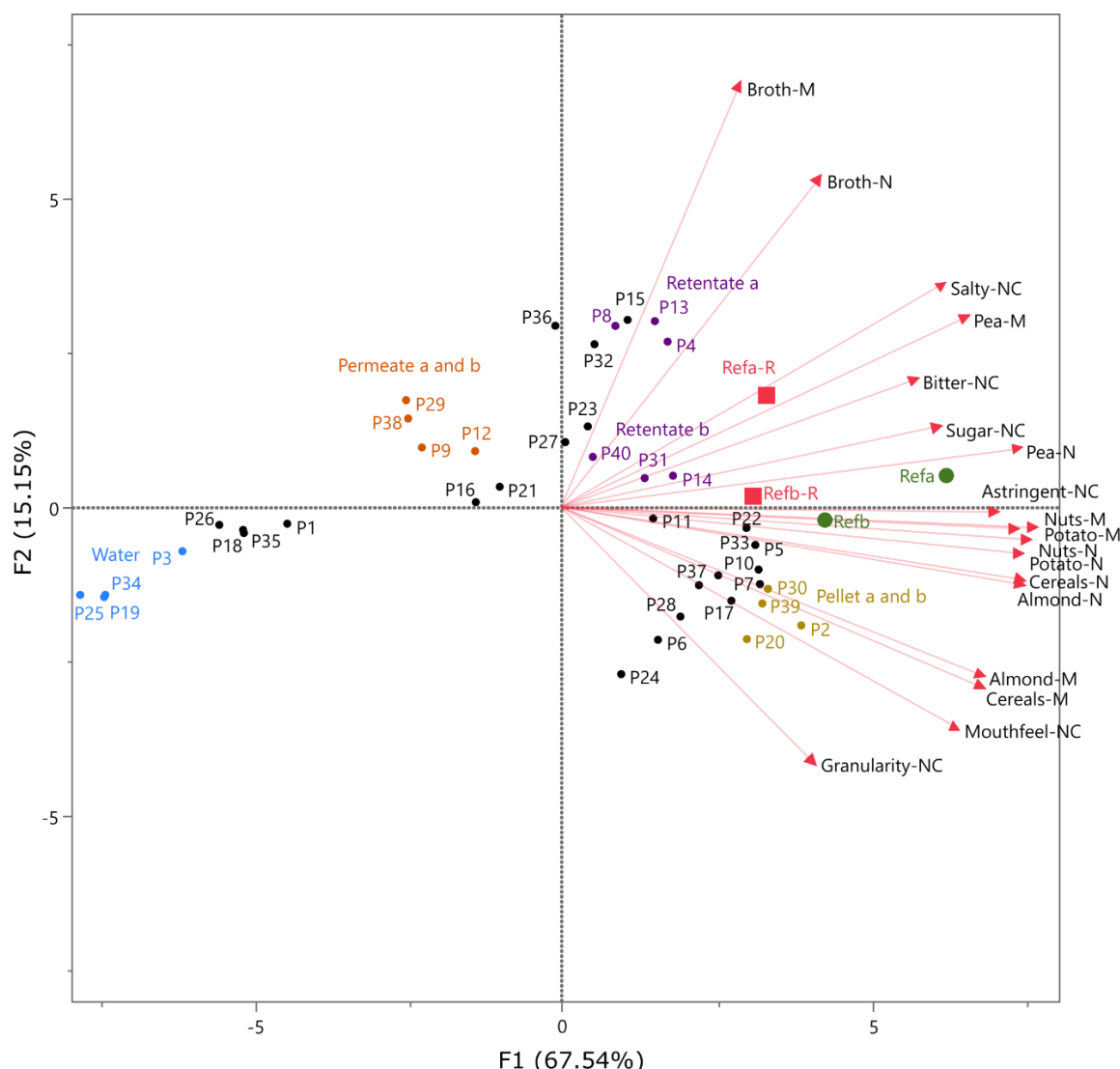


Figure 3.1.2.2: Results of the principal component analysis (PCA) examining the solutions' sensory profiles. The plot shows the loadings i.e. the correlational relationships between PCA axes 1 and 2 and the sensory attribute values in the original dataset, as well as the relative similarity of the solutions' sensory profiles. In green are the active observations corresponding to the raw products (Refa and Refb). In blue are the active observations corresponding to 100% of water. In orange are the active observations corresponding to 100% of permeate (*a* or *b*). In purple are the active observations corresponding to 100% of retentate (*a* or *b*). In yellow are the active observations corresponding to 50% of pellet (*a* or *b*). In dark are the others active observations. In red are the supplementary observations corresponding to the experimental solutions with the same composition as the reference solutions (Refa-R and Refb-R).

To build upon these results, PCA was used to visually depict the relationships among solution types and attributes (Figure 3.1.2.2.). The solutions were well distributed along axes F1 and F2, which accounted for 82.68% of the variance. Thus, maps based on the first two axes seemed to provide a good quality projection of the initial multidimensional table, even though some information might have remained

hidden in the subsequent axes. The 12 aroma attributes were clustered within one quarter of the correlation circles and thus clearly interacted in multiple ways. Aroma attributes assessed in the mouth and nose were strongly correlated ($R^2 = 0.86$ for pea, $R^2 = 0.88$ for broth, $R^2 = 0.83$ for cereals, $R^2 = 0.95$ for nuts, $R^2 = 0.87$ for almond, $R^2 = 0.89$ for potato). These results suggest that panellists assigned similar scores to aroma attributes perceived orthonasally and retronasally and that food processing in the mouth had a minor effect on olfactory perception. Orthonasal doors result from volatile compounds traveling from the external environment and through the nares to the olfactory mucosa, whereas retronasal doors result when volatile compounds travel to the olfactory mucosa after they have been released during food destructure in the oral cavity (Sun & Halpern, 2005). That said, orthonasal and retronasal responses are often similar, except in cases where there are physicochemical or sensory interactions induced by texture, taste, or in-mouth food destructure (Goldberg *et al.*, 2018). Furthermore, results for the attributes pea-M and salty-NC were also correlated ($R^2 = 0.87$), which suggests possible congruency (Oladokun *et al.*, 2017). Consequently, our results indicate that there may have been limited interactions between texture, taste, and flavour (except in the case of the attributes pea and salty) and that food oral processing had a minimal impact on these attributes. Therefore, the aroma attributes evaluated via the nose were not included in the statistical model.

The aroma attributes potato, almond, cereals, and nuts as well as the attributes astringent and mouthfeel were significantly correlated (R^2 range = 0.72–0.98). They were also correlated with the dry matter content (%) of the solutions ($R^2 = 0.97$ for mouthfeel, $R^2 = 0.88$ for cereals-M, $R^2 = 0.85$ for almond-M, $R^2 = 0.82$ for potato-M, $R^2 = 0.81$ for nuts-M, and $R^2 = 0.73$ for astringent). These results suggest that the perception of these attributes was mainly driven by dry matter content and, thus, protein concentration. However, dry matter content was not correlated with the perception of the attributes pea and bitter. It is therefore necessary to build a more complex model to understand the origin of these attributes.

Validation of the study methodology—fraction-based formulation of solutions

In this study, a mixture design was used to create a large number of solutions by combining pea protein isolate fractions. To validate this methodology, the sensory properties of the two reference solutions, created directly from the pea protein isolates, were compared with the sensory properties of two experimental solutions that were created using the isolate fractions to have the exact same compositions as the reference solutions.

PCA was used to visually depict the main differences between the reference solutions and these experimental solutions (Figure 3.1.2.2.). The results show that the two reference solutions (Refa and Refb) and the two experimental solutions (Refa-R and Refb-R) occur in relatively close proximity compared to the other solutions on the map. The distance is greater between Refa and Refa-R than between Refb and Refb-R. For the panellists, Refa was the “sensory reference”. As a result, there may be a bias in its sensory properties that is directly due to the study’s methodology.

The main difference between the reference solutions and the experimental solutions was in their perceived granularity. The experimental solutions were perceived as less granular than the reference solutions. In commercially produced isolates, proteins are highly denatured due to the extraction process (pH changes, high temperatures) and form large aggregates that are primarily structured by hydrophobic interactions (Oliete *et al.*, 2018). It is likely that these aggregates are fairly insoluble, which could be responsible for the perceived granularity of the reference solutions. When the experimental solutions were created by combining the isolate fractions, the processes that they underwent (centrifugation and filtration) might have broken up these aggregates and induced structural changes, resulting in smaller, more soluble clusters.

Construction of the optimal mixture models

In past studies, various experimental and statistical methods have been used to explore the sensory perception of food, and the choice of techniques depends on the research question, variable type and number, and food product number (Seisonen *et al.*, 2016; Yu *et al.*, 2018; Zielinski *et al.*, 2014). While classical approaches such as fractional factorial design and simple regression have been widely used,

they may be inadequate for fully describing a complex food. Thus, this study employed optimal mixture models. This approach made it possible to limit solution number, while also minimizing the degree of aliasing to ensure less collinearity among the independent variables (Yu *et al.*, 2018).

The attribute scoring data were used to develop the optimal mixture models. Model performance was tested using ANOVA (global model; F-test for significance), lack-of-fit tests (which calculate a pure-error negative log-likelihood by constructing categories for every combination of model effect values in the data), and the coefficients of determination (R^2) (Table 3.1.2.5.). The results of the ANOVA were significant: the F-ratios ranged from 23 to 520, and the p-values were below 0.01. The lack-of-fit tests were not significant for the 10 attributes examined, which means that the error for each model was smaller than the pure error associated with replication. Thus, the models developed for each sensory attribute have relevance. Since the R^2 values were between 82 and 96%, a large amount of the variation in the attribute scores was explained, so the models' results could be interpreted with confidence. These results showed a good-quality fit. The model with the best fit was the one for the attribute mouthfeel. This finding is not surprising because past research has found that models relating food product composition and perceived texture often have the greatest explanatory value (Burseg *et al.*, 2009; Cook *et al.*, 2005).

Table 3.1.2.5.: Performance of the optimal mixture models as assessed via ANOVA; lack-of-fit tests; and the coefficients of determination (R^2). F: Fisher statistic for the fixed effects. Pvalue: p-value for the Fisher test. DF: degrees of freedom. Significant p-values (threshold of 0.05) are in bold.

Sensory attributes	ANOVA		Lack-of-fit test		Coefficient of determination R^2
	F (model DF, residual DF)	Pvalue	F (model DF, residual DF)	Pvalue	
Almond-M	62.97 (17, 62)	<0.01	0.50 (8, 54)	0.85	0.95
Astringent-NC	62.42 (14, 65)	<0.01	0.94 (11, 54)	0.51	0.93
Bitter-NC	23.12 (13, 66)	<0.01	0.43 (12, 54)	0.94	0.82
Broth-M	90.44 (15, 64)	<0.01	0.94 (10, 54)	0.50	0.95
Cereals-M	70.29 (14, 65)	<0.01	0.94 (11, 54)	0.51	0.94
Mouthfeel-NC	519.98 (8, 72)	<0.01	1.04 (18, 54)	0.44	0.96
Nuts-M	57.98 (14, 65)	<0.01	1.21 (11, 54)	0.30	0.93
Pea-M	50.92 (9, 70)	<0.01	0.88 (16, 54)	0.59	0.87
Potato-M	53.68 (13, 66)	<0.01	0.49 (12, 54)	0.91	0.92
Salty-NC	33.54 (14, 65)	<0.01	1.33 (11, 54)	0.23	0.88

When the backward elimination procedure was used (p-value < 0.05 for the F-statistic), the number of significant variables in the models ranged from 8 to 18 (main effects, first-order interactions) (Figure 3.1.2.3.). Consequently, attribute perception depended on several variables (permeate type, retentate type, and pellet type) as well as on their interactions. However, scores for different attributes were explained by different sets of variables. In other words, the perceptions of different attributes (e.g., pea, nuts, almonds, bitter) could be explained by differences in solution composition. Overall, retentate type and pellet type, but not permeate type, had strong effects on attribute perception. In addition, although the experiment was designed to incorporate orthogonality among the fixed factors, some interactions were significant. The interactions with the greatest effect on solution perception were permeate *a**pellet *b* and pellet *b**water. That said, the relative importance of the interactions was minimal compared to that of the main effects. This finding clearly suggests that the perception of pea-protein-based food products is influenced by the types of compounds present as opposed to the interactions among compound types.

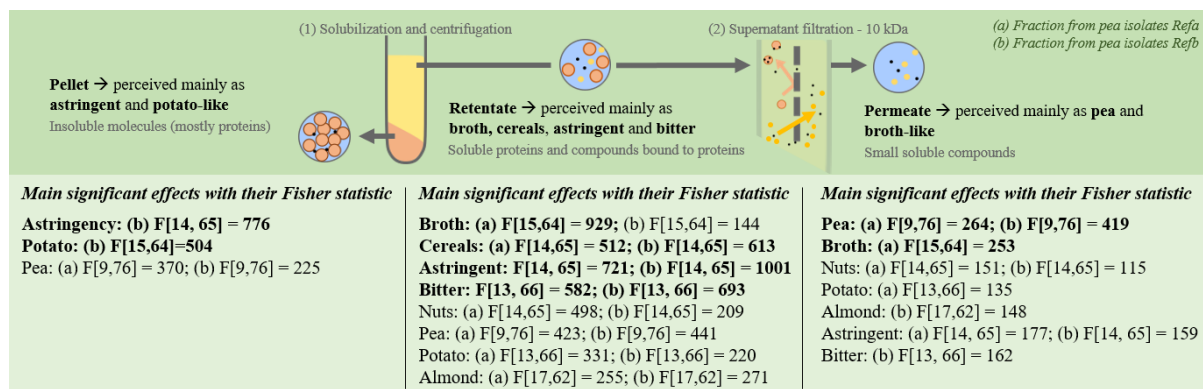


Figure 3.1.2.3.: Main significant effects identified using a backward elimination procedure (F=Fisher statistic for the fixed effects) for the six fractions: pellet (*a* and *b*), retentate (*a* and *b*) and permeate (*a* and *b*).

Solutions created from isolate-*b* fractions were perceived as more bitter and astringent, with greater mouthfeel, and stronger notes of almond, cereals, nuts, and potato. In contrast, solutions created from isolate-*a* fractions were perceived as more salty with stronger notes of pea and broth. These results suggest that isolate identity does matter, even when isolates are reduced to their fractions. Furthermore, for almost all the significant effects, the coefficients were positive. This finding means that there was a positive relationship between fraction concentration and perceived attribute intensity and thus that the perception of pea-protein-based food products is driven by compound presence rather than compound absence.

To validate the model's predictive capacity, panellists were also asked to evaluate six supplementary solutions (created with the same fractions as the main experimental solutions but using different fraction concentrations) (Table 3.1.2.1.). Although the data for these solutions were located towards the range limits of our main data set, there was overlap between the 95% confidence intervals for the observations and predictions in most cases (Table 3.1.2.6.). Predictions were least accurate for the solutions P43 and P44 (8% pellet), notably for the attributes salty, bitter, astringent, and pea. The model generated good predictions when interpolating (i.e., predicting data points that would fall within the range of our observed data). However, its predictions were of lower quality in the case of extrapolation (i.e., predicting data points outside the range of the observed data).

Table 3.1.2.6.: Observed and predicted attribute scores with the 95% confidence intervals (CIs) for the six validation solutions (two replicates performed). O = Observed. P = Predicted.

Result ± 95% CI	Salty-NC	Bitter-NC	Astringent-NC	Mouthfeel-NC	Broth-M	Pea-M	Potato-M	Almond-M	Nuts-M	Cereals-M
P41 O	2.96 ± 0.64	3.35 ± 0.61	4.73 ± 0.62	7.58 ± 0.75	2.31 ± 0.67	4.67 ± 0.55	2.83 ± 0.63	3.30 ± 0.79	4.00 ± 0.69	3.54 ± 0.68
P41 P	2.93 ± 0.57	2.81 ± 0.98	4.40 ± 0.78	9.58 ± 0.44	2.88 ± 0.65	6.03 ± 0.58	2.91 ± 0.50	2.02 ± 1.19	4.37 ± 0.88	4.28 ± 0.84
P42 O	2.86 ± 0.71	4.18 ± 0.64	5.01 ± 0.73	5.01 ± 0.70	2.10 ± 0.69	4.61 ± 0.76	2.53 ± 0.58	3.09 ± 0.77	3.09 ± 0.72	2.79 ± 0.64
P42 P	2.98 ± 0.28	3.94 ± 0.43	5.88 ± 0.34	5.36 ± 0.33	1.72 ± 0.40	3.65 ± 0.42	2.73 ± 0.22	4.20 ± 0.33	4.29 ± 0.31	4.02 ± 0.38
P43 O	2.39 ± 0.62	4.02 ± 0.75	3.31 ± 0.74	1.41 ± 0.47	1.70 ± 0.68	2.47 ± 0.79	1.34 ± 0.48	2.47 ± 0.59	1.85 ± 0.70	1.44 ± 0.43
P43 P	1.36 ± 0.23	2.46 ± 0.36	2.26 ± 0.28	1.14 ± 0.05	0.88 ± 0.38	1.12 ± 0.27	0.74 ± 0.18	1.32 ± 0.29	1.28 ± 0.27	0.94 ± 0.27
P44 O	2.87 ± 0.63	4.43 ± 0.67	3.31 ± 0.69	1.42 ± 0.40	1.6 ± 0.55	2.68 ± 0.66	1.04 ± 0.35	2.11 ± 0.58	2.16 ± 0.65	1.29 ± 0.50
P44 P	1.33 ± 0.21	2.72 ± 0.32	2.19 ± 0.22	0.86 ± 0.05	0.79 ± 0.36	0.96 ± 0.26	0.56 ± 0.14	1.99 ± 0.22	1.15 ± 0.22	1.83 ± 0.24
P45 O	3.91 ± 0.80	3.79 ± 0.66	4.19 ± 0.67	4.01 ± 0.68	5.22 ± 0.72	5.03 ± 0.71	2.51 ± 0.53	2.55 ± 0.65	3.42 ± 0.81	2.6 ± 0.57
P45 P	2.94 ± 0.61	3.65 ± 0.90	4.03 ± 0.72	4.9 ± 0.22	3.78 ± 0.58	5.22 ± 0.32	2.51 ± 0.47	3.13 ± 0.65	4.77 ± 0.66	4.12 ± 0.65
P46 O	3.82 ± 0.85	4.05 ± 0.64	4.9 ± 0.71	3.68 ± 0.6	3.16 ± 0.86	5.06 ± 0.80	2.47 ± 0.51	3.02 ± 0.68	3.47 ± 0.80	2.5 ± 0.61
P46 P	3.86 ± 0.59	4.15 ± 0.80	6.7 ± 0.50	4.08 ± 0.46	2.95 ± 0.31	4.85 ± 0.30	2.9 ± 0.31	3.59 ± 0.54	4.49 ± 0.54	4.31 ± 0.59

However, despite the low degree of collinearity among the independent variables and the incomplete orthogonality of the design, this model has helped clarify the perception of plant-protein-based foods.

Use of the models to better understand sensory perceptions

Identification of the fractions underlying beaniness

The mixture models helped clarify the origin of perceived beaniness and the respective contributions of the different isolate fractions.

The results show that the perception of the attribute cereals was largely influenced by retentates *a* and *b* (respectively: $F[14,65] = 512$ and $F[14,65] = 613$). The attribute broth was mainly affected by permeates *a* ($F[15,64] = 929$) and by retentate *a* ($F[15,64] = 253$). Attribute potato was mainly affected by pellet *b* ($F[15,64] = 504$). Finally, the perception of the attribute pea was simultaneously affected by pellets *a* and *b* (respectively: $F[9,76] = 423$ and $F[9,76] = 441$); retentates *a* and *b* (respectively: $F[9,76] = 370$ and $F[9,76] = 225$); and permeates *a* and *b* (respectively: $F[9,76] = 264$ and $F[9,76] = 419$). Retentate *a*, which had higher hexanal levels, led to more intense potato, broth, and pea attributes.

Dry matter content was similar among the fractions. However, pellets and retentates differed in their main protein type: insoluble proteins versus soluble proteins, respectively. In contrast, permeates were mainly composed of non-proteins, such as sodium, caffeic acid, and hexanal (Figure 3.1.2.1.). Hexanal was selected as the key volatile compound because recent studies have shown that hexanal have a large contribution to perception of beany notes (Trikusuma *et al.*, 2020; Bi *et al.*, 2020). Caffeic acid was selected as the key phenol compound because studies have shown that it can contribute to bitter perceptions in plant-based product (Franck *et al.*, 2007). Thus, unsurprisingly, the volatile-rich permeates contributed to the perception of the aroma attributes, as observed in previous studies. Indeed, the beaniness of pulses has been found to be strongly related to volatile composition and, notably, hexanal levels (Bott & Chambers, 2006; Vara-Ubol *et al.*, 2004). More recently, Murat *et al.* (2013) examined the volatile composition of pea isolates and pea flour and suggested that certain aldehydes, alcohols, and ketones were responsible for beaniness (Murat *et al.*, 2013). These results were confirmed recently by Bi *et al.*, who demonstrated that six aroma compounds (including 3-methylbutanoic acid and hexanal) significantly contributed to the characteristic aroma of peas and that fifteen aroma compounds (including pyrazines and pyranones) significantly contributed to the characteristic aroma of roasted peas (Bi *et al.*, 2020).

Initially, the influence of the retentates and pellets on beaniness was quite surprising. However, hexanal levels in these fractions were rather high, especially in retentate *a* (Figure 3.1.2.1.). Interactions between hexanal and proteins may be playing a role (Houde *et al.*, 2018; Wang & Arntfield, 2016). Indeed, in pea protein isolates, most volatiles are bound to proteins (Kuhn, 2004); for example, 88% of the octanal present may be bound to pea vicilin. These interactions might also be related to protein solubility (Suppavorasatit *et al.*, 2013). As proteins were present at higher concentrations in the retentates and the pellets, interactions between proteins and volatiles could explain the hexanal levels in these fractions and their effect on perceived aroma intensities. In addition, the perception of the attributes almond, broth, and pea may also have been influenced by the composition of peptides and amino acids, which may be richer in the retentates. Indeed, Henriksen showed that the bouillon note of dried sausage was related to a mixture of different amino acids and peptides and that the intensity of the potato note was positively correlated with levels of tyrosine (in both its free and peptide residue forms) (Henriksen, 1997).

Thus, the mixture models helped reveal the factors that contribute to the perception of beaniness. The results suggest that beany notes are strongly related to hexanal composition. However, according to the literature, there may also be an influence of others volatile compounds and of protein-volatile interactions as well as peptide composition.

Identification of the fractions underlying mouthfeel, bitterness, and astringency

The mixture models were also a useful tool for gaining insight into the origin of the taste and texture attributes. The results show that perceived mouthfeel intensity mainly depended on pellets *a* and *b* (respectively: $F[8, 72] = 1923$ and $F[8, 72] = 1072$). Past work found that texture was relatively balanced in hydrocolloid solutions due to the high number of factors at play (e.g., hydrocolloid type, viscosity

range, food matrix, choice of sensory evaluation technique) (van Vliet *et al.*, 2009). In this study, the ratio of dry matter content to protein content was 0.83 for pellets, 0.88 for retentates, and 0.2 for permeates. The difference in texture perception among the fractions was therefore not due to protein concentration but rather to protein type. Pea protein isolates mainly consist of globulins, which represent 65–80% of total protein concentration and belong to three major groups (legumin 11S, vicilin 7S, and convicilin 7S); some albumins are also present (Sirtori *et al.*, 2012). In addition, in commercially produced isolates, proteins tend to be highly denatured and form large aggregates primarily structured by hydrophobic interactions (Oliete *et al.*, 2018). Consequently, the process of creating new food products from isolate fractions can induce changes in this protein network. Our results suggest that different types of proteins are present in different concentrations in the pellet and retentate and that the specific pattern likely depends on protein size, solubility, and hydrophobicity. These compositional differences are probably responsible for the differences in perceived texture.

Perceived astringency mainly depended on retentates *a* and *b* (respectively: $F[14, 65] = 721$ and $F[14, 65] = 1001$) but also on pellet *b* ($F[14, 65] = 776$). Past research has indicated that the perceived astringency of foods and beverages is mainly due to the composition of phenolics, namely monomeric and polymeric phenols, such as flavan-3-ols, as has been described in wine (Damodaran & Arora, 2013; Hufnagel & Hofmann, 2008; Peleg *et al.*, 1999). Here, perceived bitterness was influenced by retentates *a* and *b* (respectively: $F[13, 66] = 582$ and $F[13, 66] = 693$). Like astringency, bitterness has been found to be influenced by the composition of phenolics, but, additionally, there is an influence of saponins (Heng *et al.*, 2006) and peptides (Aubes-Dufau *et al.*, 1995). We expected caffeic acid to mainly be present in the permeates (i.e., they are small, soluble molecules). However, retentates and pellets (especially from isolate *b*) had higher concentrations of caffeic acid, which is considered to be a marker of phenolic levels (Figure 3.1.2.1.). In plant-protein-based foods, phenols can bind to proteins via hydrophobic and hydrophilic interactions (Morton & Murray, 2001; Potter *et al.*, 1993; Zhang *et al.*, 2016). In these interactions, important roles are played by phenol chemical structure, phenol size and composition (including the number of OH groups), and food environment (e.g., pH) (de Freitas & Mateus, 2012). In this study, the fractions had different pH values (~7.5 for the retentates and pellets vs. ~9 for the permeates), which suggests that phenol-protein interactions may have been different as well. Thus, the proteins in the pellets and the retentates may also interact with phenolics, leading to differences in perceived astringency and bitterness. Numerous mechanisms can therefore be at the origin of these perceptions.

Finally, perceived saltiness is not directly in the scope of study. However, a better understanding of this perception seem relevant to the overall understanding of off-notes and the potential sensory interactions. Perceived saltiness depended simultaneously on permeates *a* and *b* (respectively: $F[14, 65] = 584$ and $F[14, 65] = 481$) and retentates *a* and *b* (respectively: $F[14, 65] = 641$ and $F[14, 65] = 273$). Solutions made from isolate *a* had higher sodium contents and were perceived as more salty. Relative to their dry matter content, the permeates and retentates had higher levels of sodium (Figure 3.1.2.1.). Previous work has found that both sodium and chloride ions are required to activate the salt receptor (van der Klaauw & Smith, 1995). However, when Frankowski *et al.* (2014) studied the sensory characteristics and composition of permeate obtained from whey ultrafiltration, they showed that, in addition to sodium, both lactic acid and potassium chloride can heighten the intensity of perceived saltiness. Past research suggests that mineral concentrations could be higher on the permeates and retentates which may also have contributed to perceived saltiness.

Thus, the mixture models provided insight into the origin of the taste and texture attributes. Our results suggest that the protein composition of the pellets and retentates influenced perceived texture. Interactions between proteins and phenolics in the pellets and retentates may have affected perceived astringency. Retentates may also be richer in phenolics, saponins, and peptides, whose presence may have impacted perceived bitterness. Finally, the permeates and retentates may have been richer in salts, heightening perceived saltiness.

Optimizing ingredient choice and product formulation

Based on these results, recommendations can be developed to improve the flavour of the pea protein isolates used in plant-protein-based food products. First, attention should be paid to ingredient optimization. Our results suggest that the filtration step was not especially effective in removing the compounds responsible for off-notes. In this regard, the centrifugation step seemed more useful: the pellets were described as less beany, bitter, and astringent than the retentates. Consequently, it could be useful to formulate plant-protein-based products using pellets. However, because pellets consist mainly of insoluble compounds, there might be a loss of functionality. Thus, employing a pellet/retentate mixture could help limit off-notes while retaining functionality. The results for the retentates also highlight the importance of protein conformation and the interactions between both proteins and aromatics as well as proteins and phenolics. These mechanisms appear to play an important role in the sensory perception of pea protein isolates and must be studied further.

The specific nature of these recommendations will depend on food type, which will, in turn, determine protein concentration and functionality, matrix type, and ingredient choice. Indeed, pea protein isolates are used in different applications, for which protein concentrations vary widely (from < 1% to > 50%, with a median of 5%). For example, they are used in sports nutrition and to replace casein and whey proteins in fermented and unfermented dairy products (Akin & Ozcan, 2017; Ben-Harb *et al.*, 2020; Schindler *et al.*, 2012); they can help enrich protein levels in baked foods, cereals, and snacks (Philipp *et al.*, 2017); and they can improve the cooking yield, water/fat binding, and sliceability of meat, fish, processed foods, soups, and sauces (Baugreet *et al.*, 2016). They are also emerging as an alternative ingredient in specialized foods, such as gluten-free products (Mariotti *et al.*, 2009) and infant formula (Le Roux *et al.*, 2020). The results of this study can help inform product formulation. For example, to improve the aroma of a product containing 3% pea protein, a mixture of pellet *b* (25%) and water (75%) would seem to be ideal (Figure 3.1.2.4.). In such a product, undesirable aromas would be relatively less intense (broth score of 1.4/10, pea score of 2.4/10, and potato score of 1.2), while desirable aromas would be relatively more intense (almond score of 4.2/10, cereals score of 3.9/10, and nuts score of 2.9/10). To provide another example, it might be helpful to decrease the bitterness and astringency of a flavoured product containing 3% pea protein; in this context, a mixture containing 74% permeate *b* and 26% pellet *a* could be useful (Figure 3.1.2.4.). This formulation should result in less intense bitterness (score of 1.6/10) and astringency (score of 1.7/10).

Here, we discuss using customized combinations of isolate fractions as a strategy for reducing the off-notes of pea-protein-based products. Past research has identified several other strategies (see the review Roland *et al.*, 2017). First, some approaches attempt to prevent the formation of certain contributing precursors (e.g., LOX, isoflavones) via cultivar selection (Stephany *et al.*, 2015) or heat treatments (which limit oxidation; Azarnia *et al.*, 2011). Other approaches try to remove or modify off-notes via soaking or heat treatments (Curti *et al.*, 2018), by influencing germination (Simons & Hall, 2018), or by solvent-based extraction (Heng *et al.*, 2006). However, such strategies often lead to a loss in functionality, which is a major drawback. Other approaches more selectively target off-notes using ultrasound technology (Miano *et al.*, 2019), radio frequency treatments (Jiang *et al.*, 2018), or enzyme treatments (Liu *et al.*, 2017). In particular, fermentation can change the volatile profiles of foods (Ben-Harb *et al.*, 2020; El Youssef *et al.*, 2020; Meinschmidt *et al.*, 2016; Schindler *et al.*, 2012). Another strategy focuses on protein-bound precursors and aims to form inclusion complexes with β -cyclodextrin (Damodaran & Arora, 2013). Filtration can also limit the presence of compounds responsible for off-notes (Yu *et al.*, 2017). The last strategy involves masking off-notes by adding sugars, salts, acids, or flavouring (Bertelsen *et al.*, 2018; Heng, 2006; Zha *et al.*, 2019). The new strategy described in this study can serve as a complement to these other techniques for improving the flavour of pea-protein-based foods.

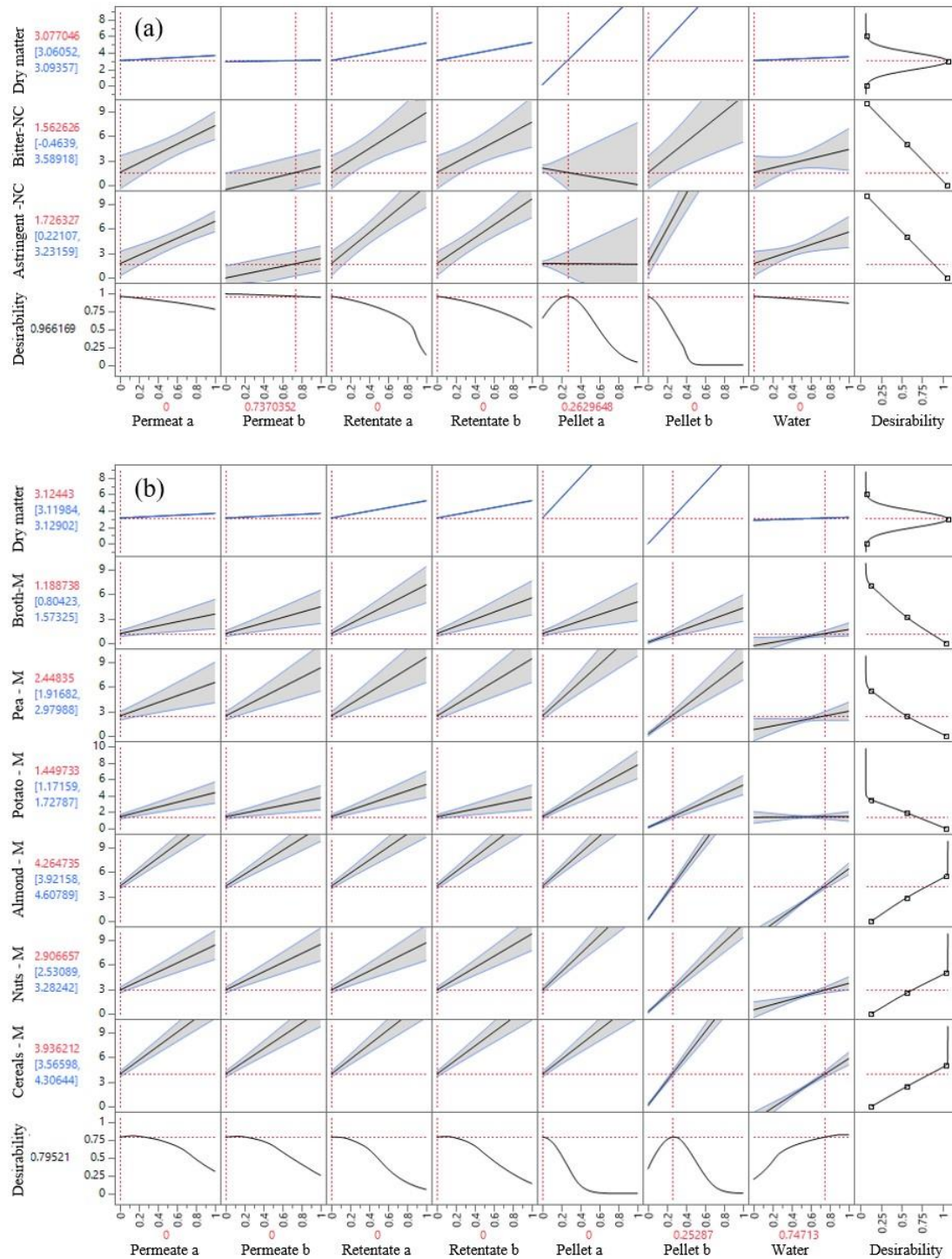


Figure 3.1.2.4.: Cross-sectional view of the predicted attribute scores as a function of a solution's fractional composition for a target dry matter content of 3%: (a) solution formulation in which astringency and bitterness are minimized; (b) solution formulation in which undesirable attributes (potato, pea, and broth) are minimized, whereas desirable attributes (almonds, nuts, and cereals) are maximized. The vertical red lines correspond to the current values of the factors (also indicated in red below the x-axes). The horizontal red lines correspond to the mean predicted scores based on the current factor values (also indicated to the left of the y-axes [95% confidence intervals in blue]). The confidence intervals are represented in grey on the plots. Overall solution desirability is shown in the last plot row and column. It was defined as the geometric mean of the desirability functions for the individual responses.

3.1.2.4. Conclusions

This study adopted an original approach: to work with fractions instead of compounds to explore how combinations of volatiles and non-volatiles affect the sensory characteristics of pea-protein-based solutions. We broke down pea protein isolates into three fractions (pellet, retentate, and permeate), which were then recombined to form different experimental solutions using a mixture design. The study yielded several key results. First, we found that panellists generally came up with repeatable and homogeneous scores for the 46 solutions during the 3-month experiment. Second, attribute intensity did not significantly differ between the reference solutions and the experimental solutions. Third, among the 18 sensory attributes initially evaluated, 10 were identified as useful for building the optimal mixture models, whose performance was validated using ANOVA and data from six supplementary solutions. The results suggest that the models effectively predicted the perception of sensory attributes based on solution composition. Fourth, these models were also used to obtain greater insight into the origin of perceived beaniness, bitterness, and astringency. Our results suggest that beaniness is a multidimensional and complex descriptor that can be expressed via other attributes: almond, broth, cereals, nuts, pea, and potato. They also indicate that attributes contributing to perceived beaniness were mainly influenced by the retentate and permeate fractions, likely because of hexanal levels. Perceived astringency was mainly influenced by the retentate and pellet fractions, while perceived bitterness was largely driven by the retentate fraction. Bitterness and astringency were associated with levels of caffeic acid content. The results of this study will thus improve understanding of how different pea protein fractions contribute to the undesirable sensory characteristics of pea-protein-based ingredients. They have also revealed that fraction-based food formulation could help reduce beaniness, bitterness, and astringency. However, it is also clearly necessary to more precisely analyse food product composition (i.e., look beyond the levels of hexanal and caffeic acid) to clarify the deeper origins of the sensory perception of foods.

Main results

- Here, we proposed an experimental strategy to study pea protein isolates perceptions: pea solutions with controlled variability were created by combining isolate fractions using a mixture design.
- The retentate fraction contributed the most to perceived beaniness, bitterness, and astringency.
- Beany aroma seemed strongly related to volatile composition.
- Protein interactions with volatiles and phenolics likely modified flavour perception.
- These results can help improve the formulation of pea-protein-based products in modifying the process.

RESULTS

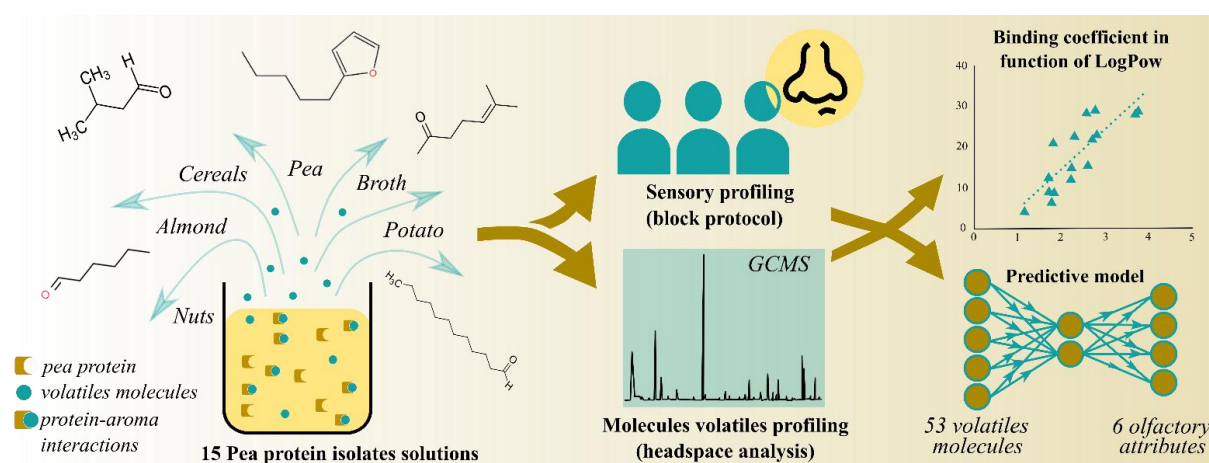
Section 2 - Chemical characterisation of pea protein isolates and first links to perceptions

Section 3.2. – Chemical characterisation of pea protein isolates and first links to perceptions

Context, objectives and presentation of section 3.2.

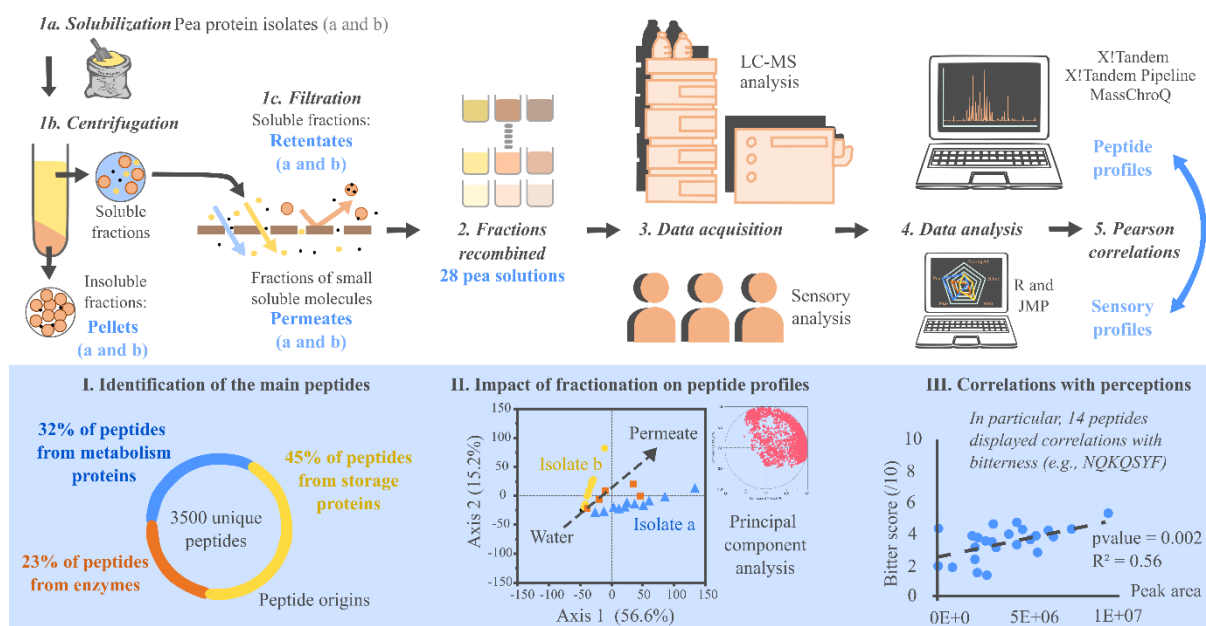
The second step of this PhD project aims to characterize the chemical composition of pea proteins, in particular the main volatile compounds; the main peptides and the main phytochemicals.

Firstly, the aim of the first work of this section (Section 3.2.1.) was to study volatile compounds composition and their interactions with proteins in these products, as well as their impact on perceptions. In short, as explained on the graphical abstract (Graphical abstract 3.2.1.), volatile compounds were identified by gas-chromatography-mass-spectrometry and protein-volatile interactions were investigated for 13 volatile molecules identified in literature as involved in beany perceptions. Relations between sensory analysis and volatile profiles were then analysed and discussed using linear (Partial-Least-Square) and non-linear (Artificial Neural Network) models.



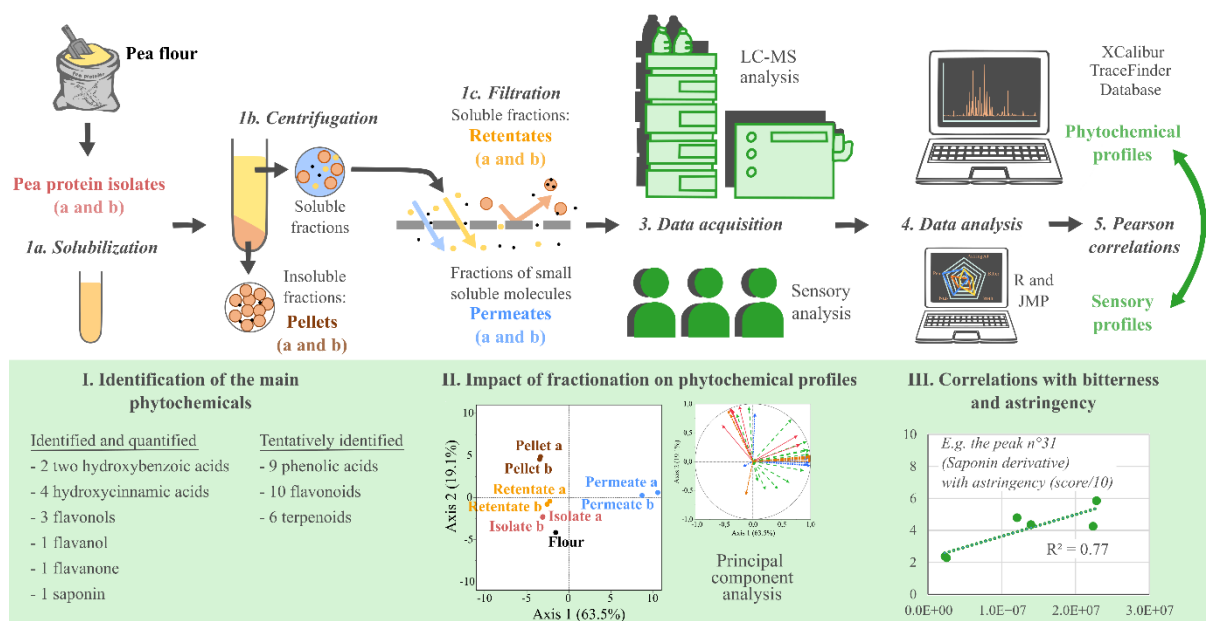
Graphical abstract 3.2.1.: Retention of volatile compounds in pea protein solutions and its impact on beany perceptions.

Secondly, the aim of the second work of this section (Section 3.2.2.) was to identify the main oligopeptides and polypeptides in pea protein solutions and to examine their correlations with sensory perceptions. In short, peptides present in pea solutions were identified and characterized using ultra high-performance liquid chromatography-mass spectrometry. Then, we examined the impact of our fraction-based formulation on peptide profile. Finally, the relationships between peptide profiles and the sensory properties of solutions were explored, with a particular focus on perceived bitterness. The graphical abstract 3.2.2. gives the main elements of this study. This work was done in collaboration with the PAPPISO platform. Results have been published and discussed in *Food Chemistry*.



Graphical abstract 3.2.2.: Identification and characterization of the main peptides in pea protein isolates using ultra high-performance liquid chromatography coupled with mass spectrometry and bioinformatics tools.

Then, the aim of the third work of this section (Section 3.2.3.) was to identify the main phytochemicals (phenolic acids, flavonoids and saponins) in pea protein solutions and examine their correlations with sensory perceptions. In short, phytochemicals profiles of the pea solutions were determined by UPLC-DAD-MS. Then, the impacts of isolates and their fractions on phytochemicals profile were studied. Finally, key phytochemical compounds were correlated to bitterness and astringency properties of pea solutions. The graphical abstract 3.2.3. gives the main elements of this study. This work was done in collaboration with the polyphenol platform (Montpellier, France). Results will be published in *Food Chemistry*.



Graphical abstract 3.2.3.: Identification and quantification of main phytochemicals from pea protein isolates and first links with perception.

Section 3.2.1. – Retention of volatile compounds in pea protein solutions and its impact on beany perceptions

3.2.1.1. Introduction

Over the last few years, plant protein ingredients are receiving much attention by industry and consumers, thanks to their nutritional values, their protein content, their environmental sustainability and their attractive price (Davis *et al.*, 2010). In particular, yellow field pea (*Pisum sativum* L.) is becoming more and more used in plant-based food (Siddique *et al.*, 2012). Its proteins are appreciated for their functional properties in food formulation: emulsifying, foaming, gelation ability and whipping capacity (Adebisi & Aluko, 2011; Gharsallaoui *et al.*, 2009). They are also appreciated for their low allergenicity and high nutritional value and for restoring nutritional balance in amino acids in cereal-based diets. However, like other pulse proteins, pea proteins have a distinct flavour, which may decrease their desirability by consumer and so their use in food formulation (Bott & Chambers, 2006; Trikusuma *et al.*, 2020). This flavour, usually called beany, is a complex flavour perception associated with diverse notes such as musty/earthy, musty/dusty, sour, starchy, powdery, green pea, nutty, and brown (Bott & Chambers, 2006).

Several studies, have already highlighted that these flavour notes are rooted in the complex composition of the aroma volatile compounds found in pulses and belonging to many chemical families: mainly aldehydes, ketones and alcohols compounds but also esters, carbonyl compounds, pyrazines, cyclic amines, furans and sulphides (Bi *et al.*, 2020; Murat *et al.*, 2013; Murray *et al.*, 1976; Trikusuma *et al.*, 2020; Whitfield & Shipton, 1966; Zhang *et al.*, 2020). They showed that these compounds are either naturally present in the seed or generated via different pathways (enzymatic and non-enzymatic degradation of lipids, amino acids, and peptides) (Baysal & Demirdöven, 2007; Damodaran & Arora, 2013; Fischer *et al.*, 2020; Sethi *et al.*, 2016) and can be modified via different processes (see review of Roland *et al.*, 2017).

Nevertheless, the mechanisms behind the beany notes of plant proteins are still poorly understood. Aroma of pea protein are still difficult to control to formulate food products with high sensory desirability. This result can be explained by the complexity of involved sensory mechanisms. Indeed, aromatic perceptions are explained through the kinetics of the aroma compounds release from the product (transport and transfer mechanisms of the aroma compounds from the product to the air phase) to the olfactory receptors in nasal cavity. These mechanisms depend on the mobility (diffusion and convective transport) and on the partition properties of aroma compounds within each phase and between them (Poling *et al.*, 2001). It depends also on the physico-chemical interactions between volatile compounds (Bott & Chambers, 2006) and between volatile compounds and the other components of the food matrix (Taylor & Roberts, 2004) - such as pea proteins (Wang & Arntfield, 2016). In addition, the link between volatiles and sensory perception is often nonlinear as illustrated by psychophysics curves used to propose the thresholds values (detection, recognition, difference, and terminal) in particular conditions (Chambers & Koppel, 2013). However, statistical methods used to study the relationships between sensory analysis and volatile measurements (regression and multivariate techniques) are mainly linear models and so do not account for thresholds values or interactions with complex matrix (Yu *et al.*, 2018; Zielinski *et al.*, 2014). One solution would be to use nonlinear models such as artificial neural networks. However these models need large datasets to handle with sensory analysis (Seisonen *et al.*, 2016), limiting their use in published studies.

In this context, the aim of this article is to study beany odour on pea protein solutions in relation to their volatiles composition. For this purpose, two lines of study are proposed here: 1) taking into account protein-volatiles interactions and 2) using linear and non-linear regressions to model odour perceptions in function of volatiles composition. To this end, the products based on the recombination of different

protein isolate fractions developed by Cosson et al (Cosson *et al.*, 2021 – Section 3.1.2.) have been reused. Volatile compound profiles were studied by gas chromatography–mass spectrometry as well as protein-volatile compound interactions. Then olfactive sensory responses were acquired by descriptive quantitative analysis with trained panellists. Finally, relations between sensory analysis and volatile measurements were analysed and discussed using linear and non-linear models.

3.2.1.2. Materials and methods

Source of materials

As standards, analytical grade aroma compounds ((E)-2-methylbut-2-enal – grade $\geq 97\%$, Hexanal – grade $\geq 98\%$, Hexan-1-ol – grade $\geq 99\%$, heptan-2-one – grade $\geq 99\%$, Heptanal – grade $\geq 95\%$, Benzaldehyde – grade $\geq 99\%$, oct-1-en-3-ol – grade $\geq 98\%$, octan-2-one – grade $\geq 98\%$, 2-Pentylfuran – grade $\geq 98\%$, Octanal – grade $\geq 99\%$, octan-1-ol – grade $\geq 99.5\%$, nonan-2-one – grade $\geq 99\%$, Decanal – grade $\geq 92\%$) were purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA).

Preparation of protein solutions

Six fractions were obtained from two pea protein isolates (protein content Nx6.25, 83% dry matter) as explained on Cosson *et al.*, (Cosson *et al.*, 2021 – Section 3.1.2.): permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b*. Then, the six different fractions were combined in various ways to formulate 15 solutions according to the concentrations described in Table 3.2.1.1. Solutions were chosen to explore a wide range of variation of this type of ingredients while being realistic in term of protein concentration (0 to 4.25%). Recombined solutions were done at 4°C in 500mL and 100mL glass bottles and stored at -20°C for a maximum of 12 month. During fractionation and recombination Good Hygiene Practices have been ensured to limit microbial contamination (coat, gloves and hygienic cap wearing; cleaning and disinfection of hands and all equipment used with absolute ethanol follow by air drying; work in 4°C chamber). The protein content was determined by Kjeldahl method (Nitrogen content x 6.25) and dry solids were determined by drying with a prepASH®219 device by a certified external laboratory (SAS IMPROVE, Amien, France).

Table 3.2.1.1.: Composition of solution as a mixing of permeate *a* and *b*, retentate *a* and *b*, pellet *a* and *b* with their dry matter content as well as their protein content. In bold repeated solution.

Solutions	Permeate <i>a</i> (%)	Permeate <i>b</i> (%)	Retentate <i>a</i> (%)	Retentate <i>b</i> (%)	Pellet <i>a</i> (%)	Pellet <i>b</i> (%)	Water (%)	MS (%)	Protein content (%)
100W	0	0	0	0	0	0	100	0.00	0.00
100Pa	100	0	0	0	0	0	0	0.20	0.03
100Pb	0	100	0	0	0	0	0	0.20	0.04
70Pb-30Ra	0	70	30	0	0	0	0	0.65	0.45
50Pb-50Rb	0	50	0	50	0	0	0	0.95	0.77
100Ra	0	0	100	0	0	0	0	1.70	1.40
100Rb	0	0	0	100	0	0	0	1.70	1.50
25Pa-25Ra-13Ia-38W	25	0	25	0	12.5	0	37.5	2.02	1.62
50Pb-25Ib-25W	0	50	0	0	0	25	25	3.19	2.64
50Ra-25Ia-25W	0	0	50	0	25	0	25	3.94	3.23
40Ra-30Ib-30W	0	0	40	0	0	30	30	4.39	3.70
50Ia-50W	0	0	0	0	50	0	50	6.00	5.05
50Ib-50W	0	0	0	0	0	50	50	6.00	5.24
Refa	38	0	34	0	28	0	0	4.10	3.53
Refb	0	40	0	36	0	24	0	3.70	3.27

Gas chromatography and mass spectrometry analysis

Qualitative analysis

Measurements of volatile compounds release were performed using a Dynamic Headspace Gas Chromatography coupled with a Mass Spectrometer (MPS: Gerstel, GC: Agilent 7890B, MS: Agilent 5977B MSD). Protocol was carried out as follows. After thawing during one night (about 15h) at 4°C,

5g of each solution was stored at 10°C on the GC-MS sample holder (maximum during 4h). Each sample was then incubated at 40°C for 3min (agitation at 500 rpm). The headspace was then purged with a constant flow of helium at 30 ml/min for 10 min at 30°C and volatile compounds were trapped on an adsorption unit (tenax polymer). The trap was dried for 6 minutes under a stream of helium to remove traces of water. Then the trap unit was desorbed from 30°C to 270°C at a rate for 60°C/min with an isotherm of 7 min in a cool injection system at -100°C. The column head injection was carried out from -100°C to 270°C at a rate of 12°C/min with an isotherm of 5 min. The GC oven temperature was programmed from 40°C with an isotherm of 5 min, to 155°C at a rate of 4°C/min, then to 250°C at a rate of 20°C/min with an isotherm of 5 min. The GC was equipped with a polar or an apolar column (respectively HP-INNOWax Agilent, 60m x 320µm x 0.25µm and DB-5, 60m x 320µm x 1µm) with a helium flow of 1.6 mL/min. The two types of chromatography column were used to identify a larger number of volatile compounds. A mass spectrometer was used to characterize volatile compounds. It was performed in the electron impact mode at 70 eV, in full scan from m/z 29 to 300 A.M.U. (Atomic Mass Unit). The ionisation source was set at 230 °C and the quad at 150 °C. The compounds were identified by comparison of their mass spectra with those of the NIST 2017 Mass Spectral Library. The retention times and the Kovats retention index were also used for the tentative identification. The data were reported as peak area for each compounds detected. Quantification data were obtained from the integration of the areas from the total ion current (TIC). MassHunter software was used for instrument control, data acquisition and data analysis.

Quantitative analyses: Standard addition method

Quantification of volatile compounds was performed using standard addition method with 13 compounds. They have been chosen for their different chemical characteristics (as described on Table 3.2.1.2.) and their possible contribution in beany notes mentioned in the literature for plant solutions.

Table 3.2.1.2.: Volatile compounds used for standard addition method, their physicochemical properties at 25°C from literature (EPI-Suite4.11), and their concentration in the initial stock aroma solution.

Volatile compounds	CAS Number	LogPow (est)*	Solubility in water (mg/L)**	Volatility (atm-m3/mole)***	[volatile] (µg/g)
Benzaldehyde	100-52-7	1.71	7.0E+04	2.7E-05	9.40
Heptan-2-one	110-43-0	1.73	4.3E+04	1.7E-04	8.15
Octan-2-one	111-13-7	2.22	9.0E+02	1.9E-04	6.70
Hexan-1-ol	111-27-3	1.82	5.9E+03	1.7E-05	7.05
Heptanal	111-71-7	2.29	1.3E+03	2.7E-04	6.85
Octan-1-ol	111-87-5	2.81	8.1E+02	2.5E-05	6.90
Decanal	112-31-2	3.76	4.4E+01	1.8E-03	7.15
Octanal	124-13-0	2.78	3.9E+02	5.1E-04	6.40
Oct-1-en-3-ol	3391-86-4	2.6	1.8E+03	2.3E-05	5.65
2-Pentylfuran	3777-69-3	3.87	4.2E+01	1.8E-02	6.40
(E)-2-Methylbut-2-enal	497-03-0	1.15	2.5E+04	1.1E-02	8.25
Hexanal	66-25-1	1.8	5.6E+03	2.1E-04	6.80
Nonan-2-one	821-55-6	2.71	1.7E+02	3.7E+00	6.75

*: estimated hydrophobic constant expressed as octanol/water partition coefficient (EPI-Suite4.11)

**: solubility in water (mg/L) at 25°C (EPI-Suite4.11)

***: volatility in water at 25°C (atm-m3/mole) (EPI-Suite4.11)

A mixture of these 13 volatile compounds was prepared in water (Evian, France). Each volatile compound was diluted at around 10 ppm (0.001 mL/100 mL, measurement carried out using a 0.001g precision balance). Concentrations are presented on Table 3.2.1.2. Standard solution was stored in amber glass bottles at -80°C before used to prevent losses. In addition before used and after defrosting, standard solution was mixed during 5 min to ensure a thorough distribution of volatile compounds. Six-point standard curves were used. Standard solution was diluted in protein solutions (initial solution Refa or pellet solution 50Ia-50W) to approximatively 0, 0.5, 0.8, 0.15, 0.30 and 0.50 µg/g. Samples were shaken slowly and stored during 12h at 4°C to allow the equilibrium of binding of volatile compounds with proteins. All samples were prepared in triplicate and stored in dark condition at -80°C in glass vial previous to analysis. Measurements of volatile compounds release were performed using a Dynamic

Headspace Gas Chromatography (DB5 column) coupled with a Mass Spectrometer as explained previously. The calibration curves for each individual compound were obtained by plotting the area response ratio of the respective standard compound and their concentration ratio. From these results, the estimations of volatile compound concentrations (on $\mu\text{g.kg}^{-1}$ of dry matter) were calculated with their standard error. The odour activity values (OAVs) of all each individual compound were then calculated as the ratio of the concentration of each compound to the respective odour threshold in water such as $\text{OAV} = C / T$ where C is the concentration of the volatile compound in the sample and T is the odour threshold of this compound, which was obtained from information available in the literature.

Binding coefficient between volatile compounds and proteins

Binding coefficients for the 13 standards were also calculated from the six-point standard curves. The percentage of volatile compounds bonded to proteins was determined from the difference between the peak areas of flavoured samples in the absence and presence of proteins such that:

$$\text{Binding coefficient (\%)} = (1 - P_s / C_s) \times 100\%$$

where P_s (mol/L) is the calculated slope between peak headspace area and the volatile compounds concentration for the protein solution (ref or pellet). C_s (mol/L) is the corresponding calculated slope in the control (water).

Sensory analysis

Pea solutions were sensory characterized as explained by Cosson *et al.*, 2021 (Section 3.1.2.) by 17 trained panellists (13 women and 4 men; mean age = 23 years old). Attributes were evaluated in blocks. The first attribute block focused on olfactory perception evaluated by nose (attribute block 1: pea, broth, nuts, almond, potato and cereals). The second block focused on taste perception and mouthfeel (attribute block 2: salty, sugar, bitter, astringent, mouthfeel, and granularity). The last attribute block focused again on olfactory perception but solutions were evaluated in mouth (attribute block 3: pea, broth, nuts, almond, potato and cereals). Each solution was evaluated in duplicate by the 17 judges. These sensory analyses were carried out in a more global research project (Cosson *et al.*, 2021 – Section 3.1.2.). However, in this article, only the volatile data evaluated by nose (attribute block 1) will be discussed.

Statistical analysis

Analyses were performed using JMP software version 13.1.0 (SAS Institute Inc., Cary, SC, USA) and R software (R Core Team, 2017). For analyses of an inferential nature, we used $\alpha = 0.05$ to determine statistical significance.

GC-MS data sets were analysed with Analysis of Variance (ANOVA) to confirm the significant differences in aroma release between the studied solutions (data not shown, p-value significant at the threshold of 0.05). They were completed by Principal Compound Analysis (PCA, centred-scaled, Pearson type) to visualize the main differences between solutions.

Sensory data were analysed with ANOVA to confirm the significant differences in odour sensory properties between the studied solutions (data not shown, p-value significant at the threshold of 0.05). Then, differences among solutions were studied by multiple product pair comparisons (LSD-Bonferroni). The relationships between sensory and volatiles data were first studied by correlation test (Pearson type, centred-scaled).

To obtain a better insight into the origin of these aromatic perceptions, two different models were developed between the volatile compound release data and the sensory data: Partial least square (PLS) modelling and artificial neural network (ANN) modelling. For the PLS regression modelling: a model using NIPALS methods was fitted with potential explanatory variables (centred-scaled). The model was fitted with 15 initial latent variables. To determine the optimum number of factors to extract, the solutions were partitioned randomly into 4 folds. In turn, each fold was used to validate the model that is fit to the rest of the data, fitting a total of 4 models. This was run 100 times. This method is best for small data sets because it makes efficient use of limited amounts of data. For the ANN regression modelling, trainings were performed with random separation of data into training (12 protein solutions), and validation (3 protein solutions) sets and run 1000 times. Because the ANN method is a relatively

flexible model method that can be easily over-fit to the applied data set, a penalty function (weight decay) was used to avoid over-fit model results. In addition, the model was trained using least absolute deviations instead of least squares to minimize the impact of response outliers (Hastie *et al.*, 2008). Different model were tests with 1 or 2 layers and several activation nodes (sigmoid, linear or Gaussian). The performances of the developed models were statistically measured by the root mean squared error (RMSE), the residual sum of squares (RSS) and the correlation coefficient (R2) and the best model was selected.

3.2.1.3. Results and discussion

Impact of fraction composition on volatile compounds profiles

Identification of volatile compounds from pea protein solutions

To better understand the mechanisms related to aroma release in pea-based solutions at the origin of perceptions, composition on volatile compounds were studied on different pea protein solutions by GC-MS analysis. A total of 79 volatile compounds (15 specific to the apolar column, 16 to the polar column and 48 common to both) belonging to 7 chemical classes are identified on the 15 solutions (Table 3.2.1.3.). Ketones, aldehydes and alcohols are the most prominent compounds in number (20/79 aldehydes, 20/79 ketones and 14/79 alcohols). Aldehydes and furan are the most prominent compounds in term of GC-MS area (for the apolar column): $1.16\text{E}+06$ for aldehydes and $8.31\text{E}+05$ for furan (mean GC-MS area for all the volatile compounds of the family). Most of these compounds were already identified on others publications. For example, 1-pent-3-ol, (E,E)-2,4-heptadienal and heptadienal are known to be key odour active volatile produced by secondary lipid oxidation (Baysal & Demirdöven, 2007; Jacobsen, 1999; Murat *et al.*, 2013; Trikusuma *et al.*, 2020). According to the literature, these compounds mainly come from endogen origin of pea seed or from oxidation of the lipids/peptides/amino acids in the seed or during the storage (Baysal & Demirdöven, 2007; Murat *et al.*, 2013; Trikusuma *et al.*, 2020).

As regards the differences between pea protein-based solutions studied, GC-MS areas from apolar column (with standard deviation) obtained for the 15 solutions and for 4 volatile compounds (pentanal, 2-pentylfuran, heptan-2-one and oct-1-en-3-ol) are plotted in Figure 3.2.1.1. For the 4 compounds illustrating different chemical families, the permeate *a*, permeate *b*, retentate *b* and refb, as well as the recombined solutions 70Pb-30Ra and 50Pb-50Rb presented the same order of magnitude and the lowest areas. In opposition for the 4 compounds, the recombined solution 50Ra-25Ia-25W presented the highest values of peak area. To complete these results, a PCA is presented (Figure 3.2.1.2.) to visualize the relationships among solutions and the 63 volatile compounds identified in the apolar column. To the PCA, the means of the 7 chemical families were added as supplementary variables to make the graph easier to read. Solutions are distributed on axes F1 and F2 with respectively 34.2% and 17.7% of the variance. The PCA therefore represents moderately the volatiles diversity of solutions on a 2-dimensional level. Along the first axis, solutions are mainly differentiated based on their composition in aldehyde, furan and ester compounds. Along the second axis, solutions are mainly differentiated based on their compositions in ketone and alcohol compounds. Solutions recombined from batch *b* present lowest areas on volatile compounds than solutions recombined from batch *a*, especially on ketones, aldehydes, alkenes and furan areas. Fractions present lowest areas on volatile compounds than initial solutions (Refa and Refb). Among them, permeate (100Pa and 100Pb) present highest areas on esters and on alcohols. Pellet (50Ia-50W and 50Ib-50W) and retentate (100Ra and 100Rb) surprisingly present equivalent overall areas of volatile compounds than permeate (100Pa and 100Pb).

Table 3.2.1.3.: Volatile compounds identified by GC-MS with polar or apolar column (respectively HP-INNOWax Agilent, 60m x 320µm x 0.25µm and DB-5, 60m x 320µm x 1µm) from pea protein isolates recombined solutions (tentative identification using NIST mass spectra library, comparison of the GC retention index – Kovats index, physicochemical properties at 25°C from literature - EPI-Suite4.11).

IUPAC name	Retention time (apolar column)	Retention time (polar column)	NIST score (apolar column)	NIST score (polar column)	Kovats index (apolar column)	Kovats index (polar column)	CAS number	Chemical family	Chemical formula	Molecular mass	Odour	LogPow (est)*	Solubility in water at 25°C (mg/L)**	Volatility Henry Law constant (atm·m ³ /mole)***
ethanol	4.88	7.5	98	98	440	929	64-17-5	alcohol	C ₂ H ₆ O	46.04	strong alcoholic ethereal medicinal	-0.31	7.92E+05	5.00E-06
propan-2-one	5.49	5.34	88	96	503	814	67-64-1	ketone	C ₃ H ₆ O	58.00	solvent ethereal apple pear	-0.24	2.20E+05	3.50E-05
pentane	5.6	3.87	92	93	500	500	8031-35-4	alkane	C ₅ H ₁₂	72.00	alkane	3.39	4.98E+01	1.25E+00
methyl acetate	6.19	5.52	86	92	531	827	79-20-9	ester	C ₃ H ₆ O ₂	74.04	green, ethereal, fruity, fresh, rum and whiskey-like	0.18	9.39E+04	1.15E-04
2-methylpropanal	7.03	NA	98	NA	554	NA	78-84-2	aldehyde	C ₄ H ₈ O	72.06	fresh aldehydic herbal green malty	0.74	3.12E+04	1.80E-04
butane-2,3-dione	7.89	NA	81	NA	560	NA	431-03-8	ketone	C ₄ H ₆ O ₂	86.00	sweet, buttery, creamy, milky	-1.34	1.00E+06	1.33E-05
butanal	8.03	6.25	98	96	596	867	123-72-8	aldehyde	C ₄ H ₈ O	72.06	pungent, green	0.88	2.39E+04	1.15E-04
butan-2-one	8.07	6.7	99	88	602	900	78-93-3	ketone	C ₄ H ₈ O	72.00	chemical-like and slightly fruity green	0.29	7.61E+04	5.69E-05
ethyl acetate	8.67	6.46	83	77	601	907	141-78-6	ester	C ₄ H ₈ O ₂	88.05	ethereal, fruity, sweet, with a grape and cherry nuance	0.73	2.99E+04	1.34E-04
oxolane	9.3	5.98	90	87	617	866	109-99-9	alkane	C ₄ H ₈ O	72.06	NA	0.46	5.45E+04	7.05E-05
3-methylbutanal	10.49	7.07	98	91	641	912	590-86-3	aldehyde	C ₅ H ₁₀ O	86.07	cocoa, almond	1.23	1.12E+04	1.23E+00
butan-1-ol	10.69	14.28	90	94	654	1145	71-36-3	alcohol	C ₄ H ₁₀ O	74.07	wine	0.88	7.67E+04	8.81E-06
benzene	10.78	7.65	96	94	662	953	71-43-2	alkene	C ₆ H ₆	78.00	aromatic	2.13	2.00E+03	5.55E-03
2-methylbutanal	10.88	6.96	96	97	665	910	96-17-3	aldehyde	C ₅ H ₁₀ O	86.07	musty, furfural and rummy, with nutty and cereal notes, and caramel and fruity undernotes	1.23	1.12E+04	2.62E-04
pent-1-en-3-ol	11.6	14.86	94	93	674	1165	616-25-1	alcohol	C ₅ H ₁₀ O	86.07	ethereal horseradish green radish chrysanthemum vegetable tropical fruity	1.12	4.53E+04	9.88E-06
pentan-2-one	11.71	NA	95	NA	680	NA	107-87-9	ketone	C ₅ H ₁₀ O	86.07	fish, pungent	0.91	2.12E+04	8.36E-05
pentane-2,3-dione	12.17	11.27	92	85	681	1060	600-14-6	ketone	C ₅ H ₈ O ₂	100.05	toasted, buttery and caramellic with marshmallow and molasses nuances	-0.85	6.16E+05	2.62E-07
pentanal	12.29	8.65	98	98	684	998	110-62-3	aldehyde	C ₅ H ₁₀ O	86.07	almond, malt, pungent	1.31	9.72E+03	1.47E-04
2-ethylfuran	12.37	7.97	94	95	720	960	3208-16-0	furan	C ₆ H ₈ O	96.06	solvent-like with a dirty musty brown earthy nuance	2.4	1.07E+03	7.88E-03
3-hydroxy butan-2-one	12.74	19.59	76	88	718	1282	513-86-0	ketone	C ₄ H ₈ O ₂	88.05	butter, cream	-0.36	8.33E+05	1.03E-05
propane-1,2-diol	14.05	NA	87	NA	745	NA	57-55-6	alcohol	C ₃ H ₈ O ₂	76.10	sweet	-0.92	8.11E+05	1.29E-08
(methyl disulfanyl) methane	14.7	NA	79	NA	785	NA	624-92-0	alkane	C ₂ H ₆ S ₂	93.99	onion, cabbage, putrid	1.77	3.74E+03	1.21E-03
pentan-1-ol	15.54	18.16	97	96	790	1255	71-41-0	alcohol	C ₅ H ₁₂ O	88.09	balsamic	1.51	2.09E+04	1.30E-05
hexan-2-one	16.66	NA	95	NA	792	NA	591-78-6	ketone	C ₆ H ₁₂ O	100.09	ether	1.38	7.75E+03	9.32E-05
hexanal	17.29	11.89	98	98	801	1084	66-25-1	aldehyde	C ₆ H ₁₂ O	100.09	grass, tallow, fat	1.78	3.53E+03	2.13E-04
butyl acetate	17.85	11.57	95	94	802	1079	123-86-4	ester	C ₆ H ₁₂ O ₂	116.00	sweet, ripe banana, tutti frutti, tropical and candy-like with green nuances	1.78	3.13E+03	2.81E-04

IUPAC name	Retention time (apolar column)	Retention time (polar column)	NIST score (apolar column)	NIST score (polar column)	Kovats index (apolar column)	Kovats index (polar column)	CAS number	Chemical family	Chemical formula	Molecular mass	Odour	LogPow (est)*	Solubility in water at 25°C (mg/L)**	Volatility Henry Law constant (atm·m ³ /mole)***
2-methylbut-2-enal	18.2	NA	80	NA	808	NA	19780-25-7	aldehyde	C ₆ H ₁₀ O	98.07	green, fruit	1.64	4.71E+03	2.03E-04
(E)-hex-2-enal	19.94	16.93	95	94	854	1220	6728-26-3	aldehyde	C ₆ H ₁₀ O	98.07	apple, green	1.58	5.26E+03	4.89E-05
4-methyloctane	20.4	NA	95	NA	862	NA	2216-34-4	alkane	C ₉ H ₂₀	128.16	NA	4.69	2.69E+00	1.00E+01
2-methylidene hexanal	20.44	14.59	87	83	NA	1160	1070-66-2	aldehyde	C ₇ H ₁₂ O	112.09	NA	2.21	1.39E+03	4.15E-04
hexan-1-ol	20.54	21.87	97	94	868	1360	111-27-3	alcohol	C ₆ H ₁₄ O	102.10	resin, flower, green	2.03	6.89E+03	1.71E-05
heptan-2-one	21.61	15.55	97	94	870	1170	110-43-0	ketone	C ₇ H ₁₄ O	114.10	cheese, fruity, coconut, waxy, green	1.98	2.15E+03	1.69E-04
2-butylfuran	21.8	13.68	91	85	889	1130	4466-24-4	furan	C ₈ H ₁₂ O	124.09	mild fruity wine sweet spicy	3.38	1.25E+02	1.39E-02
heptanal	22.26	15.66	98	91	903	1174	111-71-7	aldehyde	C ₇ H ₁₄ O	114.10	fat, citrus, rancid	2.29	1.17E+03	2.70E-04
1-(methyl disulfanyl) propane	24	NA	91	NA	928	NA	2179-60-4	alkane	C ₄ H ₁₀ S ₂	122.02	onion, garlic, tomato, potato, alliaceous and vegetative	2.86	3.59E+02	2.14E-03
benzaldehyde	25.44	27.55	97	98	960	1495	100-52-7	aldehyde	C ₇ H ₆ O	106.04	almond, burnt sugar	1.48	6.10E+03	2.67E-05
oct-1-en-3-ol	25.83	25.06	92	96	964	1445	3391-86-4	alcohol	C ₈ H ₁₆ O	128.12	mushroom, earthy, fungal, green, oily, vegetative, umami sensation and savory broth	2.6	1.84E+03	2.31E-05
octane-2,3-dione	25.97	NA	81	NA	967	NA	585-25-1	ketone	C ₈ H ₁₄ O ₂	142.10	green spicy cilantro fatty leafy cortex herbal	0.63	2.38E+04	6.13E-07
6-methylhept-5-en-2-one	26.06	21.21	93	93	988	1342	110-93-0	ketone	C ₈ H ₁₄ O	126.10	green, vegetative, musty, apple, banana and green bean-like	2.06	1.65E+03	5.11E-05
octan-2-one	26.3	19.3	95	93	966	1285	111-13-7	ketone	C ₈ H ₁₆ O	128.12	dairy, waxy, cheese, woody, mushroom and yeast	2.37	8.84E+02	1.88E-04
2-pentylfuran	26.43	17.3	94	95	985	1241	3777-69-3	furan	C ₉ H ₁₄ O	138.10	green, waxy, with musty, cooked caramellic nuances	3.87	4.18E+01	1.84E-02
octanal	26.96	19.46	97	97	984	1280	124-13-0	aldehyde	C ₈ H ₁₆ O	128.12	fat, soap, lemon, green	2.78	3.94E+02	5.14E-04
2-ethylhex-2-enal	27.16	21.09	88	84	987	1330	645-62-5	aldehyde	C ₈ H ₁₄ O	126.10	NA	2.62	5.49E+02	4.88E-04
2-ethylhexan-1-ol	27.98	26.86	96	97	1032	1491	104-76-7	alcohol	C ₈ H ₁₈ O	130.00	rose, green	2.73	1.38E+03	2.65E-05
(4R)-1-methyl-4-prop-1-en-2-ylcyclohexene	28.46	15.9	86	92	1033	1201	5989-27-5	alkene	C ₁₀ H ₁₆	136.13	lemon, orange	4.38	4.58E+00	3.19E-02
octan-1-ol	29.75	28.63	97	96	1072	1553	111-87-5	alcohol	C ₈ H ₁₈ O	130.14	chemical, metal, burnt	3	8.14E+02	2.45E-05
octa-3,5-dien-2-one (E,Z)	29.84	28.87	95	93	1071	1567	38284-27-4	ketone	C ₈ H ₁₂ O	124.09	fruit, fat, mushroom	2.08	1.62E+03	9.96E-06
1-phenylethanone	30.05	31.4	88	84	1076	1645	98-86-2	ketone	C ₈ H ₈ O	120.10	sweet pungent hawthorn mimosa almond acacia chemical	1.58	4.48E+03	1.04E-05
nonan-2-one	30.68	22.93	88	97	1081	1388	821-55-6	ketone	C ₉ H ₁₈ O	142.14	cheesy green fruity dairy dirty buttery	3.14	1.71E+02	3.67E-04
pentyl butanoate	30.68	20.39	91	95	1080	1305	540-18-1	ester	C ₉ H ₁₈ O ₂	158.13	sweet, fruity, banana, pineapple and tropical	3.32	1.02E+02	8.88E-04
octa-3,5-dien-2-one (E,E)	30.86	NA	94	NA	1095	NA	4173-41-5	ketone	C ₈ H ₁₂ O	124.09	fatty fruity hay green herbal	2.08	1.62E+03	9.96E-06
nonanal	31.33	23.1	99	93	1104	1385	124-19-6	aldehyde	C ₉ H ₁₈ O	142.1	fat, citrus, green	3.27	1.32E+02	7.34E-04
non-3-en-2-one	34.44	NA	88	NA	1122	NA	14309-57-0	ketone	C ₉ H ₁₆ O	140.12	oily spicy ketonic fermented bleu cheese waxy fatty woody	2.79	3.50E+02	6.43E-05
decan-2-one	34.64	26.36	88	88	1172	1480	693-54-9	ketone	C ₁₀ H ₂₀ O	156.15	orange floral fatty peach	3.73	4.64E+01	4.45E-04
dodecane	34.88	15.78	99	72	1200	1200	112-40-3	alkane	C ₁₂ H ₂₆	170.20	alkane	6.1	1.10E-01	8.18E+00
Decanal	35.11	26.54	95	94	1209	1484	112-31-2	aldehyde	C ₁₀ H ₂₀ O	156.20	soap, orange peel, tallow	2.79	3.50E+02	6.43E-05

IUPAC name	Retention time (apolar column)	Retention time (polar column)	NIST score (apolar column)	NIST score (polar column)	Kovats index (apolar column)	Kovats index (polar column)	CAS number	Chemical family	Chemical formula	Molecular mass	Odour	LogPow (est)*	Solubility in water at 25°C (mg/L)**	Volatility Henry Law constant (atm-m ³ /mole)***
2-phenylethyl acetate	36.35	35.49	97	97	1224	1825	103-45-7	ester	C10H12O2	164.08	sweet, honey, floral, rosy with a slight green nectar fruity body and mouth feel	2.3	7.11E+02	3.30E-06
decan-1-ol	36.55	34.5	95	78	1263	1765	112-30-1	alcohol	C10H22O	158.17	fat	4.57	2.82E+01	3.20E-05
4,6-dimethyl dodecane	36.61	NA	82	NA	1317	NA	61141-72-8	alkane	C14H30	198.24	Na	7.07	1.19E-02	1.65E+01
tridecane	37.06	NA	83	NA	1300	NA	629-50-5	alkane	C13H28	184.00	alkane	6.73	2.75E-02	2.88E+00
undecanal	37.24	NA	77	NA	1310	NA	112-44-7	aldehyde	C11H22O	170.00	waxy soapy floral aldehydic citrus green fatty fresh laundry	4.25	1.43E+01	1.34E-03
(Z)-2-butyloct-2-enal	38.24	31.69	88	90	1389	1659	13019-16-4	aldehyde	C12H22O	182.17	Na	4.59	6.50E+00	1.94E-03
dodecanal	38.68	32.9	85	86	1499	1709	112-54-9	aldehyde	C12H24O	184.00	soapy, waxy, citrus, orange mandarin	4.75	4.65E+00	1.90E-03
heptane	NA	4.41	NA	90	NA	700	142-82-5	alkane	C7H16	100.13	sweet ethereal	3.78	3.55E+00	2.27E+00
octane	NA	5.11	NA	88	NA	800	111-65-9	alkane	C8H18	114.14	gasoline	4.27	1.15E+00	3.01E+00
propan-2-ol	NA	7.33	NA	91	NA	922	67-63-0	alcohol	C3H8O	60.06	alcohol musty woody	0.28	4.02E+05	7.52E-06
2-ethyl-5-methylfuran	NA	10.24	NA	75	NA	1013	1703-52-2	furan	C7H10O	110.07	fresh gassy burnt	2.95	3.29E+02	8.69E-03
toluene	NA	10.49	NA	94	NA	1042	108-88-3	alkene	C7H8	92.06	sweet	2.54	5.73E+02	5.95E-03
ethylbenzene	NA	13.41	NA	92	NA	1136	100-41-4	alkene	C8H10	106.08	NA	3.03	2.29E+02	7.89E-03
2-methylbutyl butanoate	NA	18.56	NA	92	NA	1272	51115-64-1	ester	C9H18O2	158.13	fruity pear apricot apple tropical gooseberry spicy rummy	3.25	1.18E+00	9.60E-04
1-hydroxypropan-2-one	NA	20.15	NA	83	NA	1284	116-09-6	ketone	C3H6O2	74.04	pungent sweet caramellic ethereal	-0.78	1.00E+06	7.73E-06
(E)-hept-2-enal	NA	20.74	NA	95	NA	1318	18829-55-5	aldehyde	C7H12O	112.09	pungent green vegetable fresh fatty	2.07	1.81E+03	1.31E-04
oct-3-en-2-one	NA	23.59	NA	96	NA	1408	1669-44-9	ketone	C8H14O	126.10	earthy spicy herbal sweet mushroom hay blueberry	2.29	1.05E+03	9.58E-05
(E)-oct-2-enal	NA	24.34	NA	88	NA	1425	2548-87-0	aldehyde	C8H14O	126.10	fresh cucumber fatty green herbal banana waxy green leaf	2.57	6.13E+02	1.74E-04
heptan-1-ol	NA	25.35	NA	86	NA	1462	111-70-6	alcohol	C7H16O	116.12	musty leafy violet herbal green sweet woody peony	2.31	1.94E+03	2.34E-05
acetic acid	NA	25.96	NA	75	NA	1465	64-19-7	carboxylic acid	C2H4O2	60.02	sharp pungent sour vinegar	0.09	4.76E+05	5.48E-07
3,7-dimethylocta-1,6-dien-3-ol	NA	28.08	NA	73	NA	1537	78-70-6	alcohol	C10H18O	154.14	citrus floral sweet bois de rose woody green blueberry	3.38	6.84E+02	4.23E-05
(5E)-6,10-dimethyl undeca-5,9-dien-2-one	NA	36.01	NA	88	NA	1835	3796-70-1	ketone	C13H22O	194.2	fresh green fruity waxy rose woody magnolia tropical	4.36	8.87E+00	9.08E-04
2,6-di-tert-butyl-4-methylphenol	NA	36.80	NA	88	NA	1870	128-37-0	alcohol	C15H24O	220.2	mild phenolic camphor	5.03	5.75E+00	4.12E-06

*: estimated hydrophobic constant expressed as octanol/water partition coefficient (EPI-Suite4.11)

***: solubility in water (mg/L) at 25°C (EPI-Suite4.11)

***: volatility in water at 25°C (atm-m³/mole) (EPI-Suite4.11)

The solutions therefore have different molecule profiles. Solutions from batch *a* have a higher number of compounds than solutions from batch *b*. Furthermore, solutions with higher protein/dry matter content appear to have higher areas of volatile compounds. This is true except for pellets diluted only with water (50Ib-50W and 50Ia-50W) which have lower concentrations and highest protein/dry matter content. These different volatile compounds profiles could be due to the different protein composition of solutions. Indeed, whereas the protein content/dry matter was similar, pellets were mainly constituted of insoluble protein and retentates of soluble proteins. However, permeates were mainly constituted of "non-protein" compounds - such as minerals, phenolic compounds or volatile compounds.

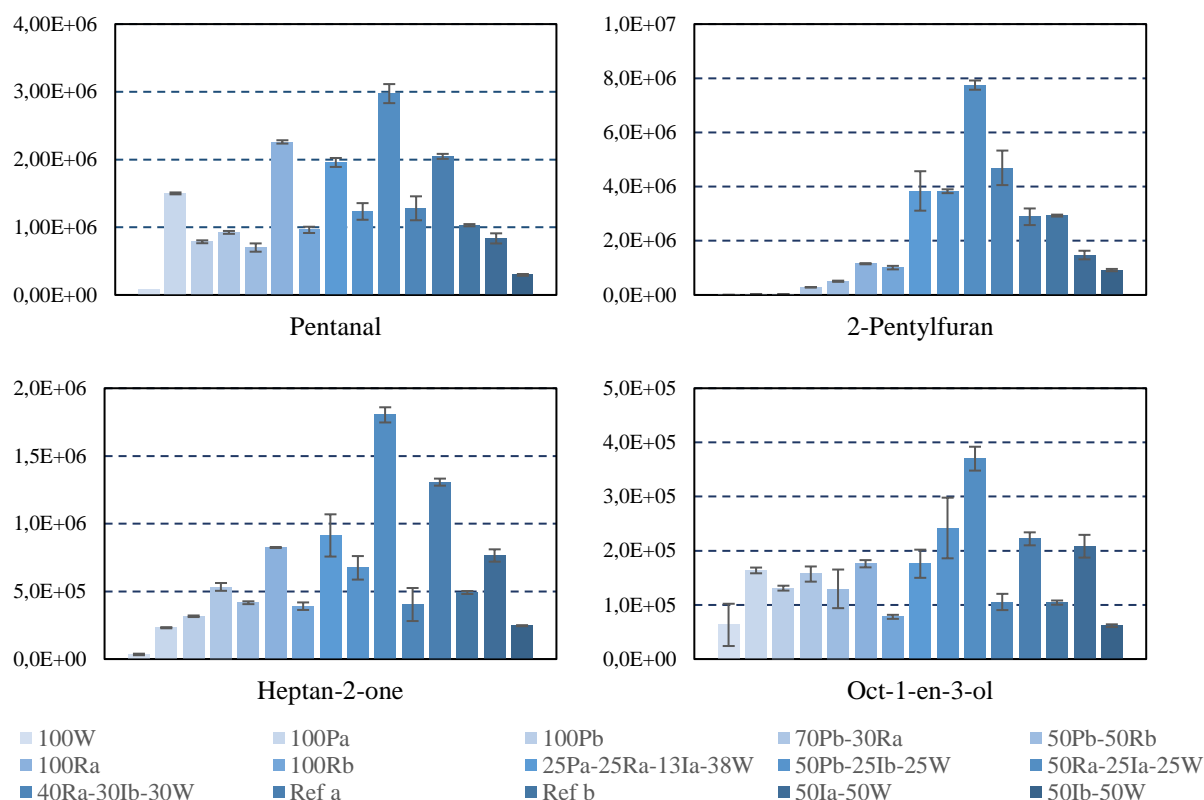


Figure 3.2.1.1: Average and standard deviation of peak areas obtained by GC-MS for 15 solutions and for 4 volatile compounds from different chemical families: pentanal, 2-pentylfuran, heptan-2-one and oct-1-en-3-ol. The colour gradient corresponds to the DM content of the samples: from light blue corresponding to water to dark blue corresponding to solutions with 6% DM.

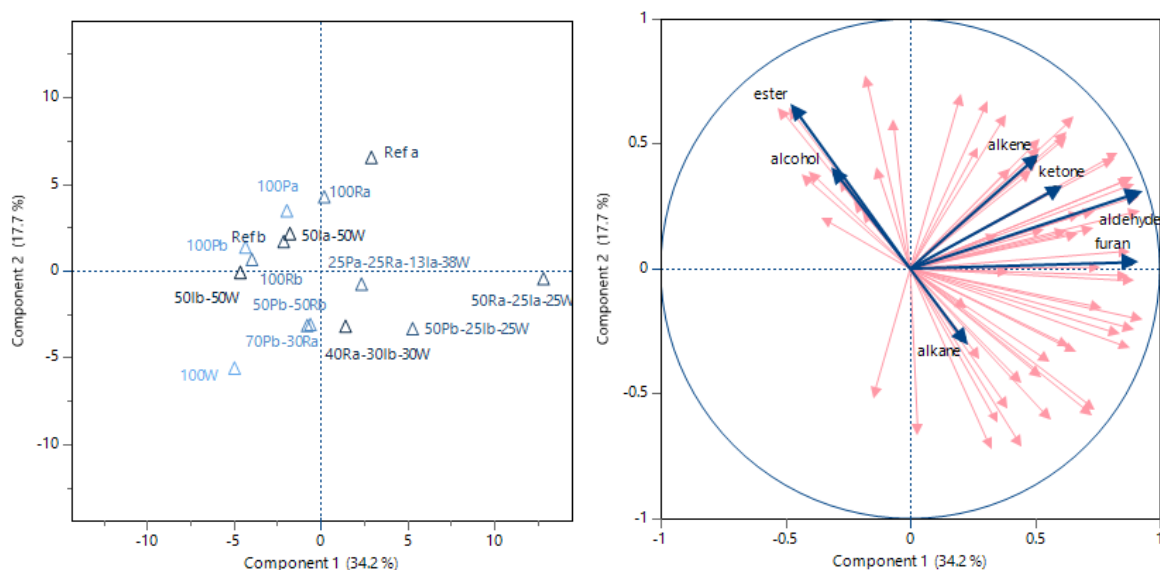


Figure 3.2.1.2: Principal component analysis of axes 1 and 2 based on the GC-MS data for the 15 solutions. In the variable figure (at right): in red are the active variable (GC-MS compounds) and in blue are the supplementary variable corresponding the mean of chemical families. In the observations figure (at left), the colour gradient corresponds to the DM content of the samples: from light blue corresponding to water to dark blue corresponding to solutions with 6% DM.

Quantification of volatile compounds in pea protein solution (Refa) and pellet (50Ia-50W)

Quantification of 13 volatile compounds were performed for pea protein solution (Refa) and pellet solution (50Ia-50W). However, for confidentiality reasons, these concentrations are not presented. In addition, from the concentrations, the OAVs of volatile compounds were calculated and listed in Table 3.2.1.4. For 7 compounds, OAVs were greater to 1. These compounds might significantly contribute to the aroma of pea protein isolates (Table 3.2.1.4.).

Table 3.2.1.4.: Comparison of the binding coefficient for initial (Refa) and pellet *a* solutions for the 13 volatile compounds with their chemical family, their type of chain, their formula, their odour threshold (ppb, in water obtained from the literature) and their odour activity value (OAV).

IUPAC name	Chemical family	Type of chain	Chemical formula	Odor threshold (ppb)	Binding Refa (%)	Binding pellet <i>a</i> (%)	OAV Refa	OAV pellet <i>a</i>
(E)-2-methylbut-2-enal	Aldehydes	Branched	C ₅ H ₈ O	3 ^a	4.4	7.1	3	2
2-pentylfuran	Furan	Aromatic	C ₉ H ₁₄ O	6 ^a	16.8	12.1	151	201
benzaldehyde	Aldehydes	Circular	C ₇ H ₆ O	390 ^a	12.5	11.6	<1	NA
decanal	Aldehydes	Linear	C ₁₀ H ₂₀ O	5 ^b	29.1	19.7	9	10
heptan-2-one	Ketone	Linear	C ₇ H ₁₄ O	140 ^a	9.2	10.9	<1	<1
heptanal	Aldehydes	Linear	C ₇ H ₁₄ O	3 ^a	22.8	17.4	2	3
hexan-1-ol	Alcohol	Linear	C ₆ H ₁₄ O	500 ^a	8.8	10.1	<1	<1
hexanal	Aldehydes	Linear	C ₆ H ₁₂ O	4.5 ^a	21.1	17.7	158	326
nonan-2-one	Ketone	Linear	C ₉ H ₁₈ O	190 ^b	21.9	17.1	<1	<1
oct-1-en-3-ol	Alcohol	Linear	C ₈ H ₁₆ O	1 ^a	15.6	14.3	4	6
octan-1-ol	Alcohol	Linear	C ₈ H ₁₈ O	190 ^c	23.1	17.7	<1	<1
octan-2-one	Ketone	Linear	C ₈ H ₁₆ O	190 ^b	15.1	14.3	<1	<1
octanal	Aldehydes	Linear	C ₈ H ₁₆ O	0.7 ^a	29.2	18.7	9	37

^aButtery *et al.*, 1999 ; ^bBelitz *et al.*, 2004 ; ^cAhmed *et al.*, 1978

To better understand the differences between volatile compositions and recombined solutions, the links between volatile compounds and protein concentration were studied. The correlation coefficients (Pearson method) were calculated between protein concentrations and peak area obtained with the apolar column. Several significant correlations were calculated for data from 13/15 solutions (except 50Ia-50W and 50Ib-50W). Six aldehydes were significantly correlated to the concentration on protein. In addition, six ketones were significantly correlated to the concentration on protein. Two alcohols were also significantly correlated to the concentration on protein. Concentrations on these volatile compounds increase when the protein concentration of solutions increase. Slopes are particularly important for furans and butanal which may means higher protein interactions for these compounds. Solutions only constituted of pellet 50Ia-50W and 50Ib-50W were not taken into account because they decrease the quality of regressions. One hypothesis could be different composition on proteins between the pellets and the other fractions, especially in terms of insoluble/soluble proteins composition.

Characterization of protein-volatiles interactions from pea protein solutions

Volatile-protein interactions has been further studied. The interactions of pea protein with volatile compounds were focused on the study of 6 aldehydes, 3 alcohol, 3 ketones, 1 furan with two different protein buffer solutions: initial solution from batch *a* (Refa) and solution mainly constituted of pellet *a* (50Ia-50W). These 13 compounds have been chosen for their different chemical characteristics (as described on Table 3.2.1.2.) and their possible contribution in beany notes mentioned in the literature for plant solutions. Table 3.2.1.4. presents the results concerning the binding coefficients for Refa and 50Ia-50W solutions (related to the protein concentration of solutions) for the 13 volatile compounds, in regard to their chemical family, their type of chain and their chemical formula. The two protein solutions were able to bind the volatile compounds used in this study and the different factors tested including chemical class, carbon number and protein source were found to significantly impact the level of volatile compound retention.

Regarding the chemical function, binding capacities of volatile compounds were higher to aldehydes than alcohols than ketones for C8, higher to aldehydes than ketones for C7, higher to aldehydes than alcohol for C5. These results are in agreement with Wang and Artnfield, who found that all proteins tested exerted higher binding capacities to aldehydes than ketones (Wang & Artnfield, 2016). A similar

trend was also reported by Heng *et al.* and Gremli (Heng, 2006). An impact of carbon chain length was also observed: retention of alcohols, linear aldehydes and ketones significantly increases within the increase in carbon number. For example, heptanal (C7) had a binding of 17.4 for Refa and decanal (C10) a binding of 19.7. These results indicate that interactions were predominantly hydrophobic in nature, as already generally observed in the literature (Andriot *et al.*, 2000; Damodaran & Arora, 2013; Damodaran & Kinsella, 1981; Wang & Arntfield, 2016). These hypothesis were confirmed by the strong correlation between binding coefficients for each volatile compounds and their corresponding hydrophobic constant (LogPow – logarithm of the octanol/water partition coefficient obtained from literature) presented on Figure 3.2.1.3. To be noted, coefficient correlations (Pearson type) were much higher for both buffer solutions without 2-pentylfuran: 0.57 (with 2-pentylfuran) vs 0.82 (without 2-pentylfuran) for 50Ia-50W; 0.68 (with 2-pentylfuran) vs 0.84 (without 2-pentylfuran) for Refa. To explain the particularity of 2-pentylfuran, a hypothesis could be steric clutter in the cycle that limits interactions with proteins.

Regarding the binding coefficients, the volatile compounds used showed an increasing amount of binding with increasing volatile compounds concentrations. These results were in agreement with literature results (Landy *et al.*, 1995). However, contrary to these studies, the concentration on protein was relatively high (4% and 6%) and the concentration of volatile compounds low (less than 10 ppm). These results suggested there is no saturation of the binding sites with the used concentrations of volatile compounds and of proteins.

Regarding protein solution type, the binding capacity was higher for Refa than for 50Ia-50W for 10/13 volatile compounds. The other 3 compounds (heptan-2-one, Hexan-1-ol, (E)-2-methylbut-2-enal) presented all as common characteristics to not be an aldehydes and to have a short length of carbon chain (inferior to C7). According the results from the literature, volatile compounds binding by proteins is very dependent on the conformational state of the proteins which affect both the binding affinity and the number of binding sites on proteins for volatile compounds (Damodaran & Kinsella, 1981). Proteins of the pellet must be more denatured with different protein composition (protein type and protein conformation). These modifications must explain the loss of solubility and so the aggregation but also the differences of protein-volatiles interactions.

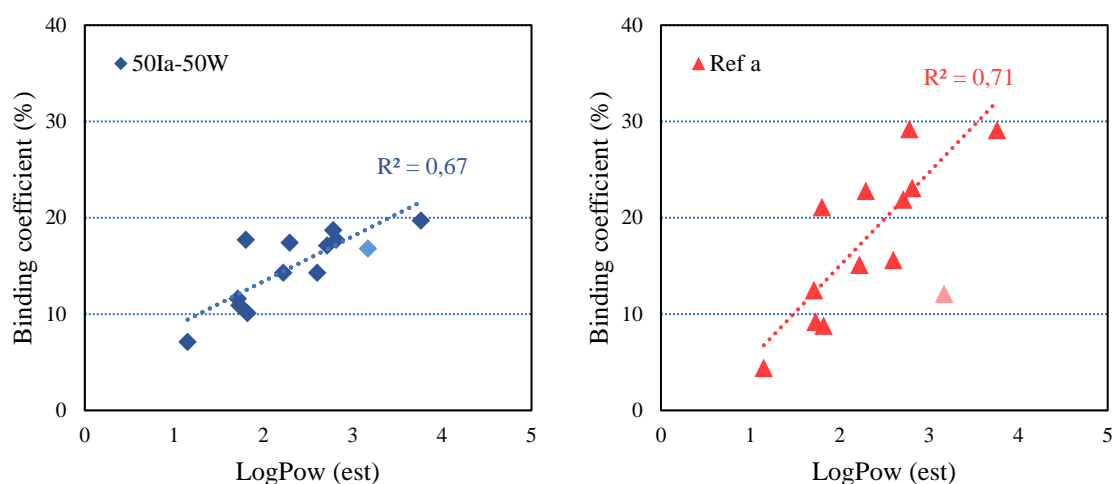


Figure 3.2.1.3.: Binding coefficient of 13 volatile compounds in function of their corresponding octanol/water partition coefficient LogPow (estimated hydrophobic constant, EPI-Suite4.11) for the two different protein buffer solutions: Refa in red (triangle) and 50Ia-50W in blue (lozenge). In light blue and light red: values for 2-pentylfuran. In dashed line: trends line with R2 for Refa and 50Ia-50W.

These results (strong correlations with protein concentration and no saturation of the binding sites with the used concentrations of volatile compounds and of proteins) are consistent with the hypothesis of protein-volatiles interactions impacting the volatile compounds release on pea protein isolates.

However, these interactions only impact about 18% of the volatile compounds (in quantity for the solution Refa, 15% for the pellet solution 50Ia-50W). Thus we can assume that they have only a low impact on the olfactory perceptions. In addition, each volatile molecule must interact differently with proteins according to its chemical characteristics, in particular its hydrophobicity, and so impacting differently the olfactory perceptions. The differences between initial solutions (Refa and Refb) and pellet solutions (50Ia-50W and 50Ib-50W) in term of volatile and sensory profile could be explained partly by differences in term of protein conformation (protein type and protein conformation). Therefore, in the following multivariate approach, protein-volatile compound interactions will not be taken into account.

Multivariate approach to reveal relationships between sensory perception of pea protein solutions and aroma profile

Correlations between sensory attributes and volatile compounds

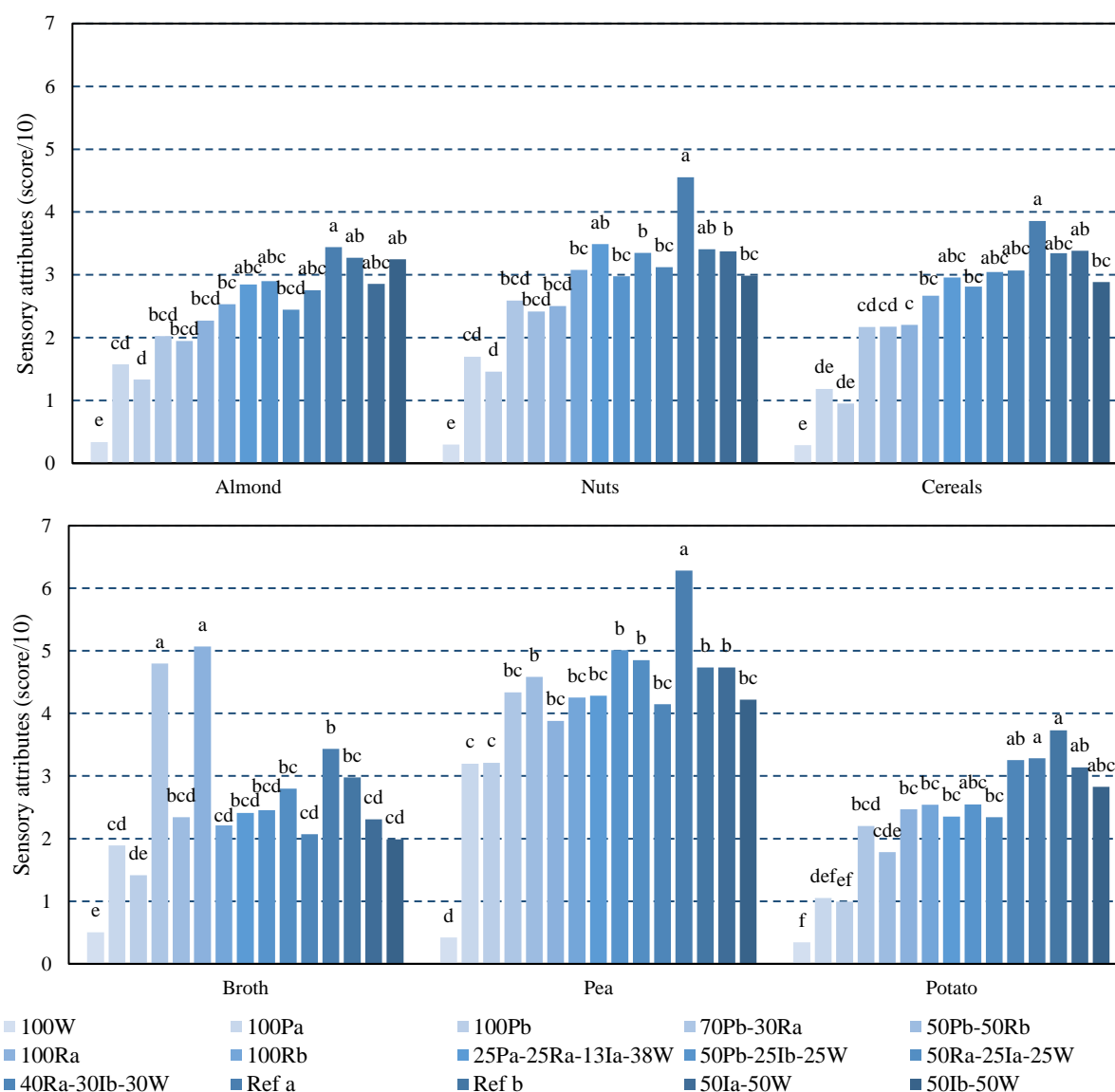


Figure 3.2.1.4.: Averaged perceived intensities (across subjects and replicates) of the 15 solutions studied for sensory attributes (p-value are significant at the threshold of 0.05 for the 6 attributes and letters corresponds to the LSD-Bonferroni group associated). The colour gradient corresponds to the DM content of the samples: from light blue corresponding to water to dark blue corresponding to solutions with 6% DM.

It has been reported that in experimental investigations of mixture aroma, a single sensory attribute describing the main character of the aroma cannot sufficiently reflect the contributions of all the volatile compounds to the aroma (Bult, 2002). In particular, it has been argued that the use of a single attribute might obscure perceptual interactions between odours (Barkat *et al.*, 2012). Thus, in our study, we decided parsed out the multiple components of beany aroma and expressed them via other terms as used in Cosson *et al.*, 2021 (Section 3.1.2.). Therefore, solutions were described by the used of 6 aroma attributes: potato, pea, cereals, broth, almond, and nuts.

For each of aromatic sensory attributes, their significant discrimination between solutions were confirmed by ANOVA results (data not shown, p-value significant at the threshold of 0.05). Their averaged perceived intensities (across subjects and replicates) are presented in Figure 3.2.1.4. Multiple product pair comparisons (LSD-Bonferroni) showed differences between solutions. As expected, solution 100W (composed only of water) presents intensities close to 0 for the six aromatic attributes. As regards nuts intensities, the initial solution Refa presents the highest values (4.55/10) and solutions composed only of permeate (100Pa and 100Pb) the lowest values (1.46/10 and 1.70/10 respectively). As regards to broth intensities, overall solutions from batch *a* present highest values, more specifically solution composed of permeate *b* and of retentate *a* (70Pb-30Ra) and solution composed only of retentate *a* (100Ra) present the highest values (4.80/10 and 5.07/10 respectively).

As regards to pea, the 14 solutions (except water 100W) are perceived globally with high values of intensity (between 3 and 6). Among them, the solution containing only permeate *a* (100Pa) presents the lowest values contrary to the initial solution Refa which presents the highest values. For potato intensities, the 15 solutions present relatively low values with maximum reach by initial solution Refa (3.3/10). For cereals intensities, the initial solutions (Refa and Refb) as well as the solution composed only of pellet *a* (50Ia-50W) present the highest values (3.9/10, 3.3/10 and 3.4/10 respectively). For almond intensities, the solutions containing only permeate (100Pa and 100Pb) present the lowest values (1.57/10 and 1.33/10 respectively) and initial solution Refa the highest value (3.44/10). Thus, as already noticed for GC-MS data, solutions from batch *a* present the highest intensity values than solutions from batch *b*.

To go further, correlation matrices (Pearson method) between sensory intensities and composition concentration were calculated. Almond, nuts, potato and cereals intensities are correlated to the concentration on protein ($R^2=0.82$, $R^2=0.72$, $R^2=0.83$, and $R^2=0.82$ respectively) with lowest intensities on permeate (100Pa and 100Pb) and highest intensities for initial solution (Refa and Refb). Attributes contributing to perceived beaniness are mainly influenced by the retentate and permeate fractions as previously shown by Cosson *et al.* (Cosson *et al.*, 2021 – Section 3.1.2.). The importance of protein concentration on beaniness intensities is in line with the protein-volatiles interactions described earlier. The volatile compounds are mainly bound to protein, modifying their release and so the perceived odour. In addition, pea, almond, nuts, potato and cereals intensities are correlated between each other's (R^2 between 0.98 and 0.79). However, broth intensity is correlated only to pea intensity ($R^2=0.53$). These results suggest that whereas beaniness is due to a mixture of volatile compounds, the individual perceptual features of each volatile compounds are less perceived, such that panellists perceived mainly the key association of volatile compounds named beaniness.

Correlation matrices (Pearson method) between sensory and aroma release data were calculated from peak area obtained with the apolar column - correlations with areas obtained from the polar column analyses, data not shown, were also analysed but do not provide additional information. Results show that 21 volatile compounds are significantly correlated to aromatic sensory notes (nuts, almond, potato, pea and cereals) as presented on Table 3.2.1.5.

These correlated volatile compounds (except one ketone) are lipophilic with positive octanol-water partition coefficient, low solubility in water (inferior at $7.6E+04$ mg/L) and high volatility (Henry Law constant inferior at $5.7E-05$ atm-m³/mole). In addition, these compounds present relatively high odour threshold (Table 3.2.1.5.). Besides, as expected by psychophysics graph, most of these correlations are positive such that intensities increase with volatile compound concentrations increase.

Table 3.2.1.5.: Correlations coefficients (Pearson method) between volatile compounds (CG-MS area) and overall aromatic intensity, pea and broth sensory attributes (scores/10) and odour threshold (ppb, in water obtained from the literature). In bold, negative correlations coefficients. *For confidentiality reasons, only the chemical family of the compounds are given here.*

Chemical family*	Pea	Potato	Almond	Nuts	Cereals	Odour threshold (ppb)
Ketone	NA	NA	NA	0.56	0.55	300 ^b
Alkene	0.59	NA	0.52	0.65	0.58	NA
Aldehyde	0.58	0.72	0.68	0.63	0.7	110 ^d
Furan	0.67	0.66	0.66	0.78	0.76	6 ^e
Aldehyde	0.65	NA	NA	0.53	NA	3 ^a
Furan	NA	NA	NA	0.58	0.6	6 ^a
Ketone	NA	-0.54	NA	NA	NA	50 ^f
Aldehyde	0.61	NA	NA	0.58	0.53	15.9 ^c
Ketone	NA	NA	NA	NA	0.52	0.19 ^g
Aldehyde	-0.7	-0.72	-0.83	-0.74	-0.72	5 ^b
Aldehyde	-0.82	-0.74	-0.86	-0.81	-0.76	24.2 ^c
Ketone	0.62	NA	NA	0.65	0.59	140 ^a
Alcohol	0.79	0.58	0.67	0.64	0.63	500 ^a
Aldehyde	0.65	0.53	0.57	0.71	0.66	4.5 ^a
Ketone	0.66	NA	NA	0.63	0.63	NA
Ketone	0.53	NA	NA	0.56	NA	200 ^b
Alcohol	NA	-0.54	NA	NA	-0.56	190 ^c
Ketone	0.6	NA	NA	0.66	0.6	190 ^b
Ketone	0.61	NA	NA	0.58	0.54	NA
Ester	NA	0.53	0.56	0.55	0.52	210 ^h
Aldehyde	NA	NA	-0.56	NA	NA	NA

^aButtery *et al.*, 1999 ; ^bBelitz *et al.*, 2004 ; ^cAhmed *et al.*, 1978 ; ^dJelen *et al.* 2013 ; ^eConception *et al.* 2017 ; ^fSchindler *et al.* 2010; ^gSiek *et al.* 1971; ^hTakeoka *et al.* 1990

The main volatile compounds are associated to the six aroma attributes (nuts, potato, pea, cereals, broth and almonds). However, each attributes is also correlated to specific compounds volatiles. However, broth intensity is illustrated by a different behaviour, without clear link to volatile compounds, suggesting either an impact of non-volatile compounds on this perception either correlation coefficients are not relevant to understand volatile compounds at the origin of broth intensity. It is commonly known that perceived aroma intensities are not determined by a single component but by a mixture of components (Laing & Jinks, 1996). The disadvantage of considering only two-by-two interactions is that the interactions between volatile compounds are not taken into account as well as the cognitive olfactory mechanisms. Besides, physiological differences between individuals make it complex the understanding of perceptions and are not taken into account here (average across panellists). Indeed, individuals do not necessarily perceive the same odour in a particular product and do not present the same sensibility. This inter-individual variability may result from many factors: genetic differences, anatomical differences, health status and age as well as experiences and semantic knowledge (Thomas-Danguin *et al.*, 2014). In particular, Barkat *et al.* (2011) suggested that the individual's characteristics of the main character of the odour in the case of blending mixtures reflects the degree of similarity between the actual odour perception of a stimulus and its internal memorized representation. These different points highlight the need to go further to study relationships between sensory perception of pea protein solutions and aroma profile, in particular to understand the specificities of broth intensity.

Regression modelling between sensory attributes and volatile compounds

To obtain a better insight into the origin of these aromatic perceptions, two different models (linear model and artificial neural network model) were developed between the volatile compounds release measured by the GC-MS data and the sensory data.

On the one hand, the relationships between sensory and volatiles data was investigated by PLS regression model. PLS is a multivariate method that establishes linear relationships between a set of predictors (volatile compounds areas) and a set of responses (sensory intensities) using latent variables.

The purpose of PLS was to establish which volatile compounds are mainly related to sensory attributes of the pea solutions. A PLS model using NIPALS methods was fitted with potential explanatory

variables (63 compounds volatiles GC-MS area from apolar column). Results show that the optimum number of latent variables is two. For this two components, the model explained 48.41% of variation in X and 70.75% of variation in Y. The predicted residual sum of squares (RSS) was 0.99. It is a measure of the discrepancy between the data and the estimation model. RSS is close to 1, indicating a relatively good fit of the model to the data. However, statistical methods such as PLS regression are linear models and so do not account for thresholds (Zielinski *et al.*, 2014; Yu *et al.*, 2017). Thus, the relationships between sensory and volatiles data was also investigated by artificial neural network model (ANN). ANN are a type of machine-learning prediction method with the ability to self-learn relationships from labelled experimental data and generalize to unlabelled situations. Several ANN model were fitted with the same potential explanatory variables (63 compounds volatiles GC-MS area from apolar column). The optimal model selected with our data presented 1 layer and 2 nodes: 1 sigmoid node and 1 linear node. As for PLS model, ANN model show globally high performance except for broth attribute as presented on Table 3.2.1.6. For training coefficient of determination are between 0.80 and 0.99 with low training error (RSS between 0.18 and 1.07) and for validation between 0.74 and 1.00 with low validation error (RSS between 0.00 and 2.13). Both models (PLS and ANN) are therefore significant but their prediction capabilities are only moderately good.

Table 3.2.1.6.: Performance of the final selected Artificial Neural Network (ANN) model to predict the 7 sensory attributes variables in function of compounds volatiles area analysed by GC-MS. RMSE: root-mean-square error. R²: coefficient of determination. RSS: residual sum of squares. Sum Freq: number of observations used. T = Training. V = Validation.

Performance	Broth		Pea		Potato		Almond		Nuts		Cereals	
	T	V	T	V	T	V	T	V	T	V	T	V
R ²	0.42	0.93	0.86	0.74	0.80	1.00	0.98	0.98	0.99	1.00	0.90	1.00
RMSE	1.20	0.17	0.30	0.84	0.34	0.06	0.13	0.13	0.12	0.04	0.24	0.01
RSS	17.41	0.09	1.07	2.13	1.42	0.01	0.21	0.05	0.18	0.00	0.66	0.00
Sum Freq	12	3	12	3	12	3	12	3	12	3	12	3

As regards important volatile compounds, PLS model shown 34 volatile compounds with variable importance projection values superior to 0.8. The variable importance projection score is a measure of a variable's importance relative to model both X and Y (Wold *et al.*, 2001); 0.8 being the commonly used criterion to say that the variable is significant. These 34 compounds include the 20 volatile compounds find previously correlated to sensory attributes. To these compounds are added 4 ketones, aldehydes, 1 alcohol, 1 furan, 2 alkanes and 1 alkene. These correlated volatile compounds are lipophilic with octanol-water partition coefficient superior to 0.

Figure 3.2.1.5. shows the correlation loadings of the 15 solutions combined with sensory evaluation and 63 volatile compounds. The Y responses (sensory attributes) are located in the lower right quadrant. The predictors (compounds volatiles) located close to the Y responses give a positive contribution to the Y estimates, while predictors located in the opposite part of the graph contribute negatively to the Y estimates. Studies show that a mixture of compounds is not a simple addition of each of its odours. Many effects are possible depending on the compounds and their initial concentration (additivity, synergy or asymmetric model).

Concerning ANN results, the most important compounds in predicting the overall sensory attributes are similar to those highlighted from PLS model. If ANN model allows to make more complex models taking into account interactions and threshold, they are however black box prediction machine with no insight into relationships between predictors and variables and which do not allow formal hypothesis tests. With ANN model, it is difficult to identify the most important variables in predicting the response (Seisonen *et al.*, 2016). And in our case they confirm the results of the PLS model but provide little additional information to understand the sensory mechanisms due to volatile compounds, perhaps because of the small number of products. In our case, we can conclude that the PLS model provides more information to understand olfactory perceptions.

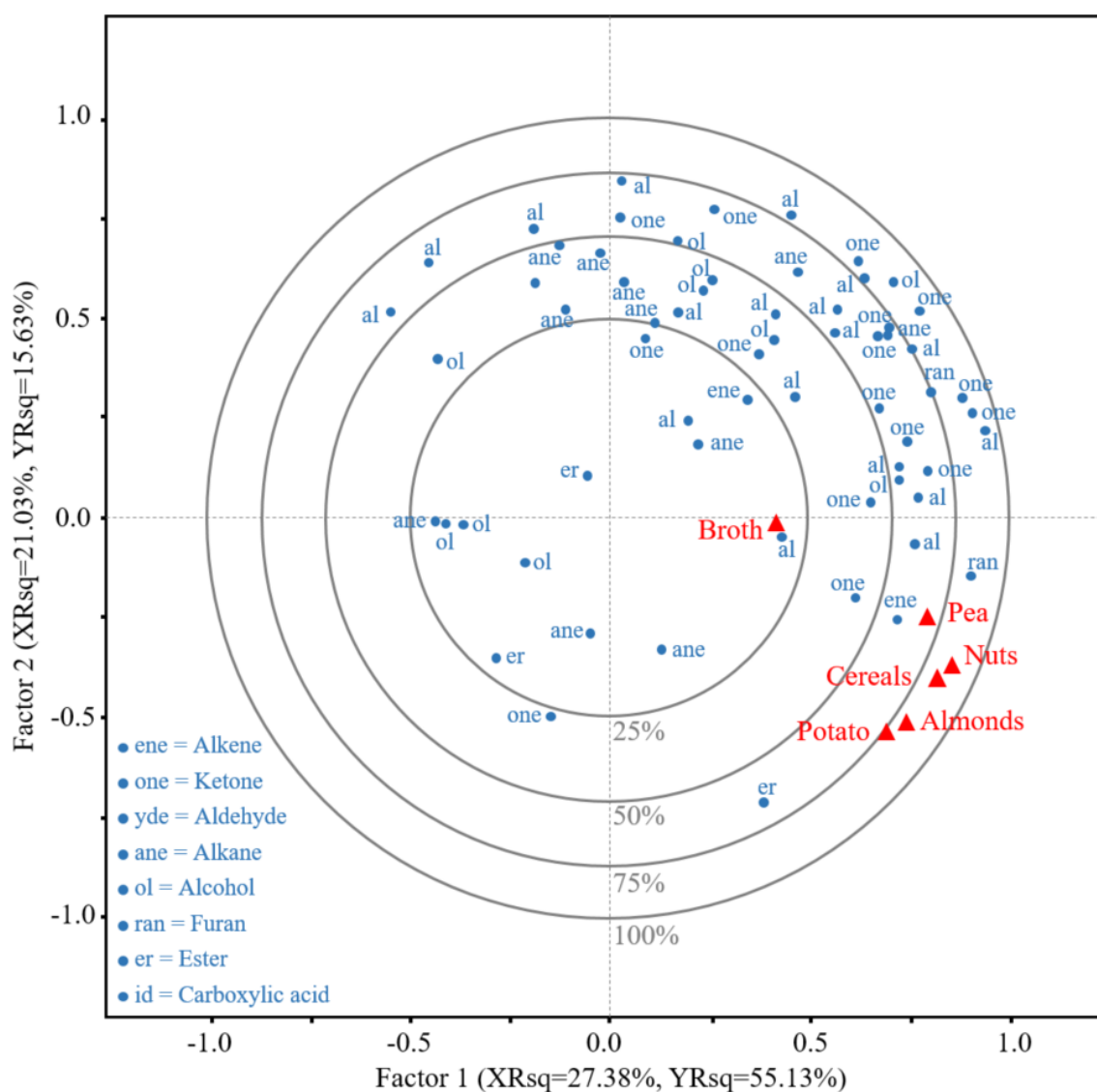


Figure 3.2.1.5.: An overview of the correlation loadings of the 15 solutions combined with sensory evaluation and 63 volatile compounds analysed by partial least squares (PLS) (centred-scale data). For confidentiality reasons, only the chemical family of the molecules are given here.

Therefore, as for two-by-two interactions, each attribute is predicted by specific compounds volatiles. However, the main volatile compounds correlated to aroma intensities are similar to the 5 attributes (nuts, potato, pea, cereals and almonds). This findings support the hypothesis that the key volatile compounds of pea protein isolates may lose some of their perceptual features in mixing and create meaningful associations that strongly contribute to the mixture odour characteristics (Paravisini & Guichard, 2016; Romagny *et al.*, 2018). Instead of other mixture where the key volatile compounds may preserve their perceptual features such that the individual odour they carry as single compounds is still identifiable within the mixture (Bult, 2002). Evaluation of this hypothesis could be done by omission experiments or reconstitution analysis to validate the actual impact of the identified volatile compounds within mixtures.

Regarding broth attribute, it is the attribute the least well explained by the PLS model as reflected in the fact that the broth point is near the 25% circle whereas the others attributes are near the 100% circle (Figure 3.2.1.5.). Similarly, the lowest correlations between experimental data and the ANN prediction data were obtained for broth attribute (for training $RSS=17.41$, $R^2=0.42$) as previously found for two-by-two interactions. As can be seen on Figure 3.2.1.6., difficulties in modelling the broth attribute seem

to be mainly due to two solutions: solution constituted only of retentate *a* (100Ra) and solution constituted of permeate *b* and retentate *a* (70Pb-30Ra).

As regards sensory data, these solutions distinguished from others with a high broth intensity: 5.07/10 for 100Ra and 4.80/10 for 70Pb-30Ra. Several hypotheses can explain these results. It could be due to very strong inter-individual differences, to a dumping effect or to other non-volatile compounds (Clark & Lawless, 1994). For example, the perception of the attribute broth may also have been influenced by the composition of peptides and amino acids, which could be richer in the retentate *a*. Indeed, Henriksen showed that the bouillon note of dried sausage was related to a mixture of different amino acids and peptides and that the intensity of the potato note was positively correlated with levels of tyrosine (in both its free and peptide residue forms) (Henriksen, 1997).

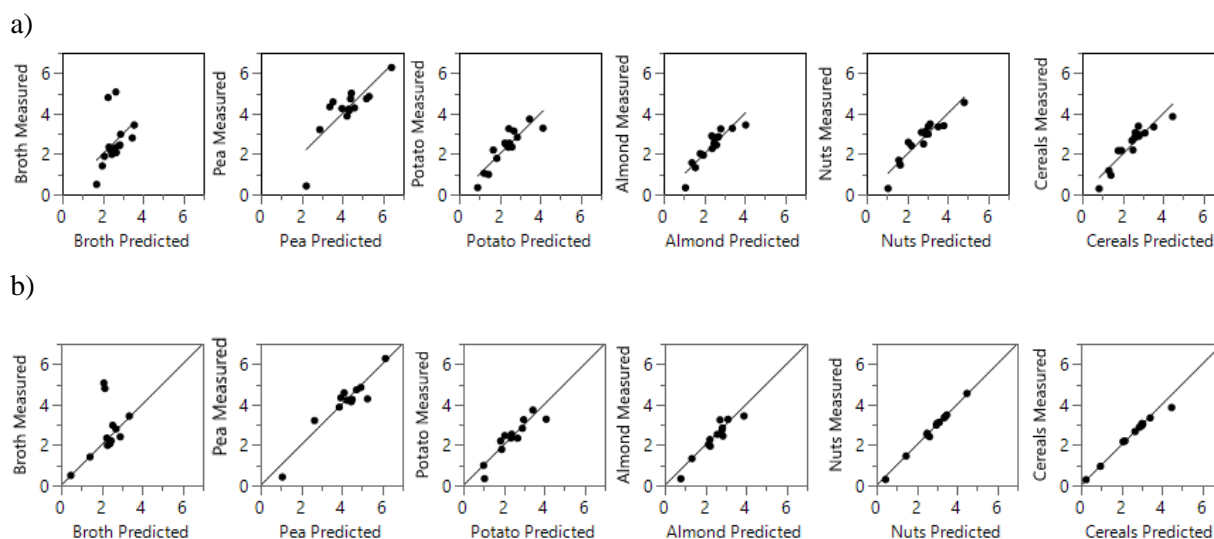


Figure 3.2.1.6.: Comparison of predicted and measured sensory attributes for the test dataset using a) the PLS model and b) the ANN model.

3.2.1.4. Conclusion and perspectives

This study adopted an original approach: to work with fractions instead of compounds to explore how combinations of volatiles and proteins affect the sensory characteristics of pea protein-based solutions. We broke down pea protein isolates into three fractions (pellet, retentate, and permeate), which were then recombined to form different experimental solutions using a mixture design. The study yielded several key results. First, a total of 79 volatile compounds were identified coming from endogen origin of pea seed or from oxidation of the lipids/peptides/amino acids in the seed or during the storage. Second, the protein-volatile interactions were mainly hydrophobic interactions and can be explained by the octanol/water partition coefficient (LogPow). However, these interactions only impact about 18% of the volatile compounds and so may have only a low impact on the olfactory perceptions. Third, the key volatile compounds are associated to the six aroma attributes (nuts, potato, pea, cereals, broth and almonds). Thus there is a common nucleus of compounds responsible for the beany perceptions. The compounds may lose some of their perceptual features in mixing and create meaningful associations that strongly contribute to the beany odour. Fourth, these key volatile compounds belong to many chemical families, mainly aldehydes, ketones and alcohols. They are mainly lipophilic but they present various solubility characteristic in water and various volatility. In order to improve the desirability of products based on pea protein isolates, it is therefore difficult to make recommendations in terms of volatile compounds to be removed. However, solutions that lead to significant changes in volatile compounds could be an interesting strategy. For example, fermentation, washing or trapping of volatile compounds and addition of flavouring. In addition, the interactions between volatile compounds and

pea proteins could be an interesting way to reduce aromatic perceptions. Indeed, by modifying the protein conformations, we could modify the interactions of volatile compounds with proteins and thus modify the aromatic perceptions.

Main results

- A total of 79 volatile compounds were identified with varied concentrations in pea protein isolates and fractions.
- Protein-volatile molecule interactions were about 18% and were mainly hydrophobic interactions, which can be explained by the octanol/water partition coefficient (LogPow).
- The key volatile compounds are associated to the perception of the six aroma attributes (nuts, potato, pea, cereals, broth and almonds). Thus there is a common nucleus of compounds responsible for the beany perceptions.
- The broth attribute is the one that stands out the most.

Section 3.2.2. – Identification and characterization of the main peptides in pea protein isolates using ultra high-performance liquid chromatography coupled with mass spectrometry and bioinformatics tools

3.2.2.1. Introduction

A major recent challenge in the agrifood industry is developing new protein sources to compensate for the anticipated future paucity of traditional animal proteins. Consequently, both the industry and consumers are focusing their attention on plant proteins. Plant protein isolates, such as those derived from peas (*Pisum sativum* L.), are often used to create foods because of their functional properties, protein content, sustainable production, and relatively low cost (Davis *et al.*, 2010). However, plant proteins, and especially isolate fractions from raw plant matter, have some drawbacks from a sensory point of view (e.g., their color, smell, and taste). It is necessary to better understand the sensory issues associated with plant proteins if we wish to develop plant protein-based foods that will be attractive to consumers.

Research on the perception of pea-based products has largely focused on the role of volatile aroma compounds in creating sensations of beaniness (Bi *et al.*, 2020) and of phenolics/saponins in creating sensations of bitterness and astringency (Heng *et al.*, 2006). However, it is important to carry out more detailed compositional analyses to clarify how foods are sensorily perceived.

Pea protein isolates are mainly composed of globulins, which are the main storage proteins in seeds. Globulins consist of two fractions that are characterized by their ultracentrifugation sedimentation coefficients: 7S (20%–40%) and 11S (20–30%). The 7S fraction is composed of vicilins and convicilins. The 11S fraction is composed of legumines (Crevieu-Gabriel, 1999). During protein isolate extraction (notably during temperature and pH changes), proteins may be naturally hydrolysed into numerous peptides of different sizes (Li & Aluko, 2010; Sirtori *et al.*, 2012). Several structural changes result because of the exposure of hydrophobic sites normally found in the protein's core (Daher *et al.*, 2020). Although such peptides remain little studied, they could potentially have properties that might serve to improve the sensory properties of plant-based products.

Indeed, specific protein fragments may elicit various sensory perceptions (e.g., sweet, bitter, umami, sour, or salty notes). Sourness and saltiness could result from the presence of charged terminal groups and/or charged side chains (Temussi, 2012). Other perceptions (sweetness, umami, and bitterness) could be explained by the presence of different peptide families. For example, certain small peptides (5–8 residues in size) can activate the TAS2R bitter taste receptors in the mouth (Aubes-Dufau *et al.*, 1995; Maehashi & Huang, 2009). These peptides tend to be hydrophobic with proline- and leucine-rich side chains, especially at their C-terminals (Kim *et al.*, 2008). They can have quite an impact: for example, 0.25 mM of a peptide (VVYPWTQRF) solution derived from bovine hemoglobin elicits the same sensation of bitterness as 0.073 mM of quinine sulfate or 21 mM of caffeine (Aubes-Dufau *et al.*, 1995). With regards to sweetness, there are no known natural peptides that result in sweet notes. However, semi-synthetic peptides, such as aspartame (the methyl ester of the aspartic acid/phenylalanine dipeptide) and neotame (a secondary amine of 3,3-dimethylbutanal and aspartame), can activate T1R2/T1R3 sweet taste receptors. In the case of umami, the umami heterodimer (T1R1/T1R3) has ligands with multiple binding sites, and thus the receptor displays low specificity and can respond to a chemically diverse range of umami molecules. More than 50 peptides (such as KGDEESLA) appear to elicit umami, but their specific functional roles remain unclear. Research on the relevant receptors has suggested that such peptides might directly lead to the sensation of umami. However, it is also possible that umami is a consequence of partial hydrolysis, which leads to sizeable concentrations of Asp or Glu (Temussi, 2012; Wang *et al.*, 2020).

Several experimental approaches have been used to study the sensory properties of protein fractions. The most common strategy to examine how specific compounds affect the sensory characteristics of products, using a combination of fractionation and omission tests (Engel *et al.*, 2002; Toelstede & Hofmann, 2008). However, peptidomics techniques are increasingly used thanks to advances being made in modern mass spectrometry and bioinformatics. These tools are ideally suited for carrying out comprehensive peptide analysis, especially when such analyses exploit the massive quantities of information currently available in genomic and transcriptomic databases. In peptidomics, different solvents and techniques are used in the fractionation, separation, and analysis of peptides (Gao *et al.*, 2019; Salger *et al.*, 2019). In such work, liquid chromatography-mass spectrometry is the most widely used analytical method. Fragmentation spectra obtained from samples are compared with theoretically expected spectra for peptide reference sequences. Sample peptides are thus assigned to the proteins that contain their sequences. Several bioinformatics tools have been developed to automate these operations, such as COMET (Eng *et al.*, 2013) or X!tandempipeline (Langella *et al.*, 2017). Information about peptide properties can be found in databases such as BIOPEP (Iwaniak *et al.*, 2016). Recently, Daher *et al.* (2020) demonstrated that peptidomics could be a valuable tool for evaluating the bitterness of protein isolates.

Thus, the aim of this study was to identify the main oligopeptides and polypeptides (5–40 amino acids long) found in pea protein isolates and to characterize their sensory properties. To this end, we used pea protein solutions and an experimental design previously employed by Cosson *et al.* (2021). The peptide profiles of the solutions were determined using ultra high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS/MS). The resulting peptides were identified, and both their physicochemical properties and their antioxidative properties were characterized. Then, we examined the impact of our fraction-based formulation strategy on peptide profile. Finally, the relationship between peptide profiles and the sensory properties of solutions (as determined in Cosson *et al.*, 2021) was explored, with a particular focus on perceived bitterness..

3.2.2.2. Materials and methods

Solution preparation

Six fractions were obtained from two pea protein isolates (protein content = nitrogen [N] content x 6.25; 83% dry matter) as explained in Cosson *et al.* (2021 – Section 3.1.2.): permeates *a* and *b*; retentates *a* and *b*; and pellets *a* and *b*. Then, these six fractions were combined in various ways to formulate 28 solutions (see the mixture design described in Cosson *et al.*, 2021 – Section 3.1.2.). This process was carried out at 4°C in 50 mL glass flasks, which were stored at -20°C. Work was performed on different groups of compound types rather than on a single compound type. Each fraction was associated with a main compound type: insoluble proteins in the case of the pellets; soluble compounds (e.g., volatiles, peptides, and phenolics) in the case of the permeates; and soluble proteins interacting with volatiles in the case of the retentates. This approach made it possible to formulate a diversity of pea protein-based solutions to obtain continuous responses and build reliable statistical models. Solutions were chosen to represent a broad spectrum of combinations while also remaining realistic in terms of protein concentrations (0–4.25%).

Overall characterization of the solutions

For each fraction, protein content was determined via the Kjeldahl method (N content x 6.25). Dry matter content (% w/w) and ash content were determined by a certified external laboratory (SAS IMPROVE, Amiens, France) via drying and calcination (prepASH®219 analysis system). Conductivity at 20°C was measured with a calibrated conductivity probe (InPro 7108-25/65-VP 3.B, M300 transmitters; Mettler Toledo, Switzerland), and pH was measured at 20°C (InPro 4801i/SG/120; Mettler Toledo, Switzerland).

Hydrophobicity index values were measured as per Kato and Nakai (1980). The reaction between the 8-anilinoanthracene-1-sulphonic acid probe (ANS) and hydrophobic amino acids (Alanine, Valine, Leucine, Isoleucine, Methionine, Phenylalanine, Tryptophan, Proline) leads to the formation of a

fluorescent complex, which is measured by spectrofluorometry. Each solution was diluted with phosphate buffer (0.053 M Na₂HPO₄·2H₂O, 0.067 M KH₂PO₄, pH = 7.0) to establish five concentration levels between 0.002% and 0.032% (wt). Then, 20 µL of an 8-anilidonaphthalene-1-sulphonic acid probe (ANS; Sigma Aldrich) was added to 4 mL of each solution; the result was thoroughly mixed for 15 min in the dark (concentration of 8 mM in the phosphate buffer). Signal intensity was measured using a spectrofluorometer (Cary Eclipse; Agilent) with excitation wavelengths of 380nm and emission wavelengths of 480nm. Protein surface hydrophobicity was then calculated as the initial slope of relative fluorescent intensity in function of the protein concentration. The relative fluorescent intensity was calculated as $(F-F_0)/F_0$ where F_0 is the fluorescent intensity values of the ANS blanks (ANS solution made with buffer - without any proteins). F is the fluorescent intensity values of the protein solutions. The slope of the relationship between protein concentration (%) and fluorescent intensity was determined via linear regression analysis. Three replicates of the analysis were performed.

Peptide identification and relative quantification

Before the UHPLC-MS/MS analysis, a sample pre-treatment procedure was applied adapted from previous work (Guillot *et al.*, 2016). Due to the nature of the samples, which are the result of several fractionation steps, it was possible to simplify the sample preparation procedure as follows. Pea solutions were centrifuged (15,000 g, 4°C, 15 min). The supernatants were filtered using a Vivaspin centrifugal concentrator (20 mL, 10 kDa; Sigma Aldrich) run at 8,000 g (30 min, 4°C). The filtrates were stored in the dark at -80°C prior to analysis.

MS was performed at the PAPPSO platform (MICALIS, INRAE, Jouy-en-Josas, France). An Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer (Thermo Fisher Scientific) coupled to an UltiMate™ 3000 RSLCnano System (Thermo Fisher Scientific) was used. Peptides were loaded into a precolumn (Acclaim PepMap C18; 5 µm particle size, 5 mm length, 300 µm ID) at a rate of 20 µL/min and were separated using a C18 column (Acclaim PepMap nanoViper; 2 µm particle size, 500 mm length, 75 µm ID) at a rate of 300 nL/min and measured over a total gradient length of 147 min with increasing buffer B (80% acetonitrile [ACN] and 0.1% formic acid) from 1 to 60 % for 115 min. Buffer A was 0.1% formic acid in 98% water. The eluted peptides were distributed throughout the gradient showing a good and an adequate peptide separation. The eluted peptides were analyzed online using the Orbitrap mass analyzer. The mass spectrometer was operated in data dependent acquisition (DDA) and positive mode ionization was performed, employing a spray voltage of 2.8 kV. Peptide ions were analyzed using a data-dependent method as follows: a full MS scan (m/z : 300–1,600; resolution: 120,000) was performed by the Orbitrap mass analyzer. Doubly and triply charged peptides underwent MS/MS analysis (collision energy: 30%; resolution: 30,000; cycle time: 3 sec).

Peptide identification was performed with X!Tandem v. 2017.2.14 (Alanine) and X!Tandem Pipeline (C++) v. 0.2.40 (Langella *et al.*, 2017) using protein sequences for *Pisum sativum* L. The main peptide identification parameters were the following: no cleavage specificity, variable methionine oxidation state, and mass tolerance for parent and fragment ions of ± 10 ppm. Peptides were retained when the E-value was ≤ 0.05 , and the presence of one peptide per parental protein was considered to enable identification. Contaminant peptides were discarded following identification using a standard proteomics contaminant database, and the false discovery rate was estimated using the reversed protein database. MassChroQ software (v. 2.2.17) was employed to perform alignment, XIC extraction, peak detection, and quantification (Valot *et al.*, 2011).

Fourteen pea solutions were analyzed using UHPLC-MS/MS: Refa, Refb, 100Pa, 100Pb, 100Ra, 100Rb, 50Ia-50W, 50Ib-50W, 50Pb-25Ib-25W, 25Pa-25Ra-13Ia-38W, 70Pb-30Ra, 40Ra-30Ib-30W, 50Ra-25Ia-25W, and 50Pb-50Rb. Among them, 100Ra, 100Rb, Refa, and Refb were performed in duplicate to assess method repeatability.

To cut down on the analysis time, we hypothesized that the solutions' peptide concentrations could be estimated from solution formulations, given that the solutions were mixtures of the fractions. We found support for this hypothesis using a subset of six of the solutions (50Pb-25Ib-25W, 25Pa-25Ra-13Ia-38W, 70Pb-30Ra, 40Ra-30Ib-30W, 50Ra-25Ia-25W, and 50Pb-50Rb). For the other thirteen solutions

(50Pa-Ia25-W25, 50Rb-50W, 40Pb-60W, 50Pb-50W, 40Pa-60Rb, 30Ia-70W, 60Ra-40W, 50Pa-25Ib-25W, 40Pa-60W, 50Pa-50Ra, 40Rb-30Ia-30W, 25Ib-75W, and 40Rb-30Ib-30W), peptide composition was calculated based on the peptide composition of the fractions. A linear equation of the following type was used:

$$A_{\text{Recombined.Products}} = A_{\text{pellet.a}} \times C_{\text{pellet.a}} + A_{\text{retentate.a}} \times C_{\text{retentate.a}} + A_{\text{permeat.a}} \times C_{\text{permeat.a}} + A_{\text{pellet.b}} \times C_{\text{pellet.b}} + A_{\text{retentate.b}} \times C_{\text{retentate.b}} + A_{\text{permeat.b}} \times C_{\text{permeat.b}}$$

where A was the area of the peptides, and C was the relative quantity of each fraction.

Before the statistical analyses were performed, the data were processed. First, the areas of each replicate were averaged (cleansing step). The areas of identical peptides with the same charges were also summed. In this study, we chose to use all a peptide's isotopes in its quantification. This decision was made for two reasons: a) the isotope distribution for a given peptide is discrete and depends mainly on the presence of heavy isotopes and b) isotope composition can be treated as "homogeneous." Consequently, using all the isotope peaks should improve the results because signal variability should decline if multiple values are used. Missing data are always a modeling concern, so we assumed that this approach would still yield a better approximation than comparing isotopes separately. Second, certain peptides were removed (first filtering step): only peptides present in at least two solutions were retained. Third, the peptide composition of 13 of the solutions was calculated as described above (calculation step). Fourth, peptides with little variation in area were removed (second filtering step): only peptides that varied at least 50% among the solutions were retained. Finally, to remove any artefacts, null values were replaced by randomly selected values between 1+E04 and 1+E05 (i.e., values corresponding to the detection threshold). The general workflow of the different steps of peptidomics analysis is illustrated below (Fig. 3.2.2.1.).

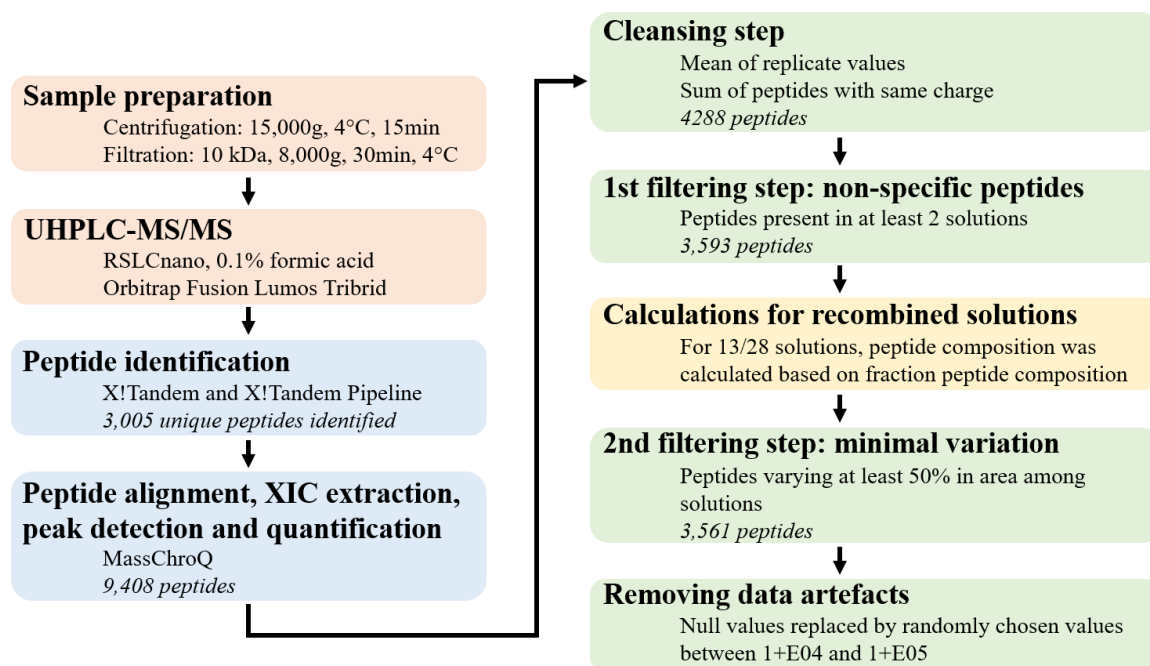


Figure 3.2.2.1.: General workflow of the different steps of peptidomic analysis: the preparation and measurement processes (in orange) to the bioinformatic analyses (in blue), the preprocessing of the data (in green), and the calculations for the recombined products (in yellow).

Characterization of peptide properties

Peptides were characterized based on nine physicochemical properties: length (number of amino acids), the GRAVY index (the grand average of hydrophobicity), bulk (the average bulkiness of the amino acids), the aliphatic index (relative volume occupied by aliphatic side chains), polarity (average polarity of the amino acids), charge (overall net charge), relative basic nature (fraction of informative positions

that are occupied by Arg, His, or Lys), relative acidic nature (fraction of informative positions that are occupied by Asp or Glu), and relative aromatic nature (fraction of informative positions that are occupied by His, Phe, Trp, or Tyr). As in Proust *et al.* (2019), these properties were computed using the `aminoAcidProperties` function of the R package “alakazam” v. 0.2.8 (Gupta *et al.*, 2015). Default settings were used for scaling and normalization. The bioactivity and sensory properties of peptides were explored via comparisons with known bioactive and taste peptides listed in the BIOPEP database (Iwaniak *et al.*, 2016). Only peptides that were more than three amino acids long were examined to avoid noise in the results. Finally, the perceptions of peptides were investigated by looking at the sensory scores of the 28 solutions evaluated by Cosson *et al.* (2021 – Section 3.1.2.).

Statistical analysis

Analyses were performed using R (R Core Team, 2019) and JMP (v. 13.1.0; SAS Institute Inc., Cary, SC, USA). For the inferential analyses, $\alpha = 0.05$ was the threshold for statistical significance. To visualize the intersections in the peptide sets among the six fractions and the two raw solutions, the function “upset” in the package UpSetR was used (Conway *et al.*, 2017). To visually explore differences in peptide profiles among the 28 solutions, we carried out principal component analysis (PCA, wide method) on a correlation matrix. To visualize the overall characteristics of the peptides, we plotted the distributions of each physicochemical property (normalized distribution, kernel density). Finally, to examine the relationships between the peptide data and the sensory data for the 28 solutions, we analyzed a correlation matrix (Pearson method).

3.2.2.3. Results and discussion

Identification and characterization of the main peptides in pea protein isolate solutions

Identification of the peptides in the pea protein solutions

After preliminary processing of the peptide data, 3,561 peptide ions (with different charges) and 3,005 unique peptides were identified. Mass ions varied in m/z (305–1395 m/z), charge (2–4), isotope number (0–5), and area (1.0E+04–1.0E+10, median = 1.4E+06). The three most common peptides were NPFIFK, FANAQPQQR, and NQKQSYF; they came from vicilins and provicilins. They likely represent favored hydrolysis sites. In addition, 348 peptides with the following modifications were identified: loss of an ammonia, usually via vicinal dehydration, ammonia rearrangement, and rehydration via ammonia release, resulting in the loss of nitrogen without any gain in oxygen (MOD:01160); oxygenation of an L-methionine residue to form a diastereomeric L-methionine sulfoxide residue (MOD:00719); replacement of a residue amino or amino hydrogen with an acetyl group (MOD:00408); and formation of a double bond via the removal of a water molecule from a residue (MOD:00704) (Jupp *et al.*, 2015).

The 3,005 peptides had origins in a wide range of proteins from three main groups (Fig. 3.2.2.2.): storage proteins (45%), enzymes (23%), and proteins derived from seed metabolism (32%). Within these groups, only the proteins with the most peptides are illustrated. The others have been grouped according to their functions. The majority of the peptides came from storage proteins and more specifically, from vicilins (18%), convicilins (4%), and legumins (11%). This result is not surprising since protein isolates are mainly composed of the latter three protein types (Crevieu-Gabriel, 1999).

It was interesting to note the presence of peptides from proteins associated with sensory off-notes. There were large quantities of peptides from lipoxygenases (7%), which catalyze the degradation of polyunsaturated fatty acids; the latter are thought to play an important role in the development of undesirable off-flavors in pulses. The initial products of lipoxygenase activity are hydroperoxides, which are further degraded into a wide range of compounds, including many that are responsible for off-flavors, such as hexanal and n-pentylfuran (Roland *et al.*, 2017). In addition, peptides from aldehyde dehydrogenase were observed. This enzyme catalyzes the oxidation of aldehydes and so can modify the composition of volatile compounds of pea protein solutions and so the sensations of beaniness. Peptides from protein that catalyzes phenolic acids modifications were observed: carotenoid cleavage

dioxygenase, chalcone synthase, gibberellin dioxygenase, and isoflavone synthase were present. Phenolics acids play also a role in the development of undesirable off-flavors in pulse (bitter and astringent notes).

The peptides displaying modifications were generally associated with three types of proteins: lipoxygenases (10% of modified peptides), histones (12% of modified peptides), and ribosomal proteins (5% of modified peptides). Peptides from these protein types could probably more sensitive to modifications during the pea processing.

Thus, these results show that a wide variety of peptides were identified. These peptides represent proteins from different families, mainly seed storage proteins. Clarifying the origin of these peptides also gives us information about the proteins present in the isolates, including which proteins may cause sensory perceptions (e.g., the lipoxygenases).

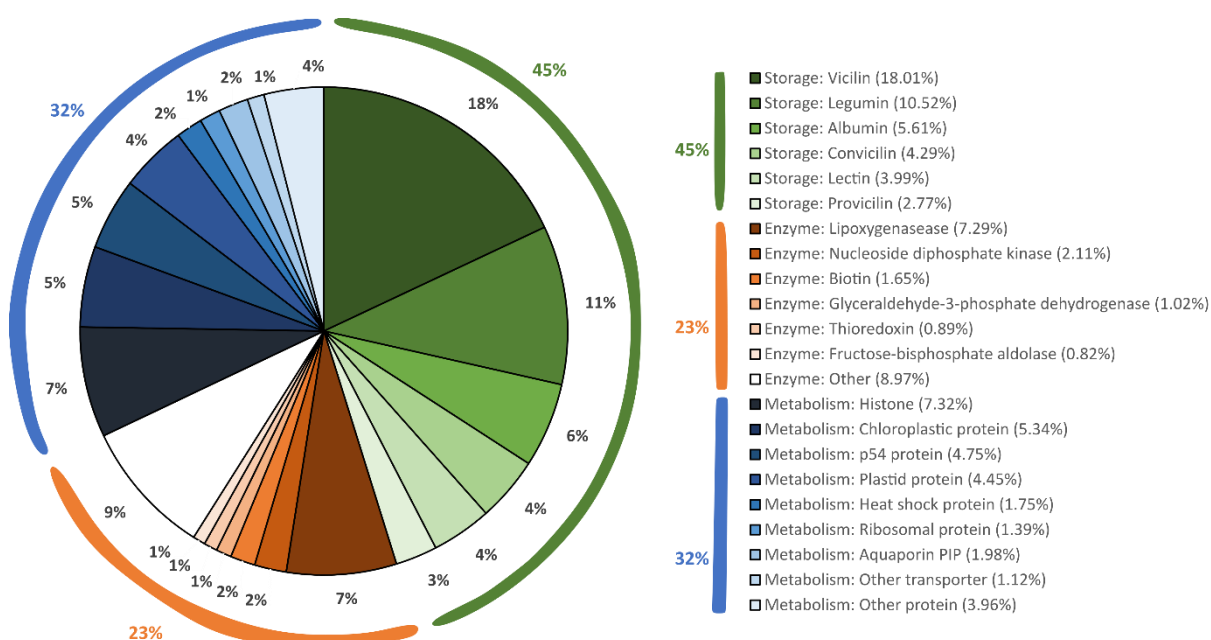


Figure 3.2.2.2.: Categorization of the 3,005 unique peptides identified via UHPLC-MS/MS based on protein origin (threshold for peptide number: 24).

Physicochemical properties of the peptides in the pea protein solutions

The peptides' physicochemical and antioxidative properties were characterized. The nine physicochemical properties were chosen with a view to comprehensively describing pea peptide diversity. The normalized distributions of the property values for the peptides are in Figure 3.2.2.3. The peptides were mostly polar and hydrophilic. The mean GRAVY index value was around -0.5, which is also the overall mean value for the 20 standard amino acids (i.e., -0.49; Kyte & Doolittle, 1982). The median net charge was close to zero. Mean bulk was around 15 Å, which is also the overall mean for the 20 standard amino acids (i.e., 15.4 Å; Zimmerman *et al.*, 1968). In terms of amino acid composition, the peptides had more aliphatic amino acids (Ala, Val, Leu, and Ile) than aromatic amino acids (His, Phe, Trp, and Tyr) or acidic amino acids (Asp and Glu). Finally, average length was 10 residues, although this observation should be interpreted with caution given the specificities of the analytical pipeline. Indeed, the upper limit on length was defined by the purification process and, more specifically, by the ultrafiltration steps; the lower limit on length (no peptides < 6 residues were detected) was a direct consequence of the chosen MS detection range (300–1,600 m/z). Thus, these results show that the main peptides in the pea protein isolates varied greatly in their physicochemical properties; however, when the overall averages were obtained, they generally corresponded to the averages for the 20 standard amino acids.

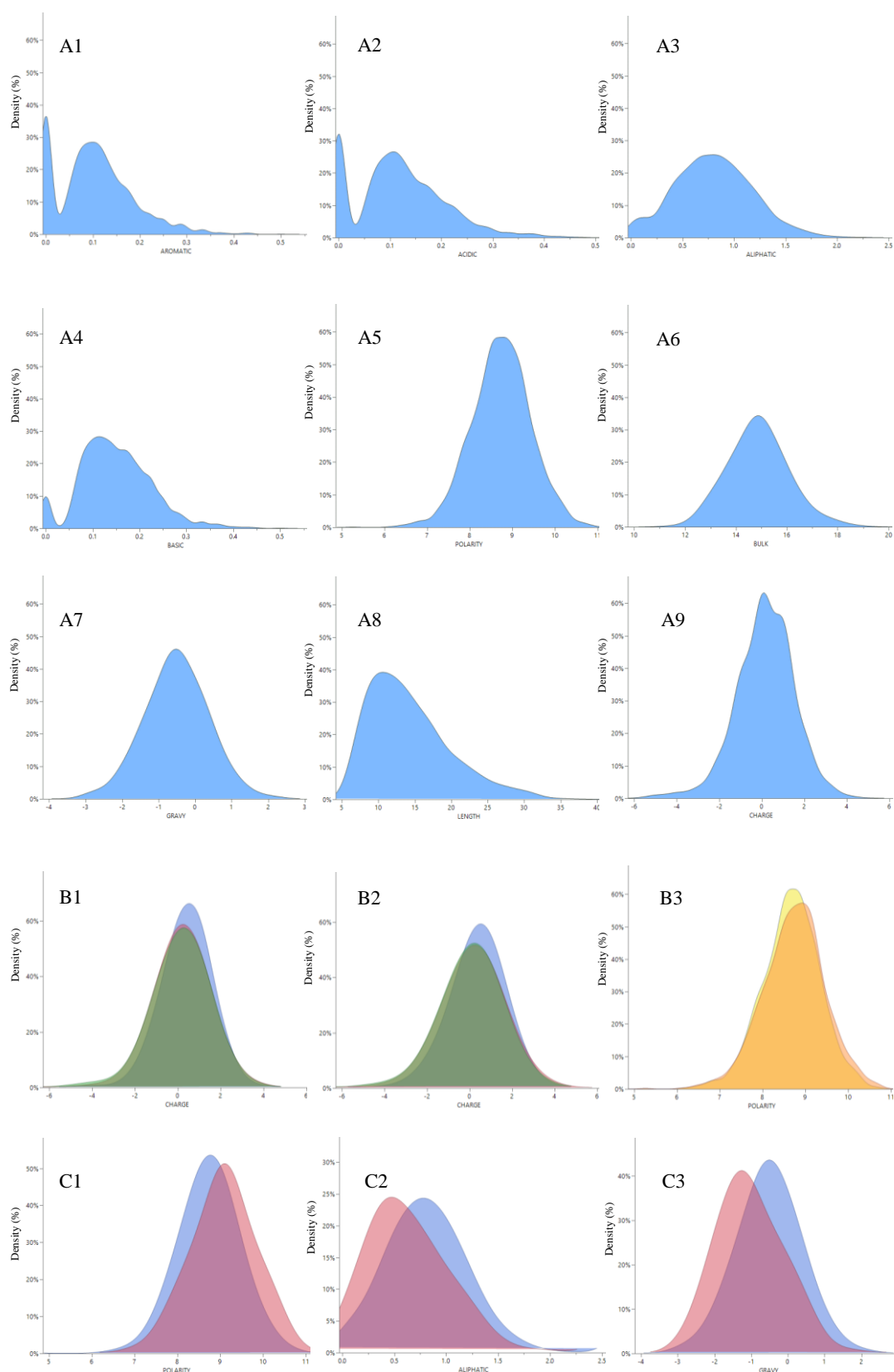


Figure 3.2.2.3: A) Distribution of the overall 3,561 peptides on the 28 solutions (normalized distribution, kernel density): A1 = Relative aromatic nature; A2 = Relative acidic nature; A3 = Aliphatic index; A4 = Relative basic nature; A5 = Polarity; A6 = Bulk; A7 = GRAVY index; A8 = Length; A9 = Charge.

B) Comparison of distributions of the overall 3,561 peptides (normalized distribution, kernel density): B1 = Charge with 50Ib-50W in blue; 100Rb in green and 100Pb in red; B2 = Charge with 50Ia-50W in blue; 100Ra in green and 100Pa in red; B3 = Polarity with Refa in yellow and Refb in orange.

C) Comparison of distributions of the overall peptides (3,561 peptides in blue) and for the peptides correlated to bitterness (275 peptides in red) on the 28 solutions (normalized distribution, kernel density): C1 = Polarity; C2 = Aliphatic index; C3 = GRAVY index.

In addition, some of the identified peptides matched with antioxidant peptides observed in pea and included in BIOPEP database (see Table 3.2.2.1.). Bioactive peptides are usually 2–20 amino acids long and have molecular masses of less than 6 kDa (Sarmadi & Ismail, 2010; Sun *et al.*, 2004). Here, eleven peptides had sequences that were homologous with those of known antioxidant peptides (BIOPEP database) previously identified in pea-protein-based solutions (Iwaniak *et al.*, 2016): ADGF; ADVFNPR; ELLI; FVPH; HLHP; KFPE; LPILR; SAEHGSLH; SGAF; YLKT; and YVGD. These peptides contained many copies of phenylalanine, an amino acid known to mediate antioxidant activity (Sarmadi & Ismail, 2010). They came from different proteins—storage proteins such as legumins; enzymes such as seed linoleate 9S-lipoxygenase-3; and metabolic proteins such as transporters. These results highlight that the diversity of peptides present in pea-protein-based solutions may have nutritional benefits and could be used to enhance the value of plant-based foods. Peptide composition should be studied further from a nutritional point of view.

Table 3.2.2.1. Peptides identified in the pea protein solutions that had sequences homologous to those of previously described antioxidant peptides (BIOPEP database) (Iwaniak *et al.*, 2016).

Antioxidative sequence	Protein	Peptide identified in this study
ADGF	Lectin	SYNVADGFTFF
		VINAPNSYNVADGFT
		VINAPNSYNVADGFTF
		VINAPNSYNVADGFTFF
ADVFNPR	Legumin L1 beta chain	HEDLAGSSQADVFNPRAGRIT
		HEDLAGSSQADVFNPRAGRITSVN
		HEDLAGSSQADVFNPRAGRITSVNSLT
		HEDLAGSSQADVFNPRAGRITSVNSLT
		HEDLAGSSQADVFNPRAGRITSVNSLTLPVLK
		HEDLAGSSQADVFNPRAGRITSVNSLTLPVLKL
		LKLHEDLAGSSQADVFNPRAGRITSVN
		LKLHEDLAGSSQADVFNPRAGRITSVNSLT
ELLI	Histone H3.2	STELLIR
FVPH	PsRT17-1	VFVPHIRTLGD
		VFVPHIRTLGDA
FVPH and SAEHGSLH	Legumin A2	SAEHGSLHKHAM(MOD:00719)FVPH
		SAEHGSLHKHAM(MOD:00719)FVPHY
HLHP	Sucrose transport protein	QLSGAFKELKRP(MOD:00719)W
KFPE	PIP1-2	M(MOD:00719,MOD:00408)EAKEEDVSLGANKFPERQPIG
		M(MOD:00719,MOD:00408)EAKEQDVSLGANKFPERQPLG
KFPE	PIP-type 7a	M(MOD:00408)EAKEQDVSLGANKFPERQPLG
LPILR	Legumin (Minor small)	LPILRN
		LPILRNL
		SGAGRISTVNSLTLPILR
		SGAGRISTVNSLTLPILRN
		SGAGRISTVNSLTLPILRNL
SGAF	Malate dehydrogenase	Q(MOD:01160)RIARISAHLHPSN
YLKT	Seed linoleate 9S-lipoxygenase-3	VKSPQKAYLKTITP
		VKSPQKAYLKTITPKFQT
		YLKTITP
YVGD	Actin-3	AYVGDEAQSKRGILT

Impact of fractionation and recombination on peptide profiles

Impact of fractionation on peptide profiles

This study adopted an original approach: the decision was made to work with fractions instead of compounds because i) we had no a priori hypothesis on which compounds would be linked to perceptions and ii) from a sensory point of view working with all compounds (e.g. with molecular

fractionation and omission tests) can be very long and difficult. We broke down the pea protein isolates into six fractions (two pellets, two retentates, and two permeates), which were then recombined to form different solutions using a mixture design. Before studying the recombined solutions, we studied the impact of fractionation on peptide profile composition.

The overall characteristics of the six fractions and the two raw solutions are presented in Table 3.2.2.2. The number of peptides per fraction was linearly correlated with the sum of the areas of the peptides (Pearson method; $R^2 = 0.83$). The permeates (100Pa and 100Pb) contained the greatest number of peptides, followed by the raw solutions (Refa and Refb). The pellets (50Ia-50W and 50Ib-50W) had the lowest number of peptides. Solutions from pea protein isolate b (Refb, retentate 100Rb, permeate 100Pb and pellet 50Ib-50W) had fewer identified peptides overall than solutions from pea protein isolate a (Refa, retentate 100Ra, permeate 100Pa and pellet 50Ia-50W). These differences could come from the processing of the two commercial products. A perspective to this work could be to study and identify the step (or steps) of the processing that generates these differences in peptide composition.

To visualize the intersections in peptide sets among the six fractions and the two raw solutions (Rfa and Refb), an UpSet plot was used (Fig. 3.2.2.4.). An higher number of peptides were observed in solutions from Refa (permeate 100Pa, retentate 100Ra, refa and then pellet 50Ia-50W) than in the respective products from Refb (permeate 100Pb, retentate 100Rb, refb and pellet b). However, there were more peptides in the raw solution from Refb than in the raw solution from Refa. Thus, the two pea protein isolates were not impacted in the same way by the pea processing: it would appear that more specific peptides were “lost” from isolate b.

To understand the effect of fractionation on the peptide profiles of the fractions, we examined the relationship between the fractions’ physical characteristics (Table 3.2.2.2.) and the sum of the areas of the peptides. There was not a significant correlation between peptide area and either dry matter content, protein content, ash content, pH, or surface hydrophobicity. However, there was a significant linear correlation with conductivity (Pearson method; $R^2 = 0.84$). Peptides (e.g., salts, which drive conductivity) are rather soluble and small in size. During the centrifugation step, they must have mostly gone into the supernatant, and then, during the filtration step, they must have passed into the permeate. Protein content and conductivity were slightly higher in isolate a than in isolate b. It can be assumed that these properties explain the higher peptide concentrations in the fractions from batch a.

The peptides’ physicochemical properties showed similar normalized distributions across fractions (Fig. 3.2.2.3.). The only notable differences occurred in charge between the pellets (50Ib-50W and 50Ia-50W) and the permeates (100Pb and 100Pa) and in the polarity between the raw solutions (Refa versus Refb). The peptides in the permeate solutions varied slightly more in charge. The peptides in the raw solution Refb were slightly more polar. Therefore, the fractionation process did not lead to peptide profiles that differed in physicochemical properties. As for the eleven peptides with sequences homologous with those of known antioxidant peptides, they occurred across the range of solutions. They were, however, most common in the raw solutions (Refa and Refb).

Table 3.2.2.2.: Overall characteristics of the six fractions and the two raw solutions.

Products	Number of peptides identified	Sum of peptide area	Dry matter content (% w/w)	Protein content (%)	Ash content (%)	Conductivity (mS/cm) at 20°C	pH at 20°C	Surface hydrophobicity index
100Pa	2586	1.31E+11	0.20	0.04	0.07	1.44	8.4	363
100Pb	1756	4.92E+10	0.20	0.04	0.04	1.16	9.3	298
100Ra	2376	7.05E+10	1.70	1.41	0.15	1.08	7.5	933
100Rb	1551	2.98E+10	1.70	1.48	0.12	0.88	7.5	1269
50Ia-50W	1565	3.72E+10	6.00	4.91	0.18	1.06	7.5	2083
50Ib-50W	809	9.26E+09	6.00	5.10	0.19	0.84	7.5	2172
Refa	2235	7.13E+10	94.00	79.05	4.14	1.09	7.5	2961
Refb	1488	2.26E+10	93.70	80.68	3.84	1.01	7.5	3504

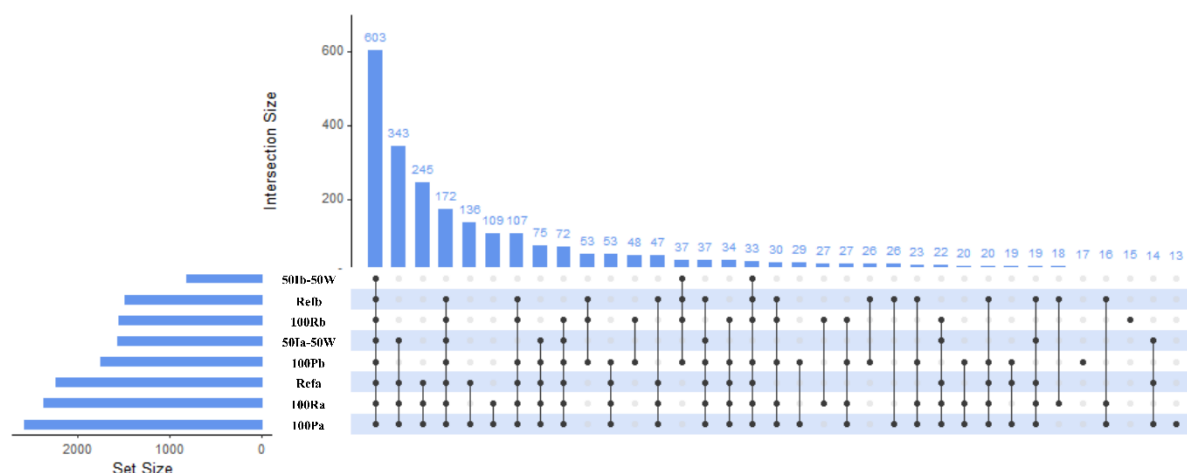


Figure 3.2.2.4.: Depiction of the intersections in peptide sets among the six fractions and the two raw solutions (UpSet plot). The blue horizontal bars show the number of peptides in each fraction/solution. The black dots and lines show the combinations of peptides that make up each cluster or subset of the fractions/solutions. The vertical histogram shows the number of peptides in each subset.

Peptide profiles of the recombined solutions

A mixture design was used to create a suite of solutions by combining the pea protein fractions in different ways. To validate this methodology, the peptide profiles of six of the recombined solutions (50Pb-25Ib-25W, 25Pa-25Ra-13Ia-38W, 70Pb-30Ra, 40Ra-30Ib-30W, 50Ra-25Ia-25W, and 50Pb-50Rb) were determined; the results were compared with the peptide profiles that were calculated using the fraction-based approach. Considering the number of values to be compared (3,561 peptides \times 6 solutions), we did not contrast the individual values of the recombined solutions but rather the distributions of the differences between their measured and calculated values. These distributions were compared to the distributions for replicates of the experimental replicate solutions (100Ra, 100Rb, Refa and Refb). The quartiles were calculated excluding any null values. The quartiles for repeated solutions were: 1st quartile—4.39E+05, median—1.18E+06, 3rd quartile—2.39E+06, and maximum—6.11E+08. The differences between the quartiles for the measured versus calculated values were as follows: 1st quartile—7.19E+05, median—1.65E+06, 3rd quartile—4.22E+06, and maximum—1.47E+09. The overall distributions were similar between replicate solutions and the data for the recombined solutions. The quartiles were slightly lower in the case of the former, but the orders of magnitude were similar. In the case of some peptides, there were significant differences between the measured and calculated values for the recombined solutions. However, these peptides were among those with the largest areas, and the relative differences were therefore small. In conclusion, it appeared that peptide profiles could be reliably estimated for the recombined solutions using fraction-based calculations.

PCA was used to visually assess the main differences among the recombined solutions (Fig. 3.2.2.5). The solutions were well distributed along axes F1 and F2, which accounted for 71.8% of the variance. Thus, the maps based on the first two axes seemed to provide a good-quality projection of the initial multidimensional table, even though some information might have remained hidden in the subsequent axes. The data for the areas of the 3,561 peptides were clustered within one half of the correlation circles and were thus clearly correlated. Overall, peptide concentrations increased from water solution (X, lower left) to the more permeate-based solutions (100 Pb and 100Pa; X upper/right). The solutions formulated with fractions from batch a (Fig. 3.2.2.5: in green) and the solutions formulated with fractions from batch b (Fig. 3.2.2.5: in blue) stand out clearly. Batch a variability is mainly found on axis 1, and batch b variability is mainly found on axis 2. Solutions formulated with fractions from both batch a and b are in the middle (Fig. 3.2.2.5: in orange). Regardless of the batch, permeates (100Pa and 100Pb) had the highest peptide concentrations. The pellets (50Ia-50W and 50Ib-50W) had the lowest peptide concentrations. The raw solutions (Refa and Refb) had intermediate peptide concentrations.

Consequently, with this experimental design, we have managed to create two different ranges of peptide concentrations. The peptides in fraction mixtures (batch a and batch b) allowed us to explore any interactions.

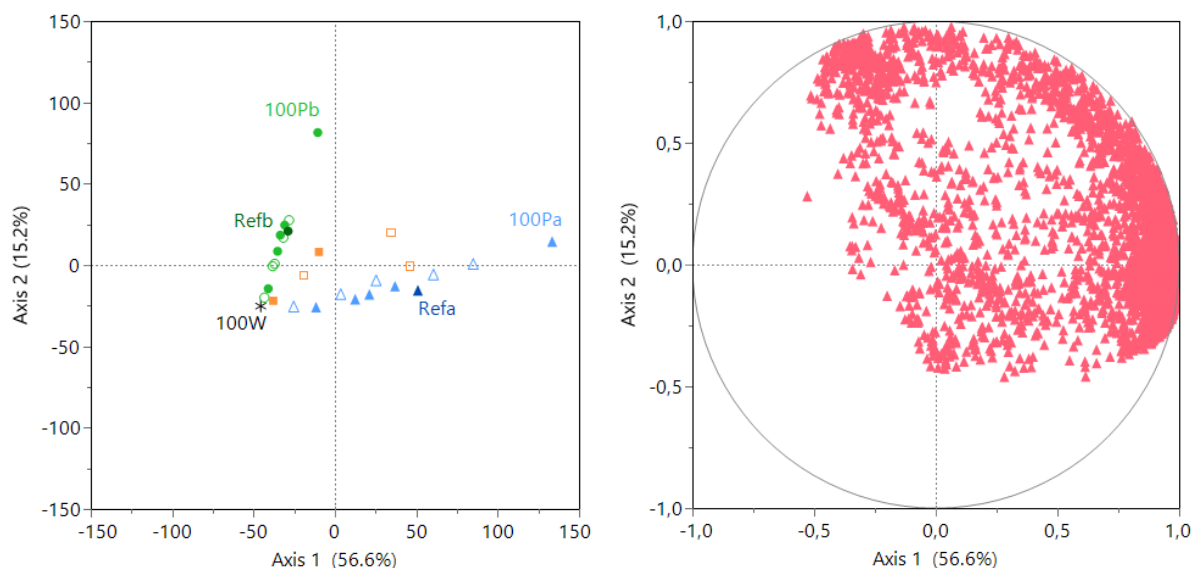


Figure 3.2.2.5.: Results of the principal component analysis (PCA, wide method) examining the peptide profiles for the 28 solutions, which were determined using a fraction-based formulation strategy and the peptides that had been identified (based on 3,561 peptides). On the left is a loading plot showing the correlational relationships between the PCA axes 1 and 2 and the peptide areas. On the right is a PCA plot with the same two axes that shows the relative similarity in the solutions' peptide profiles. The green circles are the recombined solutions created from batch b. The blue triangles are the recombined solutions created from batch a. The orange squares are the recombined solutions created from batch a and batch b. The dark star is the water solution. The solid symbols represent the measured values, and the empty symbols represent the calculated values.

Identifying factors influencing perceived bitterness

Sensory properties of the recombined solutions

The 6 different fractions were combined in various ways to formulate 28 pea protein solutions. These solutions had been used by Cosson *et al.*, in a previous sensory study to obtain greater insight into the origin of perceived beaniness (expressed via the following attributes: almond, broth, cereals, nuts, pea, and potato), bitterness, and astringency in pea-protein-based foods. Cosson *et al.*, found that the attributes contributing to perceived beaniness were mainly influenced by the retentate and permeate fractions, likely because of their levels of volatiles, which were indirectly reflected by hexanal levels. Perceived astringency was mainly influenced by the retentate and pellet fractions, while perceived bitterness was largely driven by the retentate fraction. Bitterness and astringency were associated with the levels of phenolics, which were indirectly reflected by caffeic acid content. However, this previous study concluded that a more detailed analysis of solution composition (i.e., beyond hexanal and caffeic acid levels) would be needed to uncover the more precise origins of these sensory perceptions (Cosson *et al.*, 2021).

Drawing upon the results of this previous study, the peptide profiles of the pea protein solutions were examined in tandem with the sensory profile data. The correlations between peptide areas and sensory scores were evaluated (Pearson method). Each sensory attribute (score out of 10) was correlated with the areas of several peptides (p-value < 0.05). Correlations were most common for the broth attribute (1,640 out of 3,561 peptides), followed by the salty attribute (1,277 out of 3,561 peptides). In contrast, correlations with other attributes were significantly less frequent: bitter—275 peptides; astringent—173 peptides, mouthfeel—410 peptides, pea—440 peptides, potato—246 peptides, almond—80 peptides,

nuts—135 peptides, and cereals—214 peptides. We also compared the peptides from this study with the sensory peptides listed in the BIOPEP database (Iwaniak *et al.*, 2016), but there were no noteworthy findings.

Perceived saltiness can arise from the presence of peptides with charged terminals and/or charged side chains (Temussi, 2012). However, the phenomenon can also have an indirect cause: NaCl and peptides (small soluble molecules) are likely distributed in a similar way in pea protein fractions. Furthermore, a higher salt concentration can also change how protein hydrolysis or peptide fractionation plays out, resulting in different peptide concentrations (Cheison & Kulozik, 2017).

Broth notes may be perceived when peptides have activated T1R1/T1R3 umami receptors. Indeed, umami is often described as a meaty, broth-like, or savory taste and can participate in perceived brothiness (Lioe *et al.*, 2010). Such peptides are between 2 and 11 residues long. For example, Glu-Gly-Ser-Glu-Ala-Pro-Asp-Gly-Ser-Ser-Arg was found to elicit the sensation of umami during the consumption of peanut hydrolysate (Su *et al.*, 2012). However, the idea that some peptides activate umami receptors is controversial because when such peptides are synthesized, they do not always elicit umami (Maehashi *et al.*, 1999). Another explanation could be that the sensation of umami is a consequence of the peptides' partial hydrolysis, which results in sizeable concentrations of Asp or Glu (Temussi, 2012; Wang *et al.*, 2020). Considering the number of peptides associated with the broth note (36% of the peptides), several mechanisms are likely at work. In any case, peptides appear to play a major role in the construction of brothiness.

Relationships between peptide presence and bitterness

Although bitterness is correlated with a much smaller number of peptides, it is important to discuss this sensation as well. Indeed, the bitterness of pea-protein-based foods is a major off-note in these products (Roland *et al.*, 2017). Here, among the 275 peptides correlated with bitterness, 106 were exclusively correlated with bitterness. Many peptides have the ability to activate bitter receptors (Aubes-Dufau *et al.*, 1995). Based on past research, such peptides are between 5 and 8 residues long (Aubes-Dufau *et al.*, 1995; Maehashi & Huang, 2009). Here, however, only 14 of the peptides associated with bitterness were less than 8 residues in length: SRNPIY, KRHGEW, NLQNYR, SNKFGKF, NQKQSYF, YLKGLKF, YQKSTEL, APHWNIN, AQPLQRE, ISLNKIRL, NQKQSYFA, ANAQPLQR, NAQPLQRE, and EVLSWSFH. The peptides KRHGEW, NQKQSYF, NAQPLQRE, and AQPLQRE are particularly noteworthy because they were positively correlated with the bitterest solutions. In contrast, the peptides YLKGLKF, YQKSTEL, and EVLSWSFH were negatively correlated with bitterness. The correlations are shown on the Figure 3.2.2.6.

Using BitterX software (Huang *et al.*, 2016), it was found that these eight peptides were highly likely to activate bitter receptors (either TA2R7 or T2R40; probability: 88–79%). However, to confirm that these peptides contribute to perceived bitterness in the mouth, it would be necessary to study their effects on bitter receptors in vitro or to have a sensory panel evaluate them in solution. It would also be useful to assess their concentrations relative to their perception thresholds. For example, Toelstede and Hofmann (2008) found that 12 peptides eliciting bitter sensations had recognition thresholds between 0.05 and 6.0 mmol/L.

In addition, while other groups of peptides correlated with sensory perceptions had largely overlapping characteristics, bitterness-related peptides displayed certain differences (Fig. 3.2.2.3), such as lower GRAVY values (i.e., are more hydrophilic on average), lower aliphatic values (i.e., have smaller relative volumes on average), and higher polarity (i.e., are more polar on average). These results do not concur with the results of previous studies, which have shown that such peptides are mainly hydrophobic (Kim *et al.*, 2008). In conclusion, these peptides may affect sensations of bitterness in the mouth by activating bitter receptors (i.e., the peptides displaying positive correlations), blocking bitter receptors (i.e., the peptides displaying negative correlations), or interacting with other molecules that do either (i.e., the peptides displaying positive or negative correlations).

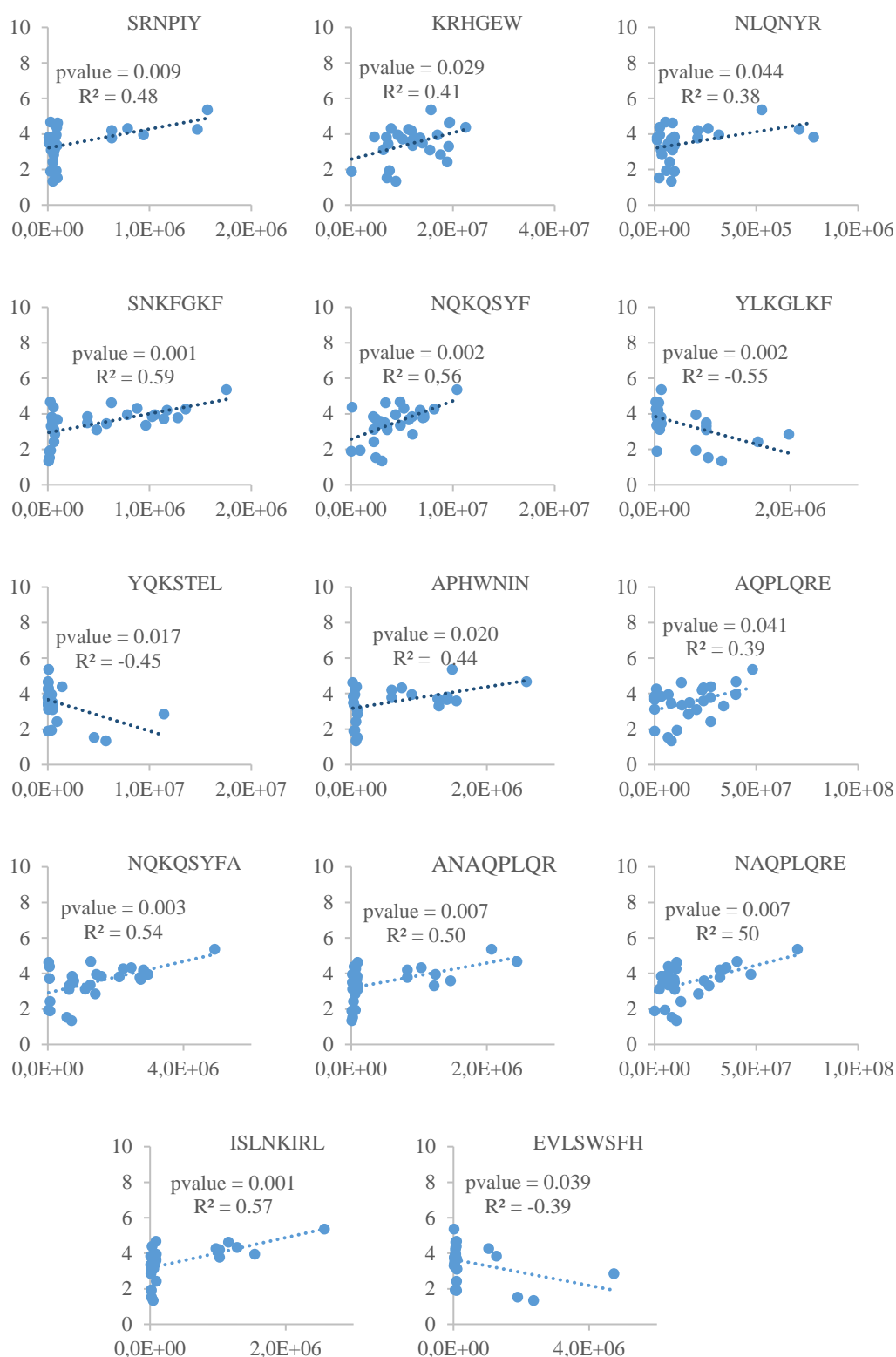


Figure 3.2.2.6.: Peptides (size < 8 residues) correlated with perceived bitterness (score out of 10); the R² values and p-values from the Pearson's correlational analysis are indicated.

3.2.2.4. Conclusion

In this study, we identified and characterized the main oligopeptides and polypeptides (5–40 amino acids long) found in pea protein solutions. We had four main findings. First, we identified a wide variety of peptides representing a range of protein families, mainly those containing seed storage proteins but also those containing proteins that can play a role in sensory perceptions, such as lipoxygenases. Second, these peptides were mostly polar and hydrophilic, and our fraction-based formulation strategy did not affect their overall physicochemical properties. Third, eleven peptides had sequences homologous with those of known antioxidant peptides. These results indicate that the variety of peptides present in pea protein solutions can have nutritional benefits. Fourth, most of the peptides in the pea protein solutions were correlated with sensory attributes. In particular, many peptides were correlated with salty and broth attributes, perhaps expressing the relationship of some peptides to umami. A lower but still significant number of peptides displayed a correlation with bitterness. These results highlight the mechanistic importance of these molecules in sensory perceptions in the mouth. Taken together, these results suggest that a better understanding of the peptide composition of plant protein isolates could help us address related sensory issues and develop plant-protein-based foods whose taste appeals more to consumers.

Main results

- 3,005 unique peptides were identified in the pea protein solutions.
- 45% of these peptides came from seed storage proteins, mainly vicilin proteins.
- Among them, 11 peptides displayed sequence homology with known antioxidants.
- As regards links with perceptions, 1,640 peptides were associated with broth scores, perhaps reflecting umami perceptions.
- In addition, 14 peptides were associated with bitter scores and were likely to activate bitter receptors.

Section 3.2.3. – Identification and quantification of main phytochemicals from pea protein isolates and first links with perception

3.2.3.1. Introduction

For several years now, one of the major challenges for the agri-food industry has been to develop new protein sources to compensate for the future lack of animal proteins. In this context, plant proteins are receiving much attention from industry and consumers. Plant protein isolates such as pea (*L. Pisum sativum*) are increasingly used as food ingredients because of their functional properties, their protein content, their good environmental sustainability and their attractive price. However, plant proteins and especially the isolate fractions of plant raw materials, possess sensory drawbacks, including olfactory and sapid off-notes which impede the development of plant protein-based foods for human consumption. Research on pea-based food products has largely focused on the role of volatile aroma compounds in creating beaniness perception. But knowledge explaining the bitterness and astringency of these products remains partial, as few studies have been carried out in comparison of beaniness perceptions.

In recent years, it has been proposed that a wide variety of phytochemicals could be responsible for the bitter and astringent perception of pea-protein isolates. In particular lipids, saponins and phenolic compounds were highlighted in the literature. Bitter lipid oxidation products that are formed either through enzymatic pathways or by autooxidation have been considered. Recently, Gläser *et al.* identified 14 lipids and lipid oxidation products on pea protein isolate fractions with high bitter taste (Gläser *et al.*, 2020). Saponins have also been associated with a bitter-astringent perception in pea protein isolates. In particular, two types of saponins have been reported: soyasaponin I and DDMP saponins (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) (Heng *et al.*, 2006; Reim & Rohn, 2015). Gläser *et al.* studied the influence of soyasaponin I on the overall bitter and astringent perceptions of pea protein isolates by calculating the Dose-over Threshold factor (ratio of the concentration to the taste threshold of the respective taste active substance). For bitterness, values were below the taste threshold indicating that the tested pea-protein isolate had limited impact whereas for astringency, they concluded that the concentration of the soyasaponin I exceeded its astringent taste threshold by a factor of 1.8. However, to our knowledge, there is no detailed studies on the composition, the content and the impact of other pea saponins on taste perceptions of pea products.

For phenolic compounds, studies on their composition and content in pea protein isolates are scarce with few links to perceptions. However, several phenolic acids were described with bitter-astringent perception on other types of plant materials. For example, caffeic acid and o-caffeoylquinic acids have been reported in coffee (Frank *et al.*, 2006) and hydroxybenzoic acids, hydroxycinnamic acids and their ester derivatives in wine (Hufnagel & Hofmann, 2008). Several flavonoids have also been associated with bitter-astringent perceptions. For example, catechin and gallic acid have been reported in red wine (Robichaud & Noble, 1990); flavan-3-ols and flavonol glycosides in cocoa (Stark *et al.*, 2006). In addition, in yellow pea, a total of 115 different structural phenolic compounds have been described in different studies (Fahim *et al.*, 2019; Neugart *et al.*, 2015; Stanisavljevic *et al.*, 2015). These phenolics were mainly glycosylated flavonols and their related compounds such as flavanols, anthocyanins, isoflavonoids (Fahim *et al.*, 2019). A large number of 3-O-glycosides of kaempferol and quercetin were also identified and quantified in pea (Neugart *et al.*, 2015; Stanisavljevic *et al.*, 2015). These compounds could therefore potentially also participate in bitterness and astringency perceptions of pea protein isolates.

Thus, the aim of this study is to identify the key phytochemicals (phenolic acids, flavonoids and saponins) from pea protein isolates and to identify links explaining sensory properties. To this end, pea flour, pea protein isolates and pea protein isolate fractions were studied. The phytochemicals profiles of the pea products were determined by UHPLC-DAD-MS. Then, the impacts of isolates and their fractions

on phytochemicals profile were studied. Finally, key phytochemicals compounds were correlated to bitterness and astringency properties of pea products.

3.2.3.2. Materials and methods

Chemical reagents

Based on literature, 12 phenolic compounds were used as standards : Kaempferol-3-O-glucoside (CAS. 480-10-4, grade analytical standard), Soyasapogenol B (CAS. 595-15-3, grade $\geq 98\%$ HPLC), Caffeic acid (CAS. 331-39-5, grade $\geq 98.0\%$ HPLC), (+)-Catechin (CAS. 225937-10-0, grade $\geq 98\%$ HPLC), trans-Ferulic acid (CAS. 537-98-4, grade $\geq 99.0\%$ HPLC), Gallic acid (CAS. 149-91-7, grade 97.5-102.5% titration), Naringin (CAS. 10236-47-2, grade $\geq 95\%$ HPLC), trans-p-Coumaric acid (CAS. 501-98-4, grade $\geq 98.0\%$ HPLC), p-Hydroxybenzoic acid (CAS. 99-96-7, grade $\geq 99\%$ FG), Quercetin-3-O-glucoside (CAS. 482-35-9, grade $\geq 90\%$ HPLC), Quercetin-3-O-rutinoside, (CAS. 207671-50-9, grade $\geq 94\%$ HPLC) and Sinapic acid (CAS. 530-59-6, grade $\geq 98\%$) were purchased from Sigma Aldrich. Acetonitrile quality Optima for HPLC-MS, Water quality Optima for HPLC-MS, Formic acid quality Optima for HPLC-MS and Methanol quality Optima for HPLC-MS were purchased from Thermo Fisher. Solutions were filtered before UHPLC analysis through PTFE filters of 13mm, porosity 0.22 μm purchased from Fisherbrand. Stock solutions of single analytes and working solutions were prepared and immediately stored in glass vial at -80°C (Vial N9 1.5 mL, 11.6x32 mm, MACHEREY-NAGEL GmbH & Co. KG).

Pea Products

Pea flour and two pea protein isolates (80% protein content Nx6.25, 83% dry matter ?) were obtained from Roquette Frères (Lestrem, France). In addition, six fractions were obtained from the two pea protein isolates, by centrifugation and membrane filtration, as explained by Cosson *et al.*, (2021 – Section 3.1.2.): pellets *a* and *b*, permeates *a* and *b*, and retentates *a* and *b*. All fractionation steps were carried out at 4°C , and stored at -20°C in 50 mL glass flasks. Dry matter content was determined via drying (prepASH®219 analysis system) and the 9 pea products obtained had the following dry matter: 92% for flour, 93.7% for isolates *a* and *b*, 6.18% for retentate *a* and *b*, 1.7% for retentate *a* and *b*, 0.2% for permeate *a* and *b*.

Preparation of standards and pea samples

Preparation of standard solutions

Solutions of 0.1 g/L of each standard were prepared in methanol/water (70/30 V/V). For Quercetin-3-O-glucoside and Quercetin-3-O-rutinoside, solubilisation was helped by 15min of sonication. Then a 0.01g/L stock solution of a mixture of all standards was prepared in methanol/water (70/30 V/V). Dilution of the stock solution with methanol yielded the working solution at concentrations of 0.05 to 15 ng/ μL for p-Hydroxybenzoic acid and of 1 to 10 ng/ μL for the other references. All stock and working solutions were filtered through a 0.22 μm PTFE filter and stored in the dark at -80°C until analysis. Calibration curves were obtained by plotting the peak areas of the compounds as a function of the concentration of the standard solution with TraceFinder software (Thermo Scientific, USA). Calibration curves were linear with R^2 values exceeding 0.99. The limit of detection (DL) was of 0.02 ng/ μL and the limit of quantification (QL) was of 8.0 ng/ μL ; except for Soyasapogenol B where DL = 0.07 ng/g and QL = 3 ng/g, and except for p-Hydroxybenzoic acid where DL = 0.05 ng/g and QL = 30.0 ng/g.

Preparation of pea samples with a high dry matter content (powders and pellets)

Samples with a high dry matter content (flour, isolates *a* and *b*, pellets *a* and *b*) (6.0g) were placed in 150 mL glass vials (Schott vial, Dutscher France) and extracted three times with 30 ml of methanol/formic acid mixture (99/1, V/V) for 2h with a magnetic stirrer at room temperature, protected from light. After extraction, supernatants were kept for 10 min at -20°C then separated by centrifugation at 4500 rpm for 20 min at 4°C (Eppendorf 5804R). The supernatant was evaporated under vacuum at 40°C and in the dark to protect sample from light (Evaporator vacuum concentrators, Jouan Thermo Electron Corporation, RC 1022). In order to remove some precipitates occurring during the concentration step, a centrifugation was performed before total evaporation of solvent (4500 rpm for 20

min at 4°C). After that, supernatants were evaporated under vacuum at 40°C protected from light until the solvent has evaporated completely. After solubilisation, in 1 mL of methanol/water mixture (80/20, V/V), samples were kept for 10 min at -20°C to promote precipitation. At that point, samples were separated by centrifugation at 4500 rpm for 20 min at 4°C. Supernatants were filtered through a 0.22 µm PTFE filter and stored in the dark at -80°C previous to analysis. All these steps of filtration and centrifugation were added to ensure obtaining a clear extract without any precipitate. Samples were prepared in triplicate.

Preparation of pea samples with a low dry matter content (permeates and retentates)

Samples with a low dry matter content (retentate a and b, permeate a and b) were prepared as explained previously except for the first step (extraction step): 6.0 g of samples were mixed to 1 ml of methanol/formic acid mixture (99/1, V/V) for 10 min at room temperature, protected from light.

Ultra-high performance liquid chromatography (UHPLC) – mass spectrometry (MS) analysis of phytochemical compounds

Chromatographic separations were performed using UHPLC system (Ultimate 3000 Thermo Scientific, USA). The analytical column used for separations was a Hypersil GOLD (100mmx2.1mmx1.9µm, Thermo Scientific). The mobile phase consisted of (A) water + 0.1% formic acid and (B) acetonitrile + 0.1% formic acid (Optima, Thermo Fisher Scientific). A gradient program at a flow rate of 0.25ml/min was used: 4 min 98% of (A), linear gradient from 98% to 70% of (A) in 26 min, linear gradient from 70% to 2% of (A) in 6 min, 9 min 2% of (A). The initial gradient pressure was 280 bar. The injection volume was 5µL and the injector temperature 7°C. The oven temperature was 25°C.

The UHPLC system was coupled to a Q Exactive Orbitrap high resolution mass spectrometer (Thermo Scientific, USA) equipped with heated-electrospray ionization probe (HESI II, Thermo Scientific, USA). The mass spectrometer was operated in negative and in positive ion modes. Parameters of the ion source were as follows: sheath gas pressure 2.4E5 Pa, auxiliary gas flow rate 10, no sweep gas flow rate, spray voltage 3KV, capillary temperature 300°C, S-lens RF Level 50V and heater temperature 300°C. MS spectra (MS and MS²) were acquired by full MS and Full MS/ddMS² range (m/z) from 85 to 1000 amu the resolution was 70000 and 17500 respectively. The system was also coupled to a diode array detector covering full range acquisition between 190 and 600 nm.

Phytochemicals were identified according to the corresponding spectral characteristics: mass spectra, accurate mass, characteristic fragmentation, UV spectrum and characteristic retention time. Xcalibur (Thermo Scientific, USA) was used for instrument control, data acquisition, and data analysis. Phytochemical quantification was obtained by integration of specific reconstructing ion current in comparison with the calibration curves with TraceFinder software (Thermo Scientific, USA). Compound discoverer (Thermo Scientific, USA) with several databases (PubChem, Phenol explorer, Flavonoid database, Arita database, NPASS database) were used to propose formula and identification of peaks.

Sensory analysis

Pea solutions were characterized by static profiling by 17 trained panellists (13 women and 4 men; mean age = 23 years old). First attribute selection were done with the help of check-all-that-apply (CATA) questionnaire. Secondly, the panellists were trained to evaluate the intensity of these attributes along an unstructured scale (range: 0–10) using external references. Thirdly, the attributes were evaluated in blocks. The first attribute block focused on olfactory perception evaluated by nose (attribute block 1: pea, broth, nuts, almond, potato and cereals). The second block focused on taste perception and mouthfeel (attribute block 2: salty, sugar, bitter, astringent, mouthfeel, and granularity). The last attribute block focused again on olfactory perception but solutions were evaluated in mouth (attribute block 3: pea, broth, nuts, almond, potato and cereals). Each solution was evaluated in duplicate by the 17 judges. Complementary details on attribute selection, panellist training and method characterization can be found in the companion papers Cosson *et al.*, 2021 (Section 3.1.2.). Here, only the bitter and astringent attributes evaluated by mouth will be discussed.

Statistical analysis

Analyses were performed using R (R Core Team, 2019) and JMP (v. 13.1.0; SAS Institute Inc., Cary, SC, USA). For analyses of an inferential nature, $\alpha = 0.05$ was the threshold for statistical significance. To visually explore differences in the results of phytochemicals profile between the nine pea products, we carried out principal component analysis (centred-reduced, Pearson n) on a correlation matrix. Phytochemicals were also investigated in relation with the sensory attributes scores of the solutions. For that, Pearson correlation matrix was performed to study links between concentration on phytochemicals and sensory scores (bitterness and astringency).

3.2.3.3. Results and discussion

Identification of phytochemicals in pea samples

This research provides an exploratory identification of main soluble phytochemicals from pea flour, pea protein isolates and pea fractions. The analysis of the materials obtained by UHPLC-DAD-MS allowed the tentative identification of a total of fifty-four compounds. These identified compounds are either native seed compounds or reaction products formed during the processes. By comparing retention times and accurate MS and MS2 data with those of reference standards, eleven compounds were unambiguously identified: two hydroxybenzoic acids, four hydroxycinnamic acids, three flavonols, two flavanols and one saponin. In addition, based on UV-vis, accurate MS and MS2, web database and literature data, nine compounds were tentatively identified as phenolic acids, ten as flavonoids, six as terpenoids and one from other family. Table 3.2.3.1 includes information about the retention times, assigned identities, UV-vis absorption, molecular formula, accurate masses, main MS data and web database for individual compounds.

Identification of phenolic acids

Standard solutions of Gallic acid (peak n°1), p-Hydroxybenzoic acid (peak n°5), Caffeic acid (peak n°8), trans-p-Coumaric acid (peak n°11), trans-Ferulic acid (peak n°12) and Sinapic acid (peak n°13) were used and analysed with ESI source in positive and negative ion modes. ESI source in negative ion mode showed better sensitivity. In addition, nine phenolic acids were tentatively identified and will be discussed in the following paragraphs in negative ion mode.

The peak n°2 presented two absorption bands characteristic of phenolic acids, one at 241sh-257 and one at 293 nm. It displayed a major molecular ion at m/z 299.0773. The fragment at m/z 137.0235 was observed on its MS2 spectrum which may correspond to the presence of a hydroxybenzoic moiety and the loss of a hexoside residu (-162). The fragment at m/z 93.0334 was also observed related to the loss of carboxylic acid function (-44). It was thus tentatively identified as p-Hydroxybenzoic hexoside or isomer. The peak n°3 presented also two absorption bands at 256 nm and at 293 nm. It displayed a major molecular ion at m/z 153.0183. The fragment ion at m/z 109.0284 was observed on its MS2 spectra which may correspond to the loss of COO. It was so tentatively identified as Protocatechuic acid, previously reported in pea (Klejdus *et al.*, 2008; Singh *et al.*, 2017).

Table 3.2.3.1.: Phytochemicals identified in pea flour, pea protein isolates and/or pea fractions with: peak number, RT [min], UV (nm), [M+H]⁺ MS1, [M+H]⁺ MS2, [M-H]⁻ MS1, [M-H]⁻ MS2, molecular weight theoretical, [M+H]⁺, theoretical, [M-H]⁻ theoretical, formula hypothesis, tentative of identification and database used for the identification.

Peak number	RT [min]	UV (nm)	[M-H] ⁺ MS1	[M+H] ⁺ MS2	[M-H] ⁻ MS1	[M-H] ⁻ MS2	Formula hypothesis	[M+H] ⁺ expected	Error (ppm)	[M-H] ⁻ expected	Error (ppm)	Classes hypothesis	Compound hypothesis	Database	Relative quantification
Phenolic acids															
n°1	3.05	253; 278	171.0291	NA	169.0134	125.0234	C ₇ H ₆ O ₅	171.0288	-1.8	169.0142	5	Hydroxybenzoic acid	Gallic acid	Standard	NEG
n°2	4.43	241sh, 257, 293	301.1021	NA	299.0773	137.0235; 93.0334	C ₁₃ H ₁₆ O ₈	301.0918	-34.2	299.0772	-0.2	Hydroxybenzoic acid	p-Hydroxybenzoic hexoside or isomer	Artia	NEG
n°3	4.45	256; 293	155.0339	NA	153.0183	109.0284	C ₇ H ₆ O ₄	155.0339	-0.1	153.0193	6.7	Hydroxybenzoic acid	Protocatechuric acid	Artia	NEG
n°4	8.71	254	254.0658	NA	252.0512	137.0235 (hydroxybenzoic – H ⁺); 132.0293 (aspartic acid – H ⁺); 93.0334 (phenol moiety)	C ₁₁ H ₁₁ NO ₆	254.0659	0.4	252.0514	0.6	Hydroxybenzoic acid	N ^p -Hydroxybenzoyl aspartic acid	PubChem	NEG
n°5	11.7	257	139.039	NA	137.0234	93.0334	C ₇ H ₆ O ₃	139.039	-0.2	137.0244	7.4	Hydroxybenzoic acid	p-Hydroxybenzoic acid	Standard	NEG
n°6	13	271; 330	343.1023	NA	341.0874	135.0442 (caffeic acid – CO ₂ – H ⁺); 179.0343 (caffeic acid – H ⁺)	C ₁₅ H ₁₈ O ₉	343.1024	0.2	341.0878	1.2	Hydroxycinnamic acid	Caffeoyl hexoside	PubChem	NEG
n°7	15.47	295	327.1085	NA	325.0928	163.0396 (cinnamic acid – H ⁺); 119.0492 (cinnamic acid – CO ₂ – H ⁺)	C ₁₅ H ₁₈ O ₈	327.1074	-3.2	325.0929	0.3	Hydroxycinnamic acid	Coumaroyl-4-O-hexoside	KnapSack	NEG
n°8	15.61	320sh	181.0496	NA	179.0342	135.0442	C ₉ H ₈ O ₄	181.0495	-0.4	179.035	4.4	Hydroxycinnamic acid	Caffeic acid	Standard	NEG
n°9	16.13	295; 320	280.0812	NA	278.067	132.0292 (aspartic acid – H ⁺); 162.0552 (loss of succinic acid)	C ₁₃ H ₁₃ NO ₆	280.0816	1.3	278.067	0	Hydroxycinnamic acid	N-Coumaroyl aspartic acid	NA	NEG
n°10	17.7	293sh, 320	310.0916	NA	308.076	132.0292 (aspartic acid – H ⁺); 193.0502 (ferric acid – H ⁺)	C ₁₄ H ₁₅ NO ₇	310.0921	1.7	308.0776	-0.1	Hydroxycinnamic acid	N-Feruloyl aspartic acid derivative	NA	NEG
n°11	19.34	220; 280	165.0546	NA	163.0391	119.0491	C ₉ H ₈ O ₃	165.0546	0.1	163.0401	5.9	Hydroxycinnamic acid	Trans-p-coumaric acid	Standard	NEG
n°12	21.08	223; 324	195.0651	NA	193.05	134.0364; 178.0265; 149.0597	C ₁₀ H ₁₀ O ₄	195.0652	0.4	193.0506	3.3	Hydroxycinnamic acid	Trans-ferric acid	Standard	NEG
n°13	21.44	223; 325	225.0757	NA	223.0608	208.0372	C ₁₁ H ₁₂ O ₅	225.0757	0.2	223.0612	1.8	Hydroxycinnamic acid	Sinapic acid	Standard	NEG
n°14	16.02	230	252.0864	NA	250.072	132.0293 (aspartic acid – H ⁺); 135.0442 (phenylacetic acid – H ⁺)	C ₁₂ H ₁₃ NO ₅	252.0866	1	250.0721	0.4	Phenylacetic acid	N-Phenylacetyl aspartic acid derivative	NA	NEG
n°15	9.34	250	268.0814	NA	266.067	132.0293 (aspartic acid – H ⁺)	C ₁₂ H ₁₃ NO ₆	268.0816	0.6	266.067	0	Other phenolic acid	Aspartic acid derivative	PubChem	NEG
Flavonoids															
n°16	21.55	266; 347	773.2117	287.0545 (kaempferol)	771.1978	609.1436 (kaempferol + 2 hexoses); 429.0825; 327.0507; 284.0237 (kaempferol); 285.0393 (kaempferol); 255.0297; 771.2020	C ₃₃ H ₄₀ O ₂₁	773.2135	2.3	771.1989	1.5	Flavonol (kaempferol derivative)	Kaempferol sophorotrioxide	Artia. KnapSack	NEG
n°17	22.13	345	755.201	287.0545 (kaempferol)	753.1879	193.0501; 255.0297; 285.0405 (kaempferol); 609.1436 (kaempferol + 2 hexoses)	C ₃₃ H ₃₈ O ₂₀	755.2029	2.5	753.1884	0.6	Flavonol (kaempferol derivative)	Kaempferol derivative	Artia	NEG

n°18	22.27	258; 345	595.1647	287.0545 (kaempferol)	593.151	488.1147; 389.2183; 285.0405 (kaempferol); 255.0297	C27H30O15	595.1657	1.8	593.1512	0.3	Flavonol (kaempferol derivative)	Kaempferol derivative	Arita	NEG
n°19	23.1	ND	697.1592	287.0545 (kaempferol)	695.1442	488.1147; 389.2183; 285.0405 (kaempferol); 255.0297; 609.1436 (kaempferol + 2 hexoses)	C30H32O19	697.1611	2.7	695.1465	3.3	Flavonol (kaempferol derivative)	Kaempferol malonyl di-hexoside	Arita	NEG
n°20	24.75	266; 347	449.1071	287.0545 (kaempferol)	447.0928	284.0323; 255.0297	C21H20O11	449.1078	1.6	447.0933	1.1	Flavonol (kaempferol derivative)	Kaempferol-3-O-glucoside	Standard	NEG
n°21	25.12	269; 346	979.2695	287.0545 (kaempferol)	977.2558	284.0237 (kaempferol); 255.0297; 609.1436 (kaempferol + 2 hexoses); 771.2020 (kaempferol + 3 hexoses)	C44H50O25	979.2714	1.9	977.2568	1.1	Flavonol (kaempferol derivative)	Kaempferol sinapoyl tri-glucoside	Arita knapsack	NEG
n°22	25.62	ND	949.2592	287.0545 (kaempferol)	947.2452	771.1990 (kaempferol + 3 hexoses); 609.1436 (kaempferol + 2 hexoses); 429.0825; 327.0507; 284.0237 (kaempferol); 255.0297; 300.0277; 389.2183	C43H48O24	949.2608	1.7	947.2463	1.1	Flavonol (kaempferol derivative)	Kaempferol feruloyl tri-hexoside	Arita	NEG
n°23	20.18	256; 267sh, 350	789.2068	303.0497 (quercetin)	787.1926	300.0276 (quercetin); 445.0775 (quercetin + hexose - H ₂ O); 271.0230; 178.9980	C33H40O22	789.2084	2	787.1938	1.6	Flavonol (quercetin derivative)	Quercetin tri-hexoside	knapsack	NEG
n°24	20.41	256; 355	627.154	303.0496 (quercetin)	625.1401	300.0276 (quercetin); 445.0775 (quercetin + hexose - H ₂ O); 271.0230; 178.9980	C27H30O17	627.1556	2.5	625.141	1.5	Flavonol (quercetin derivative)	Quercetin di-hexoside	knapsack	NEG
n°25	22.41	258; 355	611.1589	303.0496 (quercetin)	609.1456	300.0275 (quercetin); 178.9980	C27H30O16	611.1607	2.9	609.1461	0.8	Flavonol (quercetin derivative)	Quercetin-3-O-rutinoside	Standard	NEG
n°26	22.94	258; 355	465.1016	303.0496 (quercetin)	463.0885	300.0274 (quercetin); 271.0230; 178.9980	C21H20O12	465.1028	2.5	463.0882	-0.6	Flavonol (quercetin derivative)	Quercetin-3-O-glucoside	Standard	NEG
n°27	35.95	370	303.0277	NA	301.0352	NA	C15H10O7	303.0499	73.4	301.0354	0.6	Flavonol (quercetin derivative)	Quercetin aglycone	Arita	NEG
n°28	15.02	220; 279	291.086	NA	289.0714	178.998; 271.0612; 151.0389	C15H14O6	291.0863	1	289.0718	1.4	Flavanol	Catechin	Standard	NEG
n°29	25.26	224; 284	581.1872	273.0754	579.1717	271.0612; 151.0389	C27H32O14	581.1865	-1.2	579.1719	0.4	Flavanone	Naringin	Standard	NEG
n°30	23	267; 336	NA	NA	431.0981	269.0456 (apigenin); 164.0448; 271.0628	C21H20O10	433.1129	NA	431.0984	0.6	Flavone (apigenin derivative)	Apigenine-7-O-glucoside	knapsack	NEG

Terpenoids															
n°31	35.9	193- 222	925.5158	85.0291; 141.0183; 365.3195; 423.3616; 581.3833; 441.3723	923.4626	NA	C48H76O17	925.5155	-0.3	923.501	41.6	Saponin	Saponin derivative	knapsack	POS
n°32	36.06	193- 222	797.4666	85.0290; 141.0182; 365.3194; 423.3615; 581.3832; 441.3723	795.4531	NA	C42H68O14	797.4682	2	795.4536	0.7	Saponin	Saponin derivative	knapsack	POS
n°33	36.12	195- 205	943.5251	85.0293; 141.0181; 365.3193; 423.3614; 581.3831; 441.3723	941.5092	NA	C48H78O18	943.5261	1.1	941.5115	2.5	Saponin	Saponin B	Pubchem knapsack	POS
n°34	36.53	228	941.5086	85.0291; 141.0182; 365.3191; 423.3615; 581.3833; 441.3723	939.4941	NA	C48H76O18	941.5104	2	939.4959	1.9	Saponin	Saponin derivative	Knapsack	POS
n°35	37.22	226	971.5193	85.0290; 141.0184; 365.3194; 423.3617; 581.3832	969.5037	NA	C49H78O19	971.521	1.8	969.5065	2.8	Saponin	Saponin derivative	knapsack	POS

n°36	37.6	229	825.4613	85.0288; 141.0180; 365.3192; 423.3615; 581.3831	823.4426	NA	C43H68O15	825.4631	2.2	823.4485	7.2	Saponin	Saponin derivative	Knappsack	POS
n°37	40.2	228	459.3844	441.3723; 423.3618	NA	NA	C30H50O3	459.3833	-2.5	457.3687	NA	Saponin	Soyasapogenol B	Standard	POS
Others															
n°38	10.79	279- 289sh	205.0973	159.0917	203.0821	116.0495; 159.0920	C11H12N2O2	205.0972	-0.7	203.0826	2.5	Amino acid	Tryptophane	Pufochem	NEG
n°39	14.9	214; 280; 320sh	457.2171	NA	455.1774	293.1245; 179.0554	NA	NA	NA	NA	NA	NA	NA	NA	NEG
n°40	18.03	212; 283	389.1794	NA	387.1659	163.1120; 225.1129	NA	389.1806	3.1	387.1661	0.4	NA	NA	NA	NEG
n°41	24.82	193- 218sh	549.2483	NA	547.2394	161.0446; 149.0445; 89.0232	NA	549.2542	10.7	547.2396	0.4	NA	NA	NA	NEG
n°42	26.6	349	741.222	309.2784; 599.5033; 703.5117	739.207	164.0448; 271.0628	NA	741.2237	2.2	739.2091	2.8	NA	NA	NA	NEG
n°43	27	280; 340	591.1696	285.0753	589.1566	149.0446; 283.0612; 178.9980	NA	591.1708	2.1	589.1563	-0.5	NA	NA	NA	NEG
n°44	27.15	193- 218sh; 277	705.2587	NA	703.2447	161.0446; 149.0445; 89.0232	NA	705.26	1.9	703.2455	1.1	NA	NA	NA	NEG
n°45	28.48	250	591.2638	NA	589.2498	161.0446; 149.0445; 89.0232	NA	591.2647	1.6	589.2502	0.6	NA	NA	NA	NEG
n°46	30.39	258	619.2751	NA	617.2639	NA	NA	NA	NA	NA	NA	NA	NA	NA	NEG
n°47	33.38	270; 370	271.0598	NA	269.0453	223.0819; 161.0448	NA	271.0601	1.1	269.0455	0.9	NA	NA	NA	NEG
n°48	36.85	282; 333	313.0711	285.0753; 163.0391; 257.0805	311.0568	267.1967; 134.0463; 271.0612; 283.0627	NA	313.0707	-1.4	311.0561	-2.2	NA	NA	NA	NEG

The peak n°6 presented two adsorption bands at 271 nm and 330 nm. It displayed a major molecular ion at m/z 341.0874. The fragment at m/z 179.0343 was observed on its MS2 spectra which may correspond to [caffeic acid-H⁺] and only one secondary fragment at m/z 135 which may correspond to [caffeic acid-CO₂-H⁺] (Jaiswal *et al.*, 2014). It was thus tentatively identified as Caffeoyl hexoside previously reported in pea leaves (Klopsch *et al.*, 2019; Neugart *et al.*, 2015). The peak n°7 presented an adsorption band at 295 nm. It displayed a major molecular ion at m/z 325.0928. The fragment at m/z 163.0396 was observed on its MS2 spectra which could correspond to [coumaric acid-H⁺] as well as the fragment at m/z 119.0492 which could correspond to [coumaric acid-CO₂-H⁺] (Iswaldi *et al.*, 2013). It was thus tentatively identified as Coumaroyl-4-O-hexoside which has been previously reported in pea leaves (Klopsch *et al.*, 2019).

The peak n°4 presented an absorption bands at 254 nm. It displayed a major molecular ion at m/z 252.0512. Odd mass indicates the presence of an odd number of nitrogen atoms. The fragment at m/z 132.0293 was observed on its MS2 spectra which may correspond to [aspartic acid-H⁺], as well as the fragment at m/z 93.0334 which may correspond to the phenol moiety. It was tentatively identified as N-p-Hydroxybenzoyl aspartic acid, based on the work of Clifford *et al.* on amino acid-bound hydroxycinnamic acids (Clifford *et al.*, 2010). Similarly, the peaks n°9, n°10, n°14 and n°15 displayed odd mass and fragment at m/z 132.0293. The peaks n°9 and n°10 presented two adsorption bands characteristics of hydroxycinnamic acids at 295/293 nm and at 320 nm. The peak n°9 displayed a major molecular ion at m/z 278.067. The fragment ion at m/z 162.0552 could correspond to the loss of succinic acid. It was thus tentatively identified as a N-Coumaroyl aspartic acid derivative (Oracz *et al.*, 2019). The peak n°10 displayed a major molecular ion at m/z 308.0776. The fragment at m/z 193.0502 could correspond to [acide ferulic -H⁺]. It was thus tentatively identified as a N-Feruloyl aspartic acid derivative acid. The peaks n°14 and n°15 presented an absorption band at 250 nm. The peak n°14 displayed a major molecular ion at m/z 250.0720. The fragment at m/z 135.0442 may correspond to [phenylacetic acid -H⁺]. It was tentatively identified as a N-Phenylacetyl aspartic acid derivative. The peak n°15 displayed a major molecular ion at m/z 266.0670. It was tentatively identified as another Aspartic acid derivative.

Identification of flavonoids

Standard solutions of Kaempferol 3-O-glucoside (peak n°20), Quercetin-3-O-rutinoside (peak n°25), Quercetin-3-O-glucoside (peak n°26), Catechin (peak n°28) and Naringin (peak n°29) were studied using ESI source in positive and negative ion modes. ESI source showed better sensitivity in the negative ion mode for these compounds. In addition, ten other flavonoids were tentatively identified and will be discussed in the following paragraphs in negative ion mode. They presented two absorption bands characteristic of flavonoids, i.e. one in the range 211–267 nm corresponding to the phenolic nuclei and the second one in the range 347–373 nm corresponding to the conjugated system (Mabry *et al.*, 1970).

Fragment ions at m/z 287.0545 (positive ion mode) and m/z 284.0237 or 285.0405 (negative ion mode) were observed on the MS2 spectra of peaks n°16 to 22 which could corresponds to the presence of a kaempferol moiety. The peaks n°16, n°17, n°19, n°21 and n°22 displayed a MS2 fragment at m/z 609.1436 which could corresponds to [kaempferol + 2 hexoses]. In addition, the peaks n°21 and n°22 displayed a MS2 fragment at m/z 771.1990 which could corresponds to [kaempferol + 3 hexoses]. Kaempferol derivatives have been reported in pea seed coat (Duenas *et al.*, 2004; Stanisavljevic *et al.*, 2015), in pea seed (Jha *et al.*, 2019), in pea leaves (Klopsch *et al.*, 2019; Neugart *et al.*, 2015) and in pea shoots (Ferrerres *et al.*, 1995; Santos *et al.*, 2014). The peak n°16 displayed a major molecular ion at m/z 771.1978. It was tentatively identified as Kaempferol sophorotrioside which was previously identified by RMN in pea shoots (Ferrerres *et al.*, 1995). The peak n°19 displayed a major molecular ion at m/z 695.1442. From the major molecular ion, it was tentatively identified as Kaempferol malonyl dihexoside. The peak n°22 displayed a major molecular ion at m/z 947.2452. It was tentatively identified as a Kaempferol feruloyl tri-hexoside, by similarity of the Quercetine feruloyl tri-hexoside previously identified by RMN in pea shoots (Ferrerres *et al.*, 1995) and by comparison of spectra with the results of Goupy *et al.* (2013). The peak n°21 displayed a major molecular ion at m/z 977.2558. It was tentatively identified as a Kaempferol sinapoyl tri-hexoside by similarity of the Quercetine sinapoyl tri-hexoside previously identified by RMN in pea shoots (Ferrerres *et al.*, 1995). The peak n°17 displayed a major

molecular ion at m/z 753.1879 and the peak n°18 displayed a major molecular ion at m/z 593.1510. They were tentatively identified as a Kaempferol derivatives.

A fragment ion at m/z 303.0497 (positive ion mode) or at m/z 300.0276 (negative ion mode) was observed on the MS2 spectra for peaks n°23 to 27, which could corresponds to the presence of a quercetin moiety or to a flavone of the same mass. The peaks n°23 and n°24 displayed also a MS2 fragment at m/z 445.0775 which could corresponds to [quercetine + hexose - H₂O]. Like kaempferol derivatives, quercetin derivatives have been reported in pea seed coat (Duenas *et al.*, 2004; Stanisavljevic *et al.*, 2015), in pea seed (Jha *et al.*, 2019), in pea leaves (Klopsch *et al.*, 2019; Neugart *et al.*, 2015) and in pea shoots (Ferrerres *et al.*, 1995; Santos *et al.*, 2014). The peak n°23 displayed a major molecular ion at m/z 787.1926 and presented two absorption bands at 256-267sh nm and at 350 nm. It was tentatively identified as Quercetin tri-hexoside. The peak n°24 displayed a major molecular ion at m/z 625.1401 and presented two absorption bands at 256 nm and at 355 nm. It was tentatively identified as Quercetin di-hexoside. The peak n°27 displayed a major molecular ion at m/z 301.0352, an higher retention time (35.95 min) and one absorption band at 370 nm. It was tentatively identified as the Quercetin aglycone, which has been reported in pea seed coat (Stanisavljevic *et al.*, 2015) and pea seed (Jha *et al.*, 2019).

The peak n°30 presented two absorption bands at 267 nm and 336 nm. It displayed a major molecular ion at m/z 431.0981. A fragment ion at m/z 269.0456 4 was observed on its MS2 spectra which may correspond to the Apigenin moiety and at m/z 164.0448 which may correspond to an hexoside in the O position. It was tentatively identified as Apigenine-7-O-glucoside which was previously identified in pea root with a standard (Šibul *et al.*, 2016).

Identification of terpenoids

Standard solution of Soyasapogenol B (peak n°37) was studied using ESI source in positive and negative ion mode. ESI source in positive ion mode showed better sensitivity for Soyasapogenol B. It presents only one little absorption band with maximum at 228 nm and displayed a major ion at m/z 459.3844 on the MS1 spectra (positive mode). Two fragment ions at m/z 441.3723 and 423.3616 were observed on its MS2 (positive mode).

In addition, seven peaks were tentatively identified as saponins (peak n°31 to peak n°36). The seven peaks showed a better sensitivity in positive ion mode and presented only one little absorption bands with maximum between 193 nm and 229 nm characteristics of saponins (Decroos *et al.*, 2005). Their retention time were also high (between 35.9 and 37.6 min) confirming the hypothesis of very apolar compounds. The same fragment ions at m/z 85.0291, 141.0183, 365.3195, 423.3616, 581.3833 and 441.3723 were observed in their MS2 spectra (positive mode) in favour of compounds from the same family. The fragments m/z 441.3723 and 423.3616 are similar to Soyasapogenol B. The fragments m/z 581.3833 and 423.3616 are similar to DDMP Saponin (Daveby *et al.*, 1998). In addition, saponins have been previously reported in pea seed (Curl *et al.*, 1985; Daveby *et al.*, 1998; Heng *et al.*, 2006; Reim & Rohn, 2015) and the masses found for peaks n°31 to 36 correspond to saponins existing in Knappsack database for *Pisum Sativum*. In particular, the peak n°33 displayed a major molecular ion at m/z 943.5251 on the MS1 spectra (positive mode) and was tentatively identified as Saponin B (Heng *et al.*, 2006).

Finally, a last peak, which did not belong to any of the above families, have been tentatively identified. The peak n°38 displayed a major molecular ion at m/z 203.0821 in the negative ion mode. It presented a characteristics absorption band at 279-289sh nm and was tentatively identified as tryptophan.

Phytochemical profiles of pea products and identification of key bitter-astringent compounds

Quantification of phytochemicals in pea products and impact of processes

In this work, six phenolic acids, five flavonoids and one saponin were used as standards and were quantified in pea flour, pea isolates and pea fractions through their response to UHPLC-DAD-MS and calibration curves. Table 3.2.3.2 shows the concentration of the twelve standards with their standard

deviation. Quercetin-3-O-rutinoside and Catechin were identified but were not quantified due to their very low concentration in pea products. In addition, Gallic acid, Sinapic acid and Quercetin-3-O-glucoside were only quantified in the flour due to their low concentration in other pea products. Overall calculated concentrations were higher in pea flour than in pea isolates except for Kaempferol-3-O-glucoside and Soyasapogenol B for which it is the contrary. For p-Hydroxybenzoic acid, concentration differences were relatively small between protein isolates and flour. In addition, calculated concentrations were higher in pea isolates than in pea fractions except for soyasapogenol B for which concentrations were higher in pellet. It is difficult to explain these concentrations because of the different nature of the samples, due in particular to the different MS rates, and the different extraction protocols.

Concentrations vary between 0 and 2000 ng/g (i.e. 0.002 mg/g) according to the compounds and the pea products. In the literature, there is few quantitative data on phytochemicals of pea flour and pea isolates. Dvorak *et al.*, quantified several phenolic acids in pea samples with concentrations varying between 0 and 0.026 mg/g (Dvořák *et al.*, 2011). During different pea seed processing, the concentration of total phenolic acids in pea varied between 12 to 19 mg/g and total flavonoids contents between 0 to 9 mg/g (Nithiyanantham *et al.*, 2012). As regards saponins in pea flour, DDMP saponin was quantified at 1.5 mg/g (Reim & Rohn, 2015) and between 0.7 to 1.90 mg/g (Heng *et al.*, 2006). Soyasaponin I was also quantified in pea flour between 0.82 to 2.5 mg/g (Curl *et al.*, 1985) and in pea protein isolates to 1.1 mmol/kg, i.e. 1.4 mg/g (Gläser *et al.*, 2020). However, it is complicated to compare these values due to the different raw materials as well as the different extraction and analysis protocols.

Then, in order to be able to compare the products more easily, the areas were related to their dry matter content. In order to study all of the identified compounds, further work was carried out on the peak areas obtained by UHPLC-DAD-MS. Figure 3.2.3.1 shows the results of the principal component analysis (centred-reduced, Pearson n) examining the phytochemical profiles of pea flour, pea isolates and pea fractions (pellets, retentates and permeates). The solutions were well distributed along axes F1 and F2, which accounted for 82.6% of the variance. Thus, maps based on the first two axes seem to provide a good quality projection of the initial multidimensional table. Most of the phytochemical attributes were clustered within one quarter of the correlation circles along axis 1 and thus clearly are correlated. A smaller number of phytochemicals from the different families identified are orthogonal to this majority along axis 2. Related to their dry matter content, the nine products present varied profiles in phytochemicals. Permeates a and b present higher overall peak areas of phytochemicals. Flour presents higher peak areas of flavonoids. Finally, pellets a and b present higher peak areas of terpenoids.

Commercial pea protein isolates undergo significant temperature and pH changes during processing. In particular, pea proteins are usually extracted via isoelectric precipitation in which the pH is decreased to pH 4-5 (Boye *et al.*, 2010). To allow a faster flocculation, the raw extract can also be heated to denature protein (Murat *et al.*, 2013). At the end of the process, protein solutions are dried with drum drying or spray drying methods with a consequent rise in temperature. Flavonoids and most of phenolic acids are highly unstable and easily susceptible to degradation with modifications of pH, temperature, light and enzymes. Thus, overall pulses processes significantly reduce the level of phenolics (Nithiyanantham *et al.*, 2012) as observed in our results (differences between flour and isolates). Nevertheless, synergistic combinations or counteracting of several types of factors can occur, possibly explaining why a minority of phytochemicals present different behaviours. For example, oxidative reactions and formation or breakdown of compounds, leaching of water-soluble compounds and solid losses could occur during processing (Nithiyanantham *et al.*, 2012) as well as protein-compound interactions (Damodaran & Arora, 2013). According to the review of Singh *et al.*, saponins are also easily susceptible to be degraded with modifications of pH and temperature during processing (Singh *et al.*, 2017). In addition, the large number of phytochemicals in the permeates could be explained by the phenomenon of leaching of water-soluble compounds.

Table 3.2.3.2: Concentration of the 12 standards with their standard deviation on 10-3 mg/g (from extraction triplicates). The limit of detection (DL) was of 0.02 ng/μl and the limit of quantification (QL) was of 8.0 ng/μl; except for Soyasapogenol B where DL = 0.07 ng/g and QL = 3 ng/g, and except for p-Hydroxybenzoic acid where DL = 0.05 ng/g and QL = 30.0 ng/g.

Compounds	Galllic acid	p-Hydroxybenzoic acid	Caffeic acid	Coumaric acid	Ferulic acid	Sinapic acid	Kaempferol-3-O-glucoside	Quercetin-3-O-rutinoside	Quercetin-3-O-glucoside	Catechin	Naringin	Soyasapogenol B
<i>Peak number</i>	<i>n°1</i>	<i>n°2</i>	<i>n°8</i>	<i>n°11</i>	<i>n°12</i>	<i>n°13</i>	<i>n°20</i>	<i>n°25</i>	<i>n°26</i>	<i>n°28</i>	<i>n°29</i>	<i>n°37</i>
Isolate b	QL	1265.9 ± 6.9	DL	3.0 ± 1.7	3.0 ± 1.7	DL	108.9 ± 1.7	QL	QL	QL	5.6 ± 1.7	91.0 ± 10.0
Permeate a	QL	1682.0 ± 56.8	QL	8.0 ± 1.7	8.4 ± 1.7	DL	105.9 ± 1.7	QL	QL	QL	10.2 ± 1.7	93.9 ± 13.42
Permeate b	QL	170.8 ± 6.4	QL	DL	DL	QL	DL	QL	QL	QL	QL	QL
Pellet b	QL	258.5 ± 1.8	QL	2.0 ± 1.7	DL	QL	DL	QL	QL	QL	QL	QL
Pellet a	QL	72.0 ± 6.8	3.1 ± 2.3	DL	DL	QL	29.0 ± 1.7	QL	QL	QL	QL	175.6 ± 15.4
Retentate b	QL	126.5 ± 4.4	DL	1.7 ± 1.7	2.3 ± 1.7	QL	41.7 ± 1.7	QL	QL	QL	QL	188.6 ± 41.6
Retentate a	QL	54.5 ± 6.2	5.8 ± 2.1	DL	DL	QL	2.3 ± 1.7	QL	QL	QL	QL	9.4 ± 10.0
Flour	16.08 ± 1.08	1999.0 ± 18.4	90.7 ± 2.4	124.0 ± 2.2	151.3 ± 1.8	44.49 ± 1.7	62.9 ± 11.1	QL	14.8 ± 1.7	QL	81.7 ± 3.1	48.4 ± 10.0

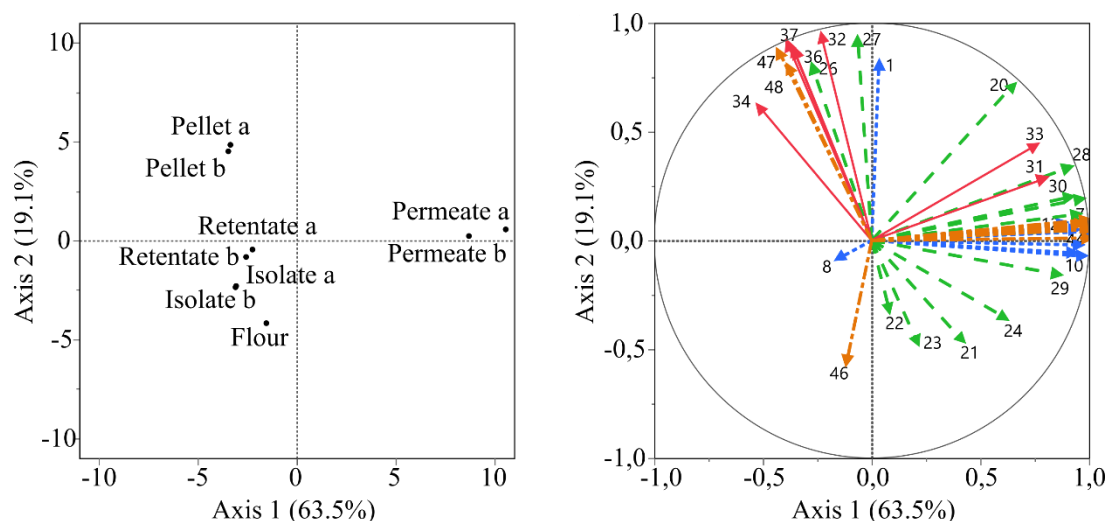


Figure 3.2.3.1.: Principal component analysis (centred-reduced, Pearson n) examining the phytochemicals profiles of pea flour, pea isolates and pea fractions (pellets, retentates and permeates). On the right is a loading plot showing the correlational relationships between PCA axes 1 and 2 and the peak area (related to the dry matter content of products) of the 48 identified phytochemicals: phenolic acids are in blue (dotted line), terpenoids are in red (solid line), flavonoids are in green (dashed line) and other compounds are in orange (densely dashdotted line). On the left is a PCA plot with the same two axes that shows the relative similarity of the 9 pea products.

Identification of the phytochemicals underlying bitterness and astringency

Phytochemical profiles of the 6 fractions were correlated to their bitterness and astringent profiles. Bitterness and astringency profiles were analysed and discussed by Cosson *et al.* (2021). However, the main results are recalled here and the Figure 3.2.3.2 summarize the results presenting the scores for the two isolates (4% dry matter), the two permeates, the two retentates and the two pellets (6% dry matter) as well as the results of the stepwise multiple comparisons procedure (Newman-Keuls test, pvalue 0.05). For the bitterness attribute, retentates and isolates showed higher scores; permeates have the lowest scores; and the products from batch a have overall lower scores for bitterness. For the astringent attribute, the differences between the products are less pronounced: pellet b has the highest score and permeates have the lowest scores.

Correlation coefficients (Pearson method) were calculated between phytochemical areas and sensory scores (p-value of 0.05). The products evaluated in sensory analysis and used for phytochemical analysis measurements were identical. According to psychophysics curves, the perception of a compound in a product depend on its concentration (Chambers & Koppel, 2013). Indeed the links between chemical compounds and sensory intensities can be linear (in supra liminar part, i.e. above threshold) or non-linear (in liminar part i.e. below threshold). Thus we chose to evaluate both linear and logarithmic correlations to cover both situations. Table 3.2.3.3 shows the correlation coefficients (Pearson method) between phytochemical compounds (UPHLC-MS peak area) and bitter and astringent intensities (scores/10) for linear and logarithmic models. In addition, linear correlations with bitter or astringent attributes are presented in Figure 3.2.3.3. Results showed that a total of eleven phenolic acids, two flavonoids, six saponins and ten non identified compounds were significantly correlated with perceptions.

Among phenolic acids, Caffeic acid (peak n°8) is correlated positively to bitterness ($R^2=0.90$) and to astringency ($R^2=0.87$). Caffeic acid is known in the literature to exhibit a very bitter persistent perception in plant products such as coffee (Frank *et al.*, 2006).

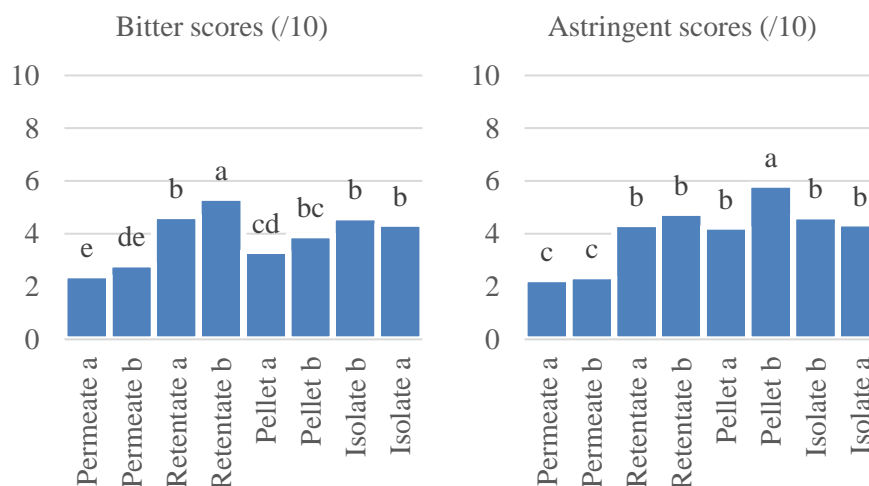


Figure 3.2.3.2.: Concentrations of bitter and astringent attributes (scores/10) as evaluated by a static profiling by a trained panel (Cosson *et al.*, 2021). Results of the stepwise multiple comparisons procedure (Newman-Keuls test, pvalue 0.05). Significant differences between groups are indicated by differences in letters.

In addition, Caffeoyl hexoside (peak n°6) is negatively correlated to bitter-astringent perceptions. Caffeic acid may be more specific to bitter receptors than their glucoside counterparts. The degradation of caffeic acid derivatives may lead to an increase in the concentration of free caffeic acid. As regards the other phenolic acids, p-Hydroxybenzoic hexoside (peak n°2), Protocatechuic acid (peak n°3), N-p-Hydroxybenzoyl aspartic acid (peak n°4), p-Hydroxybenzoic acid (peak n°5), Coumaroyl-4-O-hexoside (peak n°7), N-Phenylacetyl aspartic acid derivative (peak n°14) and Aspartic acid derivative (peak n°15) are negatively correlated to astringency. The peaks n°5, n°15 and n°4 are also negatively correlated to bitterness. Finally, N-Coumaroyl aspartic acid derivative (peak n°9) and N-Feruloyl aspartic acid derivative (peak n°10) are negatively correlated to bitterness. In the literature, a series of hydroxybenzoic acid ethyl esters and hydroxycinnamic acid ethyl esters were identified as bitter compounds in wine (Hufnagel & Hofmann, 2008) and a series of N-phenylpropenoyl-L-amino acids have been identified as key astringent compounds of roasted cocoa (Stark *et al.*, 2006). However, these ethyl esters are less polar than acids which could explain their bitterness. In addition, several hydroxycinnamic acids were studied as precursors of off-flavours in fruits (Naim *et al.*, 1992). Thus, these negative correlations may also be explained by the release of derivative compounds responsible for bitter-astringent perceptions and not identified here. However, as regards their threshold values, various values are reported in the literature according to the matrix. For example for chlorogenic, caffeic and p-coumaric acids, threshold values were reported in water between 90 and 40 mg/L, in beer between 690 and 520 mg/L and in wine between 10 and 32 mg/L (Boulet *et al.*, 2017). This is in any case much higher than the concentrations of these compounds in the isolates.

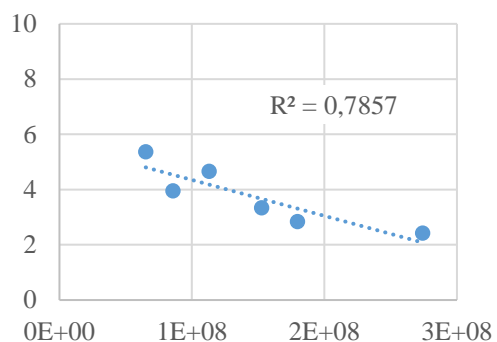
Among the flavonoids, a Kaempferol derivative (peak n°17) is negatively correlated to bitterness and astringency. Quercetin-3-O-glucoside (peak n°26) is positively correlated to astringency. Flavonoids are described in the literature as contributing to bitterness and astringency. However, the majority of these studies deals with polyphenols in red wine (Hufnagel & Hofmann, 2008). Regarding their structure, it seems that the conformation of flavonoid compounds plays a role in the activation of bitterness receptors. (+)-catechin would activate the TAS2R14 and TAS2R39 receptors while one of these isomers (-)-epicatechin would activate the TAS2R4, TAS2R5, TAS2R14 & TAS2R39 receptors (Roland *et al.*, 2017). Moreover, the molecular size of the polyphenol compounds would also play a role in the activation of bitterness receptors. Polymer with a high degree of polymerization would tend to be less bitter and more astringent whereas polymers with a low degree of polymerization would tend to be bitterer and less astringent (Sun *et al.*, 2007). In addition, the presence of galloyl groups on epicatechin can modify receptor activation (Yamazaki *et al.*, 2013) and the aglycone isomers of isoflavones, being more hydrophobic, tend to be more adapted to receptors than their glucoside counterparts. Thus, the

negative correlations could be explained by the presence of derivatives of peak n°17 (more or less glycolysed forms for example) not identified here which would play a role in bitter-astringent perceptions. The other identified flavonoids are not correlate with perceptions. However, threshold values between 0.1 and 20 mg/L have been reported for various flavones in red wine (Sáenz-Navajas *et al.*, 2010). These concentrations are much higher than the concentrations of these compounds in the isolates.

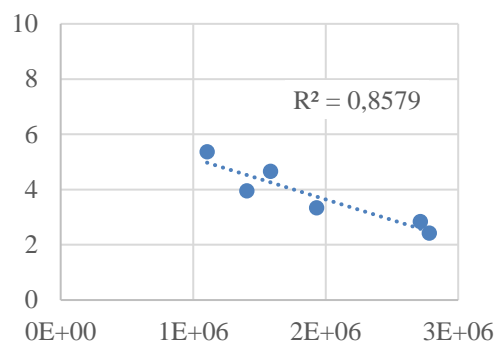
Table 3.2.3.3.: Correlations coefficients (Pearson method) between phytochemical compounds (LC-MS peak area) and bitter and astringent attributes (score/10) for linear model and logarithmic model. In bold, negative correlations coefficients. Only significative values are indicated (pvalue<0.05).

Family hypothesis	Peak number	Compound hypothesis	Linear model - Bitter		Linear model - Astringent		Logarithmic model - Bitter		Logarithmic model - Astringent	
			pvalue	R ²	pvalue	R ²	pvalue	R ²	pvalue	R ²
Phenolic acids	n°2	p-Hydroxybenzoic hexoside	NA	NA	<0.01	-0.94	NA	NA	<0.01	-0.96
	n°3	Protocatechuic acid	NA	NA	<0.01	-0.96	NA	NA	<0.01	-0.99
	n°4	N-p-Hydroxybenzoyl aspartic acid	0.04	-0.83	<0.01	-0.95	0.03	-0.85	<0.01	-0.95
	n°5	p-Hydroxybenzoic acid	0.02	-0.89	0.03	-0.87	0.01	-0.92	0.03	-0.86
	n°6	Caffeoyl hexoside	0.02	-0.88	0.01	-0.91	0.02	-0.89	0.01	-0.90
	n°7	Coumaroyl-4-O-hexoside	NA	NA	<0.01	-0.96	NA	NA	<0.01	-0.98
	n°8	Caffeic acid	0.02	0.90	NA	NA	NA	NA	0.02	0.87
	n°9	N-Coumaroyl aspartic acid derivative	0.02	-0.88	NA	NA	0.01	-0.94	NA	NA
	n°10	N-Feruloyl aspartic acid derivative	NA	NA	NA	NA	0.05	-0.82	NA	NA
	n°14	N-Phenylacetyl aspartic acid derivative	NA	NA	0.01	-0.93	NA	NA	<0.01	-0.97
	n°15	Aspartic acid derivative	0.03	-0.84	<0.01	-0.95	0.02	-0.87	<0.01	-0.95
Flavonoids	n°17	Kaempferol derivative	0.01	-0.93	0.01	-0.92	0.01	-0.94	0.02	-0.88
	n°26	Quercetin-3-O-glucoside	NA	NA	NA	NA	NA	NA	0.03	0.85
Terpenoids	n°31	Saponin derivative	NA	NA	0.02	0.88	NA	NA	0.01	0.92
	n°32	Saponin derivative	NA	NA	NA	NA	NA	NA	0.02	0.88
	n°33	Saponin B	NA	NA	NA	NA	NA	NA	0.02	0.90
	n°34	Saponin derivative	NA	NA	NA	NA	NA	NA	0.01	0.92
	n°35	Saponin derivative	NA	NA	NA	NA	NA	NA	0.04	0.83
	n°37	Soyasapogenol B	NA	NA	NA	NA	NA	NA	0.02	0.88
Others	n°39	NA	NA	NA	<0.01	-0.95	NA	NA	<0.01	-0.96
	n°42	NA	0.02	-0.90	0.01	-0.92	0.01	-0.90	0.01	-0.90
	n°43	NA	NA	NA	NA	NA	0.05	-0.81	NA	NA
	n°40	NA	NA	NA	<0.01	-0.97	NA	NA	<0.01	-0.97
	n°41	NA	<0.01	-0.97	0.03	-0.84	<0.01	-0.98	NA	NA
	n°44	NA	NA	NA	<0.01	-0.97	NA	NA	NA	NA
	n°45	NA	NA	NA	<0.01	-0.96	NA	NA	NA	NA
	n°46	NA	NA	NA	0.04	0.84	NA	NA	NA	NA
	n°47	NA	NA	NA	NA	NA	NA	NA	0.02	0.88
	n°48	NA	NA	NA	NA	NA	NA	NA	0.03	0.86

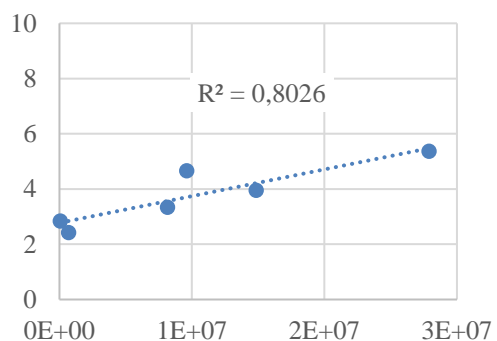
Peak n°5 (p-Hydroxybenzoic acid) in function of bitter scores



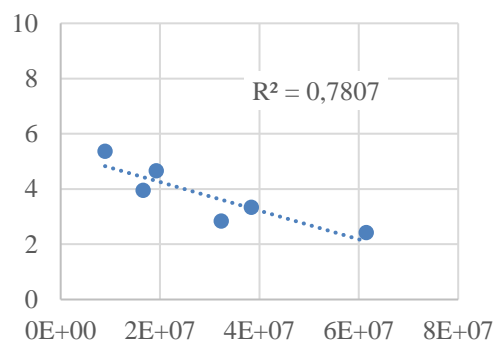
Peak n°17 (Kaempferol derivative) in function of bitter scores

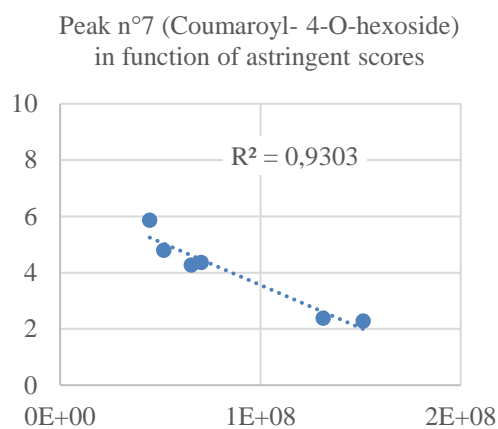
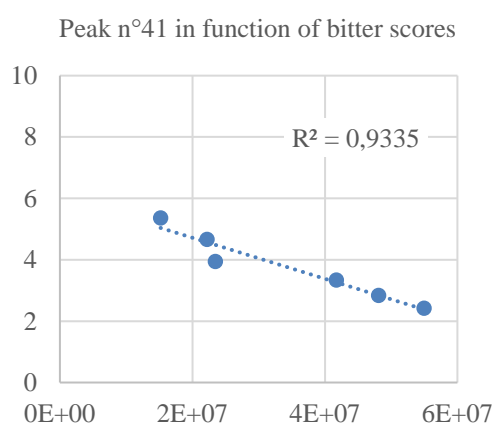
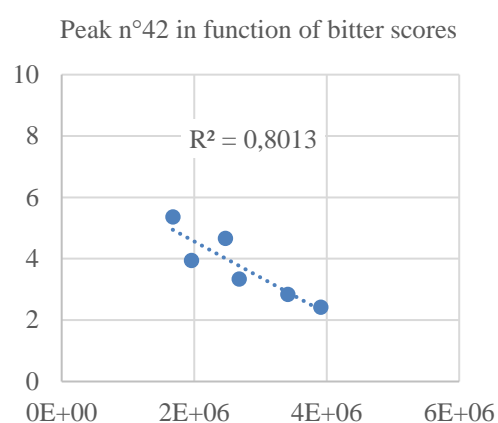
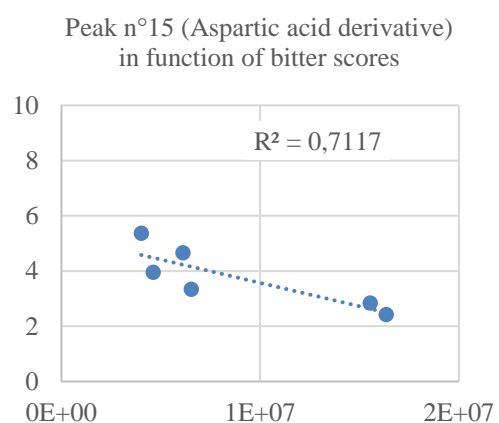
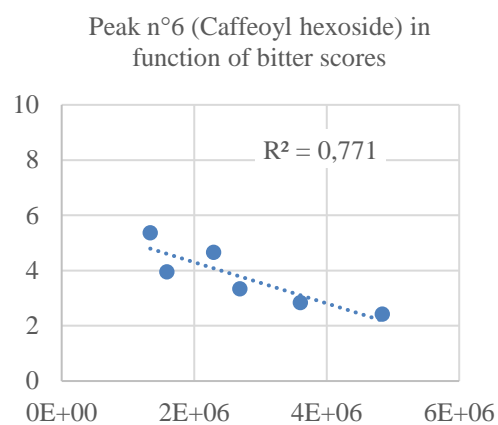
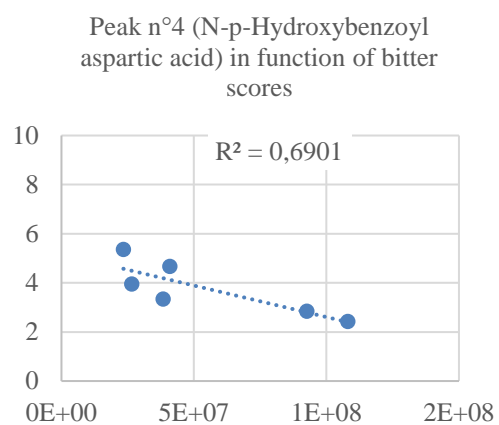


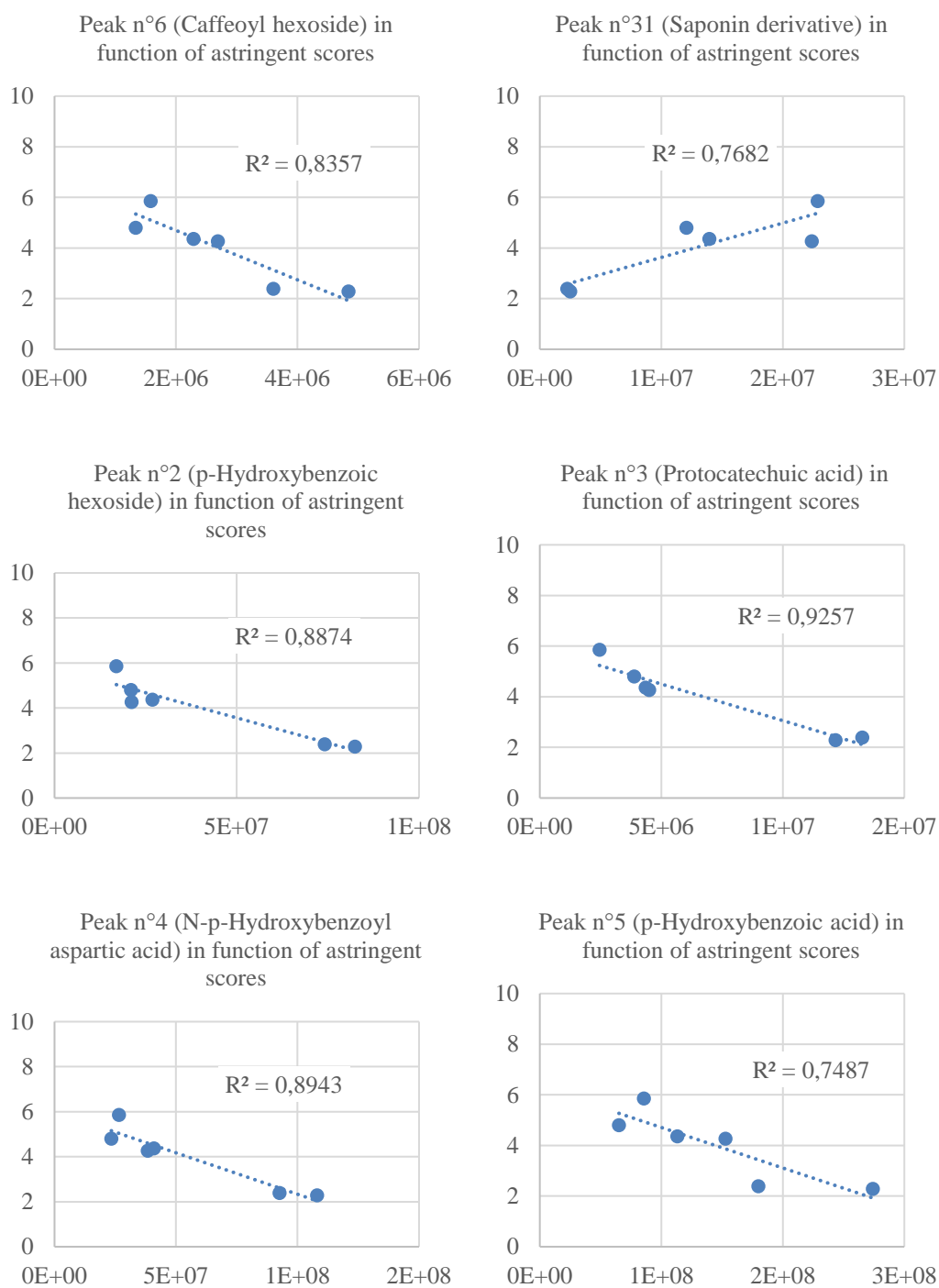
Peak n°8 (Caffeic acid) in function of bitter scores



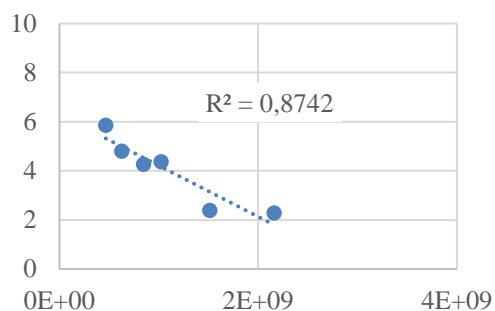
Peak n°9 (N-Coumaroyl aspartic acid derivative) in function of bitter scores



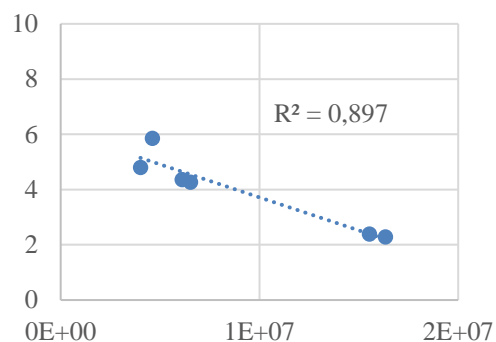




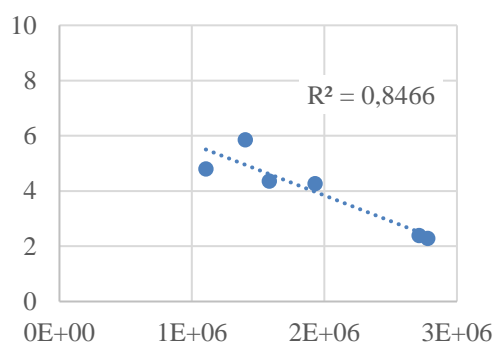
Peak n°14 (N-Phenylacetyl aspartic acid derivative) in function of astringent scores



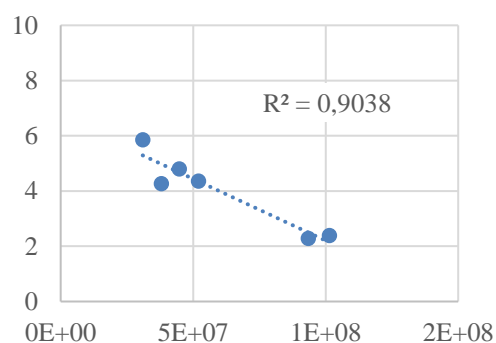
Peak n°15 (Aspartic acid derivative) in function of astringent scores



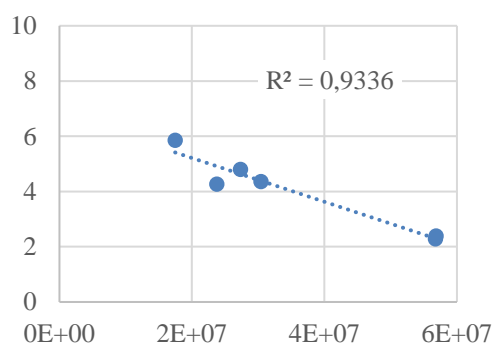
Peak n°17 (Kaempferol derivative) in function of astringent scores



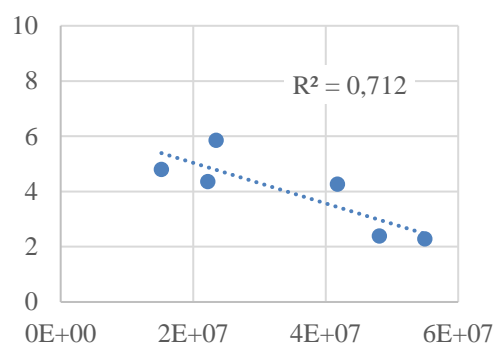
Peak n°39 in function of astringent scores



Peak n°40 in function of astringent scores



Peak n°41 in function of astringent scores



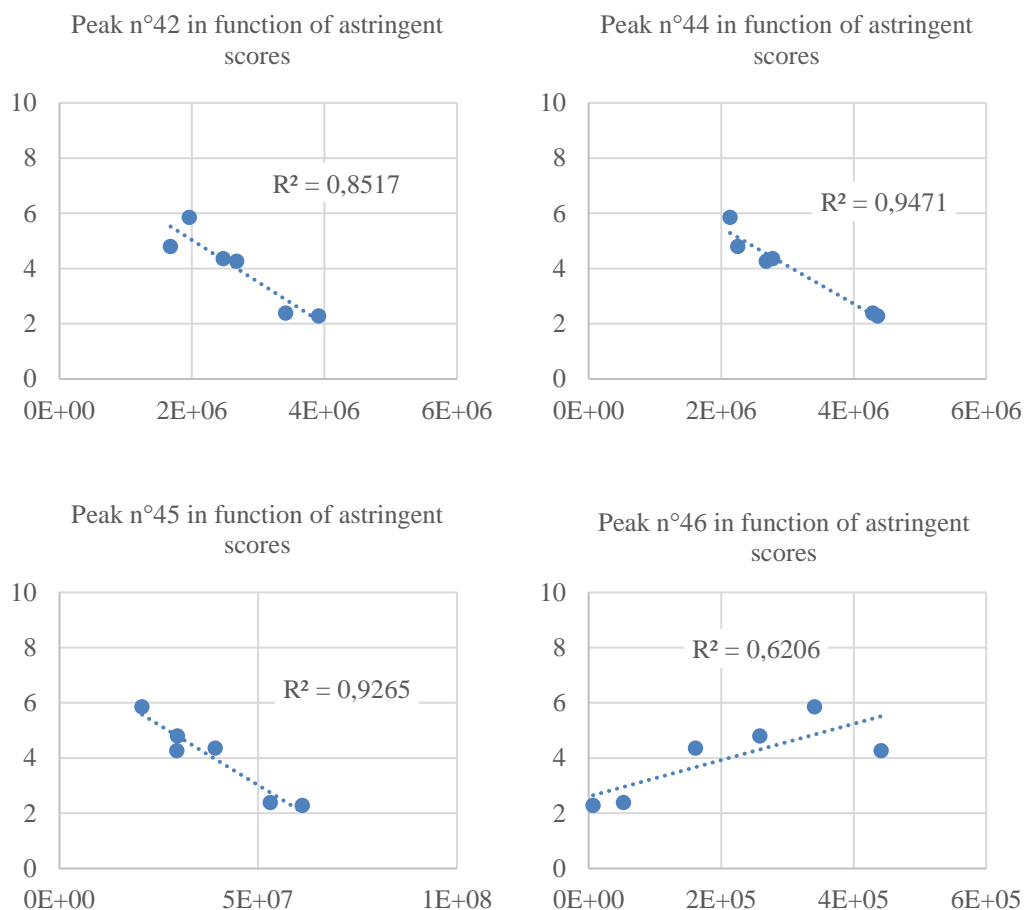


Figure 3.2.3.3.: Representative positive and negative linear correlations between phytochemicals (UHPLC-DAD-MS peak area) and bitter or astringent attributes (scores/10) with their R^2 (Coefficient of determination).

Finally, six saponins are correlated positively to astringency. Saponins are generally referred to be perceived as bitter and astringent (Heng *et al.*, 2006). To assess the impact of saponins in the pea-protein isolate, the concentrations of these compounds were calculated from the standard soyasapogenol B in pea isolates and compared to the literature values. The disadvantage of this quantification was that all saponin compounds were quantified by the same one standard compound, not by each their own standards. This is due to no commercial pea saponin compounds are available, and purification of all these saponins as standards is still difficult so far. Figure 3.2.3.4 shows the averaged concentration of the 6 saponins correlated to astringency with their standard deviation. The higher concentration is reached by Saponin B (peak n°33) with 0.05 mg/g. Considering the 4% concentration in pea protein isolate in solutions for sensory analysis, the Saponin B concentration tested by panellists was equivalent to 20 mg/L. In Heng *et al.* study, bitterness of saponins was perceived by the sensory assessors at very low concentrations values around 2 mg/L for saponins mixture (Saponin B and DDMP saponin in a ratio of 1:4) and around 8 mg/L for Saponin B (Heng *et al.*, 2006). According to this study, the concentration of Saponin B would be sufficient to be perceived by the panellists and participate in the perceptions of bitterness and astringency. However, according to Gläser *et al.*, soyasaponin I has recognition thresholds of 1.62 mmol/L for bitterness and 0.64 mmol/L for astringency (i.e. 1528 and 604 mg/L respectively) (Gläser *et al.*, 2020). The concentration of saponin alone would not be sufficient in this case to elicit bitterness and astringency but may interact with other compounds.

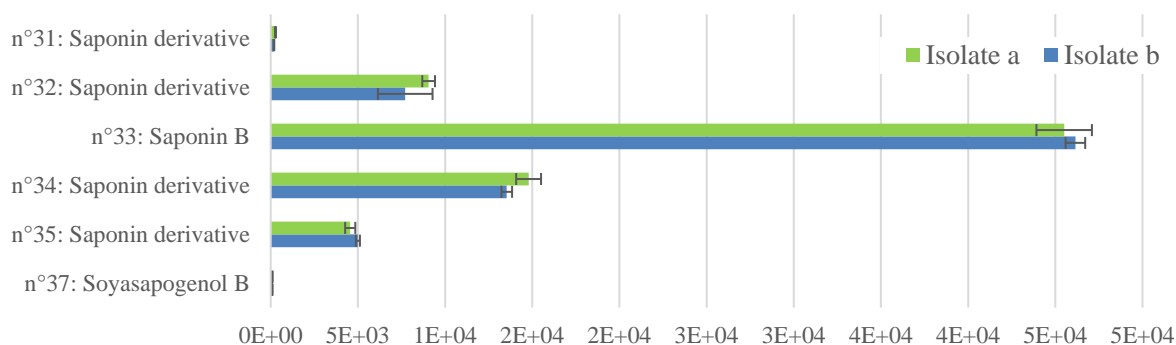


Figure 3.2.3.4.: Concentration of the 6 saponins correlated to astringency with their standard deviation on ng/g.

3.2.3.4. Conclusion

In this study, the main phytochemicals from pea flour, pea protein isolates and pea fractions were determined by ultra-high performance liquid chromatography – mass spectrometry. The study yielded several key results. First, forty-eight phytochemicals were highlighted. Fifteen compounds were tentatively identified as phenolic acids, fifteen as flavonoids and seven as saponins. In addition, comparing MS data with those of reference standards, two hydroxybenzoic acids, four hydroxycinnamic acids, three flavonols, two flavanols and one saponin were unambiguously identified and quantified. Second, peak area comparison has shown larger amounts of compounds in flour than in pea protein isolates and fractions suggesting some degradation of these compounds during the process. However, related to the dry matter content of products, permeates had larger amount of phytochemicals and could be explained by the phenomenon of leaching of water-soluble compounds. Third, the peak areas of these phytochemical compounds were linked to bitterness and astringency perceptions. Twenty-nine compounds (phenolic acids, flavonoids and also saponins) were correlated to bitterness and/or to astringency attributes. The complex mixture of these compounds (as well as other compounds, such as peptides), could participate in overall bitterness-astringency perceptions. In order to improve the desirability of products based on pea protein isolates, it is therefore difficult to make recommendations in terms of phytochemical compounds to be removed. However, solutions that lead to significant changes in phytochemical composition could be an interesting strategy, such as decrease of oxidative reactions or leach of water-soluble compounds.

Main results

- 6 phenolic acids, 5 flavonoids and 1 saponin were identified and quantified.
- 9 other phenolic acids, 10 flavonoids and 6 saponins were tentatively identified.
- Results showed larger amounts of compounds in flour than in pea protein isolates and fractions suggesting some degradation of these compounds during the process.
- 29 compounds (phenolic acids, flavonoids and also saponins) were correlated to bitterness and/or to astringency attributes and could participate in overall bitterness-astringency perceptions.

RESULTS

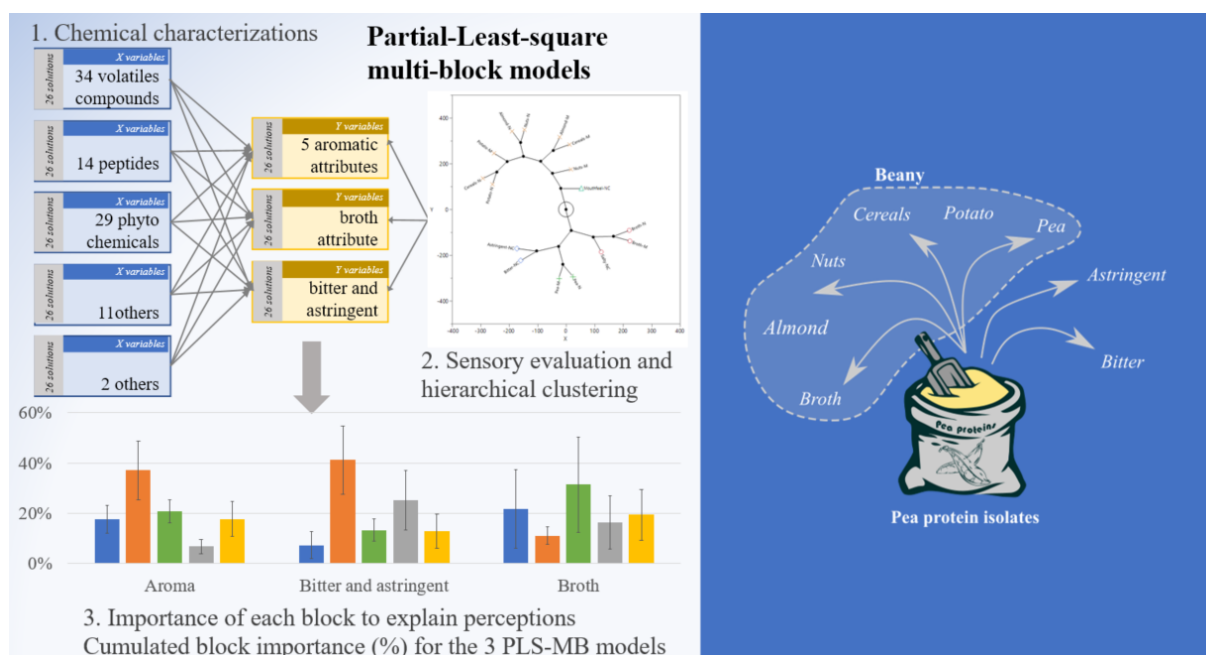
Section 3 - Relative roles of the different chemical compound families on perceptions

Section 3.3. – Relative roles of the different chemical compound families on perceptions

Context, objectives and presentation of section 3.3.

In the previous section, different types of chemical compounds were highlighted to be linked to targeted perceptions: volatile molecules for beany aroma notes, peptides and saponins for bitterness, phenolics for bitterness and astringency. The aim of the work described in the following section was to go further in understanding the complex and combined role of volatile and non-volatile compounds on overall perceptions of pea protein isolates. Then, the aim was to build a statistical model taking into account key compounds coming from different chemical families to explain sensory perceptions. In short, as explained on the graphical abstract (Graphical abstract 3.3.1.), sensory scores were pooled to chemical quantitative datasets: 63 volatile compounds, 3561 peptides and 48 phytochemicals. In addition, 12 other concentrations were acquired. Then regression analysis based on partial-least-square multi-block algorithm were used to explore the links between sensory and chemical datasets.

For confidentiality reasons, these results will not be published and will be not detailed in this manuscript.



Graphical abstract 3.3.1.: Identification through statistical modelling of volatile and non-volatile compounds responsible for perceptions in pea protein isolates

Section 3.3.1. – Identification through statistical modelling of volatile and non-volatile compounds responsible for perceptions in pea protein isolates

3.3.1.1. Introduction

As the world's population, and consequently the world's diet, increases, protein has been identified as a future limiting nutrient. Therefore, the development of new and more sustainable sources of protein is an indispensable issue. In the food industry, proteins and especially plant protein isolates are often used as food ingredients because of their functional properties (e.g., emulsification, foaming, and whipping) (Gharsallaoui *et al.*, 2009). They are used in the formulation of many types of foods, including dietary supplements, bakery and meat products. For these applications, pea protein isolates (*L. Pisum Sativum*) are receiving much attention by industry and consumers. Indeed, they are often used thanks to their protein content, their good environmental sustainability and their attractive price (Davis *et al.*, 2010).

However, the use of these ingredients is limited due to their strong sensory notes which can decrease their desirability. Sensory characterizations have highlighted the existence of three highly persistent notes: bitterness, astringency, and beaniness (Bott & Chambers, 2006; Humiski & Aluko, 2007). The mechanisms and chemical compounds underlying these perceptions are partly understood and may be due to multiple factors (Owusu-Ansah & McCurdy, 1991). Indeed, the composition of pea protein isolates is complex: they have a high protein content but also contain various peptides, volatile compounds, phytochemical compounds, complex heterosides, lipids, minerals.... All these constituents participate to the complex perception of pea-based ingredients.

Research works on the perception of pea-based products have largely focused on the role of volatile aroma compounds in creating sensations of beaniness (Bi *et al.*, 2020; Bott & Chambers, 2006; El Youssef *et al.*, 2020; Murat *et al.*, 2013; Trikusuma *et al.*, 2020; Xu *et al.*, 2020). In addition, few studies have exclusively examined the peptides which could be related to bitterness (Akin & Ozcan, 2017; Pownall *et al.*, 2010; Sirtori *et al.*, 2012). Other studies focused on the phenolics related to bitterness and astringency (Guo *et al.*, 2019; Padhi *et al.*, 2017). The role of saponins was also examined to be related to bitterness (Daveby *et al.*, 1998; Heng *et al.*, 2006; Price *et al.*, 1985). Finally, lipids and lipid oxidation products were studied and related to bitterness and astringency (Baur *et al.*, 1977; Gläser *et al.*, 2020). However, to our knowledge, no study to date has used a more global approach to examine how the complex perception of pea protein isolates arises from both volatile and non-volatile compounds and their potential interactions.

The study of the role of each family of chemical compounds on perception is necessary and a prerequisite to the study of links and interactions between volatile and non-volatile compounds. For that purpose, we first examined the role of volatile compounds composition and their interactions with proteins in pea protein isolates, as well as their impact on perceptions (Section 3.2.1.). A second part of this study identified the main peptides from pea protein isolates and studied their impact on perceptions (Section 3.2.2.). A third part of the present study identified the key phytochemicals (phenolic acids, flavonoids and saponins) from pea protein isolates at the origin of sensory properties (Section 3.2.3.). Following to these different parts, the objective of this study is to establish the relative contribution of the each group of chemical compounds present on pea protein isolates on sensory perceptions. To this end, this work was performed on the same pea protein solutions obtained by an experimental design and presented by Cosson *et al.*, (Cosson *et al.*, 2021 – Section 3.1.2.) have been used. Sensory and chemical datasets (key compounds, issued from the articles on peptides, phenol and GCMS) have been pooled in order to obtain a homogenous dataset.

The dataset was also completed by others identifications not specified here for confidentiality reasons. This large dataset was then used to model sensory attributes based on chemical compounds using PLS multi-block models. The results of the models clarified the origin of the perceived beany,

bitter, and astringent notes. **However, for confidentiality reasons, only the model will be presented here. The results that explain the perceptions will not be detailed.**

3.3.1.2. Materials and methods

The protocols of producing of pea protein solutions and their chemical and sensory characterization are described in this part (Table 3.3.1.1.). But complementary details on method characterization can be found in the others sections (Section 3.1.2.; Section 3.2.1.; and Section 3.2.2.; Section 3.2.3.).

Table 3.3.1.1.: Chemical and sensory variables used in this study with their characterization methods and their references. *The dataset was also completed by others identifications not specified here for confidentiality reasons.

Family compounds	Methods	Variables	Code	References
Sensory	Static profiling	Almond	Almond	Section 3.1.2.
		Astringent	Astringent	
		Bitter	Bitter	
		Broth	Broth	
		Cereals	Cereals	
		Nuts	Nuts	
		Pea	Pea	
		Potato	Potato	
Volatiles	GC-MS	Ketone	V1	Section 3.2.1.
		Alkene	V2	
		Aldehyde	V3	
		Furan	V4	
		Furan	V5	
		Aldehyde	V6	
		Aldehyde	V7	
		Aldehyde	V8	
		Furan	V9	
		Aldehyde	V10	
		Alkane	V11	
		Ketone	V12	
		Ketone	V13	
		Aldehyde	V14	
		Ketone	V15	
		Aldehyde	V16	
		Aldehyde	V17	
		Alkane	V18	
		Ketone	V19	
		Alcohol	V20	
		Ketone	V21	
		Aldehyde	V22	
		Ketone	V23	
		Aldehyde	V24	
		Alcohol	V25	
		Ketone	V26	
		Alcohol	V27	
		Ketone	V28	
		Ketone	V29	
		Ketone	V30	
		Aldehyde	V31	
		Ketone	V32	
		Ester	V33	
		Aldehyde	V34	
Peptides	UHPLC-MS	ANAPLQR	P*AN	Section 3.2.2.
		APHWNIN	P*AP	
		AQPLQRE	P*AQ	
		EVLSWSFH	P*EV	

		ISLNKIRL	P*IS	
		KRHGEW	P*KR	
		NAQPLQRE	P*NA	
		NLQNYR	P*NL	
		NQKQSYF	P*NQ	
		NQKQSYFA	P*NQK	
		SNKFGKF	P*SN	
		SRNPIY	P*SR	
		YLKGLKF	P*YL	
		YQKSTEL	P*YQ	
Phytochemicals	UHPLC-MS	n°2	M2	Section 3.2.3.
		n°3	M3	
		n°4	M4	
		n°5	M5	
		n°6	M6	
		n°7	M7	
		n°8	M8	
		n°9	M9	
		n°10	M10	
		n°14	M14	
		n°15	M15	
		n°17	M17	
		n°26	M26	
		n°31	M31	
		n°32	M32	
		n°33	M33	
		n°34	M34	
		n°35	M35	
		n°37	M37	
		n°39	M39	
		n°42	M42	
		n°43	M43	
		n°40	M40	
		n°41	M41	
		n°44	M44	
		n°45	M45	
		n°46	M46	
		n°47	M47	
		n°48	M48	

Pea protein solutions

Two raw pea protein isolates *a* and *b* (protein content Nx6.25, 83% dry matter) in water suspensions 4% (w/w) dry matter were used and called Refa and Refb. Six fractions were obtained from these two pea protein isolates as explained in Cosson *et al.*, (Cosson *et al.*, 2021 – Section 3.1.2.) and called permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b*. Then, the six different fractions were combined in various quantities to formulate 26 solutions according an experimental design. Their concentrations were described in Table 3.3.1.2. The interest was to work with variable quantities of groups of compounds, instead of to varying only a single compound type. Each fraction was associated with a main compound type: insoluble proteins for pellet; soluble compounds (e.g., volatiles, peptides, and phenolics) for permeate; and soluble proteins interacting with volatiles for retentate. This strategy of formulation allowed to obtain a large number of diverse pea-protein-based solutions in order to obtain continuous responses and to build reliable statistical models. Solutions were chosen to explore a wide range of composition while being realistic in term of protein concentration (0 to 4.25%).

Recombined solutions were done at 4°C in 500mL and 100mL glass bottles and stored at -20°C for a maximum of 12 month. During fractionation and recombination, Good Hygiene Practices have been ensured to limit microbial contamination (coat, gloves and hygienic cap wearing; cleaning and disinfection of hands and all equipment used with absolute ethanol follow by air drying; work in 4°C chamber).

Table 3.3.1.2.: Composition of the 26 solutions used in this study, which were created by mixing permeates *a* and *b*, retentates *a* and *b*, and pellets *a* and *b*. Refa and Refb represent the initial pea protein isolates solutions. DM: dry matter content (%). Protein content (% Nx6.25).

Solution ID	Permeate <i>a</i> (%)	Permeate <i>b</i> (%)	Retentate <i>a</i> (%)	Retentate <i>b</i> (%)	Pellet <i>a</i> (%)	Pellet <i>b</i> (%)	Water (%)	DM (%)	Protein content (%)
100W	0	0	0	0	0	0	100	0,00	0,00
25Ib-75W	0	0	0	0	0	25	75	3,09	2,62
50Ib-50W	0	0	0	0	0	50	50	6,18	5,24
30Ia-70W	0	0	0	0	30	0	70	3,71	3,03
50Ia-50W	0	0	0	0	50	0	50	6,18	5,05
40Rb-30Ib-30W	0	0	0	40	0	30	30	4,39	3,74
40Rb-30Ia-30W	0	0	0	40	30	0	30	4,39	3,63
50Rb-50W	0	0	0	50	0	0	50	0,85	0,75
100Rb	0	0	0	100	0	0	0	1,70	1,50
40Ra-30Ib-30W	0	0	40	0	0	30	30	4,39	3,70
50Ra-25Ia-25W	0	0	50	0	25	0	25	3,94	3,23
60Ra-40W	0	0	60	0	0	0	40	1,02	0,84
100Ra	0	0	100	0	0	0	0	1,70	1,40
40Pb-60W	0	40	0	0	0	0	60	0,08	0,01
Refb	0	40	0	36	0	24	0	3,70	3,27
50Pb-50W	0	50	0	0	0	0	50	0,10	0,02
50Pb-25Ib-25W	0	50	0	0	0	25	25	3,19	2,64
50Pb-50Rb	0	50	0	50	0	0	0	0,95	0,77
70Pb-30Ra	0	70	30	0	0	0	0	0,65	0,45
100Pb	0	100	0	0	0	0	0	0,20	0,04
25Pa-25Ra- 13Ia-38W	25	0	25	0	12.5	0	37.5	2,02	1,62
Refa	38	0	34	0	28	0	0	4,10	3,53
40Pa-60W	40	0	0	0	0	0	60	0,08	0,01
40Pa-60Rb	40	0	0	60	0	0	0	1,10	0,91
50Pa-25Ib-25W	50	0	0	0	0	25	25	3,19	2,64
50Pa-1a25-W25	50	0	0	0	25	0	25	3,19	2,54
50Pa-50Ra	50	0	50	0	0	0	0	0,95	0,72
100Pa	100	0	0	0	0	0	0	0,20	0,03

Sensory analysis

Pea solutions were sensory characterized by 17 trained panellists (13 women and 4 men; mean age = 23 years old) with a descriptive quantitative methodology proposed by block (Cosson *et al.* 2021 – Section 3.1.2.). 18 attributes were evaluated and organised in blocks. The first attribute block focused on olfactory orthonasal perception evaluated by nose (attribute block 1: pea, broth, nuts, almond, potato and cereals). The second block focused on taste perception and mouthfeel (attribute block 2: salty, sugar, bitter, astringent, mouthfeel, and granularity). The last attribute block focused again on olfactory retronasal perception evaluated in mouth (attribute block 3: pea, broth, nuts, almond, potato and cereals). Evaluations were performed in sensory booths, at 19°C. Each solution, coded with a 3 digit number, was evaluated in duplicate.

Chemical analysis

The nitrogen protein content was determined by Kjeldahl method (Nitrogen content x 6.25) and dry solids were determined by drying with a prepASH®219 device by a certified external laboratory (SAS IMPROVE, Amien, France).

The measurements of volatile compounds release were performed using a Dynamic Headspace Gas Chromatography coupled with a Mass Spectrometer (MPS: Gerstel, GC: Agilent 7890B, MS: Agilent 5977B MSD). MassHunter software (Agilent) was used for instrument control, data acquisition and data analysis. The detailed protocol used for these analyses are presented in Section 3.2.1.

The measurements of peptides were performed on the PAPPSO platform (MICALIS, INRA, Jouy-en-Josas, France) with an Orbitrap Fusion™ Lumos™ Tribrid™ (Thermo Fisher Scientific) coupled to an UltiMate™ 3000 RSLC nano System (Thermo Fisher Scientific). Peptide identification was performed with X!Tandem version 2017.2.142015.12.15.2 (AlanineVengeance) and X!Tandem Pipeline (C++) version 0.2.4016 (Langella *et al.*, 2016) on the protein sequence of *L. Pisum Sativum*. MassChroQ version 2.2.17 (mass Chromatogram Quantification) was used to perform alignment, XIC extraction, peak detection and quantification (Valot *et al.*, 2011). The laboratory equipment and parameters used for these analyses are presented in Section 3.2.2.

Measurements of phytochemical were performed using UHPLC system (Ultimate 3000 Thermo Scientific, USA) coupled to a Q Exactive Orbitrap high resolution mass spectrometer (Thermo Scientific, USA) equipped with heated-electrospray ionization probe (HESI II, Thermo Scientific, USA). Xcalibur (Thermo Scientific, USA) was used for instrument control, data acquisition, and data analysis. The detailed protocol used for these analyses are presented in Section 3.2.3.

The dataset was also completed by others identifications not specified here for confidentiality reasons.

Statistical analysis

Analyses were performed using JMP software version 13.1.0 (SAS Institute Inc., Cary, SC, USA) and R software (R Core Team, 2017). For analyses of an inferential nature, we used $\alpha = 0.05$ to determine statistical significance. Sensory attributes were clustered by hierarchical clustering.

Different algorithms have been proposed for PLS-MB (Biancolillo *et al.*, 2019) (Biancollilo *et al.*, 2019). The one adopted in the present work is the one proposed by the `mbpls` function of the R package “ade4” version 1.7-16 (Bougeard & Dray, 2018; Dray & Dufour, 2007).

Prior to PLS-MB analysis, the explanatory variables were standardized. In addition, data blocks were normalized to ensure that each one had the same weight in the calibration, independently of their number of variables (uniform option). The optimal model is then identified by selecting the number of latent variables with a two-fold cross-validation. The products sets are partitioned randomly into a calibration and a validation sets. In turn, each set was used to validate the model that is fit to the rest of the data. The root mean square error of calibration (RMSEc) and the root mean square error of validation (RMSEv) were calculated. This was run 500 times. The optimal model were retained as a compromise between a good fitting ability (minimization of RMSEc) and a good prediction ability (minimization of RMSEv) (Bougeard & Dray, 2018). Bootstrapping simulations (1000 times) were also applied to the three main predictive parameters (regression coefficients, cumulated variable importance index and cumulated block importance index) to provide confidence intervals, computed by the non-Studentized pivotal method (Bougeard & Dray, 2018).

3.3.1.3. Results and discussion

Construction of PLS-MB models

The general workflow of the PLS-MB analysis is described in Figure 3.3.1.1., starting from the experimental part to the statistical analyses. Steps done in previous studies are indicated in the yellow frame. Steps done in this study are indicated in the blue frame.

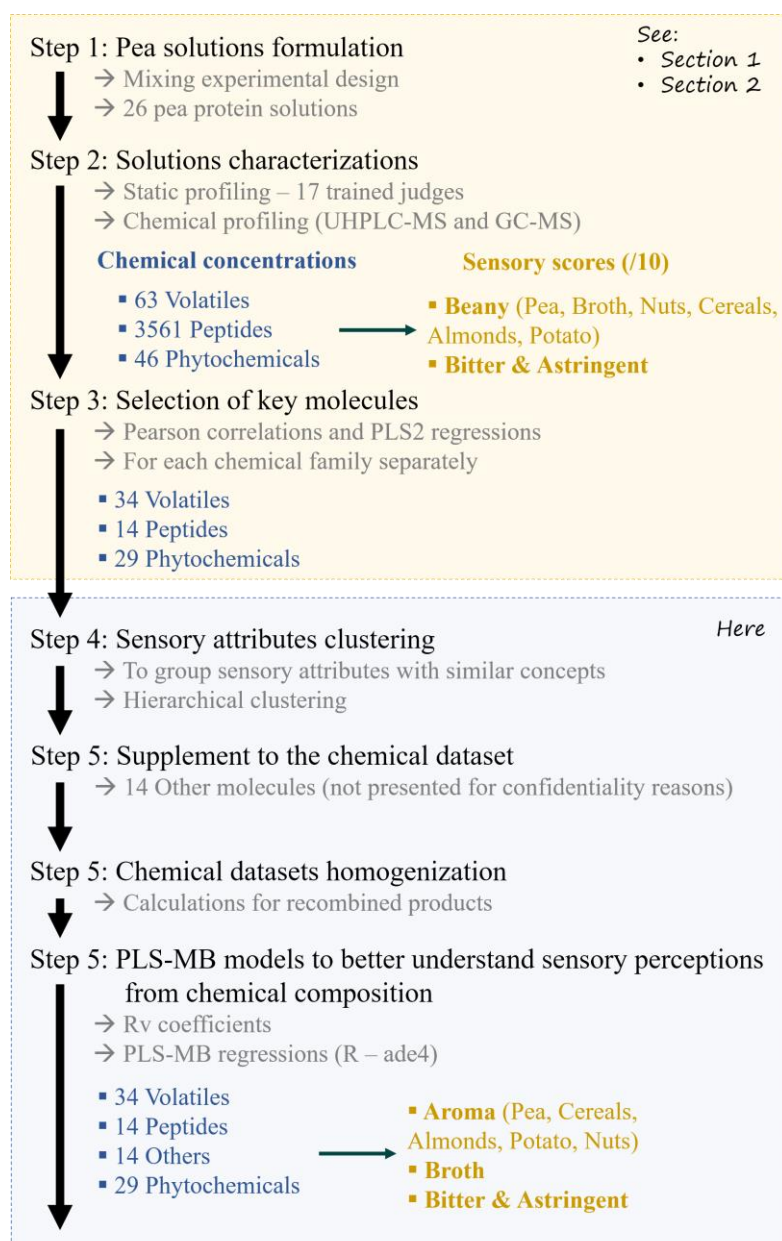


Figure 3.3.1.1.: General workflow of the PLS-MB analysis, starting from the experimental part to the statistical analyses. Steps done in previous studies are indicated in the yellow frame. Steps done in this study are indicated in the blue frames and focused on statistical links between the different datasets.

Selection of key sensory attributes and key chemical compounds

Eighteen sensory attributes were evaluated for the 26 protein solutions by the trained panel. Figure 3.3.1.2. shows the plot constellation on attributes from hierarchical clustering.

It can be observed that the sensory attributes broth and pea are quite separate from the other aromatic attributes, expressing different concepts. The bitter and astringent attributes are also quite different from the other attributes. Of the 18 sensory attributes evaluated, we chose to focus only on 8 aromatic attributes evaluated in the mouth (broth, pea, potato, almond, nuts and cereals) and bitter and astringent attributes to develop the models. Indeed, since sweet, salty granularity and mouthfeel attributes did not greatly contribute to the perceived differences among the solutions and/or are not directly within the scope of the study, these attributes were not included in the statistical model (Cosson *et al.*, 2021 – Section 3.1.2.). In addition, previous results indicated that there may be limited sensory interactions between texture, taste, and flavour and that food oral processing had only a minimal impact on solution

perceptions. We also chose to not taken into account the odour attributes evaluated via the nose in the statistical model and to study only the aroma attributes evaluated in mouth.

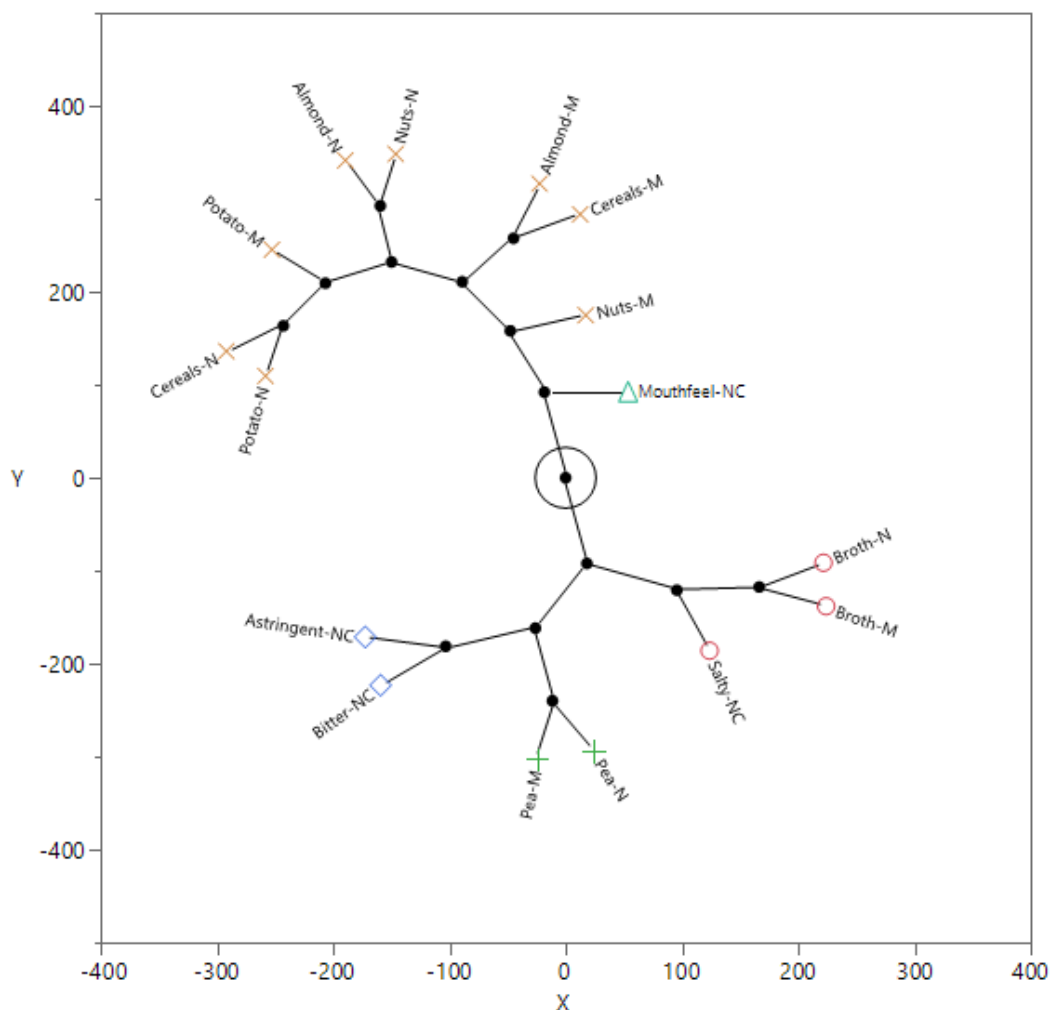


Figure 3.3.1.2.: Constellation plot from hierarchical clustering of sensory attributes. Each observation is represented by an endpoint and each cluster join is represented by a new point. The lines that are drawn represent cluster membership. The lengths of the lines represent the distance between clusters. The circle represents a cluster with all the sensory attributes.

Among all the identified chemical compounds (63 volatile GC-MS area compounds from apolar column; 48 phytochemical compounds from UHPLC-MS and 3561 peptide sequences from UHPLC-MS), only a selection of compounds was used in the model.

Thirty-four volatile compounds were selected for modelling, because these last were identified as significant in the model to discriminate the 26 solutions (Section 3.2.1.). Fourteen peptides were selected because they were identified as correlated with perceptions of bitterness and with a size inferior to 8 residues (Section 3.2.2.). Twenty-nine phytochemical compounds were selected, because they correspond to the compounds identified to be correlated to the perceptions of bitterness and astringency (Section 3.2.3.).

These reductions in the number of variables is an indispensable step in order to build a robust model. The table 3.3.1.3. presents the main results of the companion studies that allowed the selection of these variables.

Table 3.3.1.3.: Main results of the companion studies on pea protein isolates solutions: 1) mixture design, fraction-based formulation and sensory characterization; 2) identification of the main volatile compounds; 3) identification of the main peptides; and 4) identification of the main phytochemicals.

Family variables	Methods	Products	Variables	Key variables	Main results	References
Sensory	Static profiling	<ul style="list-style-type: none"> • 6 pea solutions (pure fractions) • 19 recombined solutions (fractions mixing) • 1 water 	18 sensory attributes	8 sensory attributes	<ul style="list-style-type: none"> • Panellists generally came up with repeatable and homogeneous scores. • Perceived beaniness were mainly influenced by the retentate and permeate fractions. • Perceived astringency was mainly influenced by the retentate and pellet fractions. • Perceived bitterness was largely driven by retentate fraction. 	Section 3.1.2.
Volatiles	GC-MS	<ul style="list-style-type: none"> • 6 pea solutions (pure fractions) • 6 recombined solutions (fractions mixing) 	63 volatiles molecules	34 volatiles molecules	<ul style="list-style-type: none"> • Most of the key volatile compounds correlated to aroma intensities are similar to the different aroma attributes suggesting a common nucleus of compounds responsible for the beany perceptions. • Key volatile compounds are mainly lipophilic but they present various solubility and volatility. • Protein-volatile interactions were mainly hydrophobic interactions and seem to have a low impact on the olfactory perceptions. 	Section 3.2.1.
Peptides	UHPLC-MS	<ul style="list-style-type: none"> • 6 pea solutions (pure fraction) • 6 recombined solutions (fractions mixing) 	3561 peptides	14 peptides	<ul style="list-style-type: none"> • Identified peptides were mostly polar and hydrophilic and there were no impact of the fraction-based formulation strategy on their overall physical properties. • Most of the peptides were correlated to sensory attributes of pea solutions. In particular, many peptides were correlated to broth perception expressing perhaps the umami character of some peptides • 275 peptides were correlated to bitterness but only 14 have a size inferior to 8 residues. 	Section 3.2.2.
Phytochemicals	UHPLC-MS	<ul style="list-style-type: none"> • 6 pea solutions (pure fraction) 	48 phytochemicals	29 phytochemicals	<ul style="list-style-type: none"> • 16 compounds were tentatively identified as phenolic acids, 23 as flavonoids, 13 as terpenoids and 2 compounds from other families. • Peak area comparison has shown larger amounts of compounds in flour than in pea protein isolates and fractions suggesting some degradation of these compounds during the process. • 29 molecules were correlated to bitterness and/or to astringency attributes. 	Section 3.2.3.

Datasets homogenization

Here, a mixture design was used to create 26 solutions by combining pea protein isolate fractions. If the 26 solutions were analysed by the sensory panel, only 12 solutions were analysed by UHPLC-MS to identify the peptides and by GC-MS to identify the volatile compounds. On the other hand, only 6 solutions were analysed by UHPLC-MS to identify phytochemical compounds.

To complete the datasets, we hypothesized that it would be possible to calculate the compounds area from the solution formulation. To validate this hypothesis, the peptides areas of six recombined solutions measured experimentally were compared with the peptides areas calculated from the area of fractions (50Pb-25Ib-25W, 25Pa-25Ra-13Ia-38W, 70Pb-30Ra, 40Ra-30Ib-30W, 50Ra-25Ia-25W and 50Pb-50Rb). Considering the number of values to be compared (3561 peptide sequences x 6 solutions), the distributions of the differences between calculated and measured values were compared and

presented no significant differences (Section 3.2.2.). In conclusion, calculated peptide composition of the recomposed solutions from the peptide composition of the fractions seems a good estimate.

Similarly, the volatiles composition was recalculated for the 13 last products. The volatile areas of six recomposed solutions measured experimentally were compared with the volatile areas calculated from the fraction areas (50Pb-25Ib-25W, 25Pa-25Ra-13Ia-38W, 70Pb-30Ra, 40Ra-30Ib-30W, 50Ra-25Ia-25W and 50Pb-50Rb). Standard deviations of the repeated values were compared to standard deviations of the calculated versus measured values. The different quartiles for repeated solutions were: 1st quartile $4.20\text{E}+03$, median $1.14\text{E}+04$, 3rd quartile $3.13\text{E}+04$, maximum $2.37\text{E}+06$. The different quartiles for measured versus calculated solutions were: 1st quartile $1.25\text{E}+05$, median $3.51\text{E}+04$, 3rd quartile $8.98\text{E}+04$, maximum $1.24\text{E}+07$. The quartiles are a little lower in the case of duplicate distributions but the orders of magnitude are similar. Some volatile compounds (hexanal, 2-pentylfuran, butan-2-one, pentanal and octan-1-ol) show the highest differences between the experimentally measured and the calculated solutions, but these are among the compounds with the largest areas. Their relative difference is therefore relatively small. However, it can be noted that the differences between the calculated and measured values are larger for volatile compounds than for peptides. It can be assumed that this is due to the volatile-protein interactions that are relatively important in pea protein isolates (Section 3.2.1.). A better prediction of these areas could be obtained by taking into account the binding coefficient of volatile molecules (which could be estimated by their hydrophobicity coefficient) in the recalculation of the areas. However, in order to work in a consistent way with the different datasets, we have chosen to recalculate only the concentrations from the fraction concentrations.

Similarly, the phytochemical, compositions were recalculated for the 19 last products. However, we could not validate the calculation hypothesis because no products recombined from the fractions was analysed. However, we can assume that there is little interaction between phytochemicals and pea proteins in this type of matrix (Damodaran & Arora, 2013; Potter *et al.*, 1993). It can therefore be assumed that the steps of extraction and analysis of phytochemicals are less matrix-dependent.

Before building the model, the RV coefficients (Pearson method) were calculated between each group of chemical compounds and sensory attributes. Taking into account all the physico-chemical datasets allows to improve the RV coefficients. This result is consistent with the calculation of RV coefficients that depend on the variance and covariance of the variables.

Construction of the PLS-MB models

Therefore, from these 8 sensory attributes (Y responses) and 90 chemical compounds (X explanatory variables), different statistical models were constructed. The objective of these models are to model the sensory attributes as a function of the chemical compounds in order to be able to propose hypotheses of mechanisms at the origin of the perceptions (with a focus on perception of beany, bitterness and astringency). Here, the 8 sensory attributes measure different concepts that are quite independent. In the literature, several statistical methods have been proposed to handle these situations with multiblocks. The one adopted in the present work is the one proposed by the *mbpls* function of the R package “ade4” version 1.7-16 (Bougeard & Dray, 2018; Dray & Dufour, 2007).

The objective of the PLS-MB is to model the effects of chemical composition on pea protein solutions (X explanatory variables) on the sensory perceptions of the products (Y responses). Partial least squares (PLS) are a multivariate regression methodologies that seeks to find factors (latent variables) which both capture variance and achieve correlation, by maximizing the amount of variance explained in X that is relevant for predicting Y (Westerhuis *et al.*, 1998). For the same pre-treatment, the total variance explained by classical PLS will be the same as for PLS-MB (Westerhuis *et al.*, 1998). However, PLS-MB preserve the original structure of the data and thus allows one to analyse the different tables simultaneously. In particular, the usefulness of PLS-MB lies on the ease of visualization of importance of each block of variables. In addition, PLS-MB is low sensitive to multicollinearity within explanatory blocks (Bougeard & Dray, 2018).

To build these models, the 90 chemical compounds were grouped in four blocks dependant of their chemical family origin. In addition, sensory attributes were also grouped together. Bitter and astringent

attributes, close in the plot constellation, were clustered together. Broth attribute, very different from the other sensory attributes on the plot constellation was clustered separately. The other aromatic attributes pea, potato, almond, nuts and cereals were clustered together. Thus for each of these 3 groups of sensory attributes an optimal model was selected with a two-fold cross-validation.

To evaluate the generated models, the analyses by Root mean square error of calibration (RMSE_c) and Root mean square error of validation (RMSE_v) were used. RMSE_c gives an estimate of the average deviation of the model from the data, giving us an idea on how well the model fit the data. RMSE_c can be used to measure the predictive power of a model, making it a useful tool in comparing models. The closer these indicators are to 0, the more robust the models can be considered. Table 3.3.1.4. presents these indicators for the 3 models. It can be seen that the model that best fits the data and has the most predictive power is the model to explain aromatic attributes. The model that has the lowest predictive power is the model with the broth attribute. The results suggested that a good prediction of aromatic attributes and a relative good prediction of bitterness and astringency attributes could be achieved when using chemical parameters with the developed PLS-MB regression model, in comparison with models developed in the literature on similar data sets (sensory and chemical datasets) for others PLS model (Barbin *et al.*, 2012). The results of the Broth model present lowest prediction ability. But this result is in itself interesting by showing the complexity of the mechanisms at the origin of this perception. Thus, these models will allow us to better understand the links between sensory attributes and chemical compounds.

Table 3.3.1.4.: Performances of the selected PLS multi-block model to predict the 8 sensory attributes variables in function of chemical compounds area analysed with confidence intervals (25% lower quantiles; 75% upper quantiles).

Block of attributes	Root mean square error of calibration			Root mean square error of validation		
	Mean	25%	75%	Mean	25%	75%
Block 1: Aroma (pea, almond, nuts, potato and cereals)	0,28	0,27	0,30	0,34	0,30	0,37
Block 2: Bitter and astringent	0,44	0,42	0,46	0,74	0,67	0,82
Block 3: Broth	0,54	0,48	0,59	0,92	0,79	1,08

Use of the models to better understand sensory perceptions

Similarly to variable importance in projection used in classical PLS model, cumulated block importance indexes are used to estimate the contribution and importance of the block of variables in the PLS-MB model (Figure 3.3.1.3). Expressed as a percentage, they allow to quantify the impact of each group of chemical compounds on the block of perceptions of pea protein solutions.

In addition, the cumulated variable importance index are used here to estimate the contribution and importance of each variables in the PLS-MB model (Figure 3.3.1.4). Expressed as a percentage, they allow to quantify the impact of each variable inside the group of chemical compounds on the perceptions of pea protein solutions.

However, for confidentiality reasons, only the model will be presented here. The results that explain the perceptions will not be detailed.

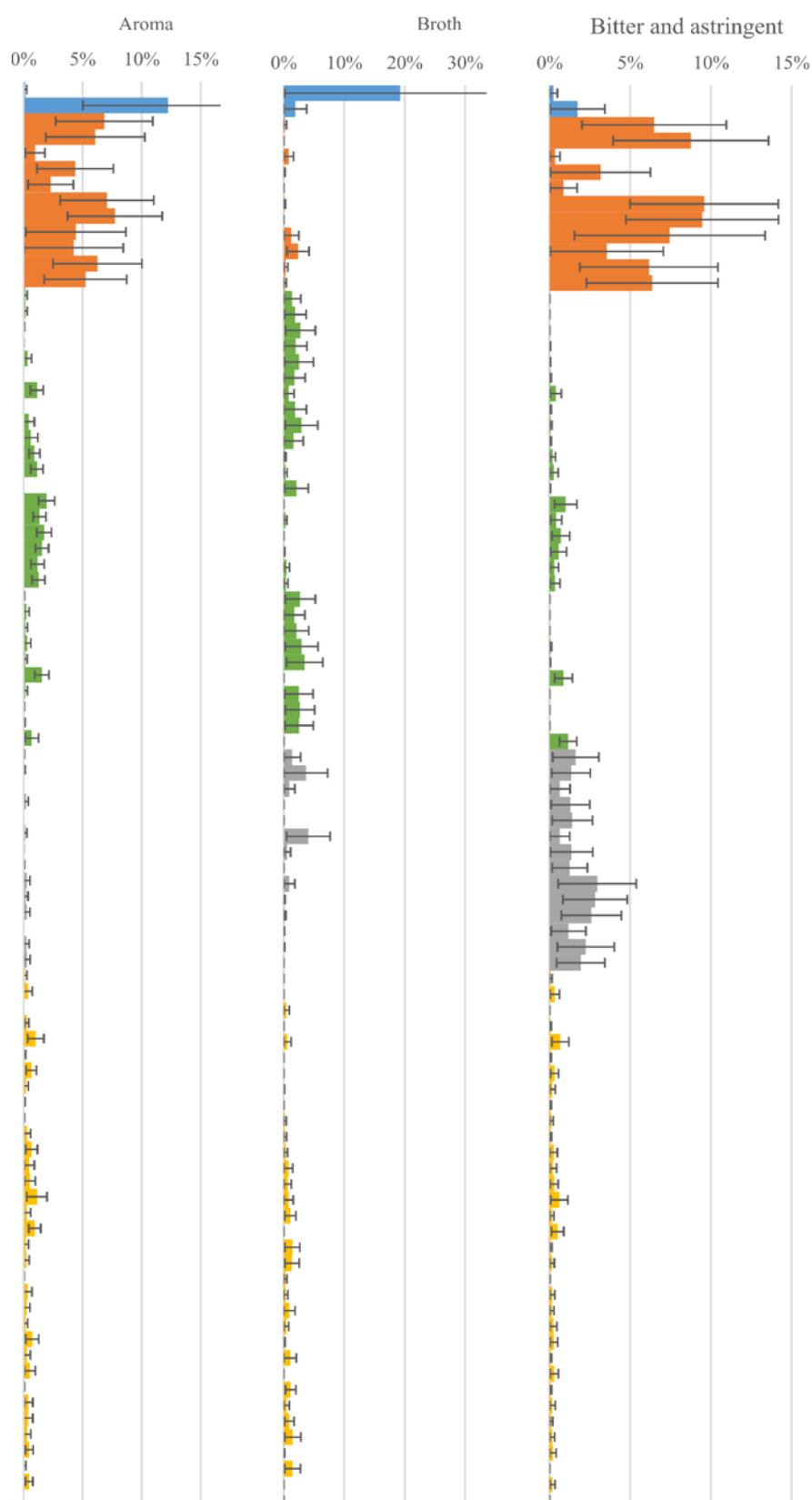


Figure 3.3.1.3.: Cumulated block importance for the first dimension (in %) with the 97.5% confidence intervals for the three PLS-MB regressions modelling sensory attributes in function of chemical composition of pea protein isolates. 1) Aroma: model in function of pea, potato, nuts, cereals and almonds sensory attributes. 2) Broth: model in function of broth sensory attribute. 3) Bitter and astringent: model in function of bitter and astringent sensory attributes.

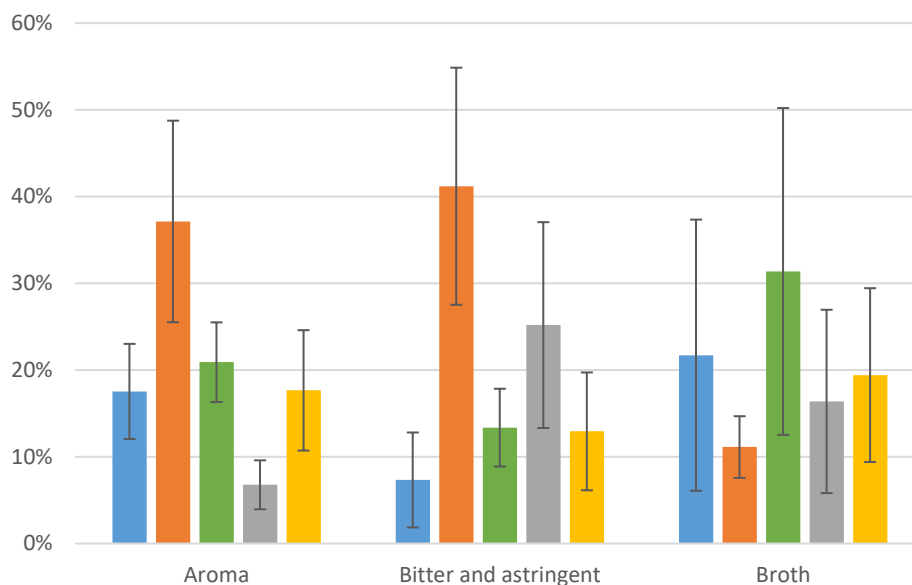


Figure 3.3.1.4.: Cumulated block importance for the first dimension (in %) with the 97.5% confidence intervals for the three PLS-MB regressions modelling sensory attributes in function of chemical composition of pea protein isolates. 1) Aroma: model in function of pea, potato, nuts, cereals and almonds sensory attributes. 2) Broth: model in function of broth sensory attribute. 3) Bitter and astringent: model in function of bitter and astringent sensory attributes.

3.3.1.1. Conclusion

This consequent dataset provides a better understanding of the sensory perceptions of pea-based products. Multi-block regression models made it possible to highlight the respective contribution of pea chemical compounds on perceptions. Unfortunately, for reasons of confidentiality, we have not been able to detail the results here.

To conclude, this study proposes an original statistical methodology to better understand the different mechanisms that occur in pea protein perceptions. This work could help in the formulation of new pea protein-based products with lower beany, bitter, and astringent perceptions and so higher desirability for consumers.

RESULTS

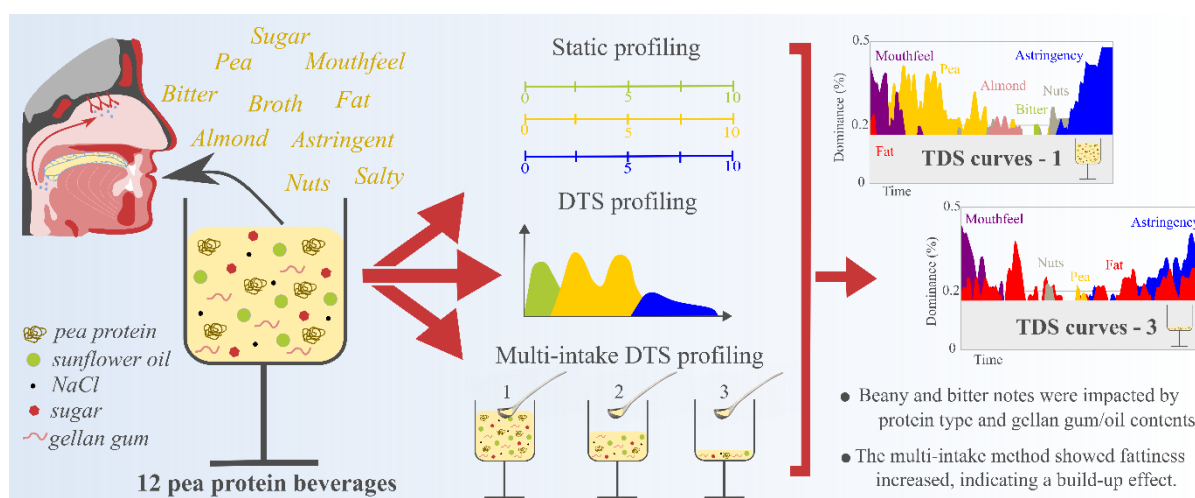
Section 4 - Impact of formulation and consumption of a whole pea based-beverage on perceptions

Section 3.4. – Impact of formulation and consumption of a whole pea based-beverage on perceptions

Context, objectives and presentation of section 3.4.

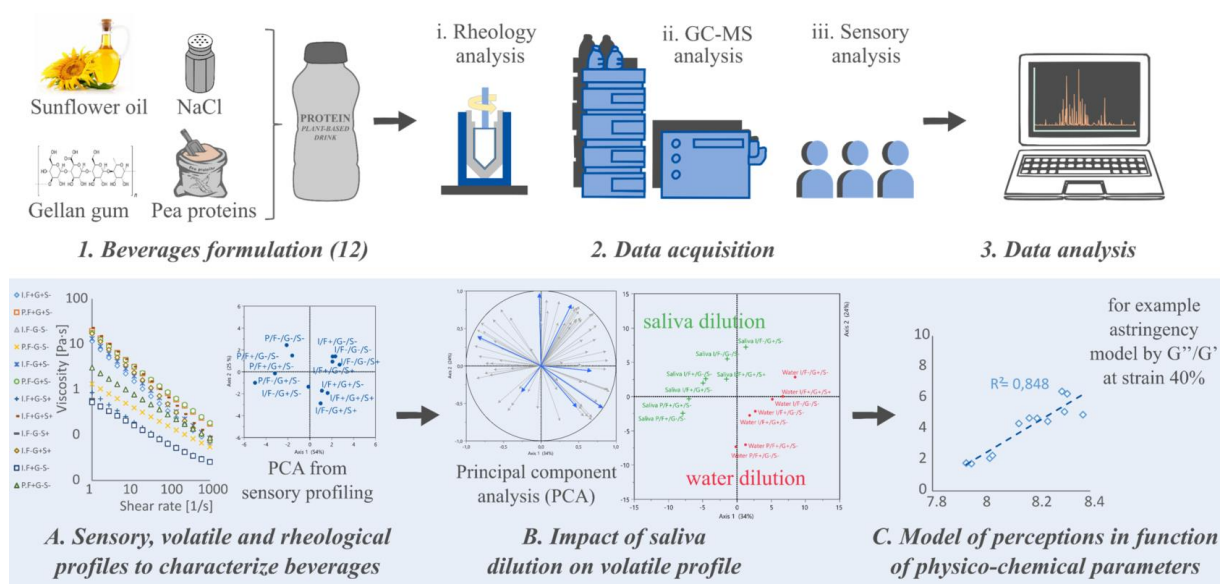
Beyond the chemical composition of the food, its structure and texture could also influence the dynamics of perception. Thus, the purpose of this fourth section is to determine how the sensory perceptions of pea protein isolates are affected by the formulation as well as the consumption of a whole product. Among the different pea applications, pea beverages were considered because they allow to vary the concentrations of various ingredients while being easy to produce in a repeatable way over a long period of time. Thus, following the previous PhD work, a pea protein isolate and its pellet were selected. They were then formulated in 12 different beverages varying in their level of salt, fat, thickeners and type of proteins.

From these beverages, the first objective was to identify the main mechanisms responsible for beany, bitter and astringent temporal perceptions and to study the interactions between sapid and olfactory perceptions (Section 3.4.1.). In short, as explained on the graphical abstract (Graphical abstract 3.4.1.), pea beverages were described and quantified using three sensory profiling methods: static block profiling with and without nose clip, mono-intake temporal dominance of sensations (TDS) profiling, and multi-intake TDS profiling. Results were published and discussed on *Foods* as presented below (Cosson, A., Souchon, I., Richard, J., Descamps, N., & Saint-Eve, A. (2020). Using Multiple Sensory Profiling Methods to Gain Insight into Temporal Perceptions of Pea Protein-Based Formulated Foods. *Foods*, 9(8), 969. <https://doi.org/10.3390/foods9080969>).



Graphical abstract 3.4.1.: Using multiple sensory profiling methods to gain insight into temporal perceptions of pea protein-based formulated foods.

The second objective was to better understand how product composition affected the sensory perception of pea protein-based beverages (Section 3.4.2.). Particular focus was placed on how rheological properties influence sensory perception, and on molecules volatiles release and on saliva's role during oral processing. In short, as explained on the graphical abstract (Graphical abstract 3.4.2.), sensory profiles of pea beverages were completed by classical rheological methods and gas-chromatography–mass-spectrometry analysis. Relation between instrumental profiles and sensory profiles were studied.



Graphical abstract 3.4.2.: Sensory perceptions of pea protein beverages explained by rheological and chemical approaches.

Section 3.4.1. – Using multiple sensory profiling methods to gain insight into temporal perceptions of pea protein-based formulated foods

3.4.1.1. Introduction

Consumers are increasingly seeking out healthy, ethically produced, and eco-friendly foods. In this context, plant proteins are proving to be a great success. Yellow field pea (*Pisum sativum L.*) is becoming a common ingredient in plant-based foods (Siddique *et al.*, 2012) because it has a low level of allergenicity and a high nutritional value. It also helps ensure the nutritional balance of amino acids in grain-based diets. Yellow field pea isolates also have desirable functional properties: they have excellent emulsification, foaming, gelation, and whipping capacities (Adebisi & Aluko, 2011; Gharsallaoui *et al.*, 2009). They are used in the formulation of many types of foods, including dietary supplements, bakery and confectionery products, beverages, yogurts, ice creams, meat products, and alternatives to meat and dairy products.

That said, consumers usually describe pea protein-based foods as having strong beany, bitter, and astringent notes, which can decrease desirability. These attributes have different chemical origins. Indeed, beaniness is the complex flavour perception associated with bean products (Bott & Chambers, 2006) and results from the complex composition of volatile aroma compounds found in pulses (Murat *et al.*, 2013). Bitterness arises from the interaction of bitter compounds (e.g., amino acids, phenolics) with the TAS2R family of receptors, which are found on the apical membranes of taste receptor cells (Maehashi *et al.*, 2009; Meyerhof *et al.*, 2010). Finally, astringency is produced by “the complex sensations due to shrinking, drawing, or puckering of the epithelium,” and it results from interactions between phenolic compounds and saliva proteins (ASTM, 1991; Gibbins & Carpenter, 2013).

To develop novel products with less pronounced beany, bitter, and astringent notes, food production companies combine pea proteins with several other ingredients (e.g., fat, salt, sugar, flavouring agents, and/or texturizing agents). However, successfully formulating new products from combinations of these ingredients can be challenging and requires a great deal of trial and error. Sensory profiling is a valuable tool in this context: it can be used to explore the impact of food composition on the perceived sensory characteristics of formulated foods, and it thus allows target food products to be obtained more quickly. Many studies have used static block sensory profiling to examine how formulation affects sensory perceptions, the physico-chemical interactions between the different constituents of the food matrix, and the interactions between perceptions of texture, sapidity, and flavour (Fontoin *et al.*, 2008; Giacalone *et al.*, 2018; Lestrinant *et al.*, 2019; Tournier *et al.*, 2007).

However, static methods cannot quantify the dynamic mechanisms that play an essential role in how consumers experience foods. Indeed, the oral processing of food includes mastication, salivation, and tongue movements, leading to a complete transformation of food in the mouth. Food transformation has major consequences on food perception and perception persistence (Di Monaco *et al.*, 2014). Retronasal aroma perception is affected by interactions among volatile compounds, and the levels of salivary compounds are not constant over the course of food consumption (Canon *et al.*, 2018). Sensations of astringency and bitterness often go hand and hand (Muñoz-González *et al.*, 2018) and slowly develop in the mouth after ingestion. They also increase following repeated exposure (Green, 1993).

Temporal sensory profiling methods are increasingly being used to take these phenomena into account and to obtain a more realistic picture of the sensory experiences elicited by food products. One widely adopted approach is the temporal dominance of sensations (TDS) method. It yields information on the sequence and duration of dominant sensations (Pineau *et al.*, 2009). The dominant sensations to occur are those that attract the most attention from consumers (Schlich, 2017). The TDS method has been used in tandem with static sensory profiling to evaluate different types of products, making it possible to identify sensory characteristics that are not picked up by one method alone. For example, this combined approach has proven to be useful for studying the persistence of gel containing odorants (Labbe *et al.*,

2009); interactions between texture and aroma in model candies (Saint-Eve *et al.*, 2011); solid foods with contrasting textural layers (i.e., fish sticks; (Albert *et al.*, 2012); interactions between olive oil composition and pureed beans and tomatoes (Dinnella *et al.*, 2012); the influence of aroma on taste and texture in an apple matrix (Charles *et al.*, 2017); and the key flavours perceived in strawberries (Oliver *et al.*, 2018).

Typically, the TDS method is applied to a single instance of food intake (i.e., one bite of a solid food or one sip of a beverage). However, in real life, food consumption involves a series of instances of food intake. Several studies have shown that repeated intake of a product can change the perception of product attributes due to sensory adaptation and/or perception persistence (Lawless & Heymann, 2010). The multi-intake TDS method can provide a sensory profile for a full portion of food. It has recently proven its utility in studies evaluating the influence of wine on cheese perception (Galmarini *et al.*, 2017) and in studies characterizing the sensory properties of an oral nutritional supplement (Thomas *et al.*, 2016), fat-free strawberry yogurts (Lesme *et al.*, 2020), and yogurts with granola (van Bommel *et al.*, 2019).

The aim of this study was to better understand how product composition affected the sensory perception of pea protein-based beverages using three different sensory profiling methods; particular focus was placed on the perception of beany, bitter, and astringent notes. Trained panellists therefore analysed lab-formulated beverages using three sensory profiling methods: static block profiling, mono-intake TDS profiling, and multi-intake TDS profiling. Analysis were centred on the effects of food composition (protein type, gellan gum content, salt content, and oil content) on texture, sapidity, and aroma as well as on the sensory interactions of flavour with taste and texture. Finally, the usefulness of a combined sensory profiling approach was discussed.

3.4.1.2. Materials and methods

Materials

Water (Evian, France), gellan gum (Texturas Ferran Adria, Spain), salt (Auchan, France), sunflower oil (Auchan, France), sugar (Daddy, France), soy lecithin (Louis Francois, France) and commercial pea protein isolates were the ingredients used to formulate the beverages. Two Thermomix® TM5™ appliances (Vorwerk, Germany) were employed to standardize product preparation.

Product preparation

In this study, different pea protein-based beverages were created in the lab. Two mixture design were used to produce a wide range of plant-beverages from different ingredients while being realistic in term of ingredient' concentrations. The first mixture design was formulated with pea protein isolates (batch *a*) and had three independent variables with two levels: sunflower oil concentration (0% or 1.5%), gellan gum concentration (0.12% or 0.5%) and salt concentration (0.08% or 0.12%). The second mixture design was formulated with pea pellets (pellet *a*) and had also two independent variables: the protein, sunflower oil concentration (0% or 1.5%) and two levels of gellan gum concentration (0.12% or 0.5%). Thus, the total number of trials was 12 (composition and ingredient concentrations are in Table 3.4.1.1.).

First, pea protein pellets were obtained as follows: water and pea protein isolates were slowly mixed together (96% [w/w] water, 4% [w/w] pea protein isolate) and then left to hydrate for 60 min at 4°C under stirring. The pellet and supernatant were separated via centrifugation at 6,000 rpm at 4°C for 10 min. The pellet was stored at 4°C for a maximum of 2 h before the beverages were made.

Second, the beverages were created using the following method: the water was mixed and heated (3 min, 50°C, and speed setting of 2.5) in one of the Thermomix appliances. The sugar, salt, pea protein (isolate or pellet), and gellan gum were then gradually mixed into the water (30 min, 50°C, speed setting of 4.5). Simultaneously, the sunflower oil was heated (1 min, 65°C, speed setting of 1.5) in the second Thermomix. The soy lecithin was then mixed into the sunflower oil (3 min, 65°C, speed setting of 2). The contents of the first Thermomix were added to the contents of the second Thermomix and combined without heating (5 min, speed setting of 5). After this step, the overall mixture was heated (6 min, 90°C, speed setting of 3.5). Immediately after preparation, the beverages were stored at 4°C until they were

used in the sensory profiling sessions. The Thermomix appliances were cleaned by filling them with a mixture of 2 L of water, 100 mL of white vinegar, and 5 mL of dishwashing liquid, which was then heated (5 min, 70°C, and speed setting of 1). The appliances were subsequently thoroughly rinsed with hot water and stored at 4°C until they were next used in order to prevent any bacterial growth.

Table 3.4.1.1.: Composition (ingredient concentrations [w/w %]) of the pea protein-based beverages used in this study. Abbreviations: I = isolate, P = pellet, F+ = 1.5% oil, F- = 0% oil, G+ = 0.5% gellan gum, G- = 0.12% gellan gum, S+ = 0.12% salt, and S- = 0.08% salt.

Product name	Protein type	Sunflower Oil (%)	Soy lecithin (%)	Gellan gum (%)	Salt (%)	Sugar (%)	Pea protein (%)	Water (%)
	(P or I)	(F+ or F-)		(G+ or G-)	(S+ or S-)			
I/F-/G-/S-	Isolate	0.00	0.00	0.12	0.08	1.00	7.00	91.80
I/F-/G-/S+	Isolate	0.00	0.00	0.12	0.12	1.00	7.00	91.76
I/F-/G+/S-	Isolate	0.00	0.00	0.50	0.08	1.00	7.00	91.42
I/F-/G+/S+	Isolate	0.00	0.00	0.50	0.12	1.00	7.00	91.38
I/F+/G-/S-	Isolate	1.50	0.10	0.12	0.08	1.00	7.00	90.20
I/F+/G-/S+	Isolate	1.50	0.10	0.12	0.12	1.00	7.00	90.16
I/F+/G+/S-	Isolate	1.50	0.10	0.50	0.08	1.00	7.00	89.82
I/F+/G+/S+	Isolate	1.50	0.10	0.50	0.12	1.00	7.00	89.78
P/F-/G-/S-	Pellet	0.00	0.00	0.12	0.08	1.00	7.00	91.80
P/F-/G-/S+	Pellet	0.00	0.00	0.50	0.08	1.00	7.00	91.42
P/F+/G-/S-	Pellet	1.50	0.10	0.12	0.08	1.00	7.00	90.20
P/F+/G+/S-	Pellet	1.50	0.10	0.50	0.08	1.00	7.00	89.82

Rheological tests were performed on each beverage to verify repeatability, and the microbial safety of the products was tested by a certified external laboratory (Eurofins Scientific, France). The products were served to the panellists at room temperature (20°C) in transparent cups (29.5 mL) identified with three-digit codes.

Experimental conditions

Sixteen panellists (15 women and 1 man, 18–39 years in age) were recruited based on their desire and availability to participate in a long-term study. Two of the panellists had participated in a study that focused on the sensory characterization of pea protein solutions the year before. The other panellists had no prior experience with pea protein-based products. The panellists were told the overall aim of the experiment. They gave their free and informed consent to participate in the study and received compensation for their participation. They were asked not to eat, drink, or smoke for at least 1 h prior to the training sessions and evaluation sessions. Panellists performed the sensory evaluations in individual booths under white light in an air-conditioned room (20°C).

Panellists had to analyse the beverages using three different sensory profiling methods: a static block method (Cosson *et al.*, 2020 –Section 3.1.1.), a mono-intake TDS method, and a multi-intake TDS (multi-TDS) method. To account for the order in which the beverages were experienced and any potential carry-over effects, beverage order was balanced across panellists using a Latin square. To reduce sensation build-up, a palate-cleansing protocol was used between beverages: panellists had to consume an apple slice, drink water, and wait 40 seconds before consuming the following beverage (Cosson *et al.*, 2020 – Section 3.1.1.). As some beverages were viscous, participants were instructed to intake beverages with spoons, instead of sipping for the three profiling methods. Sensory analysis was managed using Fizz Acquisition software (v. 2.51, Biosystemes, France).

Attribute selection and panellist training

Panellists were asked to complete a check-all-that-apply (CATA) questionnaire. It listed 30 attributes, and panellists could add more. For the final list and the validation process with the panellists, we retained the attributes that were mentioned most of the time and that allowed the products to be clearly distinguished. These 11 attributes were salty, bitter, astringent, sweet, fat, pea, almond, nuts, broth, mouthfeel, and overall aromatic intensity (Table 3.4.1.2.). As the study was conducted in French, the terms used in French as well as their translation into English are presented.

The panellists were trained to evaluate the intensity of these attributes along an unstructured scale (range: 0–10) using external references. Training took place over 10 sessions that each lasted 45 min. Afterward, panellist performance was evaluated and verified. Overall performance was assessed using ANOVA with three independent variables (product type, panellist ID, and replicate) and their first-order interactions. There was a product effect, indicating that panellists distinguished among the different beverages ($p < 0.05$). The significance of various interactions revealed whether the panellists consistently scored attributes across replicates (panellist*replicate), whether there was consistency in scoring among panellists (product type*panellist ID), and whether panellists scored products consistently across replicates (product type*replicate). The performance of individual panellists was also evaluated based on their ability to discriminate among beverages and on repeatability criteria.

Table 3.4.1.2.: Definition of the sensory attributes evaluated by the panellists.

Attributes	Attributes in French	Definition
Salty	<i>Salé</i>	A fundamental taste – sodium chloride is a typical example
Bitter	<i>Amer</i>	The fundamental taste associated with a caffeine solution
Astringent	<i>Astringent</i>	A sensation of drying out, roughening, and/or puckering that is felt in the mouth, like when consuming red wine or unripe fruit
Sweet	<i>Sucré</i>	A fundamental taste – sucrose is a typical example
Fat	<i>Gras</i>	Property relative to the perception of the quantity of fat in the product
Mouthfeel	<i>Epais</i>	The way a food feels in the mouth in relation to its viscosity
Overall aromatic intensity	<i>Intensité aromatique globale</i>	Total aroma impressions created by the product in the mouth
Pea	<i>Pois</i>	The flavour characteristic of beans and bean-based foods
Almond	<i>Amande</i>	The flavour associated with almonds
Nuts	<i>Noix</i>	The flavour associated with nuts, like walnuts or hazelnuts
Broth	<i>Bouillon</i>	The flavour associated with boiled vegetables, soup, or stock

Static block profiling

Panellists were asked to score the attributes of the 12 beverages using a static block profiling method adapted from the technique used in Cosson *et al.* (Cosson *et al.*, 2020 – Section 3.1.1.).

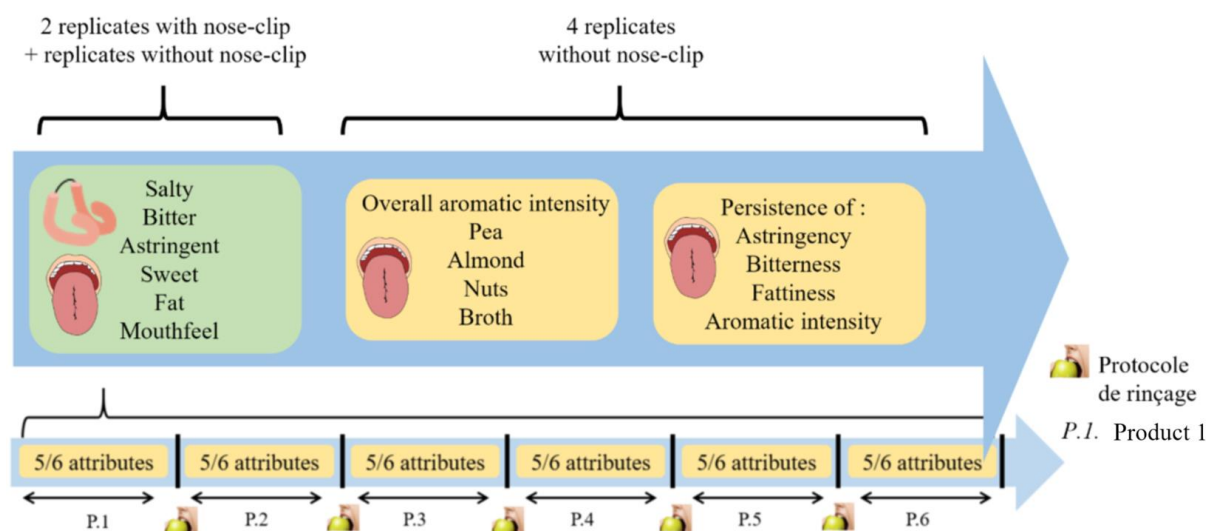


Figure 3.4.1.1.: Schematic representation of the static block profiling method used in this study.

They had to evaluate six different beverages per session and were unaware of beverage identity. They were exposed to four replicates of each product. In total, the panellists evaluated the products over 8 different sensory sessions during four weeks of evaluation. For two replicates, sapidity and texture were evaluated using a nose-clip, and aroma attributes were evaluated without using a nose-clip. For two replicates, all the attributes were evaluated without the nose-clip (Figure 3.4.1.1.). The panellists were asked to evaluate attribute intensity as during the training process (along an unstructured scale ranging

from 0 to 10). Attributes were assessed in blocks of 4, 5, and 6. First, the panellists had to evaluate sapidity and texture. Second, they had to evaluate aroma. Thirdly, they had to evaluate attribute persistence (using a shorter list of attributes).

Mono-intake temporal dominance of sensations profiling

Panellists were asked to evaluate the 12 beverages using a mono-intake TDS method—they evaluated the change in attribute intensity over a period of 120 s after taking a sip of a given beverage. Panellists were exposed to two replicates of each beverage, and they evaluated six beverages per session. In total, the panellists evaluated the products over 4 different sensory sessions during two weeks of evaluation. The attributes were the same as in the static block profiling method except for overall aromatic intensity, which was removed because it was not relevant in this method. Watery was added as an attribute, and it was described to panellists as being the opposite of the fat attribute. Another attribute was also added: “I swallowed.” All the attributes were simultaneously presented on the computer screen. Attribute order was the same for each panellist for all the mono-intake TDS sessions but was randomly assigned and balanced among panellists.

The evaluation process started as soon as the panellists took a sip of the beverage. The panellists then had to click on the attribute that they perceived as dominant, which was defined for them as “the attribute that draws the most attention.” When this dominant attribute changed, the subject had to click on the new dominant attribute. The panellist was free to choose the same dominant attribute several times or, conversely, to never select a dominant attribute. The panellists also had to click on the button “I swallowed” each time they swallowed the beverage or their saliva.

For each panellist and each beverage, the following data were collected: the time at which an attribute was selected as dominant, the specific attribute, the time that had elapsed before the panellist clicked on “I swallowed” for the first time (i.e., the panellist had largely consumed the product), and the number of times that the panellist clicked on the button “I swallowed”.

Multi-intake temporal dominance of sensations profiling

Panellists were asked to evaluate two beverages using a multi-intake TDS method; these two beverages were chosen based on the static block profiling results and the mono-intake TDS profiling results. They contained different protein types (isolate vs. pellet), had a low level of astringency persistence, and displayed different temporal sensory profiles despite having the same gellan gum, salt, and oil contents. The multi-intake TDS profiling method can be used to evaluate changes in attribute perceptions as people consume a full portion of a product (Figure 3.4.1.2.). Here, a portion was defined as 120 mL, which is equivalent to an entire ready-to-drink beverage or a serving of yogurt.

First, the panellists had to cleanse their palates. Over the course of the session, they were not allowed to consume anything except the beverage to allow for the possible cumulative effects of persistent sensations. Second, the panellists evaluated the beverages using the same general approach as in the mono-intake TDS profiling method, except that a given beverage was evaluated at three time points. The first evaluation took place after the first spoonful of the beverage was consumed (hereafter, first spoonful). The second evaluation took place after panellists had consumed 60 mL of the beverage (~ half the portion); they then had to evaluate a second spoonful of the beverage (hereafter, second spoonful). The third evaluation took place after panellists had consumed the remaining 60 mL of the beverage; they then had to evaluate a final spoonful of the beverage (hereafter, third spoonful). We thus obtained three sets of data reflecting the shift in sensations from the beginning to the end of beverage consumption. No time limits were placed on this process. Panellists were exposed to two replicates of each product. One replicate of one product was evaluated per session, resulting in a total of four sessions.

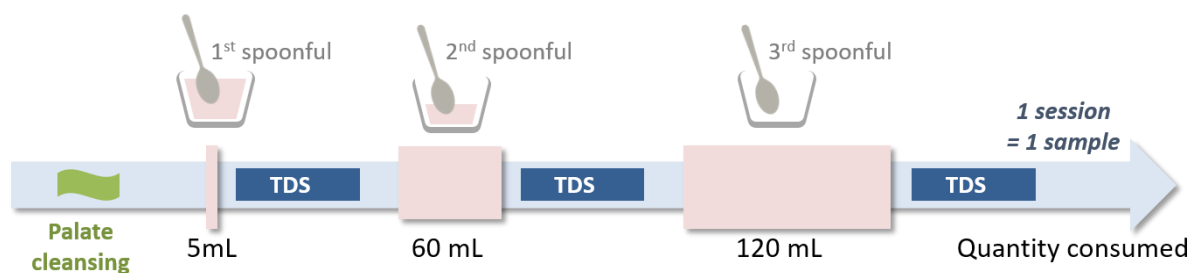


Figure 3.4.1.2.: Schematic representation of the multi-intake temporal dominance of sensations (TDS) method used in this study.

Statistical analysis

The data were automatically acquired using Fizz Acquisition software (v. 2.51; Biosystemes, 1990). Data analysis was performed using XLSTAT (Addinsoft, 2017, Paris, France) and R (R Core Team, 2019). The threshold for statistical significance was $\alpha = 0.05$. The static block profiling data were analysed using ANOVA. To assess panellist performance, ANOVA were carried out in which product type, panellist ID, and replicate were fixed effects and there were first-order interactions. Post-hoc comparisons were then performed to interpret the specific effect of product type (Newman-Keuls method). To analyse the effect of beverage composition on attribute perception, ANOVA were performed in which panellist ID, protein type, gellan gum content, salt content, oil content, and nose-clip use were fixed effects and there were first-order interactions. In the case of the mono-intake TDS profiling analyses, the time to the first instance of swallowing and the total duration of the evaluation period were extracted from the data collected during the sessions. ANOVA were performed in which product type, panellist ID, and replicate were fixed effects and there were first-order interactions. For the multi-intake TDS profiling data, the ANOVA had product type, panellist ID, replicate, and spoonful ID as fixed effects and included first-order interactions. Relative attribute dominance (i.e., the percentage of panellists who perceived a given attribute as dominant) was determined for each beverage at each time point, and the TDS curves were graphed. As suggested by Pineau *et al.* (2009), two lines were drawn on the TDS graph: one line representing the relative dominance an attribute could achieve by chance alone when considering all the attributes evaluated and one line representing the minimum relative dominance an attribute must obtain for the result to be significantly different from that expected by chance alone (binomial distribution, $\alpha = 0.05$).

3.4.1.3. Results

Panellist performance

The static block profiling data were used to examine how consistent panellists were in their scoring of attribute intensity (three-way ANOVA; Table 3.4.1.3.).

Product type was significant for 15/15 attributes, so the panellists were clearly able to distinguish among the beverages. The interactions between replicate and product type were not significant for 10/15 attributes (except for sweet, mouthfeel, persistence of bitterness, persistence of fattiness, and persistence of overall aromatic intensity). Replicate was not significant for 12/15 attributes (except for salty, pea, and persistence of overall aromatic intensity), but the interaction between panellist ID and replicate was significant for 11/15 attributes (all except bitter, mouthfeel, pea, and broth). However, in the latter case, the F-values were low compared to the F-values for the product effects. Panellist ID and the interaction between panellist ID and product type were significant for 15/15 attributes. Such interactions are common when sensory attributes are evaluated using unstructured scales, and they are difficult to control even when panellists have undergone extensive training (Jourjon *et al.*, 2005). These results nonetheless suggest that the panellists' scoring was consistent (repeatable and homogeneous) for the majority of attributes. For 3 attributes (bitter, pea and almond), there was some inconsistency between panellists, which was taken into account in the analysis of the results.

Table 3.4.1.3.: Results of the three-way ANOVA (panellist ID, replicate, and product type as fixed effects + their first-order interactions) examining consistency in panellist performance (total degrees of freedom: 739; residual degrees of freedom: 681). Significant p-values are in bold ($\alpha = 0.05$). Abbreviations: Astringent-P = persistence of astringency; Bitter-P = persistence of bitterness; Fat-P = persistence of fattiness; and Aromatic intensity-P = persistence of overall aromatic intensity.

	Panellist ID		Replicate		Product type		Panellist ID*Replicate		Panellist ID*Product type		Replicate*Product type	
	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
Salty	29.47	< 0.01	5.71	0.02	61.90	< 0.01	4.42	< 0.01	1.78	< 0.01	1.34	0.21
Bitter	34.83	< 0.01	0.01	0.92	8.44	< 0.01	1.22	0.25	2.49	< 0.01	0.43	0.93
Astringent	26.94	< 0.01	0.95	0.33	5.71	< 0.01	3.23	< 0.01	1.78	< 0.01	0.25	0.99
Sweet	23.09	< 0.01	0.92	0.34	8.14	< 0.01	2.60	< 0.01	1.64	< 0.01	2.03	0.03
Fat	10.11	< 0.01	0.49	0.49	62.77	< 0.01	1.98	0.02	2.38	< 0.01	0.91	0.53
Mouthfeel	13.79	< 0.01	1.07	0.30	358.24	< 0.01	1.58	0.07	2.00	< 0.01	3.77	< 0.01
Overall aromatic intensity	9.17	< 0.01	0.17	0.68	14.71	< 0.01	2.55	< 0.01	1.93	< 0.01	0.87	0.57
Pea	29.85	< 0.01	4.82	0.03	2.44	0.01	1.48	0.11	2.47	< 0.01	1.03	0.42
Almond	32.78	< 0.01	0.16	0.69	2.57	< 0.01	2.61	< 0.01	1.60	< 0.01	0.29	0.98
Nuts	27.21	< 0.01	3.69	0.06	5.72	< 0.01	2.84	< 0.01	2.60	< 0.01	0.84	0.59
Broth	25.34	< 0.01	0.04	0.83	41.96	< 0.01	1.01	0.45	1.76	< 0.01	0.21	1.00
Astringent-P	52.86	< 0.01	1.41	0.23	9.47	< 0.01	3.54	< 0.01	1.86	< 0.01	1.38	0.19
Bitter-P	34.61	< 0.01	0.02	0.89	2.88	< 0.01	2.93	< 0.01	1.71	< 0.01	1.91	0.04
Fat-P	79.46	< 0.01	3.19	0.07	26.28	< 0.01	1.90	0.02	2.89	< 0.01	1.90	0.04
Aromatic intensity-P	57.42	< 0.01	11.22	0.00	4.71	< 0.01	1.90	0.02	1.35	0.01	1.97	0.03

Impact of beverage composition on perceived attribute intensity

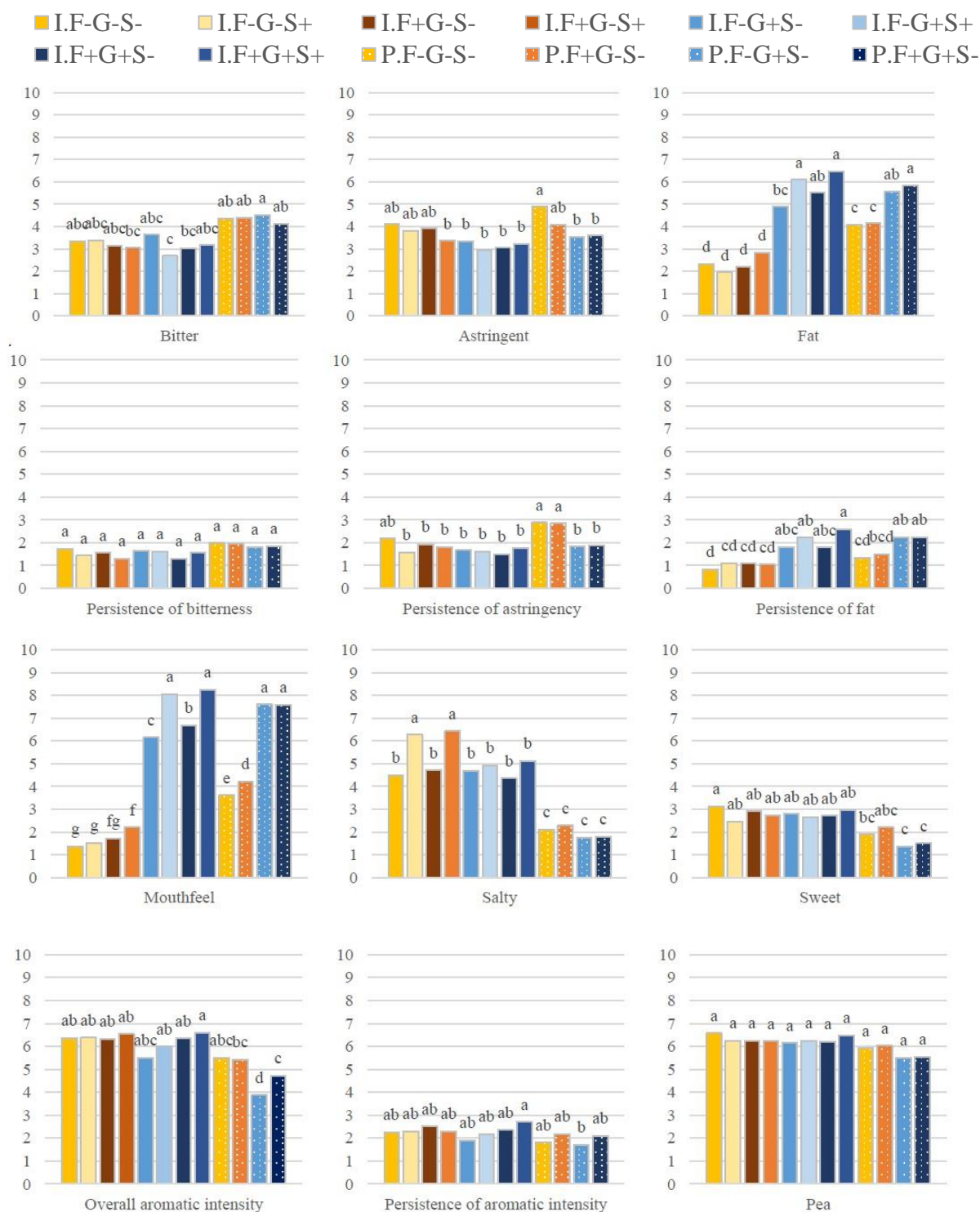
The static block profiling data were also used to examine the effects of beverage composition on attribute intensity (five-way ANOVA; Table 3.4.1.4.). The mean attribute intensities (across replicates and panellists) for the different beverages and the differences among groups (Newman-Keuls post-hoc analysis) are shown in Figure 3.4.1.3.

Table 3.4.1.4.: Results of the five-way ANOVA (panellist ID, nose-clip use, oil content, gellan gum content, salt content, and protein type as fixed effects + their first-order interactions) examining the effects of beverage composition on attribute perception using the static block profiling data (total degrees of freedom: 739; residual degrees of freedom: 716). Significant p-values are in bold ($\alpha = 0.05$). Abbreviations: Astringent-P = persistence of astringency; Bitter-P = persistence of bitterness; Fat-P = persistence of fattiness; and Aromatic intensity-P = persistence of overall aromatic intensity.

	Panellist ID		Nose-clip use		Oil content		Gellan gum content		Salt content		Protein type		Oil*Gellan gum		Gellan gum*Salt		Gellan gum*Protein type	
	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
Salty	25.39	< 0.01	4.17	0.04	0.24	0.62	27.96	< 0.01	49.64	< 0.01	241.07	< 0.01	0.73	0.39	15.56	< 0.01	1.15	0.28
Bitter	26.64	< 0.01	14.71	< 0.01	1.30	0.26	0.65	0.42	1.69	0.19	34.38	< 0.01	0.00	0.99	1.20	0.27	0.11	0.74
Astringent	22.23	< 0.01	0.86	0.35	2.39	0.12	14.33	< 0.01	2.00	0.16	5.32	0.02	2.76	0.10	0.88	0.35	0.05	0.83
Sweet	21.44	< 0.01	0.88	0.35	1.24	0.27	1.73	0.19	1.36	0.24	55.21	< 0.01	0.00	0.95	2.89	0.09	1.09	0.30
Fat	7.71	< 0.01	2.53	0.11	6.45	0.01	247.75	< 0.01	13.13	0.00	46.78	< 0.01	0.33	0.57	8.18	< 0.01	14.18	< 0.01
Mouthfeel	11.39	< 0.01	0.52	0.47	19.10	< 0.01	1769.43	< 0.01	82.71	< 0.01	233.58	< 0.01	2.03	0.15	39.03	< 0.01	23.56	< 0.01
Overall aromatic intensity	7.24	< 0.01	3.07	0.08	7.39	0.01	15.19	0.00	2.07	0.15	55.03	< 0.01	6.78	0.01	0.41	0.52	5.41	0.02
Pea	23.19	< 0.01	1.95	0.16	0.01	0.94	0.90	0.34	0.00	0.99	10.48	< 0.01	0.36	0.55	0.98	0.32	0.47	0.49
Almond	29.31	< 0.01	1.67	0.20	9.88	< 0.01	6.57	0.01	0.14	0.71	1.71	0.19	0.98	0.32	0.02	0.88	0.17	0.68
Nuts	20.15	< 0.01	1.86	0.17	12.40	< 0.01	0.99	0.32	0.69	0.41	8.99	< 0.01	6.09	0.01	1.10	0.29	3.41	0.07
Broth	23.17	< 0.01	0.82	0.36	1.10	0.29	21.10	< 0.01	31.06	< 0.01	142.49	< 0.01	0.65	0.42	0.16	0.69	11.41	0.00
Astringent-P	39.99	< 0.01	0.68	0.41	0.03	0.87	16.27	< 0.01	1.36	0.24	17.26	< 0.01	0.00	0.97	3.59	0.06	5.01	0.03
Bitter-P	29.24	< 0.01	0.04	0.84	2.47	0.12	0.06	0.80	0.83	0.36	8.16	< 0.01	0.08	0.78	3.07	0.08	0.01	0.90
Fat-P	54.04	< 0.01	3.77	0.05	1.91	0.17	118.24	< 0.01	14.35	< 0.01	21.24	< 0.01	0.00	0.96	6.11	0.01	0.02	0.89
Aromatic intensity-P	51.62	< 0.01	2.82	0.09	16.59	< 0.01	0.15	0.70	1.25	0.26	9.73	< 0.01	2.12	0.15	4.00	0.05	0.52	0.47

Sensory interactions between taste and flavour and between texture and flavour were examined. When panellists were wearing the nose-clip, they perceived the bitter and salty notes as more intense ($F=14.71$ and $F=4.17$, respectively) than when they were not wearing the nose-clip (4.00 vs. 3.45 and 3.98 vs. 3.70, respectively).

Protein type influenced the perception of 14/15 attributes (not almond). The most affected attributes were salty ($F=241.07$), mouthfeel ($F=233.58$), and broth ($F=142.49$). Compared to isolate-based beverages, pellet-based beverages were perceived as more bitter and fatty with a more pronounced mouthfeel and more persistent astringency and bitterness; they were also perceived as less salty, sweet, and aromatically intense with less persistent overall aromatic intensity.



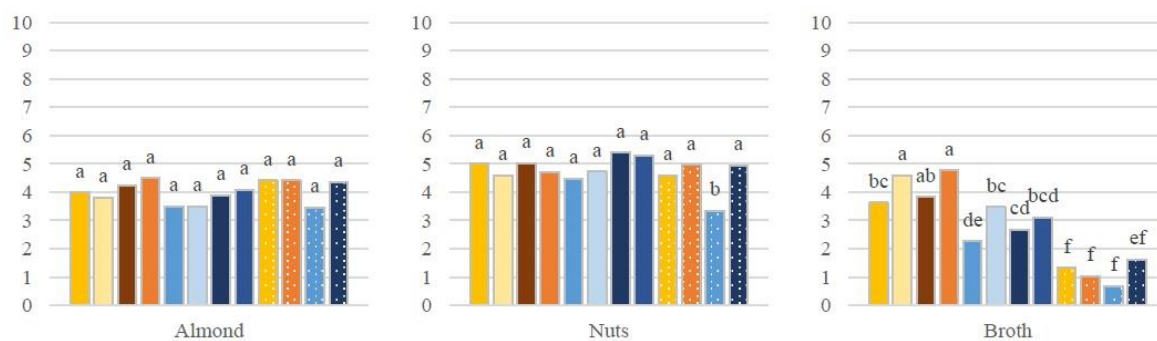


Figure 3.4.1.3: Mean attribute intensities (across replicates and panellists) of the 12 beverages containing different levels of the same ingredients as evaluated using static block profiling (differences in letters indicate significant differences among groups as revealed by Newman-Keuls post-hoc analysis). Intensity scores could range from 0 to 10. Abbreviations: I = protein from isolate (solid colour), P = protein from pellet (dotted colour), F+ = 1.5% oil content (dark colour), F- = 0% oil content, G+ = 0.5% gellan gum content (blue), G- = 0.12% gellan gum content (orange), S+ = 0.12% salt content (light colour), and S- = 0.08% salt content.

Gellan gum content (0.5% vs. 0.12%) influenced the perception of 9/15 attributes (not bitter, sweet, pea, nuts, the persistence of bitterness, or the persistence of overall aromatic intensity). The most affected attributes were mouthfeel ($F=1769.43$) and fat ($F=118.24$). Beverages with 0.5% gellan gum content were perceived as fattier with a more pronounced mouthfeel; the persistence of fattiness was also greater. These beverages were also perceived as less salty and astringent with a lower overall aromatic intensity and less persistent astringency. Their almond and broth notes were also less pronounced. Salt content (0.08% vs. 0.12%) influenced the perception of 5/15 attributes (salty, fat, mouthfeel, broth, and the persistence of fattiness). Interestingly, the most affected attributes were mouthfeel ($F=82.71$) and salty ($F=49.64$). Unsurprisingly, beverages with 0.12% salt content were perceived as saltier; they were also perceived as fattier and brothier with a more pronounced mouthfeel.

Oil content (1.5% vs. 0%) influenced the perception of 6/15 attributes (fat, mouthfeel, overall aromatic intensity, almond, nuts, and the persistence of overall aromatic intensity). The most affected attribute was mouthfeel ($F=19.10$). Consequently, oil content appeared to have more moderate effects than protein type, gellan gum content, and salt content. Compared to beverages without oil, beverages with oil were perceived as fattier with a more pronounced mouthfeel. They were also perceived as having greater overall aromatic intensity, more persistent overall aromatic intensity, and stronger notes of almond and nuts. Except in the case of protein type, beverage composition did not significantly affect the perception of bitterness. Only protein type and gellan gum content influenced the perception of astringency.

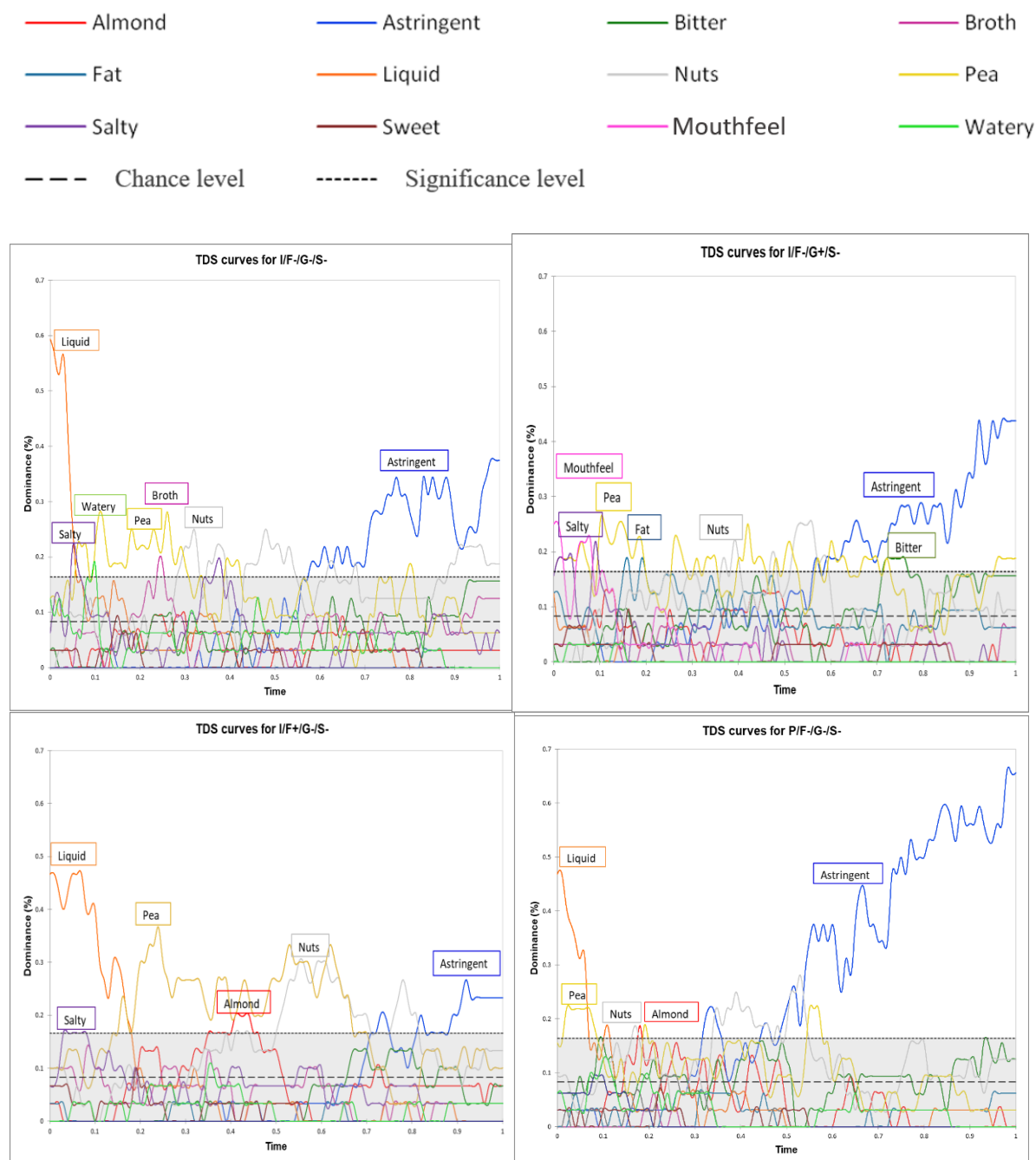
There were interactions between protein type and gellan gum content that significantly impacted 5/15 attributes (fat, mouthfeel, overall aromatic intensity, broth, and the persistence of astringency). When a beverage was made with pellet-based protein and contained 0.5% gellan gum, its fattiness and mouthfeel were perceived as more intense, whereas its overall aromatic intensity and brothiness were perceived as less intense; when the gellan gum content was lower (0.12%), the persistence of astringency was perceived as lower. There were also interactions between gellan gum content and salt content, which affected 4/15 attributes (salty, fat, mouthfeel, and the persistence of fattiness). Beverages containing 0.12% gellan gum and 0.12% salt were perceived as saltier and fattier with a more pronounced mouthfeel and more persistent fattiness. The interaction between gellan gum content and oil content significantly impacted 2/15 attributes (nuts and overall aromatic intensity). Beverages containing 0.5% gellan gum and 1.5% oil were perceived as nuttier and as having greater overall aromatic intensity. The other interactions were not significant.

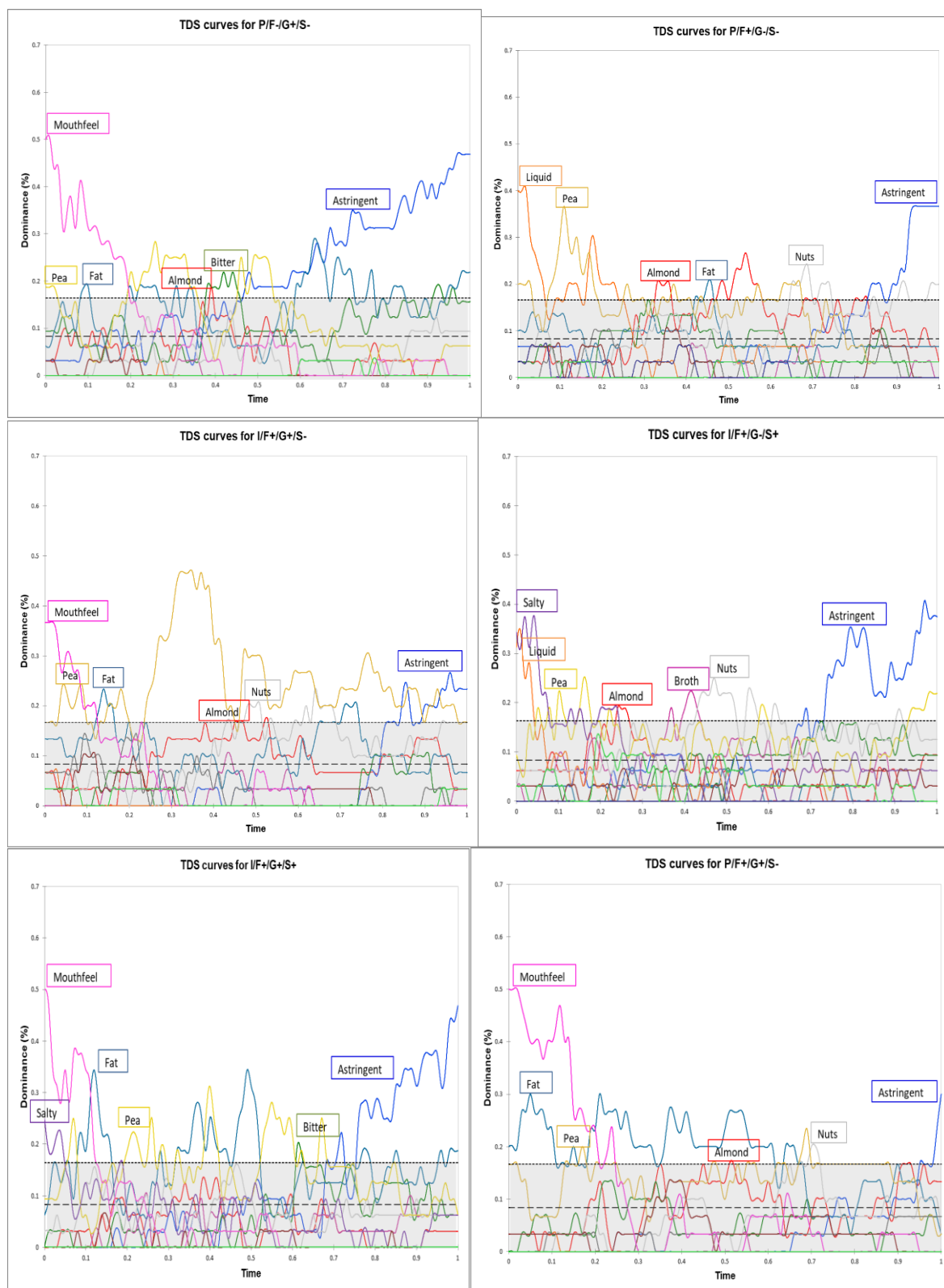
For the four attributes whose persistence was evaluated (bitter, fat, astringent, and overall aromatic intensity), the mean intensity of attribute persistence was around 2/10, which was lower than the mean intensity of the stand-alone attributes during beverage evaluation. Consequently, static block profiling

appears to provide limited information about attribute persistence, at least for the attributes tested. Furthermore, the intensities for the stand-alone attributes (blocks 1 and 2, Figure 3.4.1.1.) were strongly correlated with the intensities for attribute persistence (block 3, Figure 3.4.1.1.) ($R^2=0.84$ for astringent and the persistence of astringency; $R^2=0.81$ for bitter and the persistence of bitterness; $R^2=0.95$ for fat and the persistence of fattiness; $R^2=0.79$ for overall aromatic intensity and the persistence of overall aromatic intensity). Thus, temporal sensory profiling is needed to provide better-quality information on attribute persistence.

Results of mono-intake temporal dominance of sensations profiling

The perceived dominant attributes of the beverages across the consumption period can be seen in Figure 3.4.1.4.





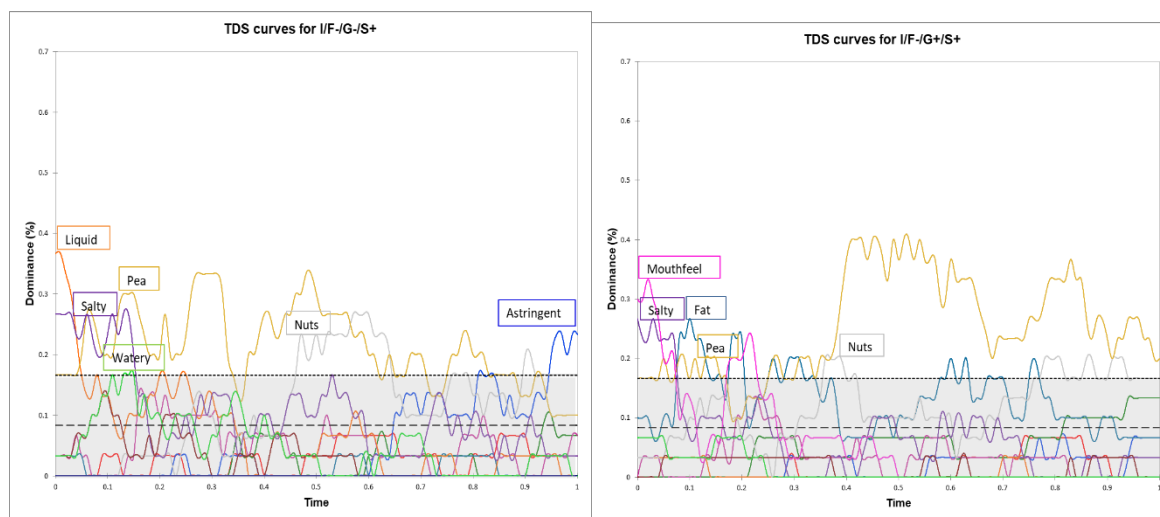


Figure 3.4.1.4: Standardized TDS curves for the 12 pea protein-based beverages used in this study. The curves depict attribute dominance over time. The two horizontal lines indicate the relative dominance an attribute could achieve by chance alone (chance level) and the minimum relative dominance an attribute needed to obtain for the result to be significantly different from that expected by chance alone (significance level). Abbreviations: I = isolate, P = pellet, F+ = 1.5% oil, F- = 0% oil, G+ = 0.5% gellan gum, G- = 0.12% gellan gum, S+ = 0.12% salt, and S- = 0.08% salt.

The first instance of swallowing is not indicated because it always occurred at the very beginning of the evaluation period (within 4.32–7.90 s of starting the 120-s period), which underscores the effect of the aftertaste on attribute dominance. Beverage composition affected the time to the first instance of swallowing and total evaluation duration (three-way ANOVA; Table 3.4.1.5.). Differences in both these dependent variables ($F=3.43$ and $F=6.51$, respectively) were observed among beverages: beverages with the least pronounced mouthfeel were swallowed the fastest (I/F-/G-/S-, I/F+/G-/S+, I/F-/G-/S+, and I/F+/G-/S- were first swallowed within 4.32–4.93 s). The beverage with the most pronounced mouthfeel was swallowed the slowest (I/F+/G+/S+ product was first swallowed within 7.90 s), and its evaluation duration was the longest. There were also marked differences among panellists in both variables (time to first swallow: range: 0–42.25 s, mean: 30.48 ± 5.11 s, and $F=35.82$; evaluation duration: range: 10.75–120 s, mean: 24.49 ± 5.90 s, and $F=158.70$). The interactions between product type and panellist ID were also significant (time to first swallow: $F=1.40$ and evaluation duration: $F=1.98$). The pronounced variability in both variables reflected the prominent differences in food oral processing among panellists.

Table 3.4.1.5.: Results of the three-way ANOVA (panellist ID, replicate, and product type as fixed effects + their first-order interactions) examining the effects of beverage type (all 12 beverages) on the time to the first instance of swallowing and the total duration of evaluation using the mono-intake TDS profiling data (total degrees of freedom: 359; residual degrees of freedom: 154). Significant p-values are in bold ($\alpha = 0.05$).

	Panellist ID		Replicate		Product type		Panellist ID*		Panellist ID* Product type		Replicate* Product type	
	<i>F</i>	<i>p-value</i>	<i>F</i>	<i>p-value</i>	<i>F</i>	<i>p-value</i>	<i>F</i>	<i>p-value</i>	<i>F</i>	<i>p-value</i>	<i>F</i>	<i>p-value</i>
Time to first swallow	35.82	<0.01	0.01	0.90	3.43	0.00	0.46	0.95	1.40	0.02	0.71	0.73
Total duration of evaluation	158.70	<0.01	1.03	0.31	6.51	<0.01	0.84	0.63	1.98	<0.01	2.31	0.01

During the evaluation period, panellists described the 12 beverages using at least 5 attributes. Specific sensory phases were also identified. In the first part of the evaluation period, for all beverages, the dominant attributes were those associated with texture and sapidity (liquid, mouthfeel, and salty). Then,

depending on the specific beverage, attributes related to aroma (almond, pea, and broth), texture (fat, watery), and sapidity (salty, bitter) were simultaneously dominant. Finally, in the last part of the evaluation period, astringency was dominant for all the beverages.

When the beverages were examined separately, the results were consistent with those obtained using static block profiling as illustrated by the high RV coefficient of 0.796 between static and TDS profiling data (Multiple Factor Analysis). For the pellet-based beverages versus the isolate-based beverages, the attributes fat, mouthfeel, and astringent remained dominant for a longer period of time, while the attributes salty, almond, pea, and broth remained dominant for a shorter period of time. The dominance of the attributes fat and mouthfeel lasted longer in beverages containing 0.5% gellan gum than in beverages containing 0.12% gellan gum. Unsurprisingly, the dominance of the attribute salty lasted longer in beverages containing 0.12% salt than in beverages containing 0.08% salt. Similarly, the dominance of the attribute fat persisted for longer in the beverages containing oil (1.5%) than in the beverages without any oil.

However, mono-intake TDS results also provided additional information, notably with regards to bitterness and astringency. Panellists seemed to barely perceive astringency in the beverages containing 0% oil and 0.12% salt (I/F-/G-/S+ and I/F-/G+/S+). This attribute was also much less dominant in beverages containing 1.5% oil and 0.08% salt (P/F+/G+/S-, I/F+/G+/S-, I/F+/G-/S-, and P/F+/G-/S-). The attribute bitter was rarely perceived as dominant; when it was, it was only in the three beverages containing the higher percentage (0.5%) of gellan gum (P/F-/G+/S-, I/F-/G+/S-, and I/F+/G+/S+). Based on these results, two beverages (I/F+/G+/S- and P/F+/G+/S-) were selected for evaluation with the multi-intake TDS method because they displayed weakly persistent astringency and different temporal profiles for the attribute pea (a contributor to beaniness).

Results of multi-intake temporal dominance of sensations profiling

In the multi-intake TDS method, panellists had to evaluate attribute dominance at three time points: once after consuming the first spoonful of beverage, once after consuming 60 mL (half) of the beverage, and once after consuming 120 mL (all) of the beverage.

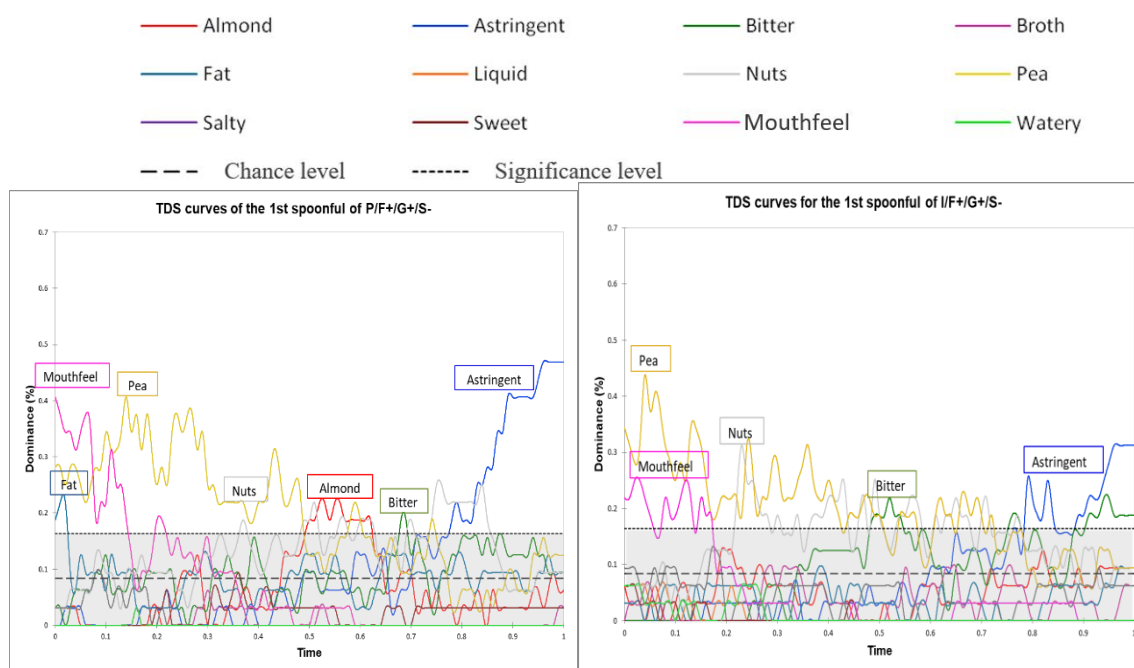
Product type affected the time to the first instance of swallowing and total evaluation duration (four-way ANOVA; Table 3.4.1.6.). Beverages differed in the time to the first swallow ($F=4.70$). The beverage with the less pronounced mouthfeel was swallowed faster (I/F+/G+/S-: 5.87 s) than the beverage with the more pronounced mouthfeel (P/F+/G+/S-: 6.73 s). There were differences in both variables among the evaluation time points (time to first swallow: $F=11.48$ and evaluation duration: $F=7.10$). Time to the first swallow was longest after the first spoonful, regardless of product type (1st spoonful: 7.55 s; 2nd spoonful: 6.12 s; 3rd spoonful: 5.23 s), as was the length of the evaluation period (1st spoonful: 48.11 s; 2nd spoonful: 44.39 s; and 3rd spoonful: 41.82 s). These results likely reflect panellist fatigue and adaptation effects.

Table 3.4.1.6.: Results of the four-way ANOVA (panellist ID, product type, spoonful ID, and replicate as fixed effects + their first-order interactions) examining the effects of beverage type (only I/F+/G+/S- and P/F+/G+/S-) on the time to the first instance of swallowing and the total duration of evaluation using the multi-intake TDS profiling data (total degrees of freedom: 191; residual degrees of freedom: 107). Significant p-values are in bold ($\alpha = 0.05$). Abbreviations: I = isolate, P = pellet, F+ = 1.5% oil, G+ = 0.5% gellan gum, and S- = 0.08% salt.

	Panellist ID		Product type		Spoonful ID		Replicate		Panellist ID * Product type		Panellist ID * Spoonful ID		Panellist ID * Replicate		Product type * Spoonful ID		Product type * Replicate		Spoonful ID * Replicate	
	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
Time to first swallow	33.07	<0.01	4.70	0.03	11.48	<0.01	0.06	0.81	4.29	<0.01	0.85	0.68	2.98	<0.01	1.94	0.15	1.06	0.31	1.24	0.29
Total duration of evaluation	61.87	<0.01	2.74	0.10	7.10	<0.01	10.54	0.00	2.15	0.01	1.81	0.02	3.42	<0.01	0.76	0.47	0.10	0.76	0.26	0.77

Attribute dominance over time for the two beverages are shown in Figure 3.4.1.5. These two beverages were selected from the evaluation with the multi-intake TDS method because they displayed weakly persistent astringency and different temporal profiles for the attribute pea. As in the results for the mono-intake TDS method, panellists described the beverages as having at least five different attributes. The sequence of dominant attributes was also similar. In the first part of the evaluation period, the dominant attributes for I/F+/G+/S- were mouthfeel and pea; for P/F+/G+/S-, they were mouthfeel and fat. Then, the attributes fat, pea, nuts, and almond were more dominant, but their relative ranks were dependent on product type and spoonful ID. In the last part of the evaluation period, the attributes astringent and bitter were dominant for I/F+/G+/S-, and the attributes astringent and fat were dominant for P/F+/G+/S-.

The results for the first spoonfuls consumed during the multi-intake TDS sessions did not fully match the results for the single spoonfuls consumed during the mono-intake TDS sessions. When I/F+/G+/S- was evaluated using the multi-intake TDS method, the attributes bitter and nuts were dominant for the longest amount of time after the first spoonful of beverage was consumed; in contrast, when the mono-intake TDS method was used, the attributes mouthfeel, fat, and pea were the most dominant. Similarly, when P/F+/G+/S- was evaluated using the multi-intake TDS method, the attributes bitter, astringent, pea, and almond were dominant for the longest amount of time after the first spoonful of beverage was consumed; in contrast, when the mono-intake TDS method was used, the attributes mouthfeel and fat were the most dominant. These contrasting results may stem from methodological differences. During the mono-intake TDS sessions, panellists evaluated a total of 12 spoonfuls of beverage at random points during a given session. Thus, these single spoonfuls do not truly correspond to the “real” first spoonfuls taken during the multi-intake TDS sessions.



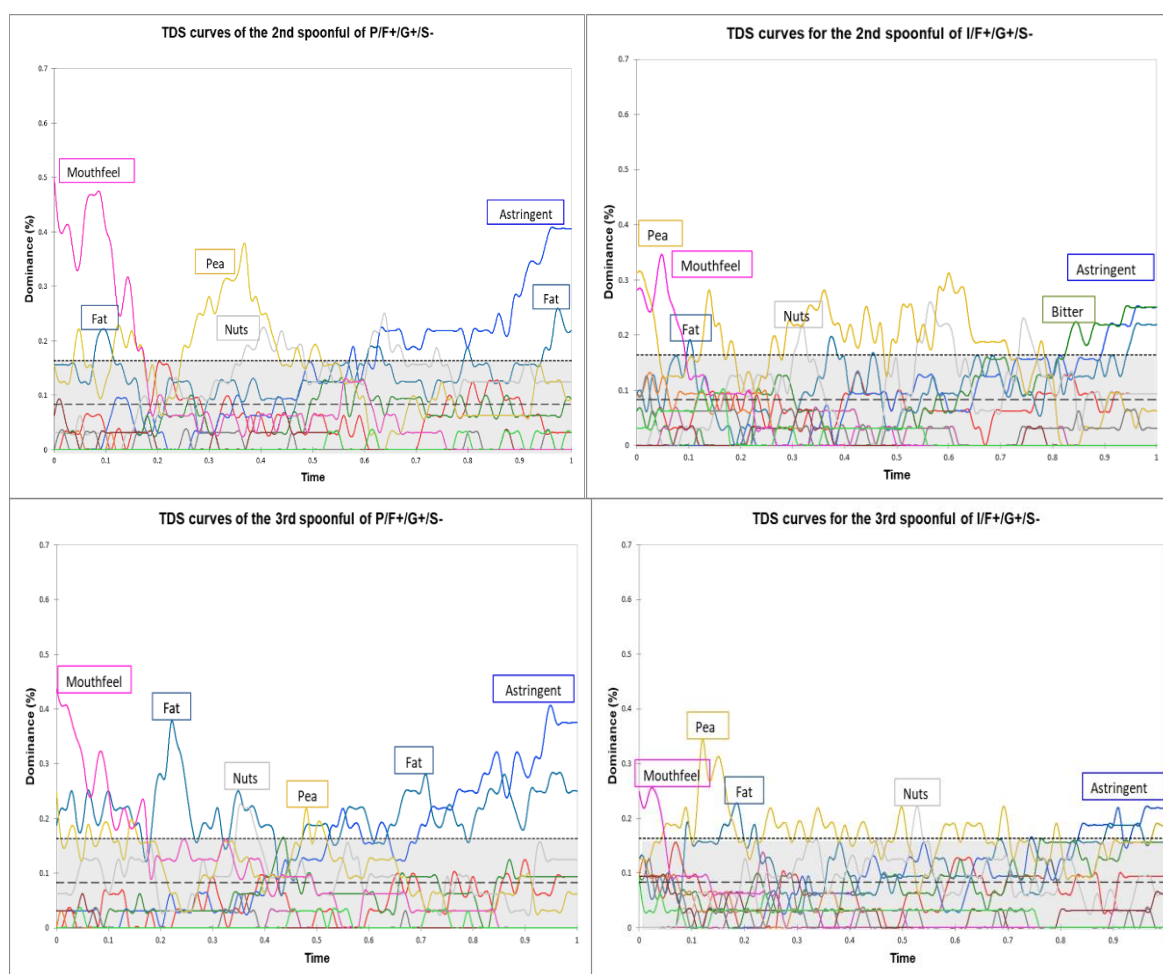


Figure 3.4.1.5.: Standardized TDS curves for the two pea protein-based beverages (I/F+/G+/S- and P/F+/G+/S-) evaluated using multi-intake TDS profiling. The curves depict attribute dominance over time (i.e., following the first spoonful, the second spoonful [after consuming 60 mL], and the third spoonful [after consuming 120 mL]). The two horizontal lines indicate the chance level and the significance level (see Figure 3.4.1.4.). Abbreviations: I = isolate, P = pellet, F+ = 1.5% oil, G+ = 0.5% gellan gum, and S- = 0.08% salt.

Attributes decreased in dominance over the course of the evaluation period for I/F+/G+/S-. Panellists perceived the beverage's attributes quite differently by the time they reached the end of consumption: for example, the dominance of the attributes pea and astringent declined between the first and the third spoonful (from 45% to 35% and from 32% to 25%, respectively). For P/F+/G+/S-, the same decline in dominance was observed for the attributes pea, nuts, and almond. However, astringency was still highly dominant at the end of the evaluation period, and the attribute fat increased in dominance over time.

3.4.1.4. Discussion

The aim of this study was to better understand how product composition affected the sensory perception of pea protein-based beverages using three different sensory profiling methods. The first part of the discussion focuses on how beverage composition affected the perception of texture and sapidity. The second part examines the perception of aroma and the sensory interactions of flavour with taste and texture. The third part addresses the importance of employing a combination of sensory profiling methods (static/temporal, mono-intake/multi-intake) when evaluating potential food products.

Perception of texture and sapidity

In this study, the composition of the pea protein-based beverages greatly impacted perceptions of texture and sapidity. When the static block profiling method was used (i.e., when sensory attributes were evaluated immediately after consumption), gellan gum content, salt content, and oil content were found to significantly increase the perceived intensity of fattiness and mouthfeel. This result suggests a relationship exists between the two attributes. Similarly, when the mono-intake TDS method was used (i.e., where sensory attributes were evaluated over a 2-min period following consumption), the attribute mouthfeel was perceived as more dominant for beverages with low gellan gum and salt contents. The attribute fat was perceived as more dominant for beverages with high gellan gum contents that also contained oil.

When beverages had a lower salt content, the perceived intensity of saltiness was lower (as measured via static block profiling) and the attribute salt was less dominant (as measured via mono-intake TDS profiling). When beverages had higher gellan gum content, the perceived intensity of astringency was lower (as measured via static block profiling), but the attribute bitter was highly dominant (as measured via mono-intake TDS profiling). Here however, in contrast to other studies, there was no significant effect of fat content on bitterness (Schoumacker *et al.*, 2017), perhaps because the differences in oil content were small (1.5% vs. 0%).

The type of protein used to make the beverage (isolate vs. pellet) also affected perceptions of texture and sapidity. Based on static block profiling, pellet-based products were perceived as being fattier, bitterer, and less salty and as having a more pronounced mouthfeel. Based on mono-intake TDS profiling, astringency was highly dominant in pellet-based products. Protein type has a compositional effect on food products. Although pea pellets and isolates both contain similar levels of total proteins, pellets are richer in insoluble proteins, while isolates are richer in minerals, carbohydrates, polyphenols, volatile molecules, and peptides. Analyses of protein extracts have identified the proteins and peptides responsible for bitterness: they have hydrophobic side chains rich in proline and leucine (Aubes-Dufau *et al.*, 1995; Shinoda *et al.*, 1987). Astringency results from saliva proteins (e.g., salivary amylase, mucin, esterase) binding with the polyphenols present in pea protein isolates and then precipitating (Charlton *et al.*, 2002; Gibbins & Carpenter, 2013; Ma *et al.*, 2014). Thus, it can be assumed here that differences in protein type were at the origin of differences in attribute perception.

As observed in previous studies, texture attributes initially dominate food perception (Le Calvé *et al.*, 2019; Pineau *et al.*, 2009; Saint-Eve *et al.*, 2011). In addition, swallowing occurs more quickly, after a few seconds (during the first part of the evaluation period), for liquid products, a result that could be explained by the oral processing dynamics of liquid foods (Salles *et al.*, 2010). While solids need to be fragmented and mixed with saliva to form a cohesive bolus, liquids can be swallowed immediately after being diluted by saliva and warmed to body temperature (Engelen, 2003). Thus, liquids usually remain in the mouth for a much shorter period of time than do solids.

The results obtained with multiple-intake TDS profiling (i.e., where the sensory attributes of a full beverage portion were evaluated) revealed a gradual decrease in the dominance of texture attributes and bitterness over time. This decrease was more pronounced for the pellet-based beverage than the isolate-based beverage. Such attributes might become less noticeable after repeated tasting due to sensory adaptation (Lawless & Heymann, 2010). There was also a gradual increase in perceived fattiness across time, which could be due to the lingering and build-up of sensations (Lawless & Heymann, 2010). These results fit with those from several other studies showing that perceptions of fattiness build up in the mouth due to fat lingering on oral surfaces (i.e., the tongue and the palate) (Appelqvist *et al.*, 2016). The persistence of the sensation of fattiness may stem from the presence of residual fat or oil in the oral cavity after swallowing, which can increase the attribute's intensity over the course of repeated ingestion (Appelqvist *et al.*, 2016).

Perception of aroma and the interactions of flavour with taste and texture

Beverage composition greatly influenced the perception of aroma. Static block profiling showed that products with greater gellan gum content were perceived as having lower overall aromatic intensity and less pronounced almond and broth notes. In contrast, mono-intake TDS profiling revealed that the

attribute pea was relatively dominant in this beverage type. The impacts of hydrocolloid solutions on the sensory perception of food depend on a large number of variables (e.g., hydrocolloid type, range of viscosity, food matrix type, choice of sensory evaluation technique). Only a few studies have explored the effects of hydrocolloids on the perception of thickened beverages (Yanes *et al.*, 2002), and, to our knowledge, none have looked at gellan gum. However, these studies generally found that an increase in beverage viscosity led to a decrease in aroma perception (Cook *et al.*, 2005; Tournier *et al.*, 2007), which is consistent with the results of this study.

Beverages containing oil were perceived as having greater overall aromatic intensity and more intense almond and nut notes based on static block profiling. When mono-intake TDS profiling was used, these beverages displayed the highest dominance of almond and the lowest dominance of broth. Past research has repeatedly shown that lipids can modify the sensory perception of food. They function as reservoirs for numerous aroma compounds, resulting in delayed release and perception (Saint-Eve *et al.*, 2009). In addition, in static block profiling, beverages with a higher salt content were perceived as displaying more intense brothiness and, in mono-intake TDS profiling, they were perceived as having the least dominant almond note. This result can be explained by sodium chloride causing the salting out of hydrophobic aroma compounds (Saint-Eve *et al.*, 2009).

Protein type influenced the perception of overall aromatic intensity: pellet-based products were perceived as less aromatic than isolate-based products, based on static block profiling. The results for mono-intake TDS profiling provided additional support for this finding: the attributes pea and nuts were perceived as less dominant in pellet-based products than in isolate-based products. Previous research has extensively examined interactions in protein-based foods between aroma compounds and proteins (Heng *et al.*, 2006). These interactions can be modified by different factors: protein conformation and composition; the properties of aroma compounds, such as hydrophobicity; and environmental conditions, such as pH (Damodaran & Kinsella, 1981). Thus, it can be assumed that the above sensory differences arose from differences in protein type and, more specifically, differences in interactions between aroma compounds and proteins.

Here, it was found that aroma attributes were dominant during the latter part of the evaluation period, based on mono-intake TDS profiling. This finding concurs with what has been seen in previous studies. During the swallowing process, the liquid bolus is first held on the upper surface of the tongue (Macqueen *et al.*, 2003). During this step, the soft palate is most often closed, and aroma compounds have limited access to the nasal cavity, which may explain why only texture and sapidity attributes were dominant during the initial part of the evaluation period. Then, the tongue generates a wave of pressure that squeezes the liquid backward through the mouth and pharynx toward the esophagus (Mackley *et al.*, 2013). Immediately after the liquid passes the epiglottis, the soft palate is re-opened (Buettner, 2002). For liquid foods, this is the first moment in which aroma compounds have access to the nasal cavity (Buettner, 2002), and the highest aroma release signal is generally observed during the first expiration after swallowing (called the swallow breath) (Linthorpe & Taylor, 2000). This series of events may explain why aroma attributes were more dominant during the latter part of the evaluation period. After a few seconds, the concentration of volatile compounds in the mouth and nasal cavity decrease significantly (Mesurole *et al.*, 2013). In contrast, non-volatile compounds remain on oral surfaces (i.e., the tongue and palate) and continue to influence perceptions (Appelqvist *et al.*, 2016), which may explain why astringency was dominant later in the evaluation period. Multiple-intake TDS profiling showed that beaniness gradually decreased over time. This decrease was more pronounced for the pellet-based beverages. However, these attributes might become less noticeable after repeated tasting due to sensory adaptation (Lawless & Heymann, 2010).

Beverage composition had a limited effect on the sensory interactions of flavour with taste and texture. However, there were some prominent taste-flavour interactions. When the panellists used nose-clips to evaluate attributes related to texture and sapidity, bitter and salty notes were perceived as less intense than when the nose-clip was not used. Beverages were also perceived simultaneously as more beany, bitter, and salty, suggesting congruent effects. These results are consistent with those found in other studies on bitter beverages. For example, cocoa flavouring enhanced bitterness in a cocoa beverage (Labbe *et al.*, 2006), and the addition of aroma compounds increased bitterness in beers (Oladokun *et*

al., 2017). Taken together, these results suggest that interactions between taste and aroma are induced by effects of congruency.

The importance of employing a combination of sensory profiling methods

Static block profiling, in which beverage attributes were evaluated immediately after consumption, revealed that the perception of beaniness was strongly affected by beverage composition. At the same time, the differences between the different attributes contributing to beaniness (pea, nuts, almond, and broth) were not very pronounced. Mono-intake TDS profiling, in which beverage attributes were evaluated over a 2-min period following consumption, provided more detailed information about differences among beverages, especially in terms of the different attributes contributing to beaniness. In particular, results suggest that pellet-based beverages were perceived as more brothy and less pea-like than isolate-based beverages. Static block profiling found that perceived astringency was moderate and the intensities for the stand-alone attributes (blocks 1 and 2, Figure 3.4.1.1.) were strongly correlated with the intensities for attribute persistence. In contrast, mono-intake TDS profiling highlighted that perceived astringency was strongly persistent over the evaluation period and that the perception of other attributes shifted. The static block profiling method made it possible to rapidly and independently evaluate attribute intensity. However, it is difficult for panellists to assess attribute dominance and intensity at the same time during TDS (Di Monaco *et al.*, 2014), and there is thus a risk of interdependence among attributes (Saint-Eve *et al.*, 2011; Teillet *et al.*, 2010). That said, static block profiling requires panellists to integrate their changing sensory perceptions over the course of oral processing to come up with a summary evaluation (Cliff & Heymann, 1993), and it is hard to control the point in the oral process at which products are evaluated. Thus, it makes sense to jointly use static block profiling and TDS profiling to obtain a better understanding of attribute intensity and dominance in food products.

Conventionally, in TDS profiling, different attribute families (taste, texture, and aroma) can be evaluated during different parts of a study (Lesme *et al.*, 2020). Here, however, the choice was made to evaluate the different attribute families at once. Although the influence of listing attributes from different families in the same list remains unknown (Di Monaco *et al.*, 2014), this methodological approach makes it possible to assess all the attributes simultaneously and to identify specific sensory phases. The initial part of the evaluation period was dominated by texture attributes. Then, depending on the product and the panellist, different attributes became dominant. Finally, in the latter part of the evaluation period, astringency became dominant.

The results obtained with the multi-intake TDS profiling method underscore that quantifying sensory experiences over time could provide additional information about how consumers perceive foods. For example, perceived fattiness became more dominant over the course of consumption, while other attributes (except astringency) became less dominant, perhaps because repeated tasting led to sensory adaptation. Previous research using multi-intake TDS profiling found that attributes related to texture and sapidity gradually increased over time but that there was no intake effect on how long aroma attributes remained dominant (Galmarini *et al.*, 2017; Lesme *et al.*, 2020; Thomas *et al.*, 2016; van Bommel *et al.*, 2019). However, in these studies, panellists evaluated multiple spoonfuls of product in a row. In contrast, the present study had panellists evaluate spoonfuls of beverage at three distinct time periods, corresponding to the beginning, the middle, and the end of the consumption of a full product portion. Another study that examined temporal changes in attribute perceptions during the consumption of an entire portion of an oral nutritional supplement found that there were differences in the aroma attribute “praline” over time (Thomas *et al.*, 2016).

These findings raise questions regarding the ideal number of spoonfuls and amount of product that should be consumed by panellists. Here, it seemed to be more useful to have panellists evaluate spoonfuls taken at specific moments during the consumption of a full beverage portion than to have panellists consume several spoonfuls of beverage in a row. In other contexts, it could make more sense to evaluate multiple spoonfuls consumed *ad libitum*, such as when the goal is to investigate the effect of sensory-specific satiety, which is a decrease in attribute perception for a specific food following repeated exposure (Hetherington & Havermans, 2013). Nevertheless, both these methodologies (i.e., consumption of a full portion or *ad libitum* consumption) share the disadvantage that only one replicate

of one product can be evaluated per session. Thus, in addition to being time consuming, there is a risk of failing to pick up on differences among products. For this reason, it is important to explore how spoonful number and the amount of product consumed influence the results obtained.

Finally, for food production companies, improving methods for characterizing the sensory profiles of products is key to better understanding consumers' experiences. This study did not take into account temporal hedonic profiles. However, it could be interesting to combine descriptive and hedonic analyses with multi-intake TDS profiling. This approach could provide further insight into pea protein-based products, leading to their improvement (Thomas *et al.*, 2016; van Bommel *et al.*, 2019).

3.4.1.5. Conclusions and perspectives

In conclusion, this study's use of three methods—static block profiling, mono-intake TDS profiling, and multi-intake TDS profiling—helped clarify how the composition of pea protein-based beverages affected sensory perceptions. The static block profiling method, in which beverage attributes were evaluated immediately after consumption, revealed that the perception of beaniness depended on protein type; it was higher when the pea protein source was an isolate than when it was a pellet. Perceived beaniness also increased when gellan gum content was lower and oil content was higher. The mono-intake TDS profiling method, in which beverage attributes were evaluated over a 2-min period following consumption, showed that beverages differed markedly in the dynamics of their aroma attributes. In particular, almond notes were more dominant and pea notes were less dominant in pellet-based beverages than in isolate-based beverages. These characteristics were accentuated from one spoonful to the next. Perceptions of astringency and bitterness were mainly impacted by protein type and gellan gum content. While static block profiling found a moderate level of perceived astringency, mono-intake TDS profiling highlighted that astringency was strongly persistent and that this persistence seemed to be limited by gellan gum and salt contents. The use of the nose-clip during static block profiling indicated that there were few interactions of flavour with texture and taste. It also yielded evidence of a weak effect of congruency between the bitter/salty notes and the beany note. Specific sensory phases were also identified: texture attributes were more prominent during initial consumption, and astringency was more prominent during later consumption. Finally, the multi-intake TDS profiling results suggest that, over time, the perception of fattiness built up and the perception of beaniness shifted because of sensory adaptation. Thus, taken together, this study's findings have enhanced understanding of sensory perceptions of pea protein-based beverages under conditions that more closely resemble those associated with real-life consumption. They also provide clues for reformulating pea protein-based products to reduce beaniness, bitterness, and astringency.

Main results

- The static block and mono-intake TDS profiling methods yielded complementary results about the impact of beverage composition on attribute perceptions.
- Static block profiling revealed that beaniness was mainly affected by gellan gum and oil content and that bitterness and astringency were mainly affected by protein type and gellan gum content.
- Mono-intake TDS profiling highlighted the dynamics of beaniness and the strong persistence of astringency, and its results suggested that higher gellan gum and salt contents could limit this persistence.
- Multi-intake TDS profiling found that, over the course of the consumption of a full product portion, beaniness and bitterness decreased, indicating an adaptation effect, while fattiness increased, indicating a build-up effect.

Section 3.4.2. – Sensory perceptions of pea protein beverages explained by rheological and chemical properties

3.4.2.1. Introduction

Consumers are increasingly seeking out healthy, ethically produced, and eco-friendly foods. In this context, plant proteins are proving to be a great success and yellow field pea (*Pisum sativum* L.), in particular, is becoming a common ingredient in plant-based foods (Siddique *et al.*, 2012). This last is used in the formulation of many types of foods, including fortified beverages, imitation milk and sports drink (Boye *et al.*, 2010). However, consumers usually describe pea protein-based foods as having strong beany, bitter, and astringent notes, which can decrease their desirability. These distinct and persistent perceptions challenge their use as beverages ingredients (Lam *et al.*, 2018).

Particular attention has been paid in literature to the beany perceptions of pea protein-based products, to understand the volatile compounds at their origin. Murat *et al.* investigated the key volatile compounds of pea flour and pea protein isolates using three extraction methods and GC-MS-O (Murat *et al.*, 2013). Azarnia *et al.* compared the volatile compounds in peas of different cultivars and under storage conditions (Azarnia *et al.*, 2011). Bi *et al.*, investigated the effects of roasting on variations in the key volatile compounds in peas by recombination and omission experiments (Bi *et al.*, 2020). Trikusuma *et al.*, characterize changes in the aroma profile of a pea protein beverage as impacted by UHT processing and storage (Trikusuma *et al.*, 2020). Zhang *et al.*, investigated the volatile composition of pea milk with GC-O-MS, and calculated odour activity values of key volatile compounds (Zhang *et al.*, 2020). Nevertheless, none of these studies, take into account the impact of the food oral process and the role of saliva on these particular perceptions.

However, during oral processing, beverages are subject to a range of mechanical and chemical transformations which can modify greatly perceptions (Foster *et al.*, 2011). It is a dynamic process involving mechanical deformation processes by mastication and movements between the tongue and the palate (Stokes *et al.*, 2013); particle hydration and lubrication by saliva to form a bolus (Mishellany *et al.*, 2006); and enzymatic degradation by salivary enzymes (Pagès-Hélary *et al.*, 2014, Buettner *et al.*, 2002). During this process, volatile compounds can be adsorbed at the surface of the oral mucosa, before being progressively desorbed and released into the oral cavity after the equilibrium has changed due to food swallowing (Buettner *et al.*, 2002). They can also be enzymatically converted to new compounds in the oral cavity by salivary enzymes (Buettner, 2002; Pagès Hélary, *et al.*, 2014) or by cellular enzymes (Robert-Hazotte *et al.*, 2019). In addition, volatile compounds release, and so aromatic perceptions, depend also of the mobility (diffusion and convective transport) and on the partition properties of aroma compounds on the matrix (Poling *et al.*, 2000; Deleris *et al.*, 2016). They depend also of their interactions with hydrophobic constituents of the matrix, such as lipids, polysaccharides and proteins (Relkin *et al.*, 2004).

Thus, complex olfactory perceptions can depend on oral process between food and individual and on physico-chemical interactions, particularly with saliva. In this context, the objective of this part of work is to quantify these different interactions in order to better understand the origin of perceptions of pea-based beverages and how these perceptions can be impacted by the formulation. For this purpose, different pea protein-based beverages (containing pea-proteins, salt, sunflower-oil, gellan gum, sugar and soy-lecithin) were formulated and sensory described by a trained panel. Their texture was characterized by rheological methods and aroma release by gas-chromatography–mass-spectrometry in different conditions (with no dilution, dilution with water and dilution with saliva). Relationships between rheological data and sensory data were discussed taking into account some oral processing factors implied for liquid products. In addition, the ability of volatile compounds to predict aroma perceptions was discussed taking into account the food dilution with saliva.

3.4.2.2. Materials and methods

Product preparation

In this study, 12 types of pea protein-based beverages were created in the lab (composition and ingredient concentrations are in Table 3.4.2.1.). Water (Evian, France), gellan gum (Texturas Ferran Adria, Spain), salt (Auchan, France), sunflower oil (Auchan, France), sugar (Daddy, France), and soy lecithin (Louis Francois, France) were the other ingredients used to formulate the beverages. Two Thermomix® TM5™ appliances (Vorwerk, Germany) were employed to standardize product preparation.

Table 3.4.2.1.: Composition (ingredient concentrations [w/w %]) of the pea protein-based beverages used in this study as well as the pH and the power law index and consistency index measured by steady shear stress. Abbreviations: I = isolate, P = pellet, F+ = 1.5% oil, F- = 0% oil, G+ = 0.5% gellan gum, G- = 0.12% gellan gum, S+ = 0.12% salt, and S- = 0.08% salt.

Product name	Protein type	Sunflower Oil (%)	Soy lecithin (%)	Gellan gum (%)	Salt (%) (NaCl)	Sugar (%)	Pea protein (%)	Water (%)	pH	Power law index	Consistency index
	(P or I)	(F+ or F-)		(G+ or G-)	(S+ or S-)						
I/F-/G-/S-	Isolate	0.00	0.00	0.12	0.08	1.00	7.00	91.80	7.95	0.55	0.51
I/F-/G-/S+	Isolate	0.00	0.00	0.12	0.12	1.00	7.00	91.76	7.88	0.51	0.59
I/F-/G+/S-	Isolate	0.00	0.00	0.50	0.08	1.00	7.00	91.42	7.91	0.25	12.09
I/F-/G+/S+	Isolate	0.00	0.00	0.50	0.12	1.00	7.00	91.38	7.94	0.21	15.19
I/F+/G-/S-	Isolate	1.50	0.10	0.12	0.08	1.00	7.00	90.20	7.88	0.54	0.56
I/F+/G-/S+	Isolate	1.50	0.10	0.12	0.12	1.00	7.00	90.16	7.86	0.49	0.72
I/F+/G+/S-	Isolate	1.50	0.10	0.50	0.08	1.00	7.00	89.82	7.84	0.26	10.26
I/F+/G+/S+	Isolate	1.50	0.10	0.50	0.12	1.00	7.00	89.78	7.87	0.21	20.44
P/F-/G-/S-	Pellet	0.00	0.00	0.12	0.08	1.00	7.00	91.80	7.73	0.51	1.94
P/F-/G+/S-	Pellet	0.00	0.00	0.50	0.08	1.00	7.00	91.42	7.66	0.34	16.76
P/F+/G-/S-	Pellet	1.50	0.10	0.12	0.08	1.00	7.00	90.20	7.74	0.50	2.15
P/F+/G+/S-	Pellet	1.50	0.10	0.50	0.08	1.00	7.00	89.82	7.64	0.33	16.32

Two different types of pea protein (isolates and pellet) were evaluated. First, pea protein pellets were obtained as follows: water and pea protein isolates were slowly mixed together (96% [w/w] water, 4% [w/w] pea protein isolate) and then left to hydrate for 60 min at 4°C under stirring. The pellet and supernatant were separated via centrifugation at 6000 rpm at 4°C for 10 min. The pellet was stored at 4°C for a maximum of 2 h before the beverages were made.

Second, the beverages were created using the following method: the water was mixed and heated (3 min, 50°C, and speed setting of 2.5) in one of the Thermomix appliances. The sugar, salt, pea protein (isolate or pellet), and gellan gum were then gradually mixed into the water (30 min, 50°C, speed setting of 4.5). Simultaneously, the sunflower oil was heated (1 min, 65°C, speed setting of 1.5) in the second Thermomix. The soy lecithin was then mixed into the sunflower oil (3 min, 65°C, speed setting of 2). The contents of the first Thermomix were added to the contents of the second Thermomix 2 and combined without heating (5 min, speed setting of 5). After this step, the overall mixture was heated (6 min, 90°C, speed setting of 3.5). Immediately after preparation, the beverages were stored at 4°C until they were used in the sensory profiling sessions. The Thermomix appliances were cleaned by filling them with a mixture of 2 L of water, 100 ml of white vinegar, and 5 mL of dishwashing liquid, which was then heated (5 min, 70°C, and speed setting of 1). The appliances were subsequently thoroughly rinsed with hot water and stored at 4°C until they were next used in order to prevent any bacterial growth.

Before all sensory analysis, the microbial safety of the products was controlled by a certified external laboratory (Eurofins Scientific, France). The products were served to the panellists at room temperature (20°C) in transparent cups (29.5 ml) identified with three-digit codes.

Texture analysis

A rheometer (MCR301, Anton Paar, Graz, Austria) equipped with sanded coaxial cylinders (diameter of 27 mm) was used to test the gels' rheological properties. The beverages were carefully poured into

the cylinder, which was kept at 20 °C. Different tests were subsequently carried out on the samples. Three replicates were done for each experiment and each product.

(1) Steady shear rate (1 to 1000 1/s, 15 measurement points) were performed at 20 °C using rotation mode. From the shear rate and the shear stress data, the flow curves were plot using logarithmic scales and modelled with a power law ($\tau = \gamma k^n$ such as τ = shear stress, γ = shear rate, n = power law index and k = consistency).

(2) Strain sweeps (0.001–100%, 1 Hz) were performed at 20 °C. From the shear stress data, the loss modulus [Pa] and the storage modulus [Pa] were calculated.

(3) Frequency sweeps (10–0.01 Hz, 1%, 11 measurement points) were performed at 20 °C. From the shear stress data, the loss modulus [Pa] and the storage modulus [Pa] were calculated.

Volatile compounds analysis

To analyse the aroma compounds released from the pea protein beverages, GC-MS analysis was performed. A Dynamic Headspace Gas Chromatography coupled with a Mass Spectrometer (MPS: Gerstel, GC: Agilent 7890B, MS: Agilent 5977B MSD) was used to quantify aroma compounds released from the different beverages. Protocol was carried out as follows. After thawing during one night (about 15 hours) at 4°C, 5g of each product was stored at 10°C on the GC-MS sample holder. Each sample was then incubated at 40°C for 3min (agitation at 500 rpm). The headspace was then purged with a constant flow of helium at 30 ml/min for 20 min at 30°C and aroma compounds were trapped on an adsorption unit (tenax polymer). The trap was dried for 6 minutes under a stream of helium to remove traces of water. Then the trap unit was desorbed from 30°C to 270°C at a rate for 60°C/min with an isotherm of 7 min in a cool injection system at -100°C. The column head injection was carried out from -100°C to 270°C at a rate of 12°C/min with an isotherm of 5 min.

The GC oven temperature was programmed from 40°C with an isotherm of 5 min, to 155°C at a rate of 4°C/min, then to 250°C at a rate of 20°C/min with an isotherm of 5 min. The GC was equipped with an apolar column (DB-5, 60m x 320µm x 1µm) with a helium flow of 1.6 mL/min. A mass spectrometer was used to characterize aroma compounds. It was performed in the electron impact mode at 70 eV, in full scan from m/z 29 to 300 A.M.U. (Atomic Mass Unit). The ionisation source was set at 230 °C and the quad at 150 °C. The compounds were tentatively identified by comparison of their mass spectra with those of the NIST 2017 Mass Spectral Library. The data were reported as peak area for each compounds detected. Quantification data were obtained from the integration of the areas from the total ion current (TIC). MassHunter software was used for instrument control, data acquisition, and data analysis.

Impact of saliva on volatile compounds analysis

Samples (2.5g) were diluted at 50% with either water (Evian, France) or saliva (Commercial human saliva, pooled from 10 donors - 5 female and 5 male, BioIVT, United Kingdom). Samples were shaken slowly and stored during 2h at 4°C to allow complete solubilisation and equilibrium for the interactions within volatile and non-volatile compounds. All samples were prepared in duplicate and stored in dark condition at -80°C in glass vial previous to analysis. Measurement of aroma compounds release was conducted using the same GC-MS. The protocol was exactly as described previously except that the headspace was purged with a constant flow of helium at 30 ml/min for 20 min at 30°C.

Sensory analysis

The pea protein solution sensory characterization can be found in the companion papers (Cosson *et al.*, 2020; Section 3.4.1.). However, the main parameters are recalled here for sake of clarity. Pea solutions were characterized by sixteen panellists (15 women and 1 man, 18–39 years in age). Panellists performed the sensory evaluations in individual booths under white light in an air-conditioned room (20°C). The attributes were generated and selected with a check-all-that-apply (CATA) questionnaire. A final list of 11 attributes was used to characterise the intensities of beverages: salty, bitter, astringent, sweet, fat, pea, almond, nuts, broth, mouthfeel, and overall aromatic intensity. The panellists were trained to evaluate the intensity of these attributes along an unstructured scale (range: 0–10) using external references. Training took place over 10 sessions that each lasted 45 min.

Panellists were asked to score the attributes of the 12 beverages using a static block profiling method. They had to evaluate six beverages per session and were unaware of beverage identity. To account for

the order in which the beverages were experienced and any potential carry-over effects, beverage order was balanced across panellists using a Latin square. To reduce sensation build-up, a palate-cleansing protocol was used between beverages: panellists had to consume an apple slice, drink water, and wait 40 seconds before consuming the following beverage (Cosson *et al.*, 2020; Section 3.4.1.). Sensory analysis was managed using Fizz Acquisition software (v. 2.51, Biosystemes, France). Panellist's performances were evaluated and controlled. Overall performance was assessed using ANOVA with three independent variables (product type, panellist ID, and replicate) and their first-order interactions. There was a product effect, indicating that panellists distinguished among the different beverages ($p < 0.05$). The significance of various interactions revealed whether the panellists consistently scored attributes across replicates (panellist*replicate), whether there was consistency in scoring among panellists (product type*panellist ID), and whether panellists scored products consistently across replicates (product type*replicate). The performance of individual panellists was also evaluated based on their ability to discriminate among beverages and on repeatability criteria.

Statistical analysis

Data analysis was performed using XLSTAT (Addinsoft, 2017, Paris, France) and JMP (JMP software version 13.1.0 (SAS Institute Inc., Cary, SC, USA)). For analyses of an inferential nature, we used $\alpha = 0.05$ to determine statistical significance.

To analyse the profiling results, we carried out PCA (Principal component analysis) on correlation matrix; the data were averaged across replicates and panellists (centred, Pearson type).

To analyse rheological data, the dynamic viscosity was calculated and plotted in function of the shear rate according to the formulations of pea beverages. The power law index and consistency index from the power law model were also calculated. To analyse effects of the samples composition on the power law index, ANOVA (analysis of variance) were performed in which pea protein origin, oil content, gellan gum content, and salt content were fixed effects. The loss modulus and the storage modulus and their ratio were calculated and plotted in function of the strain or the frequency according to the formulations of pea beverages. Finally, correlations coefficients and their corresponding p-value (Pearson method) were calculated between rheological parameters and mouthfeel/astringent sensory intensities.

To analyse GC-MS data, hierarchical cluster analysis (HCA, two-way clustering, Ward method) was performed on GC-MS data. Dendrogramms were plotted with colour map. To analyse impact of dilution with saliva on GC-MS results, we carried out PCA (Principal component analysis) on correlation matrix; the data were averaged across replicates (centred, Pearson type). To visually explore differences in the results obtained using sensory analysis and GC-MS analysis, we carried out multiple factor analysis (MFA) (centred by group; Pearson type); the data from both methods were averaged across replicates (and panellists). Finally, correlations coefficients and their corresponding p-value (Pearson method) were calculated between volatile compounds (CGMS area) and overall aromatic intensity, pea and broth sensory attributes.

3.4.2.3. Results and discussion

Chemical and rheological characterization of pea beverages

Rheological properties of different pea beverages

In this study, three common rheological tests were done: steady shear test, strain sweep test and frequency sweep test. The analysed set of beverages presented two concentrations of NaCl, gellan gum and sunflower oil as well as two different types of pea protein (isolates and pellet).

Results from the steady shear test are presented on Figure 3.4.2.1. with the dynamic viscosity plotted in function of the shear rate according to the formulations of pea beverages. Results show monotonic increase in viscosity with the increase in shear rate: products with higher gellan gum content and less salt content present the highest value of viscosity. A power law was successfully used to link the shear

stress to the shear rate and allowed to determine two characteristic indexes: the power law index and consistency index. These indexes were calculated for each pea beverage (Table 3.4.2.1.).

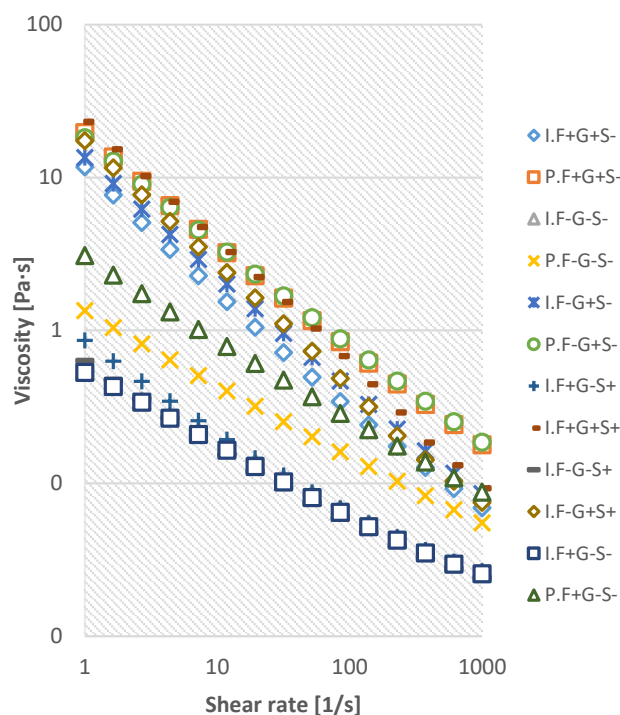


Figure 3.4.2.1.: Dynamic viscosity [Pa.s] in function of the shear rate [1/s] according to the formulations of pea beverages.

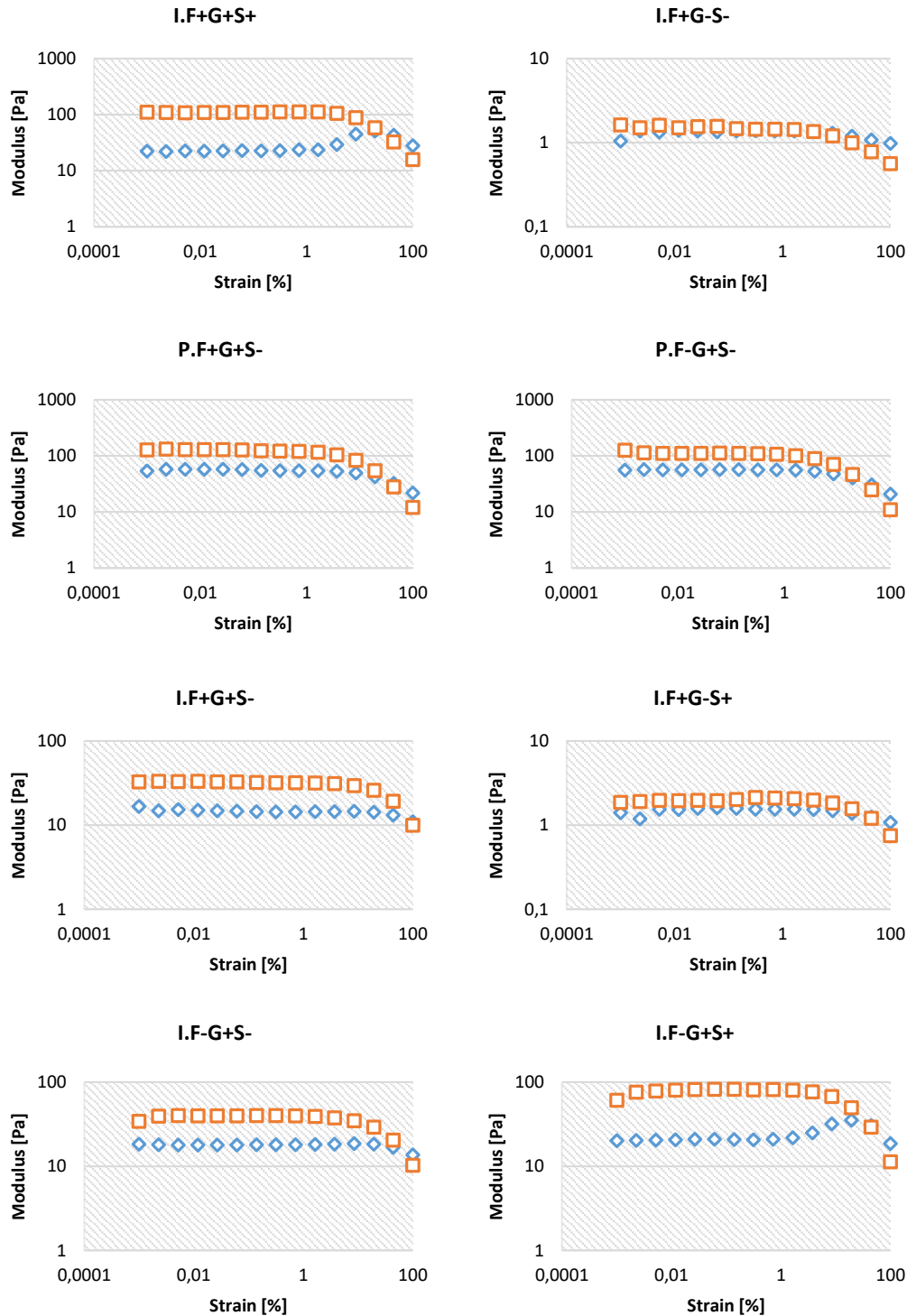
The power law index is inferior to 1 for the 12 beverages. Therefore beverages are shear thinning. The effects of the composition on the power law index was also compared through ANOVA (Table 3.4.2.2.). The pea protein origin, the gellan gum content and the salt content have a significant impact on the power law index whereas the oil content have no significant effect. Gellan gum content is responsible of the most significant effect, whereas pea protein origin the least significant. Samples containing 0.5% of gellan gum are more shear thinning than samples containing 0.12%. According to the literature, the gelation mechanism and the solution-gel transition of gellan gum are strongly influenced by cosolutes such as minerals, polyols or carbohydrates (Miyoshi, Nishinari, 1999; Sworn *et al.*, 2009). So, it could explained why salt content and protein type modified the rheological properties of the pea beverages.

Table 3.4.2.2.: Results of the ANOVA examining effects of the samples composition on the power law index (n). (Total degrees of freedom: 11 ; residual degrees of freedom: 7. Significant p-values are in bold ($\alpha = 0.05$)).

Source	F	p-value
Pea protein origin	11.36	< 0.01
Oil content	2.27	0.13
Gellan gum content	1895.14	< 0.01
Salt content	37.09	< 0.01

To characterize the beverages, their strain-dependent behaviour was also examined by oscillatory measurements (strain sweep test). Results are presented on Figure 3.4.2.2. They showed monotonic increase of the shear stress when the strain increase. The storage modulus [G'] and the loss modulus [G''] shown constant values for strain around 0.1% (linear viscoelastic region). G'' value represents the energy dissipated (i.e. the viscous portion) and G' value represents the stored energy, (i.e. the elastic portion) (Meyers and Chawla, 1999). For most pea beverages $G' > G''$, except for pea beverages with isolates as protein type and less gellan gum content where $G' = G''$. Thus these samples have a gel-like structure which indicate the presence of a network structure. Indeed, the proposed gelation mechanism of gellan

gum is based on the model domain which assumes the formation of distinct junction zones and disordered flexible polymer chains connecting adjacent junction zones (Sworn *et al.*, 2009). Gel character is often found in polysaccharides such as gellan which react gel-like under static conditions and exhibit very low viscosities at high shear rates (Lam *et al.*, 2017). Besides, pea beverages with isolates as protein type, higher gellan gum content and less salt content present a pronounced G'' maximum at the end of the viscoelastic region. It means that the microstructural reorganization before breakdown entails energy dissipation leading to an increase of G'' values up to a peak value at the beginning of the non-linear response (Hellriegel *et al.*, 2013). After the cross over point ($G' = G''$), beverages present a fluid behaviour ($G'' > G'$) and the food material start to flow. In addition, pea beverages with isolates as protein type, higher gellan gum content and higher salt content present higher ratio G'/G'' i.e. a more elastic behaviour.



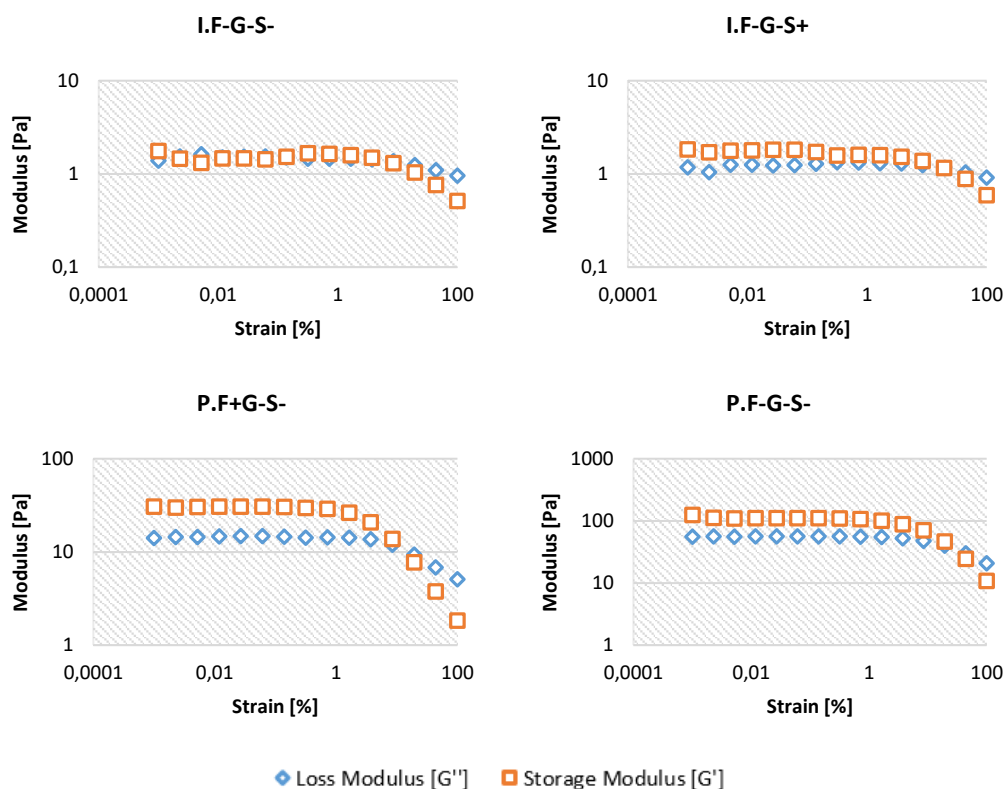


Figure 3.4.2.2.: Loss modulus (in blue) and storage modulus (in red) [Pa] in function of the strain [%] according to the formulations of pea beverages.

To characterize the beverages, their frequency-dependent behaviour was finally examined by oscillatory measurements (frequency sweep test). Results shown monotonic increase of the shear stress when the frequency increase. The ratio G'/G'' in function of the frequency according to the formulations of pea beverages is presented Figure 3.4.2.3. Results show few variations of the ratio G'/G'' according to the frequency. At 10 Hz, beverages with isolates as protein type present higher G'/G'' than beverages with pellet as protein type. But, results show more variation between the pea beverages at 0,01 Hz: beverages with isolates as protein type, higher gellan gum content and higher salt content present much more higher G'/G'' .

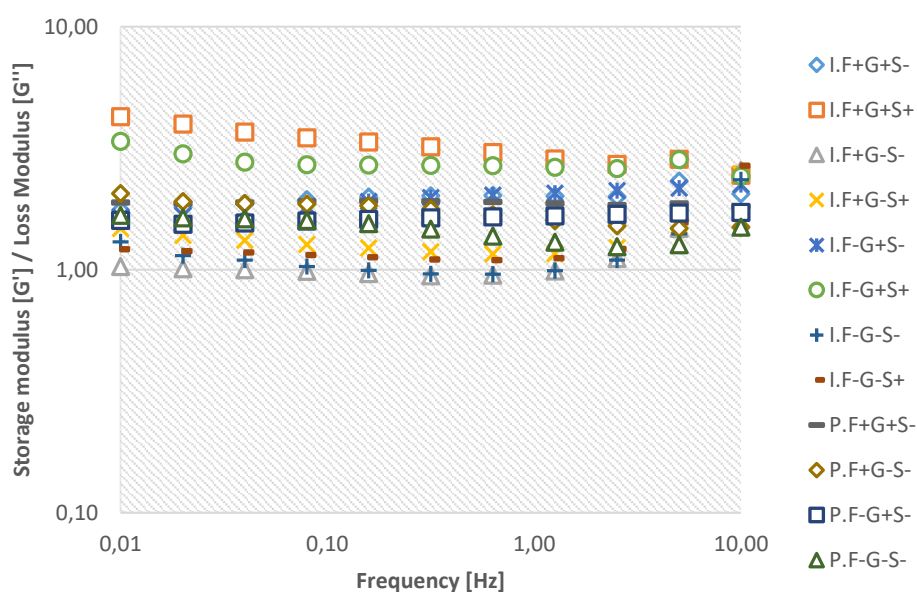


Figure 3.4.2.3.: Storage modulus [G'] / Loss Modulus [G''] in function of the frequency [Hz] according to the formulations of pea beverages.

Volatile compounds profiles of different pea beverages

In this study, composition on volatile compounds were studied by GC-MS analysis on the 12 beverages previously described. A total of 71 volatile compounds belonging to 8 chemical classes were identified (Table 3.4.2.3.). The distribution of these compounds is presented on a heat map associated to an AHC on products and on compounds (Figure 3.4.2.4). Ketones and aldehydes are the most prominent compounds in number (ketones: 18 compounds, aldehydes: 19 compounds). Ketones, aldehydes, alcohols and furan families are the most prominent compounds in term of GC-MS area (which is linked mainly to the concentration and on the less extend to the volatility and hydrophobicity of the compounds in the beverages): aldehydes $1.86\text{E}+06$, alcohol $9.74\text{E}+05$, ketones $9.56\text{E}+05$, furan $6.05\text{E}+05$ (mean GC-MS area for all the volatile compounds of the family).

Most of the compounds identified were also known on others publications. For example, on the 78 compounds identified by Murat *et al.* (2013) on pea protein isolates, 39 volatile compounds are similar with this study, including (E,E)-2,4-heptadienal known to be important odour active volatile secondary lipid oxidation products (Jacobsen, 1999). On the 21 compounds studied by Trikusuma *et al.*, (2019) on pea protein beverage, 7 volatile compounds are similar. On the 72 compounds identified by Bi *et al.* (2020) on roasted pea protein isolates, 22 volatile compounds are similar including 2-pentylfuran and 1-octanol. These differences in composition are not surprising because the studied products are different in terms of composition and sourcing as well as in term of extraction protocols and analytical methods. This result shows that the profile in volatile compounds could be highly variable according to the process, the formulation and the analytical conditions. To be noted, in the literature, the main origin of these compounds is indicating coming from endogen origin of pea seed or from oxidation of the lipids/peptides/amino acids in the seed or during the storage (Murat *et al.*, 2013; Trikusuma *et al.*, 2019). For example, they could come from the oxidation of unsaturated lipids and phospholipids (Cowan *et al.*, 1973) or the oxidation because of the lipoxygenase (Baysal and Demirdöven 2007) which could be accelerated by oxidation and auto-oxidation (Damodaran and Arora 2013).

Besides, the volatile profiles of products did not changed much according to the beverages composition (oil content, salt content, gellan gum content and protein type) and these variations are highly depending of the chemical compounds. Globally, beverages with higher gellan gum content present lower volatiles release. The strength of the gels being higher for beverages with higher content of gellan gum, we can assume that there is less open space located between the gel chains resulting in higher obstacles to the free diffusion of volatile compounds and higher interactions with proteins (Gostan *et al.* 2004; Wang & Arttnfield, 2016). This result is constituent with sensory results which shown lower aromatic perceptions with higher gellan gum concentration. In addition, globally beverages with pellet present lower concentrations on volatile compounds. Although pea pellets and isolates both contain similar levels of total proteins, pellets are richer in insoluble proteins and they have a different mineral composition. Previous research has examined interactions in protein-based foods between volatile compounds and proteins and these interactions can be modified by different factors: protein conformation and composition as well as the environmental conditions, such as pH and NaCl composition (Damodaran & Kinsella, 1981). These modifications of interactions can result on modifications on volatile release. This result is also constituent with sensory results which shown lower aromatic perceptions with pellet.

But beverages present few variation of concentrations on volatile compounds according to the concentration in oil. In the literature, the role of lipids is often described as a role of reservoir for the volatile compounds. Indeed, volatile compounds mainly hydrophobic are less released in the vapour phase from oil than from water resulting in their delayed release (Voilley and Etiévant, 2006; Paravisini et Guichard 2016). Beverages present also few variation of concentrations on volatile compounds according to the concentration on NaCl. Usually, an increased amount of NaCl induces an increase in the air/water partitioning of most aroma compounds, due to a salting-out effect (Endo *et al.* 2012). Indeed, NaCl ions mobilising water compounds for their hydration, less water is available for the solubilisation of volatile compounds and they are therefore more released into the vapour phase (Rabe *et al.* 2003). But, NaCl can modify the structure of proteins and so their interactions with volatile compounds resulting also on modifications on volatile release. This low impact of the NaCl content on volatile compounds is also constituent with sensory results which shown low impact of NaCl content on aromatic perceptions.

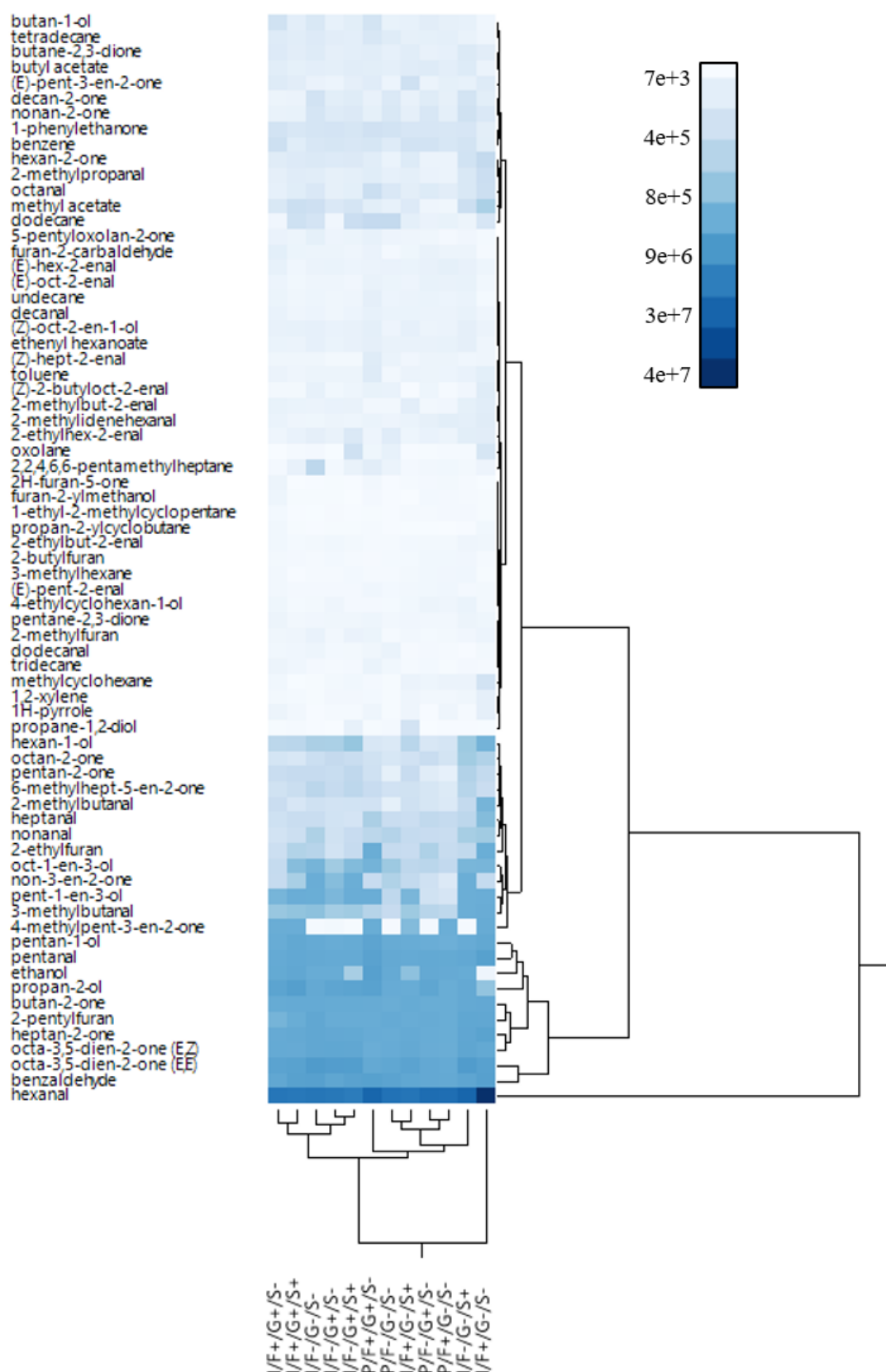


Figure 3.4.2.4.: Hierarchical clustering (ward method) of the 12 beverages analysed by GC-MS and the 71 volatile compounds identified.

Table 3.4.2.3.: Volatile compounds identified by GC-MS with apolar column (DB-5, 60m x 320µm x 1µm) from pea protein isolates beverages (tentative identification using NIST mass spectra library, comparison of the GC retention index – Kovats index, physicochemical properties at 25°C from literature - EPI-Suite4.11).

IUPAC name	Retention time	NIST score	Kovats index	CAS number	Chemical family	Chemical formula	Molecular mass	Odour	LogPow (est)*	Solubility**	Volatility***
ethanol	4.9	98	440	64-17-5	Alcohol	C ₂ H ₆ O	46.04	strong alcoholic ethereal medical	-0.31	7.92E+05	5.00E-06
propan-2-one	5.5	88	503	67-64-1	Ketone	C ₃ H ₆ O	58.00	solvent ethereal apple pear	-0.24	2.20E+05	3.50E-05
propan-2-ol	5.8	93	515	67-63-0	Alcohol	C ₃ H ₈ O	60.10	dry alcoholic woody musty	0.05	4.02E+05	8.10E-06
methyl acetate	6.2	86	531	79-20-9	Ester	C ₃ H ₆ O ₂	74.04	green, ethereal, fruity, fresh, rum and whiskey-like	0.18	9.39E+04	1.15E-04
2-methylpropanal	7.0	98	554	78-84-2	Aldehyde	C ₄ H ₈ O	72.06	fresh aldehydic herbal green malty	0.74	3.12E+04	1.80E-04
propan-1-ol	7.1	98	560	71-23-8	Alcohol	C ₃ H ₈ O	60.10	alcohol, pungent	0.25	2.72E+05	7.41E-06
butane-2,3-dione	7.9	81	560	431-03-8	Ketone	C ₄ H ₆ O ₂	86.00	sweet, buttery, creamy, milky	-1.34	1.00E+06	1.33E-05
butan-2-one	8.1	99	602	78-93-3	Ketone	C ₄ H ₈ O	72.00	chemical-like and slightly fruity green	0.29	7.61E+04	5.69E-05
2-methylfuran	8.5	95	605	534-22-5	Furan	C ₅ H ₆ O	82.10	ethereal green cocoa nutty almond coffee	1.85	3.41E+03	5.93E-03
ethyl acetate	8.7	83	608	141-78-6	Ester	C ₄ H ₈ O ₂	88.05	ethereal, fruity, sweet, with a grape and cherry nuance	0.73	2.99E+04	1.34E-04
oxolane	9.3	90	617	109-99-9	Alkane	C ₄ H ₈ O	72.06	NA	0.46	5.45E+04	7.05E-05
3-methylbutanal	10.5	98	641	590-86-3	Aldehyde	C ₅ H ₁₀ O	86.07	cocoa, almond	1.23	1.12E+04	1.23E+00
butan-1-ol	10.7	90	654	71-36-3	Alcohol	C ₄ H ₁₀ O	74.07	wine	0.88	7.67E+04	8.81E-06
benzene	10.8	96	662	71-43-2	Alkene	C ₆ H ₆	78.00	aromatic	2.13	2.00E+03	5.55E-03
2-methylbutanal	10.9	96	658	96-17-3	Aldehyde	C ₅ H ₁₀ O	86.07	musty, furfural and rummy, with nutty and cereal notes, and caramel and fruity undertones	1.23	1.12E+04	2.62E-04
3-methylhexane	11.4	94	670	589-34-4	Alkane	C ₇ H ₁₆	100.20	NA	3.71	2.32E+01	1.64E+00
pent-1-en-3-ol	11.6	94	674	616-25-1	Alcohol	C ₅ H ₁₀ O	86.07	green radish vegetable rummy truffle oily resinous	1.12	4.53E+04	9.88E-06
pentan-2-one	11.7	95	680	107-87-9	Ketone	C ₅ H ₁₀ O	86.07	fish, pungent	0.91	2.12E+04	8.36E-05
propan-2-ylcyclobutane	12.0	88	690	872-56-0	Alkane	C ₇ H ₁₄ O	98.19	NA	3.52	3.39E+01	3.39E-01
pentane-2,3-dione	12.2	92	681	600-14-6	Ketone	C ₅ H ₈ O ₂	100.05	toasted, buttery and caramellic with marshmallow and molasses nuances	-0.85	6.16E+05	2.62E-07
pentanal	12.3	98	732	110-62-3	Aldehyde	C ₅ H ₁₀ O	86.07	almond, malt, pungent	1.31	9.72E+03	1.47E-04
2-ethylfuran	12.4	94	735	3208-16-0	Furan	C ₆ H ₈ O	96.06	solvent-like with a dirty musty brown earthy nuance	2.40	1.07E+03	7.88E-03
3-hydroxybutan-2-one	12.7	76	718	513-86-0	Ketone	C ₄ H ₈ O ₂	88.05	butter, cream	-0.36	8.33E+05	1.03E-05
Methylcyclohexane	13.9	92	720	108-87-2	Alkane	C ₇ H ₁₄	98.19	NA	3.61	2.84E+01	4.30E-01
propane-1,2-diol	14.0	87	745	57-55-6	Alcohol	C ₃ H ₈ O ₂	76.10	sweet	-0.92	8.11E+05	1.29E-08
3-methylbutan-1-ol	14.1	98	736	123-51-3	Alcohol	C ₅ H ₁₂ O	88.15	whiskey, malt, burnt	1.16	4.16E+04	1.41E-05
(E)-pent-3-en-2-one	14.2	96	739	625-33-2	Ketone	C ₅ H ₈ O	84.12	musty, stale water and phenolic with a fishy and shell fish nuance	0.52	4.62E+04	1.34E-05
2-methylbut-2-enal	14.5	97	753	497-03-0	Aldehyde	C ₅ H ₈ O	84.06	green, fruit	1.15	1.34E+04	1.20E-04
pyridine	14.6	90	740	110-86-1	Pyridine	C ₅ H ₅ N	79.10	sickening sour putrid fishy amine	0.65	7.30E+05	1.10E-05
(methylsulfonyl) methane	14.7	79	785	624-92-0	Alkane	C ₂ H ₆ S ₂	93.99	onion, cabbage, putrid	1.77	3.74E+03	1.21E-03
1H-pyrrole	14.8	87	760	109-97-7	Pyrrole	C ₄ H ₅ N	67.09	sweet warm nutty ethereal	0.75	3.12E+04	1.80E-05
(E)-pent-2-enal	15.1	89	759	1576-87-0	Aldehyde	C ₅ H ₈ O	84.12	green, waxy and fruity	1.09	1.50E+04	3.22E-05
pentan-1-ol	15.5	97	766	71-41-0	Alcohol	C ₅ H ₁₂ O	88.09	balsamic	1.51	2.09E+04	1.30E-05
toluene	15.9	90	773	108-88-3	Alkene	C ₇ H ₈	92.14	paint	2.73	5.73E+02	6.64E-03
1-ethyl-2-methylcyclopentane	16.5	82	776	3726-46-3	Alkane	C ₈ H ₁₆	112.21	NA	4.01	1.17E+01	4.50E-01
hexan-2-one	16.7	95	792	591-78-6	Ketone	C ₆ H ₁₂ O	100.09	ether	1.38	7.75E+03	9.32E-05

IUPAC name	Retention time	NIST score	Kovats index	CAS number	Chemical family	Chemical formula	Molecular mass	Odour	LogPow (est)*	Solubility**	Volatility***
4-methylpent-3-en-2-one	17.2	97	787	141-79-7	Ketone	C6H10O	98.14	potato bin, raw and baked potato, jimica with raw vegetative, nutty and dirt like nuances	1.37	8.04E+03	3.67E-05
hexanal	17.3	98	801	66-25-1	Aldehyde	C6H12O	100.09	grass, tallow, fat	1.78	3.53E+03	2.13E-04
2-ethylbut-2-enal	17.8	97	808	19780-25-7	Aldehyde	C6H10O	98.14	NA	1.64	4.71E+03	1.17E-004
butyl acetate	17.9	95	802	123-86-4	Ester	C6H12O2	116.00	sweet, ripe banana, tutti frutti, tropical and candy-like with green nuances	1.78	3.13E+03	2.81E-04
furan-2-carbaldehyde	18.9	88	815	98-01-1	Furan	C5H4O2	96.08	brown, sweet, woody, breadly, nutty, caramellic with a burnt astringent nuance	0.41	5.36E+04	3.77E-06
furan-2-yl methanol	19.8	81	850	98-00-0	Alcohol	C5H6O2	98.10	burnt, sweet, caramellic, brown	0.28	2.21E+05	7.86E-08
(E)-hex-2-enal	19.9	95	854	6728-26-3	Aldehyde	C6H10O	98.07	apple, green	1.58	5.26E+03	4.89E-05
2-methylidene hexanal	20.4	87	842	1070-66-2	Aldehyde	C7H12O	112.09	NA	2.21	1.39E+03	4.15E-04
hexan-1-ol	20.5	98	868	111-27-3	Alcohol	C6H14O	102.10	resin, flower, green	2.03	6.89E+03	1.71E-05
1,2-xylene	21.0	95	876	95-47-6	Alkene	C8H10	106.17	geranium	3.16	2.24E+02	5.18E-03
heptan-2-one	21.6	97	870	110-43-0	Ketone	C7H14O	114.10	cheese, fruity, coconut, waxy, green	1.98	2.15E+03	1.69E-04
2-butylfuran	21.8	91	889	4466-24-4	Furan	C8H12O	124.09	mild fruity wine sweet spicy	3.38	1.25E+02	1.39E-02
Cyclooctatetraene	22.0	93	880	629-20-9	Alkene	C8H8	104.15	NA	3.08	7.73E+01	8.88E-04
heptanal	22.3	98	899	111-71-7	Aldehyde	C7H14O	114.10	fat, citrus, rancid	2.29	1.17E+03	2.70E-04
2H-furan-5-one	22.5	93	876	497-23-4	Ketone	C4H4O2	84.07	buttery	-0.60	4.18E+05	9.74E-06
(Z)-hept-2-enal	24.9	85	927	57266-86-1	Aldehyde	C7H12O	112.09	NA	2.07	1.81E+03	6.43E-05
heptan-1-ol	25.3	92	969	111-70-6	Alcohol	C7H16O	116.20	herb	2.62	1.94E+03	1.88E-05
benzaldehyde	25.4	97	960	100-52-7	Aldehyde	C7H6O	106.04	almond, burnt sugar	1.48	6.10E+03	2.67E-05
phenol	25.6	98	980	108-95-2	Alcohol	C6H5OH	94.11	phenol	1.46	2.62E+04	3.33E-07
oct-1-en-3-ol	25.8	92	980	3391-86-4	Alcohol	C8H16O	128.12	mushroom, earthy, fungal, green, oily, vegetative, umami sensation and savory brothy	2.60	1.84E+03	2.31E-05
ethenyl hexanoate	26.0	89	996	3050-69-9	Ester	C8H14O2	142.20	NA	2.69	4.12E+02	1.20E-03
6-methylhept-5-en-2-one	26.1	93	988	110-93-0	Ketone	C8H14O	126.10	green, vegetative, musty, apple, banana and green bean-like	2.06	1.65E+03	5.11E-05
octan-2-one	26.3	95	997	111-13-7	Ketone	C8H16O	128.12	dairy, waxy, cheese, woody, mushroom and yeast	2.37	8.84E+02	1.88E-04
2-pentylfuran	26.4	94	985	3777-69-3	Furan	C9H14O	138.10	green, waxy, with musty, cooked caramellic nuances	3.87	4.18E+01	1.84E-02
2,2,4,6,6-pentamethyl heptane	26.6	84	997	13475-82-6	Alkane	C12H26	170.20	NA	5.94	1.51E-01	9.35E+00
(Z)-oct-2-en-1-ol	26.8	93	1039	18409-17-1	Alcohol	C8H16O	128.21	fatty oily sweet fruity	2.59	1.86E+03	2.73E-05
octanal	27.0	97	1001	124-13-0	Aldehyde	C8H16O	128.12	fat, soap, lemon, green	2.78	3.94E+02	5.14E-04
2-ethylhex-2-enal	27.2	88	1007	645-62-5	Aldehyde	C8H14O	126.10	NA	2.62	5.49E+02	4.88E-04
4-ethylcyclohexan-1-ol	27.4	81	1000	4534-74-1	Alcohol	C8H16O	128.22	NA	2.55	2.02E+03	2.55E+00
(2E,4E)-hepta-2,4-dienal	27.4	88	1000	4313--03-5	Aldehyde	C7H10O	110.07	fried	1.86	2.81E+03	1.41E-05
2-ethylhexan-1-ol	28.0	96	1032	104-76-7	Alcohol	C8H18O	130.00	rose, green	2.73	1.38E+03	2.65E-05
(4R)-1-methyl-4-prop-1-en-2-ylcyclohexene	28.5	86	1033	5989-27-5	Alkene	C10H16	136.13	lemon, orange	4.38	4.58E+00	3.19E-02
1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane	28.7	74	1032	470-82-6	Alkane	C10H18O	154.00	minty camphoreous cooling eucalyptus medicinal	2.74	3.32E+02	1.10E-04
(E)-oct-2-enal	29.4	90	1060	2548-87-0	Aldehyde	C8H14O	126.10	green, nut, fat	2.57	6.13E+02	7.34E-05
octan-1-ol	29.8	97	1072	111-87-5	Alcohol	C8H18O	130.14	chemical, metal, burnt	3.00	8.14E+02	2.45E-05
octa-3,5-dien-2-one (E,Z)	29.8	95	1075	38284-27-4	Ketone	C8H12O	124.09	fruit, fat, mushroom	2.08	1.62E+03	9.96E-06
1-phenylethanone	30.1	88	1076	98-86-2	Ketone	C8H8O	120.10	must, flower, almond	1.58	4.48E+03	1.04E-05
nonan-2-one	30.7	88	1081	821-55-6	Ketone	C9H18O	142.14	cheesy green fruity dairy dirty buttery	3.14	1.71E+02	3.67E-04
octa-3,5-dien-2-one (E,E)	30.9	94	1096	4173-41-5	Ketone	C8H12O	124.18	fatty fruity hay green herbal	2.08	1.62E+03	9.96E-06
undecane	31.1	91	1100	1120-21-4	Alkane	C11H24	156.31	alkane	5.74	2.57E-01	1.93E+00
nonanal	31.3	99	1104	124-19-6	Aldehyde	C9H18O	142.10	fat, citrus, green	3.27	1.32E+02	7.34E-04

IUPAC name	Retention time	NIST score	Kovats index	CAS number	Chemical family	Chemical formula	Molecular mass	Odour	LogPow (est)*	Solubility**	Volatility***
non-3-en-2-one	34.4	88	1122	14309-57-0	Ketone	C ₉ H ₁₆ O	140.12	oily spicy ketonic fermented bleu cheese waxy fatty woody	2.79	3.50E+02	6.43E-05
decan-2-one	34.6	88	1172	693-54-9	Ketone	C ₁₀ H ₂₀ O	156.15	fermented cheesy	3.73	4.64E+01	4.45E-04
dodecane	34.9	99	1200	112-40-3	Alkane	C ₁₂ H ₂₆	170.20	alkane	6.10	1.10E-01	8.18E+00
decanal	35.1	95	1209	112-31-2	Aldehyde	C ₁₀ H ₂₀ O	156.20	soap, orange peel, tallow	2.79	3.50E+02	6.43E-05
2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-one	36.4	82	1255	6485-40-1	Ketone	C ₉ H ₁₂ O ₂	150.22	minty	2.71	3.67E+02	7.73E-05
tridecane	37.1	83	1300	629-50-5	Alkane	C ₁₃ H ₂₈	184.00	alkane	6.73	2.75E-02	2.88E+00
(Z)-2-butyloct-2-enal	38.2	88	1361	13019-16-4	Aldehyde	C ₁₂ H ₂₂ O	182.17	NA	4.59	6.50E+00	1.94E-03
5-pentylloxolan-2-one	38.3	92	1360	104-61-0	Ketone	C ₉ H ₁₆ O ₂	156.12	coconut creamy waxy	2.08	1.20E+03	1.69E-04
dodecanal	38.7	85	1401	112-54-9	Alkane	C ₁₂ H ₂₄ O	184.00	soapy, waxy, citrus, orange mandarin	4.75	4.65E+00	1.90E-03
tetradecane	38.7	88	1399	629-59-4	Alkane	C ₁₄ H ₃₀	198.39	mild waxy	7.20	9.19E-03	9.20E+00

*: estimated hydrophobic constant expressed as octanol/water partition coefficient (EPI-Suite4.11)

**: solubility in water (mg/L) at 25°C (EPI-Suite4.11)

***: volatility in water at 25°C (atm-m³/mole) (EPI-Suite4.11)

Effect of saliva dilution on volatile compounds release

In this study, we chose to focus on the impact of saliva to study the impact of food oral process, which can have an impact on aroma release, and by consequence on perception. For that, dilution by water or saliva on volatile compounds profiles was studied by GC-MS methods. Indeed, during eating, volatile compounds are released directly from food to the air but also from food to saliva. In the literature, the role of saliva on volatile compounds release is highlighted as an important mechanisms for sensory perceptions (Canon *et al.*, 2018).

Among the 12 studied beverages, 7 beverages were characterized with water dilution and saliva dilution to observe the impact of the main factors that can affect the release of aromas in interaction with saliva. Firstly I/F+/G+/S-, I/F-/G-/S-, I/F+/G-/S- and I/F-/G+/S- were analysed to study the impact of sunflower oil concentration (1.5% versus 0%), of gellan gum concentration (0.5% versus 0.12%) and their interactions. P/F+/G+/S- and P/F+/G-/S- were also analysed to study the impact of protein type (isolates versus pellet) and its interaction with gellan gum concentration. Finally I/F+/G+/S+ were analysed to focus on the impact of NaCl concentration (0.12% versus 0.08%).

A total of 73 volatile compounds were identified and released from beverages diluted with water or saliva including 57 volatile compounds common to the previous analysis (Table 3.4.2.3.). Nineteen volatile compounds were identified on the saliva alone (i.e. diluted with water). These compounds were already identified on saliva in the literature. They could have several endogenous or exogenous origin. Pentane-2,3-dione, butane-2,3-dione and phenol could come from bacterial activity in mouth (Soini *et al.*, 2010; Milanowski *et al.*, 2016). 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane, ethanol and 2-ethylhexan-1-ol could come from commercial products such as cosmetics, fragrances, detergents (Milanowski *et al.*, 2016). Others compounds could dissolve into the saliva from breathe such as propan-2-one (Lindinger *et al.*, 1997) and consumed foods (Milanowski *et al.*, 2016). Among these compounds, 11 present significant differences between GC-MS areas from the beverages diluted with water and/or diluted with saliva: 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane, butan-1-ol, ethanol, ethyl acetate, propan-1-ol, propan-2-one, butane-2,3-dione, octan-1-ol, phenol, 1-methyl-4-(1-methylethenyl)-cyclohexene and 2-ethylhexan-1-ol.

To visualize the main differences in volatile compounds profiles between pea beverages diluted by water or by saliva, a PCA is presented in Figure 3.4.2.5. On the figure, the mean of each chemical family were added as additional variables. Results showed that beverages diluted by saliva and by water are well distributed on axis F1 representing 34% of the variance. Beverages with different compositions are distributed on axis F2 representing 24% of the variance. The effect of saliva on volatile compounds profile has a higher impact than the composition of the studied beverages. The release of 73 volatile compounds was significantly impacted by the nature of the dilution by saliva or by water. This effect was dependent of the chemical structure of the volatile compounds.

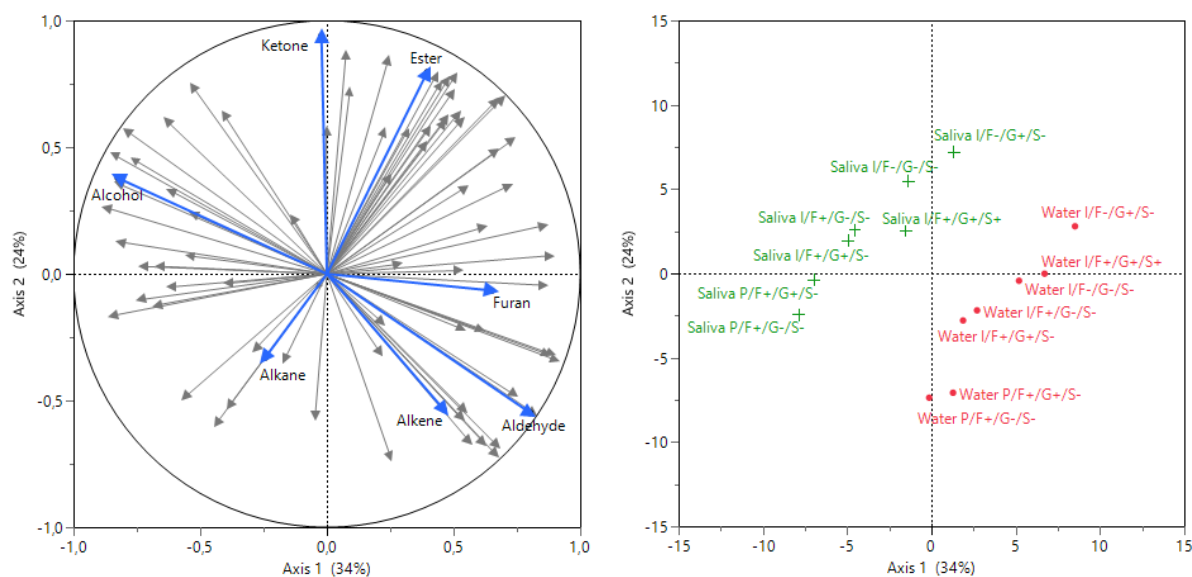


Figure 3.4.2.5.: Results of the principal component analysis (PCA) examining the beverages' GC-MS area profiles diluted by saliva or by water. On the left is a loading plot showing the correlational relationships between PCA axes 1 and 2 and the volatile compounds. In blue are the supplementary variables (mean chemical family). On the right is a PCA plot with the same two axes that shows the relative similarity of the beverages' GC-MS area profiles. Beverages diluted with water are in red. Beverages diluted with saliva are in green.

First, most of the identified aldehydes such as ((E)-oct-2-enal or heptanal) presented higher areas in beverages diluted by water than in beverages diluted by saliva. In opposition, most of alcohols (such as hexan-1-ol, pent-1-en-3-ol or 3-methylbutan-1-ol) present higher areas in beverages diluted by saliva.

These results are in agreement with the data of literature, which described the complex role of saliva on volatile compounds release. Previous researches reported both direct mechanisms (molecular interactions, enzymatic conversion, salting-out effect, dilution) involving salivary components (salts, proteins including enzymes, microbiota) and indirect mechanisms (changes of aroma diffusion through modification of the physicochemical properties of the food matrix) (Ployon *et al.*, 2017; Pérez-Jiménez 2018; Muñoz-González *et al.*, 2018). In addition, these effects seem to be impacted by the saliva pre-treatment, the nature of food matrices, the used analytical methods and the chemical structure of the volatile compounds. For example, results showed that the extent of enzymatic degradation varies according to the chemical structure of the volatile compounds suggesting the involvement of hydrophobic effects (Pagès-Hélary *et al.*, 2014, Buettner *et al.*, 2002). According to the study of Otake, Roozen, & Burger (1998), in presence of saliva or water in water/oil emulsions, the release of hydrophilic compounds was lower in the air phase above the emulsions. In contrast, hydrophobic compounds would be mostly solubilized in the oil phase, thus saliva/water addition did not impact on their partitioning with air.

For example, if we compare two chemically similar compounds hexanal and hexan-1-ol. The chemical properties of these compounds are relatively similar: mass of 100 g/mol for hexanal and 102 g/mol for hexan-1-ol, hydrophobicity (LogPow) of 1.8 for hexanal and 2.0 for hexan-1-ol, solubility in water at 25°C of 3E+03 mg/L for hexanal and 7E+03 mg/L for hexan-1-ol; volatility (Henry Law constant) of 2E-04 atm-m³/mole and 1,71E-05 atm-m³/mole for hexan-1-ol). However the area of hexanal is higher on beverages diluted by water and the area of hexan-1-ol is higher on beverages diluted by saliva. For example for beverage I/F+/G+/S- the area of hexanal was 6.71E7 for beverage diluted with water and 2.48E7 for beverage diluted with saliva. For the same beverage I/F+/G+/S- the area of hexan-1-ol was 8.71E05 for beverage diluted with water and 1.25E7 for beverage diluted with saliva. In order to explain these differences, the most likely hypothesis is that they are due to enzymatic activities of the saliva or the microbiota.

All these results confirmed that release behaviours were strongly molecule-dependent. They highlighted the fact that different types of interactions with mucosa and/or saliva were involved, depending on the molecule's properties. These results are in agreement with previous studies, which notably showed the key role of interaction between ethyl propanoate, 2-nonanone and (Z)-3-hexen-1-ol and mucosa (air/mucosa partition coefficient) on the persistence of aroma compound in exhaled air (Délérès *et al.*, 2016).

In addition, previous results can be found concerning the impact of food composition. The release of compounds are differently impacted in function of the nature of each compound and the composition. Globally beverages with pellet present lower areas on volatile compounds. For example, they present lower 3-methylbutanal areas. Beverages with different oil content present different profile on volatile compounds. For example, they present lower 4-methylpent-3-en-2-one areas. In addition, globally beverages with higher salt content present higher areas on volatile compounds. For example, they present higher propane-2-one areas. However, beverages diluted were less impacted by gellan gum concentration. We can assume that the strength of the gels are similar after dilution for the 7 beverages and allow a similar free diffusion of volatile compounds (Gostan *et al.* 2004).

Identifications of the role of chemical and rheological parameters explaining perceptions

Effect of formulation factors on sensory perceptions of pea beverages

To better understand how product composition affect the sensory perception of pea protein-based beverages, the 12 beverages were sensory described by a trained panel. The perception of these pea protein beverages were evaluated and described in companion papers (Cosson *et al.*, 2020; Section 3.4.1.). However, the main results are recalled here for sake of clarity. A PCA was used providing a way to visualize the relationships among products and sensory attributes (Figure 3.4.2.6). Overall four groups of beverages are distinguished (confirmed by AHC). Along the first axis, beverages are divided in two groups corresponding to the protein type (pellet versus isolate). Pellet-based beverages are perceived as being fattier, bitterer, and less salty, less aromatic and as having a more pronounced mouthfeel. Along the second axis, beverages are separated in two groups corresponding to the gellan gum content (0.12% and 0.5%). Beverages with greater gellan gum content are perceived as having lower overall aromatic intensity, less pronounced almond and broth notes, higher fattiness and mouthfeel notes as well as lower astringent notes. In addition and consistently, beverages with the highest salt content were perceived as more salty.

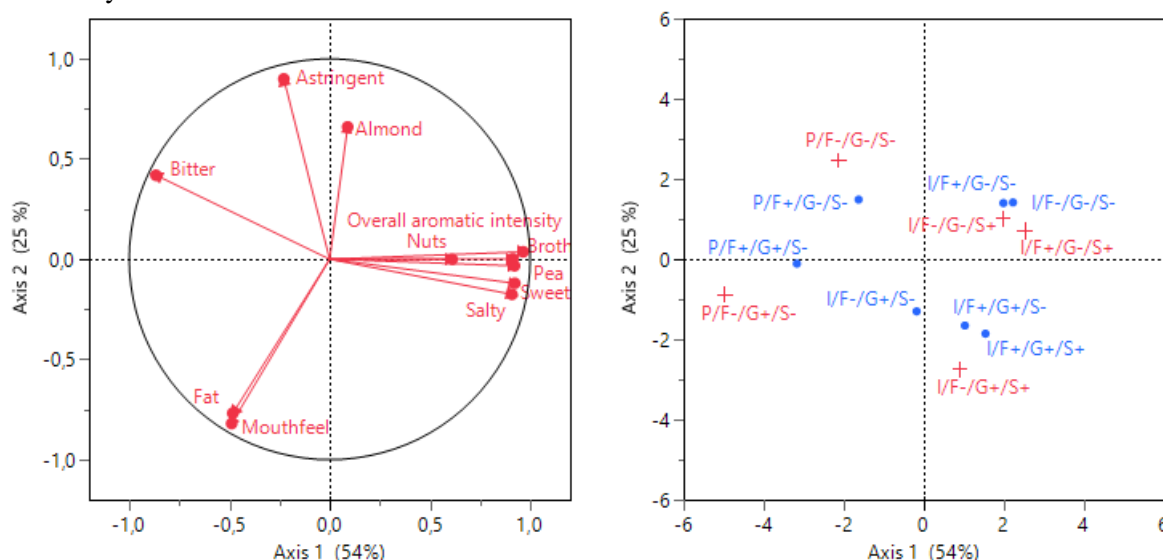


Figure 3.4.2.6.: Results of the principal component analysis (PCA) examining the beverages' sensory profile. On the left is a loading plot showing the correlational relationships between PCA axes 1 and 2 and the sensory attributes. On the right is a PCA plot with the same two axes that shows the relative similarity of the beverages' sensory profiles. In blue are the 7 beverages diluted by saliva/water during the GC-MS analysis.

RV coefficients (Pearson method) were calculated between sensory intensities of all beverages and physicochemical variables: 1) composition variables (sunflower oil, soy lecithin, gellan gum and salt), 2) rheological parameters (issued from steady shear test (Power law index, Consistency index, n/k , pH, Viscosity at 50s⁻¹), strain sweep test (G''/G' Strain 0.001 %, G''/G' Strain 0.06%, G''/G' Strain 44% and G''/G' Strain 100%) and frequency sweep test (G''/G' at 10s⁻¹, G''/G' at 0.01 Hz)); and 3a) volatile areas without dilution, 3b) volatile areas when a dilution by water was used, 3c) volatile areas when a dilution by saliva was used. For correlation with volatile release, the dataset included only the 7 beverages studied.

The coefficients obtained are relatively high: 0.74 for rheological group, 0.56 for volatile -no dilution-group, 0.70 for volatile compounds for volatile -water dilution- group, 0.65 for volatile - saliva dilution-group, 0.49 for composition group (Josse *et al.*, 2008). It can thus be observed that the sensory dataset is closer to the data set of rheological parameters followed by the correlation with volatile compounds area. These results show that texture perceptions are prominent in the perceptions of these pea beverages. The interactions of texture perceptions with sapid (bitter in particular) and aromatic (beany) perceptions are surely not negligible. The results concerning the difference between volatile datasets diluted by saliva or water in correlation with perception is surprising since the closest volatile dataset is the one diluted with water. This result is in disagreement with the study of Neyraud and Dransfield (2003), who showed that the concentration of taste compounds (NaCl and sugar) dissolved in saliva was higher correlated to taste perception than the initial concentration of tastants in the food material.

To understand our results, we need to consider four parameters: the food composition, the chemical compounds characteristics, the GC-MS parameters, and the saliva dilution. Each of these parameters impacts the release of volatile compounds.

As regards the food composition, as previously discussed, the release of volatile compounds is formulation-dependent. As regards chemical compounds characteristics, the release of the compounds depends on their hydrophobicity and volatility. As regards the GC-MS parameters, we had an extraction time of 20 min and we used non-limiting teenax cartridges. The aim was to minimize the interactions with saliva and matrix effects, which is not the case in real conditions. One hypothesis to explain our results would be that these parameters are not well representative of what happens during the food oral processing.

As regards, saliva dilution, different sub-parameters could impacted our results. First, preliminary saliva treatments are likely to affect the structure of saliva proteins. For example, freezing can lead to the denaturation of proteins and the formation of non-native protein aggregates (Pérez-Jiménez 2018). Changes in the structure of salivary proteins can impact their interactions with volatile compounds and thus their release. Secondly, the value of dilution (here 50%) impact also volatile compounds release. To discuss this dilution coefficient, it is necessary to take into account the dynamic of the oral process. At the beginning of the food oral process, the saliva film is thin and there is few product-saliva dilution. During the swallowing process, the saliva film become thicker and the product-saliva dilution increase (De Loubens *et al.*, 2011). According to the study of Doyennette *et al.* (2011), when considering the consumption of a liquid product, approximately 10% of the initial solution was kept in the final diluted mixture. Thus, to go further, it would be interesting to modify this conditions of dilution. Finally, a last parameters was not taking into account here: the oral mucosa also both impacts the kinetic of aroma compounds release and metabolizes volatile compounds (Ployon *et al.*, 2020, Délérís *et al.*, 2016).

Specific association between rheological measurements and sensory perceptions

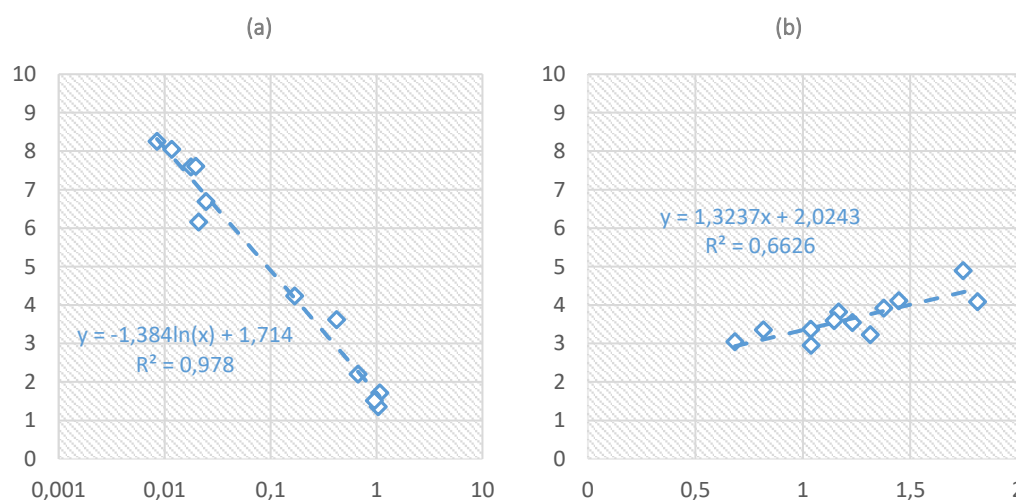
In order to focus and detail the role of the rheological parameters in perception, two-by-two correlations were calculated (Pearson method) for the 7 beverages diluted (Table 3.4.2.4.) and the significant correlations considered as the most relevant are plotted in Figure 3.4.2.7.

Table 3.4.2.4.: Correlations coefficients and associated pvalue (Pearson method) between rheological parameters and mouthfeel and astringent sensory attributes (scores/10).

		Mouthfeel		Astringent	
		pvalue	coefficient	pvalue	coefficient
Steady shear test	Power law index (n)	<0.01	-0.92	<0.01	0.77
	Consistency index (k)	<0.01	0.96	0.03	-0.62
	n/k	<0.01	-0.94	0.12	0.47
	Viscosity 50s ⁻¹ [Pa·s]	<0.01	0.92	0.13	-0.47
Frequency sweep test	G''/G' at 10 Hz	0.53	0.20	0.08	0.53
	G''/G' at 0,01 Hz	<0.01	-0.80	0.17	0.42
Strain sweep test	G''/G' at 0,001%	0.02	-0.67	0.08	0.53
	G''/G' at 0,06%	<0.01	-0.89	0.09	0.51
	G''/G' at 100%	0.68	-0.14	0.01	0.75
	G''/G' at 44%	0.26	-0.35	<0.01	0.81

The highest correlations concerned the mouthfeel attribute. This last is well correlated to parameters from the steady shear test but also to a lesser extent to parameters from the frequency sweep test (G''/G' at 0,01 Hz) and the strain sweep test (G''/G' at 0,001% and G''/G' at 0,06%). Constitutively, in the literature, initial thickness perception has been found to be correlated to viscosity measurements made at shear rates around 50 s⁻¹ (Joyner, 2018; Stokes *et al.*, 2013). However, several studies highlighted that other rheological parameters may be also important. Indeed, food is exposed to a range of shear and deformation processes during oral processing. Especially, Stokes *et al.*, highlight that full rheological characterisation of foods, including high shear viscosity, normal stress differences (non-linear viscoelasticity) and extensional viscosity, could be interesting (Stokes *et al.*, 2013).

Besides, astringent intensity was correlated to a lesser extent to parameters from the steady shear test (power law index and consistency index). Astringent attribute is also correlated to parameters from the strain sweep test (G''/G' at 100% and G''/G' at 44%). In the literature, astringent perceptions are more likely to be compared to friction coefficient (Rossetti *et al.*, 2009). While the shear rate used is perhaps not relevant to astringency perception, it may well be the determining factor explaining mass transfer, from the bulk to the receptors, of phenolic compounds responsible of astringency. Besides, in a previous study (Cosson *et al.*, 2020; Section 3.4.1.), TDS results showed that astringency was dominant for all the beverages in the last part of the evaluation period. In this period, the material properties of the food bolus may then be drastically different from those measured in the rheometer, because of exposition to a range of shear and deformation processes but also because of changes induced by mixing/interacting with saliva and from temperature (Stokes *et al.*, 2013). To continuously study the different phases of oral processing, *in-situ* and non-destructive methods have also been proposed in the literature (Foegeding *et al.*, 2017, Mantelet *et al.*, 2020). These methods could be interesting to study astringency of pea beverages with different saliva dilution.

**Figure 3.4.2.7.:** (a) Mouthfeel intensity (scores/10) in function of log(n/k). (b) Astringent in function of G''/G' at strain 40%.

Specific association between volatile compounds and sensory perceptions

In order to distinguish more precisely the volatile compounds with the greatest impact, two-by-two correlations were calculated (Pearson method) with the 7 beverages not diluted, diluted with water and diluted with saliva. Table 3.4.2.5. presents the significant correlations (p -value < 0.05). Thirty-two volatile compounds were significantly correlated to aromatic sensory notes. Seventeen volatile compounds were correlated to almond attribute, fourteen to broth attribute, eight to pea attribute, four to overall aromatic intensity and three to nuts attribute. These volatile compounds have known flavours (Table 3.4.2.3.). Two-thirds of these correlations are negative. Among them, some compounds been identified as being particularly relevant to pea flavour such heptan-2-one (Trikusuma *et al.*, 2019), octa-3,5-dien-2-one (Murat *et al.*, 2013) and hexan-1-ol (Bi *et al.*, 2020).

Table 3.4.2.5.: Correlations coefficients (Pearson method) between sensory attributes (scores/10) and volatile compounds (CG-MS area, beverages undiluted, diluted with water and diluted with saliva). Negative correlations are in bold. C = Correlation coefficient.

***For confidential reasons, only the chemical family of the compounds are given here.**

Sensory attributes	Beverages diluted	IUPAC name	C
Almond	saliva	aldehyde	0.80
	saliva	aldehyde	-0.76
	water	aldehyde	-0.92
	saliva	aldehyde	-0.89
	water	aldehyde	-0.84
	water	alcohol	-0.81
	saliva	aldehyde	-0.84
	saliva	furan	-0.77
	saliva	alcane	0.85
	water	alcane	0.83
	water	ester	0.76
	water	furan	-0.76
	saliva	ketone	-0.77
	water	ester	-0.78
	water	ketone	-0.76
	saliva	ketone	-0.78
	water	ketone	-0.82
	not	ketone	-0.79
	saliva	ketone	-0.77
	water	ketone	-0.83
	saliva	ketone	-0.79
	water	ketone	-0.78
	saliva	ketone	-0.87
	saliva	alcane	-0.83
Overall aromatic intensity	not	aldehyde	-0.81
	not	aldehyde	-0.76
	saliva	alcohol	-0.77
	water	alcohol	-0.83
Broth	not	aldehyde	-0.87
	not	aldehyde	-0.83
	not	aldehyde	0.76
	water	ketone	0.80
	not	aldehyde	0.80
	saliva	aldehyde	0.80
	not	ketone	0.92
	not	ketone	0.86
	not	alcohol	0.86
	water	alcohol	0.77
	not	ketone	0.79
	not	ester	0.93
	not	ketone	0.76
	saliva	ketone	0.76
	saliva	ketone	0.76
	not	ketone	0.84
	saliva	ester	-0.87
	water	ketone	-0.76
	not	alcohol	0.83
Nuts	not	aldehyde	-0.77
	not	aldehyde	-0.82
	water	aldehyde	-0.86
	not	aldehyde	-0.83
	saliva	aldehyde	-0.83
	water	aldehyde	-0.84
	water	ketone	-0.75
	saliva	alcohol	-0.84
	water	alcohol	-0.88
	saliva	alcohol	-0.78
	water	aldehyde	-0.78
	water	aldehyde	-0.78
Pea	not	aldehyde	-0.77
	not	aldehyde	-0.82
	water	aldehyde	-0.86
	not	aldehyde	-0.83
	saliva	aldehyde	-0.83
	water	aldehyde	-0.84
	water	ketone	-0.75
	saliva	alcohol	-0.84
	water	alcohol	-0.88
	saliva	alcohol	-0.78
	water	aldehyde	-0.78
	water	aldehyde	-0.78

These results allowed us to discuss on i) compounds effects and ii) saliva effect. First, as regards compounds effect, these results confirm that perceived aroma perceptions are not determined by a single component. And so the identification of the overall sensory-relevant volatile compounds profile should be needed (Diez-Simon *et al.*, 2019). Secondly, as regards saliva effect, different correlations are observed for undiluted beverages, diluted with saliva or water. For example, octa-3,5-dien-2-one is correlated with almond perceptions regardless of the dilution of beverages. However, hexan-1-ol is correlated only with beverages undiluted or diluted with water, whereas 2-methylidenehexanal is correlated only with beverages diluted with saliva. These results confirm the hypothesis that the impact of saliva must be molecularly dependent. It is therefore difficult to predict the impact of saliva on aromatic perceptions. Finally, as a prospective, it will be interesting to take into account the dynamics of release of volatile compounds during food oral process. Indeed, in a previous study (Cosson *et al.*,

2020; Section 3.4.1.), TDS results showed specific aroma phases for the beverages. In the first part of the evaluation period, pea attribute is more often dominant. Then, almond attribute is more often dominant. Finally, in the last part of the evaluation period, nuts attribute is more often dominant. These results suggest a dynamic release of volatile compounds (and therefore probably a different impact of saliva depending on these compounds) which is not taken into account here. To go further, it will be interesting to study volatile compounds with dynamic analytics methods such as PTR-MS (Ployon *et al.*, 2020).

3.4.2.1. Conclusion

In conclusion, this study's use of three types of approach—sensory profiling, rheological profiling and GC-MS profiling—helped to clarify how the composition of pea protein-based beverages affected sensory perceptions. The 12 pea protein beverages studied permits to explore a wide range of ingredients while being realistic in term of plant beverage products with variation on NaCl content, on gellan gum content, on oil content and on protein type (isolates and pellets). As regards texture perceptions, mouthfeel and astringent perceptions were significantly impacted by the gellan gum content. Rheological properties allow a good understanding of these perceptions. As regards aromatic perceptions, these studies allowed us to discuss 1) the formulation effect, and 2) the individual effect via the saliva dilution. First, the composition and release of volatile compounds were only relatively little affected by studied formulation: products with low hydrocolloid content present highest volatile release. Secondly, volatile compounds releases were significantly modified when beverages were diluted by saliva in comparison to water. In term of aroma perceptions prediction, the study of the food saliva-dilution provides additional information and seems necessary for understanding sensory perceptions of pea protein-based foods during oral processing. Therefore, the formulation factor that appears to be most relevant for decreasing off-notes in pea protein products appears to be the concentration of texturizing ingredients.

Main results

- Products with a low hydrocolloid content present the highest volatile release and were perceived as the lowest intense in aroma perception.
- Volatile compounds releases were significantly modified when beverages were diluted by saliva in comparison to water.
- In term of aroma perceptions prediction, the study of the food saliva-dilution provides additional information.
- Mouthfeel and astringent perceptions were significantly impacted by the gellan gum content. Rheological properties allow to model these perceptions.

CHAPTER 4

GENERAL DISCUSSION

CHAPTER 4 - GENERAL DISCUSSION

The results presented in the chapter 3 allowed us to improve our understanding, both in terms of sensory and chemical knowledge of pea protein isolates, as well as on the methodological level. The objectives of the present chapter are to integrate and discuss all the results obtained on the role of the main groups of chemical compounds and role of formulation on sensory perceptions as well as the different methodologies employed. The main conclusions drawn in the previous chapters are presented in order to be discussed on i) the knowledge sharing between solutions and beverages, ii) the impact of inter-individual variability on perceptions and iii) the reflective analysis on strategy and used methods.

4.1. How to explain the off-notes of pea-based products from the composition?

To begin with, to understand the role of composition on perception, a multidisciplinary strategy combining sensory, physico-chemical and statistical analysis has been developed on pea protein isolates. 46 pea-protein-based solutions were formulated by combining the different fractions (insoluble, soluble fraction, and soluble fraction with low molecular weight) and sensory and physicochemical properties were characterised. The main results on the perception of the fractions are illustrated in Figure 4.1.1. They showed that beaniness was mainly influenced by the retentate and permeate fractions. Bitterness was mainly influenced by the retentate fraction, whereas astringency was influenced by the retentate and pellet fractions.

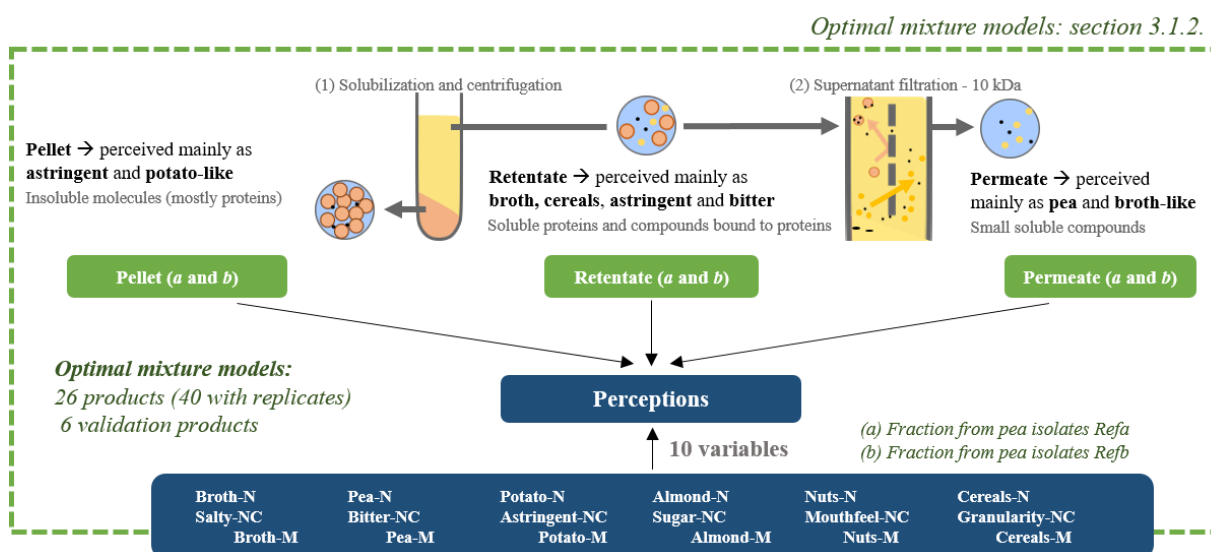


Figure 4.1.1.: Diagram of the main results of the model of pea perceptions in function of the pea fractions composition.

Then, the main groups of compounds in pea protein isolates were identified: volatile compounds, peptides and phytochemicals (phenolics and saponins). Relations between sensory profiles and compounds profiles were analysed using different statistical methods. A second diagram, synthesizing the links between the perceptions and the fractions is presented in Figure 4.1.2.

A total of 79 volatile compounds were identified, including 34 volatile compounds correlated to “beany” aroma. The large majority of these 34 compounds was similarly correlated to the different notes contributing to beany, suggesting a common nucleus of compounds responsible for the beany perceptions. A total of 3500 peptides were identified including 14 peptides with sequences inferior to 8

residues and correlated to bitter perceptions. A total of 48 phytochemicals were tentatively identified as phenolic acids, flavonoids or terpenoids, including 29 compounds correlated to bitterness and astringency perceptions. But they were also correlated to the aromatic perceptions which could be due to their indirect role by modifying pH and ionic strength.

As regards the relative role of each group of components, for reasons of confidentiality, we are not allowed to discuss the results here (Figure 4.1.2.).

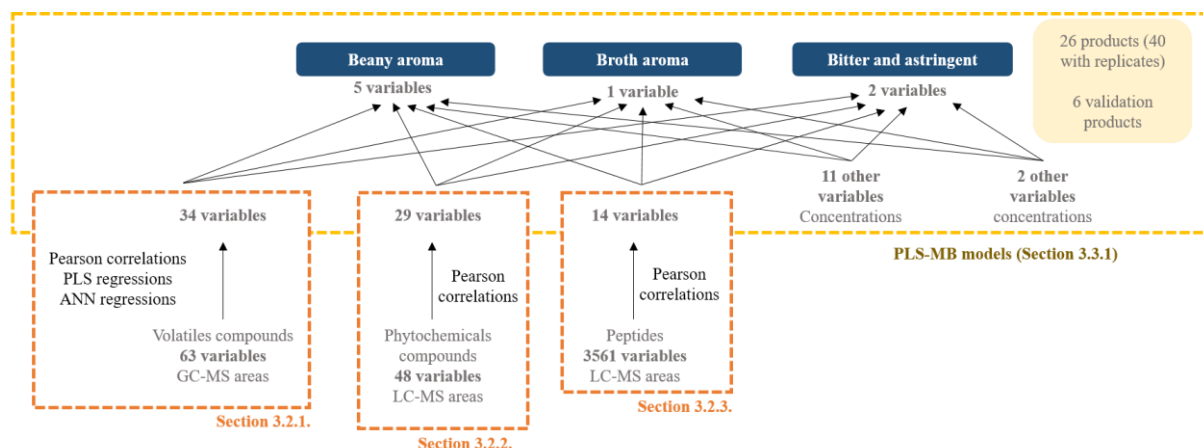


Figure 4.1.2.: Diagram of the main results of the model of pea perceptions in function of the chemical composition.

In addition, this work has provided some knowledge on these pea protein isolates composition. As regards peptides identification, we identified a wide variety of peptides representing a range of protein families, mainly those containing seed storage proteins but also those containing proteins that can play a role in sensory perceptions, such as lipoxygenases. These peptides were mostly polar and hydrophilic. Moreover, eleven peptides had sequences homologous with peptides known as being antioxidant. As regards phytochemical compounds, larger amount of these compounds can be found in flour than in pea protein isolates and fractions highlighting maybe the degradation of these compounds during the process. However, related to the dry matter content of products, permeates had larger amount of phytochemicals. Concerning the volatile compounds, 79 were identified coming from endogen origin of pea seed or from oxidation of the lipids/peptides/amino acids in the seed or during the storage. In addition, the protein-volatile interactions were mainly hydrophobic interactions and could be explained by the octanol/water partition coefficient (LogPow). However, these interactions could only impact about 18% of the volatile compounds and so may have only a low impact on the olfactory perceptions.

Secondly, this work contributed to better understand how product composition affected the sensory perception of pea protein-based beverages, varying in protein type (pellet vs. isolate) and their content of gellan gum, salt and oil. Relations between sensory profiles and chemical/physico profiles were discussed in regards to the food oral processing. A third diagram, summarizing the results obtained in relation to the formulation and dynamics of perceptions, is presented in Figure 4.1.3.

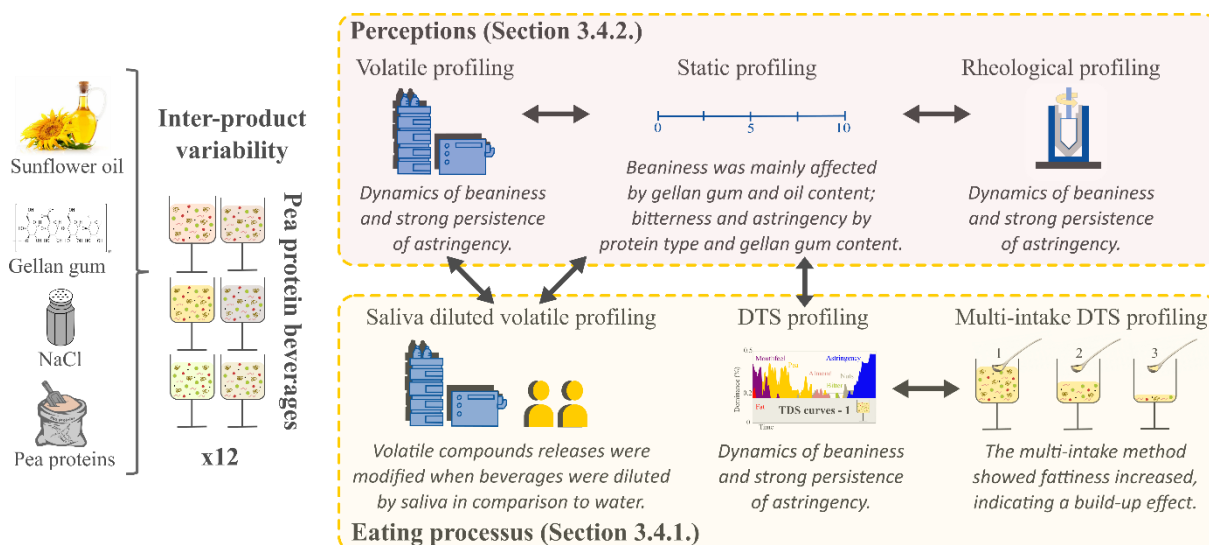


Figure 4.1.3.: Diagram of the main results of the study on pea beverages. TDS: Temporal dominance of sensations.

Concerning the methodologies, sensory profiling and TDS methods yielded complementary results about the impact of beverage composition on perceptions. Static block profiling revealed that beaniness was mainly affected by gellan gum and oil content and that bitterness and astringency were mainly affected by protein type and gellan gum content. In addition, mono-intake TDS profiling highlighted the dynamics of beaniness and the strong persistence of astringency. The results suggested that higher gellan gum and salt contents could limit its persistence. Taken into account the consumption of a full portion of product, results of multi-intake TDS profiling showed that beaniness and bitterness decreased during the consumption, indicating a possible adaptation effect, while fattiness increased, indicating a build-up effect. As regards texture perceptions, mouthfeel and astringent perceptions were significantly impacted by the gellan gum content. Rheological properties were clearly related to these perceptions. Concerning aromatic perceptions, the individual effect via the saliva dilution were evaluated in addition to the formulation effect. Products with low hydrocolloid content present highest volatile release. Volatile compounds releases were significantly modified when beverages were diluted by saliva in comparison to water. In term of aroma perceptions prediction, the study of the food saliva-dilution provides additional information and seems necessary for understanding sensory perceptions of pea protein based foods during oral processing. Therefore, the formulation factor that appears to be the most relevant for decreasing off-notes in pea protein products seems to be the concentration of texturizing ingredients.

Thus, this PhD work provides some results which could be used to reformulate pea protein products to improve their perception. In closing, the following diagram present a summary of the main results listed previously on knowledge of pea protein isolates ingredients and pea protein based-products (Figure 4.1.4.). We have seen with these works that it is possible to modify perceptions at several levels. First, it is possible to modify perceptions at the level of molecular composition. The bitter and astringent attributes are mainly explained by the composition of phytochemicals and peptides. The beany perceptions are mainly explained by the volatile composition. We could therefore limit these perceptions by preventing the formation of these compounds during the process. It is also possible to modify the perceptions at the level of the fraction composition. Retentates are perceived as mainly as broth, cereals, astringent and bitter. The filtration step was so not especially efficient in removing the compounds responsible of these off-notes. However, the pellet are perceived mainly as astringent and potato-like. The centrifugation step seem so more useful to decrease these off-notes. It is also possible to modify the perceptions from the food composition. Beaniness, bitterness, astringency and their persistence can be decrease by adding gellan gum, sugar, salt or oil. It is also possible to mask off-not by adding texturing agents, sugars acid of flavouring. Finally, the perception of these off-notes depends of the individuals. Thus it could be interesting also to educate consumer on the benefits and the taste of the pea protein, to

promote new recipes and new industrial products or to train chefs in collective catering to promote pea based products.

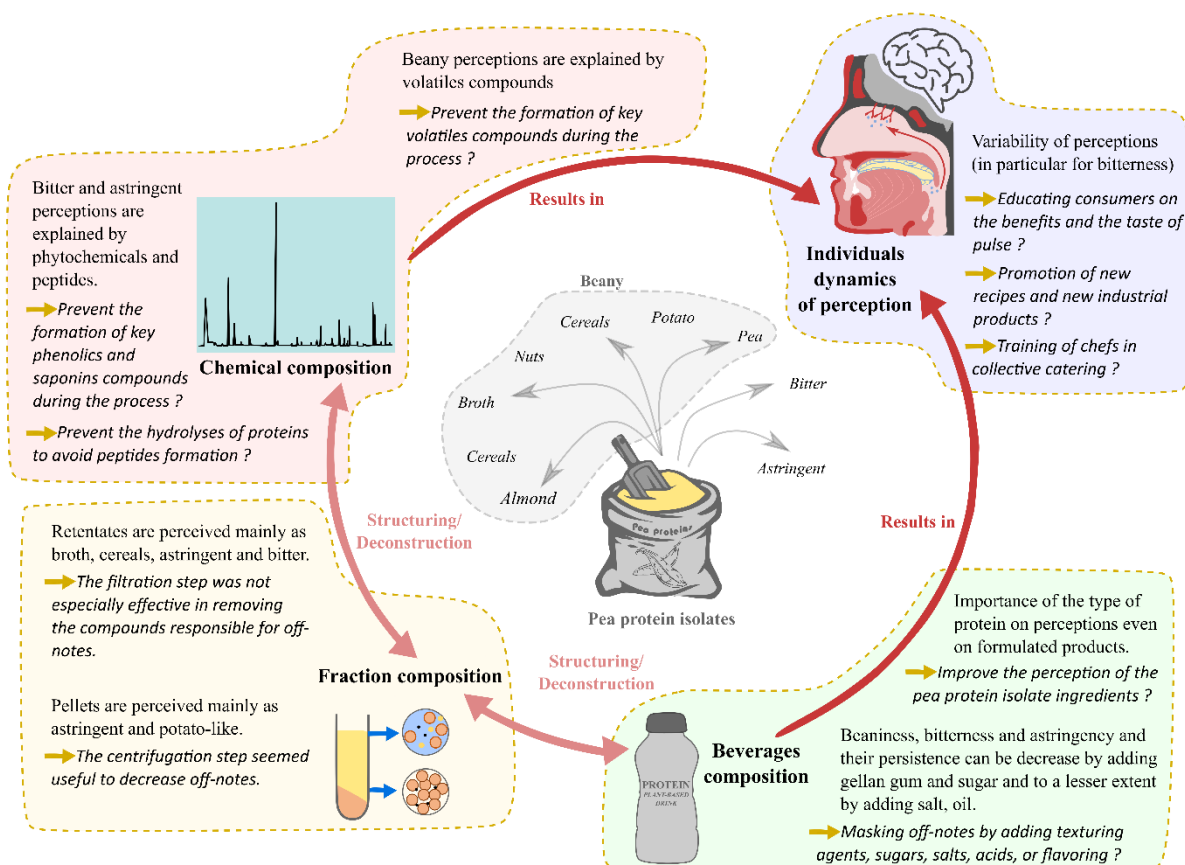


Figure 4.1.4.: Diagram of the main results of this work and propositions which could be envisaged to decrease pea protein off-notes and increase the desirability of pea based products.

4.2. What is the impact of the food (beverages vs solutions) on the results?

4.2.1. Solutions vs beverages: sensory results

The sensory profiles of protein solutions and formulated products were different, showing the clear impact of formulation on perception. The impact of the use of two different panels for the solutions and beverages could also explain the differences of perception. Hence, in the following paragraphs we chose to relatively compare the results issued of the two panels/products types to gain more insights about pea proteins perceptions.

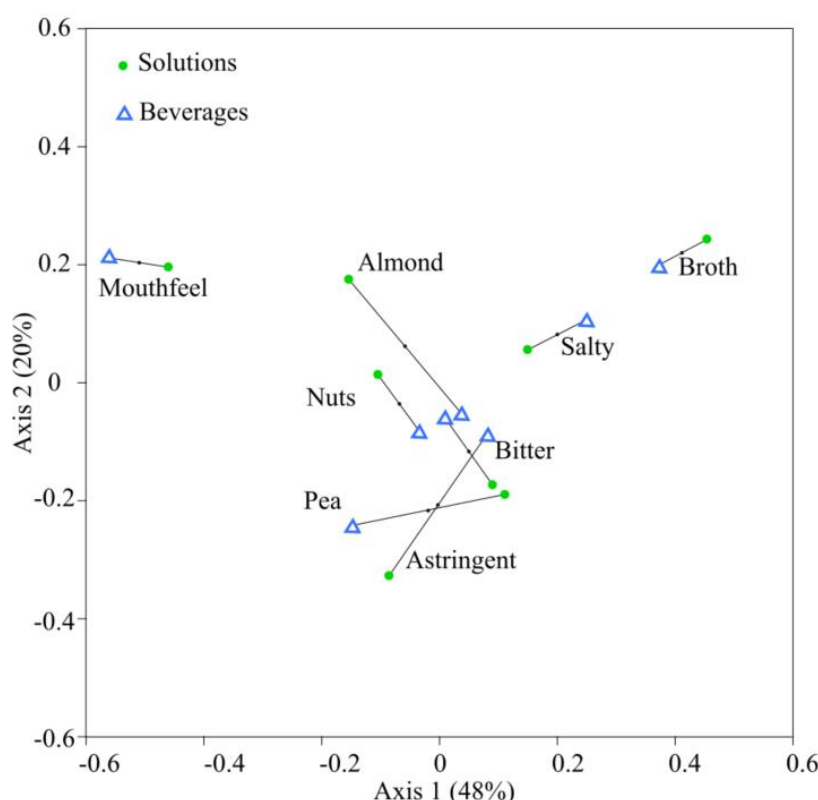


Figure 4.2.1.: Results of the Generalised Procrustes Analysis (GPA)¹ examining the common sensory attributes used by the two panels/products type for axis 1 and 2. In blue (triangle) are the attributes evaluated for the pea beverages. In green (circle) are the attributes evaluated for the pea recombined solutions.

¹ Generalized Procrustes Analysis (GPA) was performed on all sensory data to visually compare the panels' positioning of the 8 common attributes. As explained by Elgaard et al., 2019, data was averaged over both replicates and panellists, and analysed with the GPA function in the FactoMineR package (Lê et al., 2008). The tolerance level for solution convergence was set to 10E-10. The maximum number of iterations was set to 200. The data was scaled. RV coefficients were extracted, to numerically investigate the panels' similarities. A permutation test was performed on the GPA solution to investigate the strength of the results with the GPA.test function (RVAideMemoire package). The number of permutations was set to 500 (Elgaard et al., 2019).

GPA was performed to visually investigate the positioning of 8 common sensory attributes in the sensory space by the two panels (Figure 4.2.1.). The two panels used the sensory attributes in a similar manner, since their individual panel means are placed closely together in the sensory space. The RV coefficient of 0.58 between the two panels confirmed the relative similarity of the two panels. The GPA plot shows that mouthfeel, almonds, nuts, salty and broth attributes were well discriminated from each other by both panels. They therefore represent different sensory dimensions. To go further in the comparison of the panels, as the panels seem to have used sensory attributes relatively in the same way, the results of the products were compared by PCA (Figure 4.2.2.). Beforehand, these data were reduces-centred by data sets to avoid different scaling-used by panels. These results allow us to compare relatively the

spaces of products. We observed more variability within solutions than beverages. In particular, four solutions (the most diluted with water) stand out clearly. This could suggest that there is a perception threshold at which the products are perceived to be significantly more intense (psychophysical curve). For beverages, four products, formulated with pellet, are also clearly perceived different.

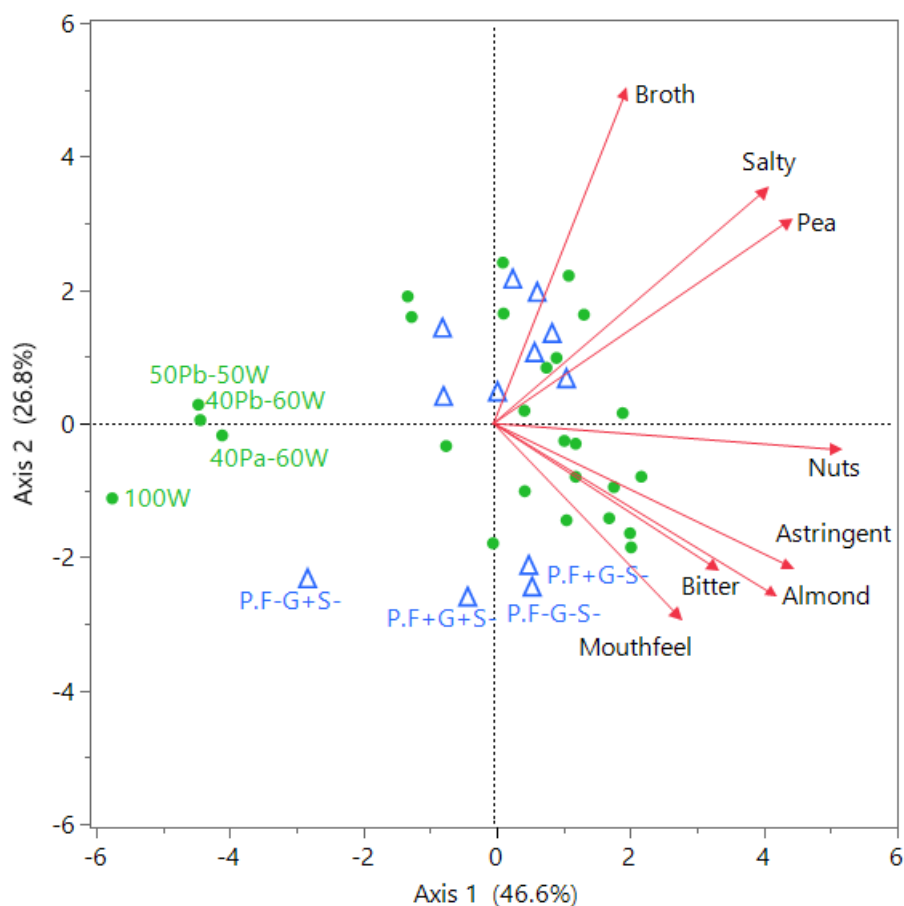


Figure 4.2.2.: Results of the principal component analysis (PCA) examining the sensory profiles. The plot shows the loadings i.e. the correlational relationships between PCA axes 1 and 2 and the sensory attribute values (in common for both products datasets). In blue (triangle) are the pea beverages. In green (circle) are the pea recombined solutions. In order not to overload the graph, only the most relevant legends have been added.

4.2.2. Solutions vs beverages: volatile compounds results

In a same way, we compared volatiles profiles obtained by GC-MS analysis from both sets of products. However, one should be cautious about the results of this comparison as the volatile compounds in the two datasets are derived from slightly different GC-MS parameters (extraction of 10 min for the pea solutions versus 20 min for the pea beverages). Of all the identified volatile compounds, 34 were common to both sets of products. Beforehand, these data sets were reduced-centred and represented with a principal component analysis (Figure 4.2.3.).

A similar variability on aroma release is observed between pea solutions and pea beverages. Most of the variability is on axis 1 (39.5%) carried by the majority of compounds. These axes can differentiate the samples with high GC-MS areas such as I/F+/G-/S-, Refa or 50Ra-25Ia-25W and products with lower areas such as water (100W) or P/F+/G-/S-, P/F-/G+/S-, P/F-/G-/S-. Here again a clear distinction is made between products formulated with pellets. The differences between Refa and 50Ia-50W seem similar to the differences between beverages formulated with Refa (I) and pellet (P) with the exception of the product P/F+/G+/S-. The differences between pea beverages and pea solutions can be explained by the

impact of i) the formulation (hydrocolloids content, interactions with lipids and matrix, salting out effect...) and of ii) the oral process (time of swallowing, viscosity of the products...).

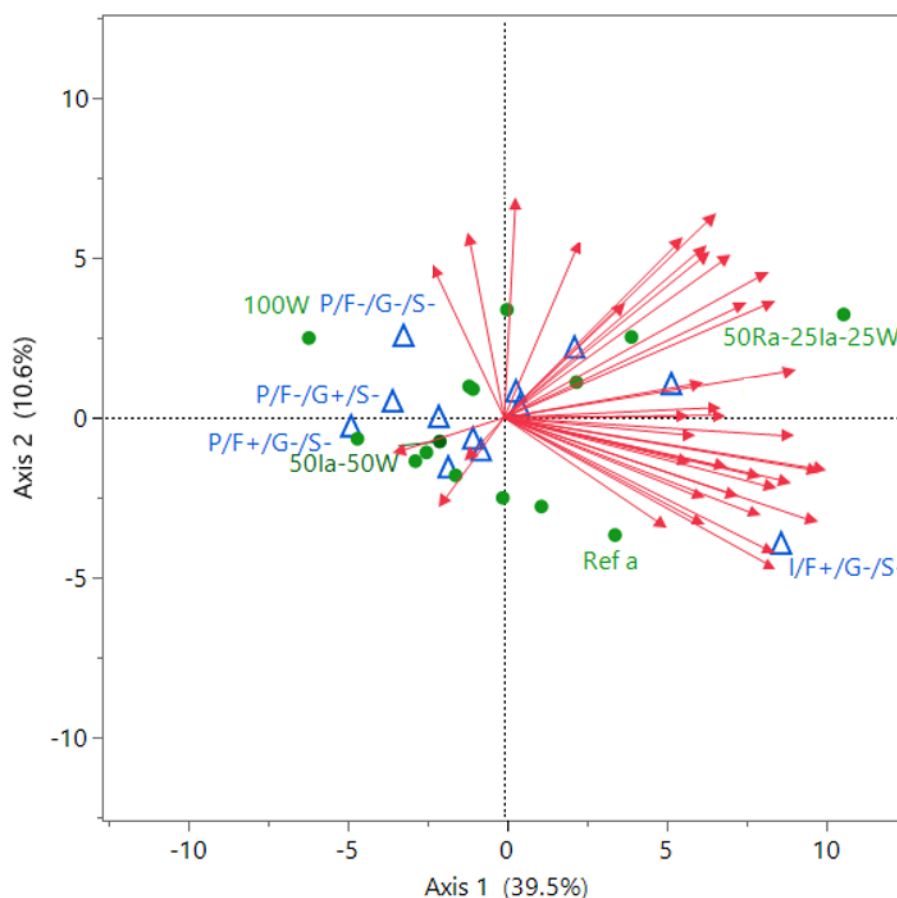


Figure 4.2.3.: Results of the principal component analysis (PCA) examining the volatile compounds profiles for both type of products. The plot shows the loadings i.e. the correlational relationships between PCA axis 1 and 2 and the volatile compounds GC-MS areas attribute values (in common for both products datasets). In blue (triangle) are the pea beverages. In green (circle) are the pea recombined solutions (50Ia-50W in dark green). In order not to overload the graph, only the most relevant legends have been added.

4.2.3. Solutions vs beverages: sensory interactions

In addition to other effect, sensory interactions seem to play a key role in the perceptions of pea protein isolates. While it is complicated to measure sensory interactions in the case of aromatic attributes, sensory interactions could be evaluated for flavour and texture/sapid attributes by using a nose-clip for the pea solutions (solutions P1 to P6, Section 3.1.1.) and pea beverages. The Figure 4.2.4. presents the Newman-Keuls post-hoc comparisons examining effects of using nose-clip for significant attributes (ANOVA, data not shown) for the evaluation of 6 pea solutions and 12 pea beverages by block protocol profiling. Salty and bitter notes are significant perceived different for both types of products and show the same trends. Products evaluated with nose clip have lower scores. For solutions, the astringent attribute presents a significant effect and shows lower scores in presence of the nose clip. For beverages, the overall aromatic and fat-P attributes are significantly perceived differently and have higher scores with the presence of nose clip. Thus these results confirm the existence of sensory interactions within formulated products or solutions. Zhang *et al.* (2016) reported interactions between beany and bitterness perceptions in soya isolates. So, pea aroma perception could increase the bitterness perception in the studied products. The modification of the overall aromatic attribute suggests a congruent effect of the panellists in the evaluation of beverages.

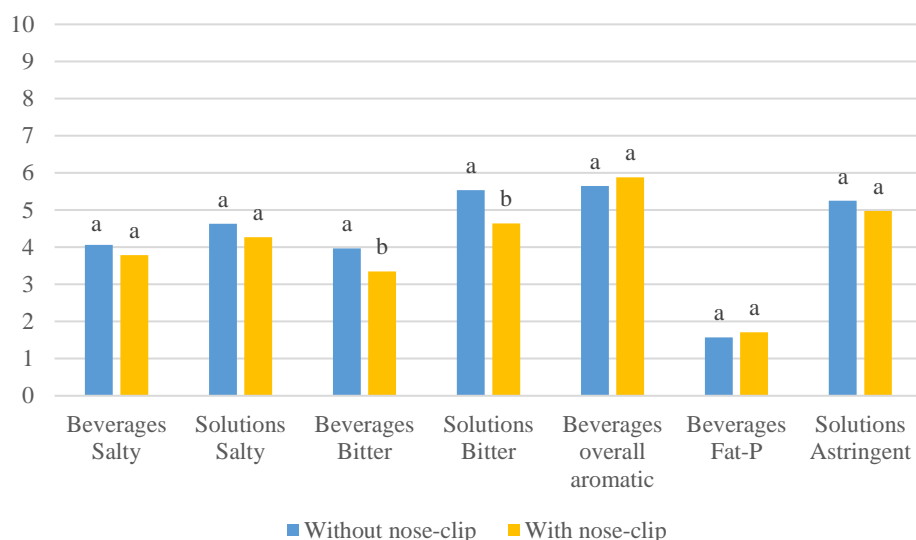


Figure 4.2.4.: Results from the Newman-Keuls post-hoc comparisons examining effects of using nose-clip for significant attributes (ANOVA, data not shown) for the evaluation of 6 pea solutions and 12 pea beverages by block protocol profiling. Significant differences are indicated by differences in letters ($\alpha = 0.05$).

4.3. What is the impact of inter-individual variability on perceptions?

Inter-individual variability in perception is largely known and must be taken into account. During the different works of the PhD, we chose to work a small group of healthy adults (16 and 17 panellists) to conduct the sensory evaluation. This number is classically used for secretive tests in the literature (usually between 10 and 20 panellists), but we would to characterise our panellists in regards to the population with as example their evaluation of sensibility with PROP test. Despite the small sample size, differences were observed between our panellists in terms of duration before swallowing, changes in perceptions (TDS) or bitterness sensitivity (Prop Test). These differences were not taken into account in the work setting, because we averaged results of panellists to study the link with physicochemical data. However, one strategy could have been to take this variability into account by studying groups of panellists to construct separate models. This clustering stems from (i) consumers' eating habits, (ii) physiological characteristics and/or (iii) genotypic characteristics.

4.3.1. Panellists physiological inter-variability express via TDS parameters

Chewing time is one of the criteria frequently found in the literature to distinguish between panellists' chewing behaviours (Jourdain *et al.*, 2017). The chewing duration can induce differences in the physicochemical properties of the bolus at the origin of different dynamics of texture perceptions. Here, we did not have the opportunity to measure this parameter. However, the TDS gives us some indirect parameters: time of first swallow, time of last swallow, time of last sensation. If we look at the parameters from the TDS, two groups of panellists can be distinguished on the hierarchical cluster as shown on Figure 4.3.1 (green and red). The green cluster group the panellists with the longer time of perception, the longer first swallow time and the longer last swallow time. To be noted, there is not correspondence between these clusters of panellists and their PROP status.

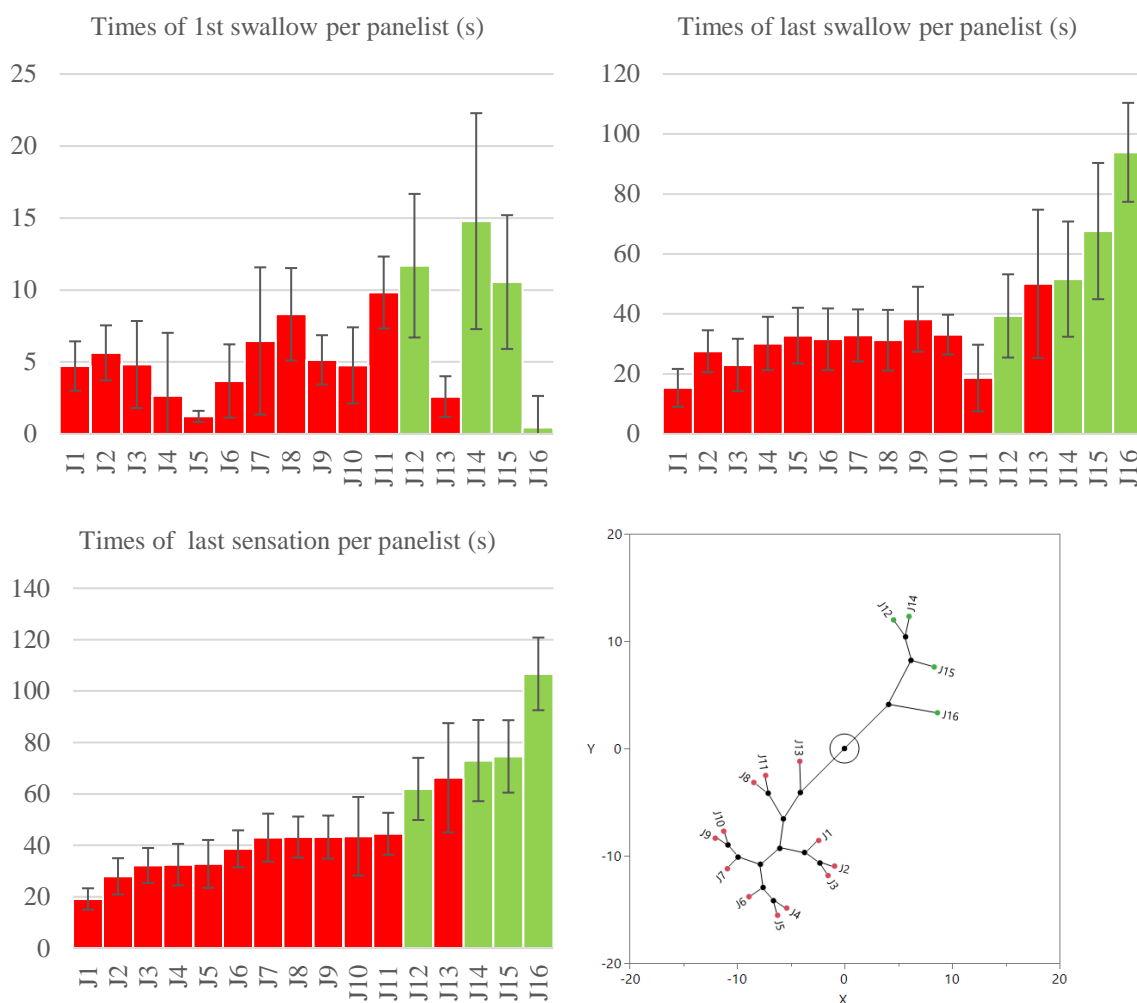


Figure 4.3.1.: Panellists (16) physiological inter-variability express via Temporal Dominance of Sensation profiling parameters: time of first swallow (s), time of last swallow (s), time of last sensation (s). Constellation plot from hierarchical clustering of TDS parameters from panellists. Each observation is represented by an endpoint and each cluster join is represented by a new point. The lines that are drawn represent cluster membership. The lengths of the lines represent the distance between clusters. The circle represents a cluster with all the panellists.

The impact of these clusters was studied on sensory scores by ANOVA (Table 4.3.1). We observed that the cluster effect is significant for 7 attributes (almond, aromatic intensity-P, astringent, broth, fat, mouthfeel and overall aromatic intensity) and for the interaction cluster*product for the fat attribute. The panellists of cluster green noted the products more almond, more broth, fatter and more aromatic. And they rated the products as less astringent and less mouthfeel. It can be assumed that for the green group, the products are kept longer in the mouth. This means that the aroma compounds could have more time to be released and the fat to cover the oral surfaces (i.e., the tongue and the palate) (Appelqvist *et al.*, 2016).

Table 4.3.1.: Results of the ANOVA examining cluster of panellists and product effects during the experiment where attribute intensity evaluated in mouth for the 12 pea beverages was evaluated using the static profiling protocol. Significant p-values are in bold ($\alpha = 0.05$).

Attributes	Cluster of panellists		Cluster*Product		Product	
	F	pvalue	F	pvalue	F	pvalue
Almond	17.58	<0.01	0.97	0.47	1.13	0.34
Aromatic intensity-P	8.11	<0.01	0.45	0.93	1.75	0.06
Astringent	22.01	<0.01	0.31	0.98	2.41	<0.01
Astringent-P	0.82	0.36	0.14	1.00	3.10	<0.01
Bitter	0.28	0.60	0.65	0.79	3.33	<0.01
Bitter-P	0.81	0.37	0.49	0.91	1.00	0.44
Broth	9.38	<0.01	1.24	0.26	20.96	<0.01
Fat	6.31	0.01	2.98	<0.01	39.31	<0.01
Fat-P	0.02	0.89	0.67	0.77	7.09	<0.01
Mouthfeel	4.08	0.04	1.30	0.22	182.23	<0.01
Nuts	1.16	0.28	1.25	0.25	2.52	<0.01
Overall aromatic intensity	4.38	0.04	1.58	0.10	9.86	<0.01
Pea	0.02	0.88	0.82	0.62	0.69	0.75
Salty	0.02	0.88	1.78	0.05	30.40	<0.01
Sweet	0.00	0.96	0.51	0.90	4.04	<0.01

4.3.2. Panellists genotypique inter-variability express via the Prop Test

Bitterness sensitivity (PROP Test) is also one of the criteria frequently found in the literature to distinguish between panellists' behaviour (Dinehart *et al.*, 2006). Some studies have shown that, compared to lower bitter sensitive panellists (i.e. Low Prop Tasters), higher bitter sensitive panellists (i.e. High Prop Tasters) do perceive a variety of compounds with greater intensity (Fischer *et al.*, 2015). Here, we found that PROP status affected evaluations of bitterness as well as evaluations of other sapid and flavour attributes (6 pea solutions, Section 3.1.1.). In particular, LPTs seemed to have a longer time discriminating among sample types for a number of attributes; the only attribute for which there was evidence of discrimination was a texture attribute. We used the beverages data to examine whether panellist PROP status influenced sensory evaluation. The ANOVAs results showed that PROP status influenced the evaluation of all the attributes (PROP status significant for 15/15 attributes). The Newman-Keuls post-hoc comparisons results (Figure 4.3.2.) showed the variation of intensity scores by PROP status. In particular, there are significant rating differences for the attributes bitter, sweet, broth, fat-P and aromatic intensity-P.

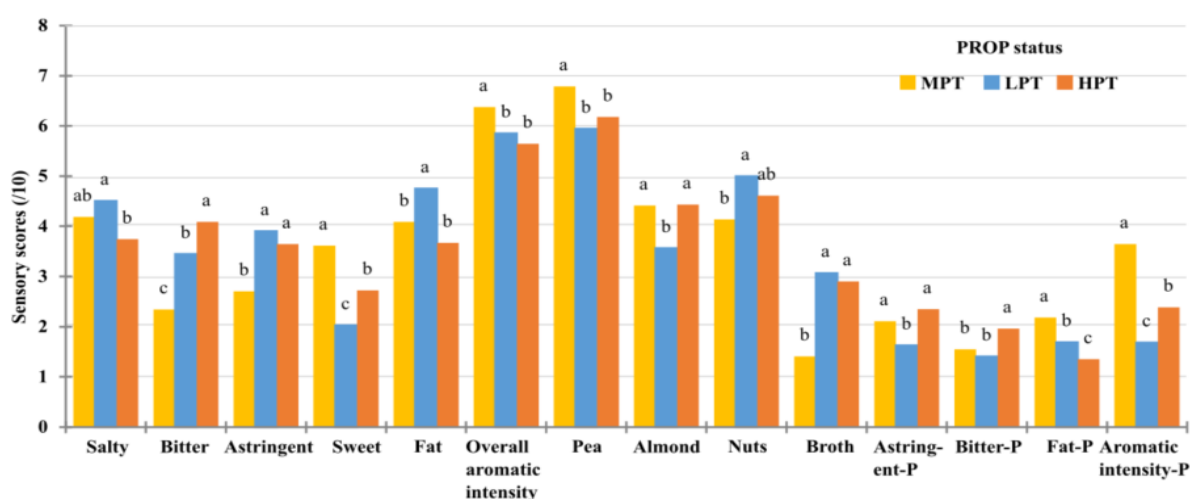


Figure 4.3.2.: Results of the sensory scores evaluated for the 12 beverages and from the Newman-Keuls post-hoc comparisons examining effects of PROP status where HPT = high PROP taster group, MPT = medium PROP taster group, and LPT = low Prop taster group. Significant differences between are indicated by differences in letters.

To study the impact of panellists having different PROP status on formulated products, some ANOVA were performed in which oil, gellan, protein and salt content were fixed effects (Table 4.3.2.). We observed that according to the PROP status, the ingredients did not have the same impact. For example, the oil concentration impacts the perceptions of almond, astringency and fat only for the high PROP taster group. On the other hand, the gellan concentration does not impact astringency perceptions for the high PROP taster group. Thus, the sensitivity to bitterness measured by the PROP test seems to be an interesting variable to take into account for a better understanding of the mechanisms at the origin of food perceptions related to the diversity of individuals.

Table 4.3.2.: Results of the ANOVA examining formulation effects (oil, gellan gum, protein and salt content) according to the PROP status during the experiment where attribute intensity evaluated in mouth for the 12 pea beverages solutions was evaluated using the static profiling protocol. Significant p-values are in bold and in blue ($\alpha = 0.05$). HPT = high PROP taster group. MPT = medium PROP taster group. LPT = low Prop taster group.

pvalue	Oil content			Gellan content			Protein content			Salt content		
	HPT	LPT	MPT	HPT	LPT	MPT	HPT	LPT	MPT	HPT	LPT	MPT
Almond	0.01	0.25	0.84	<0.01	0.29	0.74	0.58	0.15	0.43	0.86	0.86	0.83
Aromatic intensity-P	0.01	0.20	0.09	0.91	0.44	0.78	0.47	0.12	0.03	0.24	0.48	0.47
Astringent	0.01	0.57	0.70	0.13	<0.01	<0.01	0.02	0.87	0.40	0.72	0.28	0.45
Astringent-P	0.73	0.62	0.84	0.10	0.02	<0.01	<0.01	0.09	0.12	0.20	0.57	0.19
Bitter	0.55	0.93	0.06	0.76	0.85	0.05	<0.01	<0.01	0.13	0.43	0.69	0.38
Bitter-P	0.18	0.94	0.34	0.57	0.68	0.22	<0.01	0.76	0.26	0.84	0.83	0.25
Broth	0.65	0.73	0.77	<0.01	<0.01	0.94	<0.01	<0.01	0.08	<0.01	0.02	0.21
Fat	<0.01	0.10	0.38	<0.01	<0.01	<0.01	<0.01	<0.01	0.12	0.04	0.06	0.01
Fat-P	0.96	0.36	0.20	<0.01	<0.01	<0.01	0.21	0.01	0.01	<0.01	0.43	0.17
Mouthfeel	0.02	<0.01	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Nuts	0.40	0.05	0.00	0.11	0.72	0.20	0.14	0.18	0.13	0.43	0.92	0.60
Overall aromatic	0.13	0.09	0.13	0.10	<0.01	0.49	<0.01	<0.01	<0.01	0.52	0.16	0.94
Pea	0.84	0.66	0.84	0.85	0.47	0.16	0.31	0.54	<0.01	0.67	0.97	0.37
Salty	0.41	0.30	0.87	0.05	0.00	0.13	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
Sweet	0.21	0.33	0.27	0.03	0.41	0.19	<0.01	<0.01	<0.01	0.26	0.66	0.82

4.3.3. Panellists' eating habit inter-variability

As regards consumers' eating habits, a questionnaire was asked to the pea solutions panellists about their eating habits. Among the 17 panellists, 1 panellist never cooked, 5 panellists rarely cooked, 5 panellists occasionally cooked and 6 panellists daily cooked. In addition, 1 panellist did not like legumes and can eat them only on some exceptions (in restaurants, at friends' homes), 7 panellists preferred cereals to legumes but can eat them occasionally, 9 panellists like legumes and eat them regularly.

Table 4.3.3.: Results of the ANOVA examining cluster and product effects during the experiment where attribute intensity evaluated in mouth for the 26 pea recombined solutions was evaluated using the block profiling protocol. Significant p-values are in bold ($\alpha = 0.05$).

Attributes	Cluster		Cluster*Product		Product	
	F	pvalue	F	pvalue	F	pvalue
Almond	22.94	<0.01	2.12	<0.01	29.77	<0.01
Astringent	0.27	0.60	0.70	0.87	26.13	<0.01
Bitter	0.87	0.35	0.95	0.53	14.86	<0.01
Broth	2.57	0.11	3.27	<0.01	45.57	<0.01
Cereals	58.86	<0.01	1.10	0.33	39.45	<0.01
Mouthfeel	0.56	0.45	1.18	0.24	102.72	<0.01
Nuts	18.13	<0.01	0.97	0.51	29.42	<0.01
Pea	2.63	0.11	1.16	0.27	41.87	<0.01
Potato	70.45	<0.01	1.99	<0.01	37.45	<0.01
Salty	13.25	<0.01	0.45	0.99	22.95	<0.01

Clusters of panellists were performed on their food habits and related to their perception evaluation of pea solutions (Table 4.3.3.). The first cluster of panellists regrouping 8 panellists, seems do not like legumes or prefer cereals to legumes and eat them occasionally (Cluster “occasionally”). The second cluster regrouped the 9 panellists which liked legumes and eat them regularly (Cluster “Regularly”). To be noted, there is not correspondence between these clusters of panellists and their PROP status. We observed that the cluster effect is significant for 5 attributes (almond, cereals, nuts, potato and salty) and for the interaction cluster*product for the potato, broth and almond attribute. The cluster “Regularly” overall noted the products with higher scores.

4.3.4. Panellists inter-variability and chemical variability

Another challenge of the present work was to take into account the inter-individual variability measured in sensory analysis (here i.e. sensory repetitions) in statistical models. For chemical measurements, the variability due to the measuring instruments is different. This raises the question of how to deal with repetitions. Here we have chosen to always work with averaged data. However, it would have been conceivable to work with the repetitions for the sensory data and to try to relate them to the averaged chemical data. For example, the PLS-MB model was built again but considering all the sensory repetitions (by copying the averages of the chemical data) (exactly same methods as explained on Section 3.3.1.). Table 4.3.4. presents the model indicators (Root mean square error of validation and of calibration) for the 3 models for both datasets. Results are similar. It can be seen that the models that best fit the data and have the most predictive power are the models to explain aromatic attributes and then the models to explain bitter and astringent attributes. However, overall model with repeated sensory data less fit the data and have a lower predictive power. Nevertheless, with repeated sensory data, there is less differences between root mean square error of validation and root mean square error of calibration. We can assume that this is due to the largest "rows" in the model.

Table 4.3.4.: Performances of the selected PLS Multi-Block model to predict the 8 sensory attributes variables in function of chemical compounds area analysed taking into account either sensory averages or sensory repetitions. RMSE = Root mean square error.

Attributes	Sensory repetitions		Sensory average	
	RMSE calibration	RMSE validation	RMSE calibration	RMSE validation
Aroma	0.40	0.40	0.28	0.34
Bitter	0.64	0.64	0.44	0.74
Broth	0.86	0.85	0.54	0.92

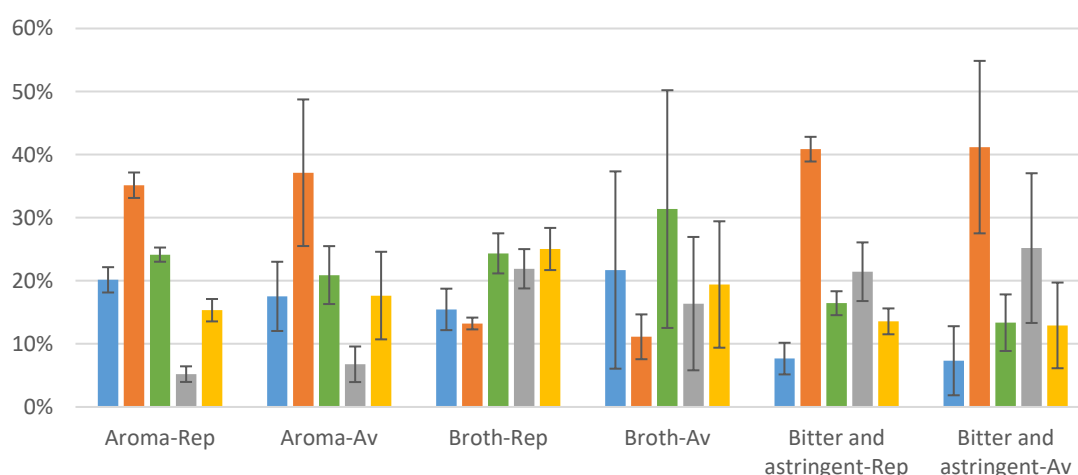


Figure 4.3.3.: Cumulated block importance for the first dimension (in %) with the 97.5% confidence intervals for the three PLS-MB regressions modelling sensory attributes in function of chemical composition of pea protein isolates. 1) Aroma: model in function of pea, potato, nuts, cereals and almonds sensory attributes. 2) Broth: model in function of broth sensory attribute. 3) Bitter and astringent: model in function of bitter and astringent sensory attributes. Rep: model with repeated sensory data. Av: model with averages sensory data.

Cumulated block importance indexes are also used to estimate the contribution and importance of the block of variables in the PLS-MB models (Figure 4.3.3.). Expressed as a percentage, they allow to quantify the impact of each group of chemical compounds on the block of perceptions of pea protein solutions. Results are similar for both datasets. However, here the standard deviations are much smaller in the case of repeated sensory data. Taking into account individual variability thus provides interesting additional information to better understand the mechanisms behind off-notes of pea protein isolates.

In order to go further, to the discussions of the previous paragraphs, the questions of individual and collective performance of the panels, of the understanding of the attributes could have been discussed. Furthermore, in addition to the reflection on the variability of the panelists, the variability of the physicochemical measurements, although much lower than the variability of the panelists, could also have been discussed. These different points underline the importance of taking into account all the characteristics of a study and not forgetting, when interpreting the results, the limitations of these studies.

4.4. Reflexive analysis of the choice of methods for the study of the links between composition and perceptions

To better understand the mechanisms behind the perceptions of pea protein isolates, a design-based experimental strategy was combined with a strategy of statistical modelling of sensory attributes based on compositional data. This strategy made it possible to highlight groups of compounds that participate in the mechanisms at the origin of perceptions. It also allows to make hypotheses on these mechanisms. This strategy is necessary as a first step because it allowed to refocus research on specific groups even if it is not precise enough to understand the individual impact of each molecule. For this, it would be necessary to complete our analyses. For example, we could use the strategy of omission (Engel *et al.*, 2002). Besides, to confirm the taste/aroma of an individual molecule we could predict how molecule could be perceived based on knowledge of their chemical structure and knowledge of aroma/taste receptors (Huang *et al.*, 2016). Another possibility would be to test these compounds on cell-based expression system for human receptors (Bouysset *et al.*, 2020).

In addition, the second most important strategic choice of these PhD works was used to create a large number of solutions by combining pea protein isolate fractions. To validate this strategy, the sensory properties of the two reference solutions, created directly from the pea protein isolates, were compared with the sensory properties of two experimental solutions that were created using the isolate fractions to have the exact same compositions as the reference solutions. The main difference between the reference solutions and the experimental solutions was in their perceived granularity. These results allowed us to make the hypothesis that when the experimental solutions were created by combining the isolate fractions, the processes that they underwent (centrifugation and filtration) might have broken up these aggregates and induced structural changes, resulting in smaller and more soluble clusters.

In addition, this strategy allowed us to think about calculated compound concentrations from their formulation. The proposed calculation was based on a linear combination of the dry matter area of compounds and the relative quantity of each fraction. This model has been particularly efficient for the characterization of peptides. To increase the robustness of the model for volatile compounds, it would be interesting to take into account the interactions with proteins in the calculation. To do this, it would be necessary to redo the volatiles analyses on all mixtures because the interactions are very molecule-dependent.

Besides, one of the challenges of this PhD was to manage the limited number of products (number of rows) compared to the number of identified compounds (number of columns). As a result, the models built are less powerful and it is more difficult to take into account the interactions. However, this problem is recurrent in descriptive sensory studies, because when working with panellists, it is always difficult to obtain large data sets. Nevertheless, this study allowed us to work on ways to select the most relevant data such as clustering methods and preliminary models such as PLS regressions.

As regards the pea solutions, in order to have a large number of products in a same range of perceptions, the choice taken in the present study was to work with only two commercial pea protein isolates and to create a controlled variability. Therefore, an original approach based on the recombination of different fractions from raw products was considered. This original strategy of using recombined products has yielded interesting results to model perceptions. However, this approach is insufficient to extrapolate to the diversity of commercial protein isolates. To go further, it would be interesting to validate the developed models on other pea protein isolates.

Concerning the beverages, Pea protein isolates are used as ingredients in many foods, including dietary supplements, bakery and meat products. Among these applications, we decided for this project to study pea protein beverages, for which large formulations could be tested (salt, sugar, fat, texturizing agent). This food application was chosen while being a key application and easy to produce in a repeatable way over a long period of time (6 months). However, it would also have been interesting to study other formulated products such as plant cakes or plant meat analogues for which the cooking step leads to very significant modifications of the composition and sensory perceptions (in particular due to Maillard reaction, product oxidation,...). Besides, it would have been interesting to also study other formulation factors such as sugar content.

Regarding the methods of chemical characterization of pea protein isolates, the results showed that the most impacting parameters to understand the perceptions of protein solutions were protein concentrations. However, other characterizations of protein isolates also seem to impact the perceptions of protein isolates: volatile compounds impacting mainly aromatic perceptions, phytochemical compounds impacting mainly bitterness and astringency perceptions; and peptides impacting mainly bitterness and broth perceptions. However, in the literature other compounds not studied here have been highlighted as potential bitter or beany compounds. In particular bitter lipid oxidation products that are formed either through enzymatic pathways or by autoxidation have been considered (Baur *et al.*, 1977). Gläser *et al.* identified 14 lipids and lipid oxidation products on pea protein isolates fractions with high bitter taste (Gläser *et al.*, 2020). In addition, in yellow pea, a total of 115 different structural phenolic compounds have been described in different studies (Fahim *et al.*, 2019; Stanisavljevic *et al.*, 2016; Neugart *et al.*, 2015). With the extraction and analysis conditions used here, we might be able to extract and identify only a part of these compounds. For example, MS spectra were acquired by full MS range (m/z) from 85 to 1000 AMU limiting the identification of compounds with a greater mass than 1000 AMUS such as saponins. In addition, several peaks could not be identified due to coalitions and limited knowledge on these compounds. Similarly, the identification of peptides was probably not exhaustive. However, this study is an essential first step and will allow later more in-depth studies of these compounds.

Many other methods were tried during this PhD work, but they proved to be less relevant to meet the objective set and therefore were not pursued. Thus, the characterization of isolates by 2D and 1D electrophoresis; the characterization of volatile compounds by GC-MS olfactometry and by PTR-MS; the characterization of the denaturation of isolates by nano DSC and DLS; the characterization of free peptides and total peptides were not continued. However, these laborious works to characterize pea protein isolates have allowed the capitalization of knowledge on these products. They will also allow the optimization of future work on similar products.

To conclude, this PhD work has highlighted the importance of the choice of extraction and analysis methods to characterize the composition of pea protein isolates. Moreover, although the results obtained can only be applied to the product area studied, the different methods could be transposed to other product areas. One of the challenges of future work is to design methods for the extraction of compounds, (volatiles, peptides, phytochemical compounds) that correspond to the compounds released during the oral process. This would allow a better modelling of dynamic sensory attributes and thus go further in the understanding of perceptions.

GENERAL CONCLUSION AND PERSPECTIVES

General conclusion and perspectives

Developing a food offer rich in plant products appreciated by consumers is a major challenge to move towards a more sustainable food system. Indeed, pea off-notes (bitter, astringent and beany notes) are a critical limit for the incorporation of high levels of plant proteins in food and for their acceptability of products by consumers. In this context, the objectives of this PhD project were i) to identify the role of composition on sensory perceptions, ii) to establish the role of food formulation, sensory interactions and oral process on perceptions.

For this, a multidisciplinary strategy combining sensory analysis, physico-chemistry and statistics was set up. A mixing plan was built to create a range of solutions from 6 fractions (permeates *a* and *b*, retentates *a* and *b*, pellets *a* and *b*) derived from commercial pea protein isolates. Then, 23 solutions were formulated by combining these fractions and analysed by a sensory profile and different chemical composition characterisation: volatile compounds, peptides, phytochemical compounds (phenolic acids, flavonoids and terpenoids). The relationships between sensory and chemical profiles were analysed using Pearson correlations, linear (Partial-Least-Square) and non-linear (Artificial Neural Network) models. As main results, 79 volatile compounds were identified, including 34 correlated to beany notes; 3500 peptides including 14 correlated to bitterness; and 48 phytochemical compounds including 29 correlated to bitterness and astringency. In addition, the relative role of each group of compounds on perceptions were analysed by multi-block PLS models. These results made it possible to quantify the relative role of compounds on all studied perceptions.

In a second step, the role of pea protein isolates was investigated in real foods in order to understand the influence of formulation on perceptions. For this purpose, 12 beverages were formulated, varying in pea protein type and gellan gum, salt and oil contents. They were analysed by temporal sensory profiling methods, rheological measurements and volatile compound release measurements. The results showed that beany, bitter and astringent perceptions are mainly driven by protein type and gum gellan content. Rheological properties allowed a prediction of texture and astringency perceptions. Volatile compounds release were strongly affected by the presence of saliva showing the role of the oral process on perceptions.

Different research perspectives can be suggested to this work. The use of model foods could be very interesting. For example, it would be valuable to work on beverages with similar textures to the beverage of the thesis, which could be flavoured in beany note by adding “beany” volatile molecules and with different compounds as variable of formulation. The temporal sensory evaluation of these beverages could allow decomposing the effect of each compounds and the interactions with the aromatic notes of beany. The second perspective deals with the dynamic aspect of perceptions. Indeed, the perceptions of pea protein isolate based-products in the mouth, such as beany aroma, bitterness and astringency, cannot be explained only by the chemical composition of products or by their structure. They also depend on dynamic phenomena, resulting from the destructuring of products during the oral process, which is highly dependent on each individual. Taking into account individual differences and the dynamics of the mechanisms is therefore a challenge. Thus, a perspective would be to integrate the predictive approach data from analyses allowing to characterize dynamic phenomena. On line analysis of in-nose release of aroma compounds during consumption of pea based products would help to address this question. First tests using Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) have been made during the PhD thesis, but the sensitivity of the device used was not sufficient. It could be interesting to go further into this issue. At the same time, the use of the time-intensity profile method during consumption of the products would provide data in the form of intensities of the descriptors during consumption, the form of which would allow an easier modelling of these perceptions according to the composition. The third research perspectives deals with the modelling. The PLS-MB allowed to identify the main group of compounds involved in perceptions. However, a simplification of the data set has first been necessary to perform the modelling. These simplifications have been performed through linear regressions and other mathematical methods could perhaps be applied in order to better taking into account interactions between compounds during the step of data reduction.

Thus, this PhD work highlights the key role of interactions between the different constituents present in isolates or formulated products to explain perceptions. They also provide clues to reformulate pea protein products to reduce sensory off-notes. Based on these results, recommendations on combinations of isolate fractions, on chemical compounds composition, and on formulation composition can be developed to improve the flavour of the pea protein isolates used in plant-protein-based food products. First, attention should be paid to ingredient optimization. Our results suggest that the filtration step as used in this work, was not effective enough in removing the compounds responsible for off-notes. A longer diafiltration would be more efficient, but probably complex due to the fouling of membrane. The centrifugation step seems to be a more efficient process as the pellets were described less beany, bitter, and astringent than the retentate for protein solutions. Consequently, it could be used as a strategy of reformulation by using plant-protein-based products based on pellets. Because pellets consist mainly of insoluble compounds, they might lose functionality. Thus, employing a pellet/retentate mixture could help limit off-notes while retaining functionality. However, this strategy needs to be validated for formulated products. We can also recommend to prevent the formation of some contributing compounds during the process (phenolics and saponins, volatiles from oxidation process, peptides from proteins hydrolyses). Thirdly, as regards formulation, its effect is low in relation to the type of protein. In order to improve products based on pea protein isolates, it is therefore critical to improve the perception of the pea protein isolate ingredients. However, beaniness, bitterness and astringency and their persistence can be decreased by adding texturing agents. It may be also recommended to reduce the beany notes by using fermentation or other process (thermal treatment, pH modification).

Therefore, the specific nature of these recommendations are clearly dependant on food type, which could determine protein concentration and functionality, matrix type, and ingredient choice. Other strategies for reducing the off-notes of pea-protein-based products could also be envisaged, such as cultivar selection (Stephany *et al.*, 2015), ultrasound technology (Miano *et al.*, 2019), radio frequency treatments (Jiang *et al.*, 2018) or enzyme treatments (Ben-Harb *et al.*, 2020; El Youssef *et al.*, 2020). The strategies proposed here can serve as a complement to these other techniques for improving the perceptions of pea-protein-based foods. To go further, it would be interesting to complete this study with liking and perception by consumers, from the first stages of conception to the evaluation of final products.

Lastly, it would be interesting to carry out similar studies on other types of pulse such as soybean or lupin proteins. If similar compounds were identified as also participating in the perceptions of beany or perceptions of bitterness or astringency, this would allow us to extend our results to other plant proteins. This would also allow crossover studies and a better chance of identifying common molecules responsible for off-notes. Indeed, food industry are working on the development of new products enriched with different plant proteins, suitable to consumers. Understanding the perceptions of pulse in general is a key step to help the industry in the development of food products. These new products, more appreciated, could help to change the image of plant proteins. It is therefore necessary to continue research work in nutrition, processes, food behaviour and sensory analysis to develop this sector and be in line with the expectations and needs of consumers. With a positive image (good for health and eco-friendly), plant proteins have a strong potential for growth in diets.

RESUME EN FRANCAIS

RESUME EN FRANCAIS

Introduction

Face à la croissance démographique et la crise climatique à venir, comment proposer un régime alimentaire plus riche en protéines végétales? L'utilisation de **légumineuses telles que le pois** dans les aliments permettrait de contribuer à l'évolution de cette offre (Siddique *et al.*, 2012). Cependant, les légumineuses ont encore du mal à se développer dans l'alimentation humaine en raison de verrous technologiques, nutritionnels et sensoriels. En particulier, elles sont encore fortement limitées par des **défauts sensoriels** (Roland *et al.*, 2017). La revue de la littérature a mis en évidence trois principaux types de défauts associés aux protéines végétales : des notes aromatiques dites « **beany** », des notes **d'amertume** et des notes **d'astringence**. Ces notes sont décrites comme très persistantes et difficiles à évaluer rendant difficiles la formulation des produits alimentaires à base d'isolats de protéines de légumineuses.

Concernant leurs origines, l'étude de la littérature a montré qu'un grand nombre de molécules tel que des molécules volatiles, des peptides, des phytomicronutriments mais également des lipides, des minéraux et des glucides, sont susceptibles de participer aux mécanismes à l'origine de ces perceptions (Gläser *et al.*, 2020; Humiski & Aluko, 2007; Murat *et al.*, 2013). Ces molécules sont soit intrinsèques à la graine, soit générées lors des différentes étapes de fractionnement des matières premières ou lors de la transformation finale du produit. Jusqu'à présent, la majorité des travaux de recherche ont porté sur le rôle des **molécules volatiles** (issues principalement de l'oxydation des lipides) à l'origine des sensations de « **beany** » (Bi *et al.*, 2020; Murat *et al.*, 2013; Trikusuma *et al.*, 2020). D'autres travaux se sont intéressés à la composition en **phytomicronutriments**, notamment des **saponines**, comme contributeur des perceptions **d'amertume et d'astringence** (Heng *et al.*, 2006). Cependant, aucune étude n'a utilisé une approche globale pour comprendre le rôle des molécules volatiles et non volatiles des isolats protéiques de pois et de leurs interactions potentielles sur ces perceptions complexes. Pour identifier les molécules clés à l'origine des perceptions, des **méthodes de fractionnement moléculaire** et des tests d'omission sont généralement utilisés (Engel *et al.*, 2002). Ces méthodes ne prennent pas en compte les différentes interactions et sont dépendantes de la connaissance fine de la composition de ces isolats protéiques de pois, qui reste encore à approfondir, notamment concernant la composition en peptides ou en phytomicronutriments. Le récent développement des technologies (**spectrométrie de masse et bio-informatique**) doit pouvoir permettre des identifications plus précises de ces composés.

Différents mécanismes permettent d'expliquer la perception : i) mécanismes directs par l'activation des récepteurs par les molécules (Matoba & Hata, 1972), ii) mécanismes indirects par le biais **d'interactions** entre les constituants de la matrice alimentaire (Wang & Arntfield, 2016, Guichard, 2002), ou d'interactions physiologiques et/ou cognitives (Keast & Breslin, 2003). Si dans la littérature, des travaux existent sur les interactions sensorielles et physico-chimiques qui peuvent moduler les perceptions d'amertume et d'astringence, les **dynamiques des perceptions** restent peu étudiées dans le cas des produits à base de protéines végétales.

Dans ce contexte, les travaux menés dans le cadre de ce doctorat visaient à répondre à la question de recherche suivante : "Quels sont les principaux mécanismes physiques, chimiques et sensoriels qui expliquent les notes sensorielles des isolats protéiques de pois ? » **Différents objectifs ont alors été identifiés. Le premier cherchait à évaluer le rôle des principaux groupes de molécules chimiques (molécules volatiles, peptides, phytomicronutriments, minéraux) sur les perceptions des isolats protéiques. Le second objectif était d'évaluer le rôle de la formulation des aliments, des interactions sensorielles (interactions chimiques, physiologiques et cognitives) et du processus oral sur la libération de ces molécules lors de la consommation d'aliments et sur les perceptions temporelles.**

Une stratégie originale et multidisciplinaire a donc été entreprise combinant analyse sensorielle, physico-chimie et statistique. Tout d'abord, un protocole de profil sensoriel adapté à ces produits très persistants a été développé pour décrire au mieux les perceptions des isolats protéiques de pois (Section

1.1., Figure 1). Ensuite une stratégie expérimentale basée sur un plan d'expérience a été développée pour créer une "diversité contrôlée" de produits (Section 1.2., Figure 1). La composition chimique (molécules volatiles, peptides et phytomicronutriments) des produits obtenus a été étudiée et des premiers liens entre ces molécules et les perceptions du pois ont été identifiées (Section 2, Figure 1). Pour aller plus loin dans la compréhension du rôle complexe et combiné des molécules volatiles et non volatiles sur les perceptions globales des isolats protéiques de pois, un modèle statistique prenant en compte l'ensemble des différentes molécules chimiques a été construit (Section 3, Figure 1). Au-delà de la composition chimique de l'aliment, sa structure et sa texture peuvent également influencer les perceptions, et notamment leurs dynamiques. Ainsi, la dernière partie de cette thèse a cherché à comprendre le rôle de la formulation sur les perceptions des isolats protéiques de pois au cours de la consommation d'une portion entière de produit. Pour cela, des boissons à base de protéines de pois ont été étudiées, en variant leur niveau de sel, de matière grasse, d'épaississants et le type de protéines utilisées (Section 4, Figure 1).

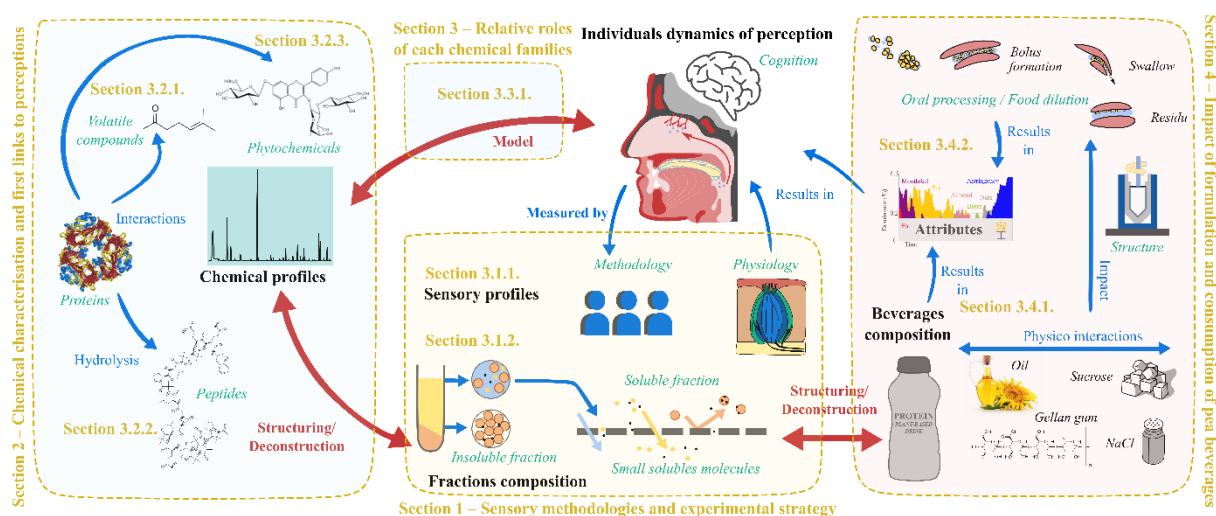


Figure 1: Représentation schématique de l'approche expérimentale du travail de doctorat. Les étiquettes des sections correspondent au « Chapitre 3 – Résultats » du manuscrit de thèse.

Méthodologies sensorielles et stratégie expérimentale pour étudier les perceptions des isolats protéiques de pois

Caractérisation sensorielle des isolats protéiques de pois

Afin d'étudier les mécanismes à l'origine des propriétés sensorielles des produits à base de protéines de pois, il a été nécessaire dans un premier temps de décrire et quantifier leurs perceptions. La revue de la littérature a montré que les isolats protéiques de pois sont généralement décrits comme étant « beany », amer et astringent avec une forte persistance (Bott & Chambers, 2006; Humiski & Aluko, 2007). Cependant, il n'existe pas de définition consensuelle des perceptions de « beany » et lors d'une évaluation sensorielle la forte rémanence des produits influence fortement l'évaluation sensorielle des produits suivants (fatigue sensorielle). Ainsi, l'objectif des premiers travaux étaient d'optimiser le protocole d'évaluation des produits à base de pois lors de l'utilisation de la méthode de profil sensoriel.

Pour ce faire, un protocole de **profil sensoriel par évaluation de blocs d'attributs** (« Block profiling ») a été développé comme présenté Figure 2. Les produits y sont présentés de manière monadique et séquentielle. Pour chaque séquence d'évaluation, le premier échantillon de la série sert de référence. Le protocole de rinçage a également été travaillé afin d'optimiser les conditions d'évaluation de ces isolats protéiques. Le protocole par bloc s'est révélé plus performant (sur des critères de discrimination, répétabilité et homogénéité) pour évaluer les échantillons présentant des perceptions persistantes et intenses, tels que l'amertume ou l'astringence, et des perceptions multidimensionnelles, tels que le « beany ». Les attributs ont été évalués avec et sans pince-nez afin de limiter les interactions entre les sapides/textures et les arômes afin de dissocier les interactions cognitives des interactions chimiques.

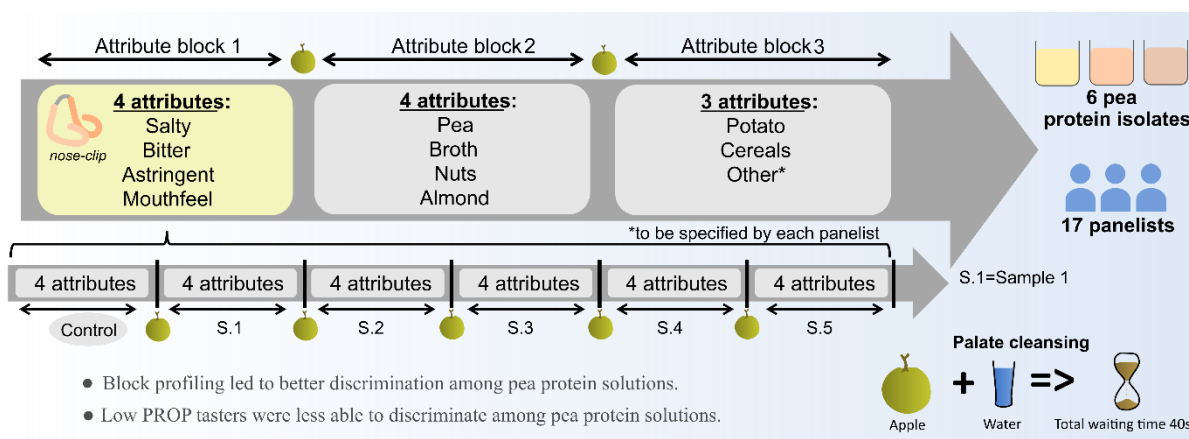


Figure 2: Bloc protocole utilisé pour la caractérisation sensorielle d'isolats protéique de pois.

Les résultats confirment que « **beany** » est un descripteur multidimensionnel et complexe que l'on peut définir par les notes « amande », « bouillon », « céréales », « noix », « pois » et « pomme de terre ». En termes de caractérisation des produits, des résultats similaires ont été obtenus avec le protocole classique par rapport au protocole en bloc. Toutefois, les performances des panélistes (reproductibilité et homogénéité) ont été meilleures avec le protocole en bloc. En outre, la **sensibilité à l'amertume des panélistes** (test **6-n-Propylthiouracil** dit « **PROP** ») a été évaluée afin de mieux caractériser le panel utilisé. Ici, les résultats ont montré que la sensibilité au PROP affecte la capacité à discriminer et évaluer l'amertume ainsi que les évaluations d'autres attributs sapides et aromatiques. Ce statut caractérisant la « sensibilité au PROP » pourrait ainsi aider à sélectionner les panélistes en fonction de leur sensibilité sensorielle. L'intérêt de l'utilisation de ce nouveau protocole de profil au service de l'évaluation sensorielle des légumineuses a été présenté, discuté et publié dans *Food Quality and Preference* (Cosson *et al.*, 2020).

Construction d'un plan de mélange à base de fractions pour mieux comprendre les perceptions

L'apparente complexité des mécanismes à l'origine des défauts sensoriels des légumineuses nous a conduit à mettre en place une stratégie originale impliquant un plan de mélange basé sur le fractionnement des isolats protéiques de pois et permettant de créer une gamme de solutions à base de pois avec des compositions variées. Trois fractions ont été obtenues à partir d'isolats protéiques de pois commerciaux : une **fraction insoluble** (appelée **culot**), une **fraction soluble** (appelée **rétenant**) et une **fraction soluble contenant des composés d'un poids moléculaire inférieur à 10 kDa** (appelée **perméat**). Chaque fraction est composée principalement d'une famille de molécules : les protéines insolubles pour le culot ; les composés solubles de faible masse molaire pour le perméat ; et les protéines solubles pour le rétenant. Ensuite, **26 solutions** (40 avec les produits répétés) et **6 produits de validations** ont été formulés en combinant ces différentes fractions selon un **plan de mélange**. Les perceptions de ces solutions ont été caractérisées et quantifiées par profil sensoriel par bloc (« Block profiling ») et un **modèle a ensuite été construit** en combinant ces données comme présenté en Figure 3.

L'étude des résultats a montré que les panélistes ont globalement obtenu des scores répétables et homogènes au cours des trois mois d'évaluations, comme le souligne l'utilisation d'une référence constante et de produits répétés. Par ailleurs, les produits recombinaison ont pu être comparés avec les produits initiaux. Les intensités perçues entre ces 2 produits n'étaient pas significativement différentes, signifiant un impact **faible des procédés de fractionnement/recombinaison sur les perceptions** de « beany », d'amertume et d'astringence et validant l'approche utilisée. Parmi les 18 attributs sensoriels initialement évalués, 10 ont permis d'établir un modèle de prédiction robuste. Les performances de ces modèles ont ensuite été validées par analyse de variance et l'évaluation de six solutions supplémentaires.

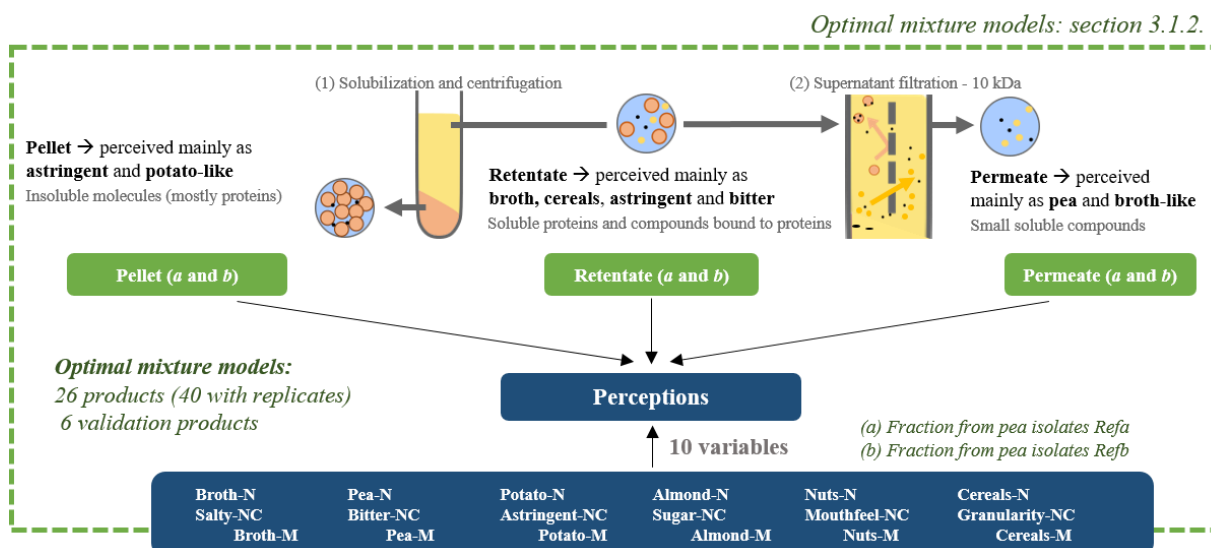


Figure 3: Diagramme des principaux résultats du modèle de perception des isolats protéiques de pois en fonction de la composition des fractions de pois.

Les résultats suggèrent que les différentes notes contribuant aux perceptions de « **beany** » sont **principalement influencées par les fractions de rétentat et de perméat**. L'astringence est quant à elle **plutôt influencée par les fractions de rétentat et de culot**. Cette stratégie d'étude a donc permis de mieux comprendre comment différentes fractions d'isolats protéiques de pois contribuent aux caractéristiques sensorielles indésirables de ces ingrédients. Elle a également révélé que formuler des aliments à l'aide de ces fractions protéiques serait une stratégie intéressante pour réduire les défauts de ces ingrédients. Cependant, pour comprendre plus finement les mécanismes, il est nécessaire d'analyser précisément la composition des fractions et des solutions recombinées et ainsi aller plus loin pour identifier les molécules à l'origine des perceptions. Les résultats ont été publiés et discutés dans *Food Research International* (Cosson *et al.*, 2021).

Caractérisation chimique des isolats protéiques de pois et premiers liens avec les perceptions

Identification et quantification des principales molécules volatiles et premiers liens avec les perceptions de « beany »

Afin d'étudier la composition en molécules volatiles des isolats de pois et des fractions, des mesures par **chromatographie en phase gazeuse couplée à la spectrométrie de masse** ont été réalisées comme présentés dans la Figure 4. Pour une sélection de molécules identifiées dans la littérature comme étant clés dans les perceptions de « beany », les interactions protéines-composés volatils ont été étudiées. Les relations entre les données sensorielles et les données chimiques ont ensuite été analysées et discutées à l'aide de modèles linéaires (**Régression des moindres carrés partiels**) et non linéaires (**Réseaux de Neurones**).

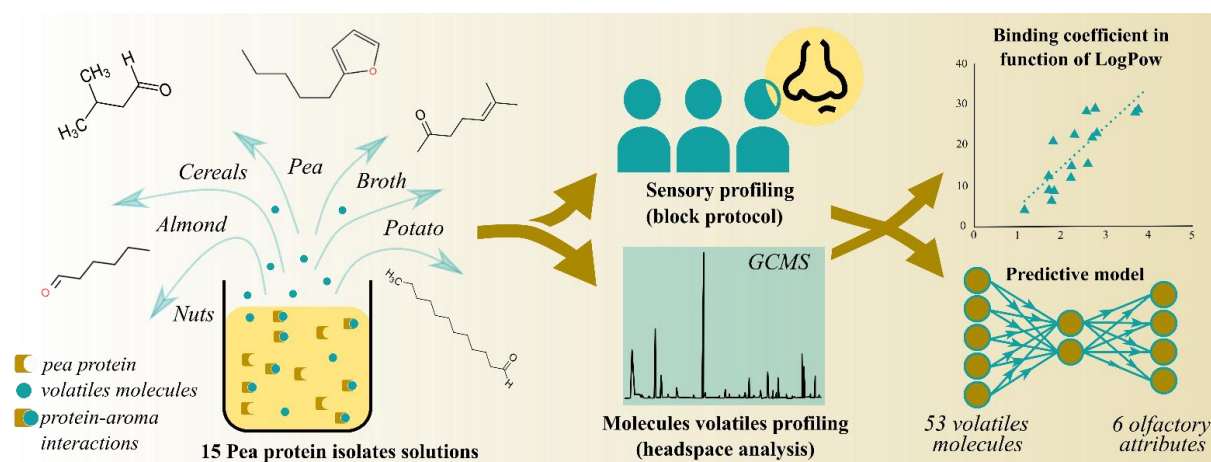


Figure 4: Libération des molécules volatiles dans les solutions protéiques de pois et impact sur les perceptions aromatiques.

Un total de **79 molécules volatiles** a été identifié. Ces molécules proviennent principalement de l'oxydation des lipides/peptides/acides aminés dans les graines ou lors de la transformation/stockage des isolats. Les résultats ont montré que les **interactions protéines-composés volatils** sont principalement hydrophobes et peuvent être expliquées par le coefficient de partage octanol/eau (LogPow). Cependant, les interactions entre molécules et protéines ne semblent impacter la rétention des composés d'arôme en moyenne que de 18% comparé à l'eau, ce qui suggère qu'elles n'impactent que peu les perceptions olfactives.

Concernant l'impact de la composition et de la libération des molécules volatiles sur les perceptions, les résultats ont montré que **la majorité des molécules volatiles sont associées aux six attributs aromatiques** (« noix », « pomme de terre », « pois », « céréales », « bouillon » et « amandes »). On peut supposer que ces molécules peuvent créer des associations qui contribuent fortement aux notes « beany ». Ces molécules volatiles clés appartiennent à différentes familles chimiques, principalement des aldéhydes, des cétones) et des alcools. Elles sont principalement hydrophobes mais présentent des caractéristiques de solubilité dans l'eau et de volatilité variées. **Afin de réduire les défauts des produits à base d'isolats de protéines de pois, il est donc difficile de cibler spécifiquement certaines molécules volatiles.** Ainsi, les solutions qui permettraient de modifier la composition globale des molécules volatiles pourraient constituer une stratégie intéressante. On peut par exemple imaginer que la fermentation, le lavage ou le piégeage des molécules volatiles seraient des solutions intéressantes, comme l'ajout d'arômes pour masquer les défauts. En outre, jouer sur les interactions entre les molécules volatiles et les protéines de pois par la formulation des produits alimentaires pourraient être un moyen intéressant et efficace de réduire les perceptions aromatiques. En effet, en modifiant les conformations des protéines, les interactions des molécules volatiles avec les protéines pourraient être modifiées et par conséquent modèleraient les perceptions aromatiques. Pour des raisons de confidentialité, ces résultats n'ont pas pu être publiés pour l'instant et sont en discussion avec la société Roquette.

Identification et quantification des principaux peptides et premiers liens avec les perceptions

Afin d'étudier une partie des molécules non volatiles des isolats protéiques de pois, les principaux oligopeptides et polypeptides (de 5 à 40 acides aminés) ont été identifiés et caractérisés par **chromatographie liquide à ultra haute performance et spectrométrie de masse**. Ensuite, l'impact du fractionnement et des recombinaisons sur ces compositions en peptides a été étudié. Les relations entre les profils de peptides et les propriétés sensorielles des solutions protéiques ont été analysées, en particulier les liens aux perceptions d'amertume. L'ensemble de la démarche et les principaux résultats sont présentés dans la Figure 5.

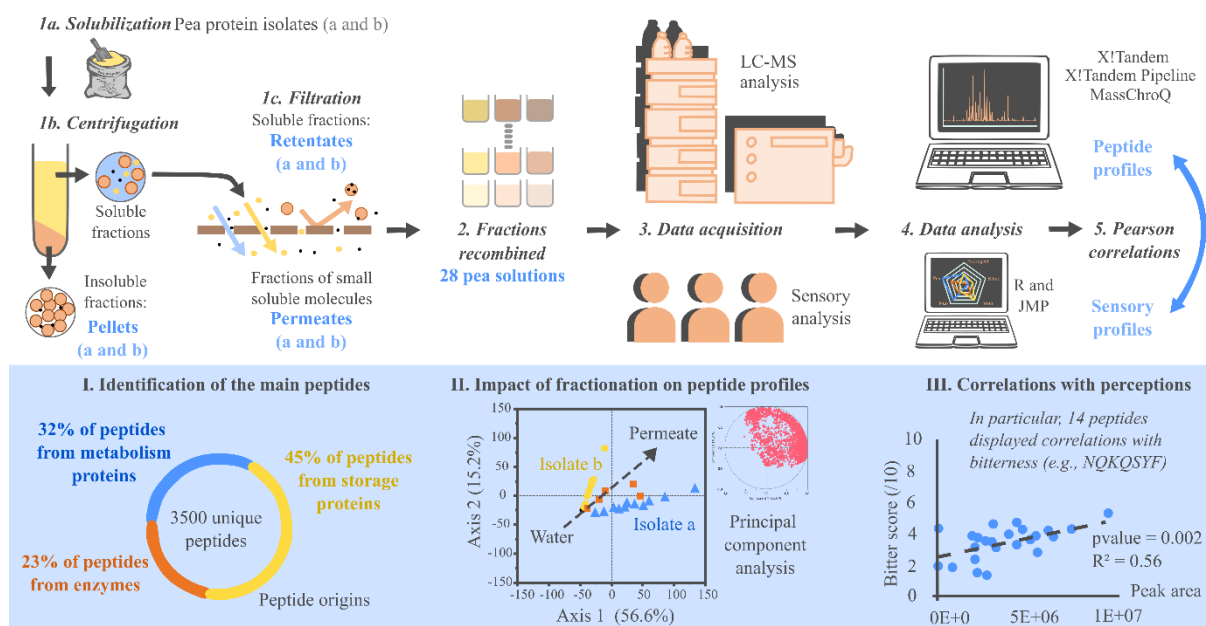


Figure 5: Identification et caractérisation des principaux peptides dans les isolats protéique de pois par chromatographie liquide à ultra haute performance couplée à la spectrométrie de masse.

Une **grande variété de peptides a été identifiée**, représentant une série de familles de protéines : principalement des protéines de stockage des graines mais aussi des protéines pouvant jouer un rôle dans les perceptions, comme les lipoxygénases. Ces peptides sont pour la plupart polaires et hydrophiles. Onze peptides ont été identifiés comme ayant des séquences homologues à celles de peptides antioxydants connus. Concernant les liens aux données sensorielles, la plupart des peptides présents dans les solutions de protéines de pois présentent une corrélation significative avec au moins un attribut sensoriel. En particulier, **de nombreux peptides ont été corrélés positivement avec les attributs « salé » et « bouillon », exprimant peut-être la relation de certains peptides avec les récepteurs responsables des perceptions umami**. Un nombre plus faible mais néanmoins significatif de peptides présente également des corrélations significatives avec les intensités d'amertume. En particulier, **14 peptides avec une taille inférieure à 8 acides aminés sont positivement corrélés à l'amertume** et donc sont potentiellement capable d'activer les récepteurs à amertume. Les résultats ont été publiés et discutés dans *Food Chemistry* (Cosson *et al.*, 2022).

Identification et quantification des principaux phytomicronutriments et premiers liens avec les perceptions d'amertume et d'astringence

Dans un troisième temps, les principaux phytomicronutriments (acides phénoliques, flavonoïdes et saponines) ont été identifiés par **spectrométrie de masse à haute résolution** dans les solutions de protéines de pois. L'impact des procédés de fabrication des isolats et du fractionnement a été étudié en comparant les profils de la farine, des isolats et des fractions. Enfin, les principaux phytomicronutriments ont été corrélés aux propriétés d'amertume et d'astringence des solutions de pois. L'ensemble de la démarche et les principaux résultats sont présentés dans la Figure 5.

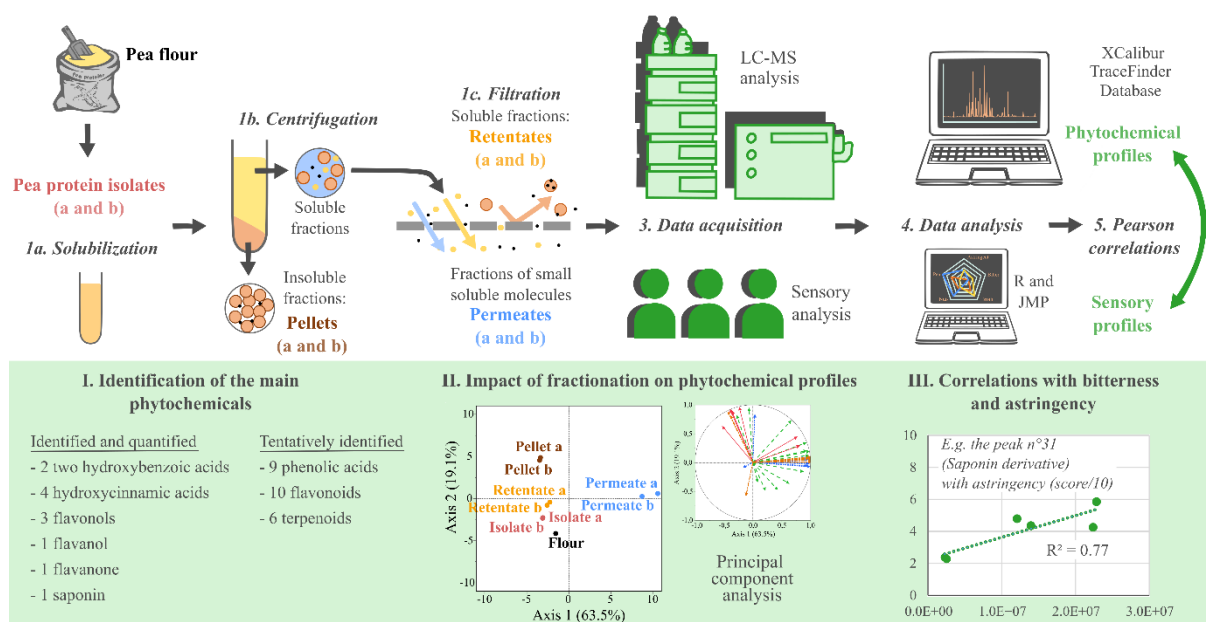


Figure 6: Identification et quantification des principaux phytomicronutriments des isolats protéiques de pois et premiers liens avec les perceptions.

Ainsi, **quarante-huit molécules** ont été identifiées dans les produits. Parmi elles, quinze ont été potentiellement identifiées comme des **acides phénoliques**, quinze comme des **flavonoïdes**, sept comme des **terpénoïdes**. En comparant les données de la masse avec celles des références, deux acides hydroxybenzoïques, quatre acides hydroxycinnamiques, trois flavonols, deux flavanols et une saponine ont été identifiés et quantifiés sans ambiguïté. Concernant l'impact des procédés, la comparaison des aires des pics des molécules a montré de plus grandes quantités de molécules dans la farine que dans les isolats et fractions de protéines de pois, ce qui suggère une certaine **perte ou dégradation de ces molécules au cours des procédés**. Cependant, par rapport à la teneur en matière sèche des produits, les perméats contiennent une plus grande quantité de phytomicronutriments que les autres fractions. Par ailleurs, **vingt-neuf molécules (acides phénoliques, flavonoïdes et saponines) ont pu être corrélées à des attributs d'amertume et/ou d'astringence**. Un article est en préparation et sera soumis à *Food Chemistry* en Septembre 2021.

Rôles relatifs des différentes familles de molécules chimiques sur les perceptions

Différents types de molécules chimiques ont été identifiés et quantifiés afin de comprendre l'origine des perceptions: des molécules volatiles pour les notes de « beany », des peptides et des saponines pour l'amertume, des molécules phénoliques pour l'amertume et l'astringence. Afin d'aller plus loin dans la **compréhension du rôle complexe et combiné de ces différents blocs de molécules (volatiles et non volatiles) sur les perceptions** des isolats protéique de pois, des modèles statistiques de régression ont été utilisés. Pour cela, les données sensorielles quantitatives (intensités sur 18 attributs) ont été reliées avec les données chimiques quantitatives (63 molécules volatiles, 3561 peptides et 48 phytomicronutriments). Ces données chimiques ont été complétées par d'autres types de mesures non décrite ici pour des raisons de confidentialité. Ensuite, une **régression basée sur l'algorithme des moindres carrés partiels en multi-blocs** (Multi Block-Partial Least square régression) a été construite pour explorer les liens entre les ensembles de données sensorielles et chimiques comme présenté dans la Figure 7.

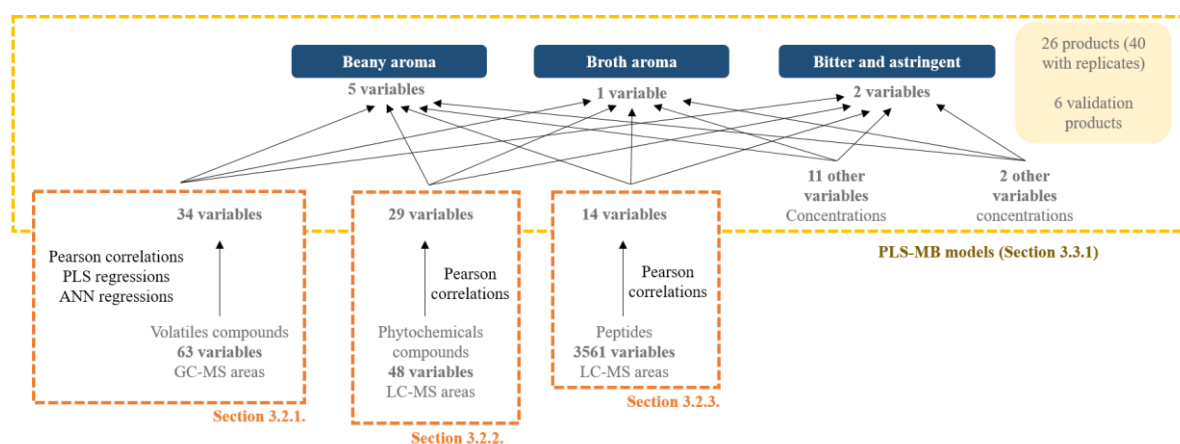


Figure 7: Identification par modélisation statistique des molécules volatiles et non volatiles responsables des perceptions dans les isolats protéiques de pois.

Ces travaux ont conduit à la rédaction d'une publication qui n'a pu être pour l'instant soumise, car celle-ci est en discussion avec le service de valorisation de la société Roquette qui a financé ce travail.

Impact de la formulation et de la consommation d'une boisson entière sur les perceptions

Au-delà de la composition chimique des aliments, leur structure et leur texture peuvent également influencer la dynamique des perceptions des produits alimentaires formulés à base d'isolat protéique de pois (Guichard, 2002; Saint-Eve *et al.*, 2011). Ainsi, l'objectif de cette quatrième partie était de déterminer comment les perceptions des isolats protéiques de pois sont affectées par la formulation. Parmi les différentes applications des isolats protéiques de pois, les boissons protéiques ont été choisies pour cette étude, car elles permettent de faire varier les concentrations de divers ingrédients tout en étant faciles à produire et reproductible sur une longue période.

Ainsi, suite aux résultats des travaux précédents, un isolat protéique de pois et son culot ont été sélectionnés comme ingrédients protéiques pour la formulation des boissons. Ainsi, **12 boissons** variant selon leur **niveau de sel, de matière grasse, d'épaississants (gomme de gellane) et de type de protéines (culot et isolat) ont été formulées**. Ces boissons végétales ont été décrites sensoriellement par l'utilisation de trois méthodes sensorielles : **profil statique** avec et sans pince-nez, **dominance temporelle des sensations (TDS)** en mono-bouché et TDS en **multi-bouché** (portion de 120mL : évaluation de la première bouchée, de la bouchée à 60mL (moitié de la portion) et de la dernière bouchée à 120mL). L'ensemble de la démarche et les principaux résultats sont présentés dans la Figure 5.

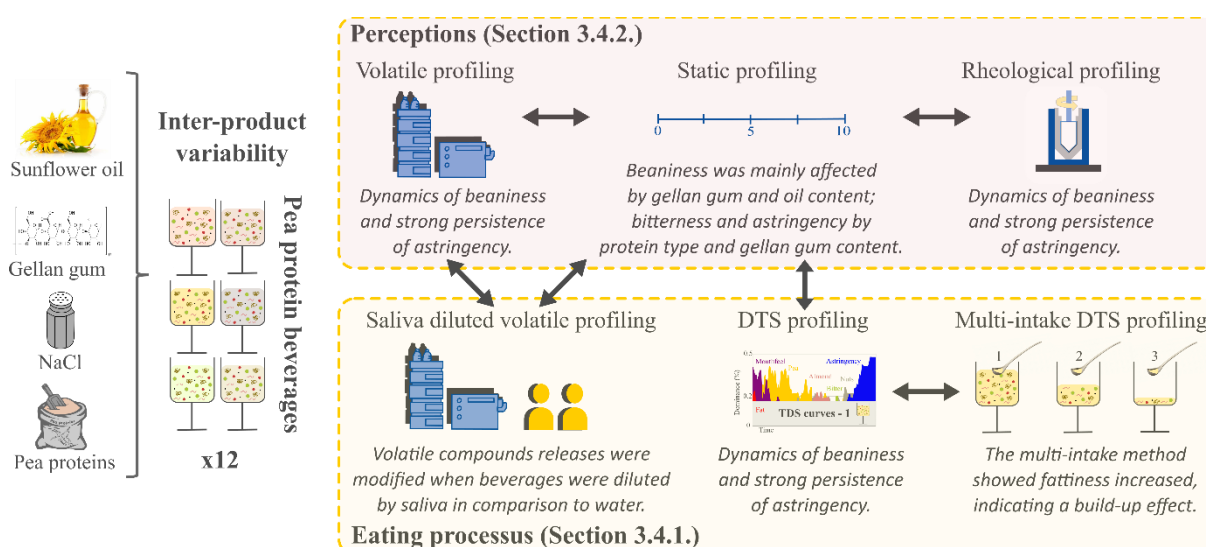


Figure 8: Diagramme des principaux résultats de l'étude sur les boissons à base de protéines de pois. TDS : Dominance temporelle des sensations. Les étiquettes des sections correspondent au « Chapitre 3 – Résultats » du manuscrit de thèse.

Complémentarité des méthodes de profil sensoriel pour mieux comprendre les perceptions temporelles des boissons formulées

Les résultats obtenus par profil (« Block profiling ») a révélé que les **perceptions de « beany » dépendent principalement du type de protéines, de la teneur en gomme gellane** et en huile. L'utilisation du pince-nez lors du profil statique a indiqué qu'il n'y a que **peu d'interactions** entre les attributs sapides et de textures et les attributs aromatiques. La méthode TDS utilisée sur une seule bouchée, et sur une période de 2 minutes après l'introduction en bouche, a montré que les boissons diffèrent nettement dans les **séquences de perception**. Par ailleurs, les perceptions d'astringence et d'amertume sont très persistantes et principalement influencées par le type de protéine et la teneur en gomme gellane. Des **phases sensorielles spécifiques** ont également été identifiées : les attributs de texture sont plus importants lors de la première bouchée, et l'astringence est plus marquée lors de la dernière bouchée. Enfin, les résultats TDS obtenus lors de la consommation d'une portion entière de produit (**multi-bouchées**) suggèrent qu'au fil du temps, la **perception de la matière grasse s'est accrue** (effet d'accumulation) et les perceptions aromatiques ont diminué (adaptation sensorielle). Ainsi, les résultats de cette étude ont permis de mieux comprendre les perceptions des boissons à base de protéines de pois dans des conditions qui ressemblent davantage à celles associées à une consommation réelle. Ils fournissent également des pistes pour reformuler les produits à base de protéines de pois afin de réduire les défauts sensoriels. Les résultats ont été publiés et discutés dans *Foods* (Cosson *et al.*, 2020).

Les perceptions des boissons expliquées par des approches rhéologiques et chimiques

Le deuxième objectif de cette partie du travail était de mieux comprendre comment la composition du produit affecte les perceptions des boissons à base de protéines de pois. Un accent particulier a été mis sur la façon dont les propriétés rhéologiques influencent les perceptions, les molécules volatiles libérées et le rôle de la salive lors du processus oral. Pour cela, les profils sensoriels des boissons ont été complétés par des **caractérisations rhéologiques** classiques et par des **analyses par chromatographie en phase gazeuse et spectrométrie de masse**. Les relations entre les profils instrumentaux et les profils sensoriels ont été étudiées.

En ce qui concerne les perceptions de texture, l'« épaisseur » des solutions et l'astringence sont significativement influencées par la teneur en gomme de gellane. Les propriétés rhéologiques permettent d'expliquer les perceptions de texture. En ce qui concerne les perceptions aromatiques, l'effet de la formulation a été pris en compte, mais également le rôle des individus en intégrant l'impact lié à la dilution de l'aliment par la salive. Les résultats ont permis de montrer que **la composition et la libération des molécules volatiles n'ont été que relativement peu affectées par les formulations étudiées** : les produits à faible teneur en gellane présentent la plus forte libération de molécules volatiles. Ainsi, le facteur de formulation qui semble être le plus impactant pour diminuer les défauts sensoriels dans les produits à base de protéines de pois semble être la concentration des texturants. Deuxièmement, la libération de molécules volatiles au-dessus des échantillons a été **sensiblement modifiée lorsque les boissons sont diluées par la salive par rapport à l'eau**. En termes de prédiction des perceptions aromatiques, l'étude de la dilution des aliments par la salive fournit des informations supplémentaires et semble nécessaire pour comprendre les perceptions des aliments à base de protéines de pois lors du processus oral.

Conclusions et perspectives

Pour évoluer vers un système alimentaire plus durable, développer une offre alimentaire riche en produits végétaux appréciés par les consommateurs est un enjeu majeur. Pour répondre à cet enjeu, mes travaux de doctorat apportent à la communauté scientifique et industrielle des connaissances nouvelles ainsi que des outils et des approches méthodologiques originales (sensorielles, analytiques, statistiques) pour mieux comprendre les perceptions des protéines de pois. Ils ouvrent sur des pistes pour revisiter

les procédés de fractionnement du pois et aider à la mise en œuvre des ingrédients protéiques végétaux dans des aliments afin de diminuer leurs défauts sensoriels.

Ainsi, à partir des solutions de pois, 79 molécules volatiles ont pu être identifiées, dont 34 corrélées aux notes « beany » ; 3500 peptides dont 14 corrélés à l'amertume ; et 48 phytomicronutriments dont 29 corrélés à l'amertume et l'astringence. Les modèles multi-blocs ont permis d'identifier le rôle relatif de ces blocs de molécules de nature différente sur les perceptions « beany », amère et astringente. A partir des boissons à base de pois, les résultats ont montré que les notes « beany » et d'amertume dépendent principalement du type de protéine et de la teneur en gellane. L'astringence, fortement persistante, peut être limitée par la formulation. Enfin, les profils en molécules volatiles, affectés par la présence de salive, ont permis de montrer le rôle clés du processus oral sur ces perceptions. Sur la base de ces résultats (et selon le type d'aliment), des recommandations sur les combinaisons de fractions d'isolats, sur la composition des molécules chimiques et sur la composition des formulations ont été développées pour améliorer la saveur des isolats protéique de pois utilisés dans les produits alimentaires. Ces travaux ont été valorisés au travers de publications dans des revues à comité de lecture (4 publiées et 2 à soumettre), des communications orales (4), sous forme de poster dans des congrès internationaux (5), mais également des communications orales chez l'industriel partenaire de la thèse.

Différentes perspectives de recherche peuvent être proposées suite à ce travail. La première concerne l'aspect dynamique des perceptions. En effet, les perceptions en bouche des produits dépendent également de phénomènes dynamiques, résultant de la déstructuration des produits au cours du processus buccal, qui est fortement dépendant de chaque individu. Ainsi, une perspective serait d'intégrer les données de l'approche prédictive des analyses permettant de caractériser les phénomènes dynamiques. La seconde perspective de recherche porte sur la modélisation. La régression Partial Least Square multi-bloc a permis d'identifier les principaux groupes de molécules impliquées dans les perceptions. Toutefois, d'autres modèles statistiques pourraient peut-être être appliquées afin de mieux prendre en compte les interactions entre les molécules. Enfin, il serait intéressant de mener des études similaires sur d'autres types de légumineuses comme les protéines de soja ou de lupin. Cela permettrait de réaliser des études croisées et d'avoir de meilleures chances d'identifier les molécules communes responsables des défauts sensoriels. En effet, l'industrie alimentaire travaille sur le développement de nouveaux produits enrichis avec différents types de protéines végétales. La compréhension des perceptions des légumineuses en général est donc une étape clé pour aider les industriels dans le développement de ces produits alimentaires. Par conséquent, il est nécessaire de poursuivre les travaux de recherche en nutrition, en procédés, en comportement alimentaire et en analyse sensorielle pour développer ce secteur et être en phase avec les attentes et les besoins des consommateurs. Avec une image positive (bonne pour la santé et respectueuse de l'environnement), les protéines végétales ont un fort potentiel de croissance dans les régimes alimentaires.

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Titre : Des protéines de pois pour les aliments de demain: de la composition chimique aux modèles statistiques, pour comprendre les mécanismes à l'origine des perceptions de beany, d'amertume et d'astringence

Mots clés : composé d'arômes, composés phytochimiques, peptidomique, perception, formulation, modélisation

Résumé : Face à la croissance démographique et la crise climatique à venir, comment proposer un régime alimentaire plus riche en protéines végétales? L'utilisation de légumineuses telles que le pois dans les aliments permettrait de contribuer à l'évolution de cette offre. Cependant, l'amertume, les notes aromatiques «beany» et la rémanence de ces ingrédients sont des freins à leur utilisation. L'objectif de ce doctorat était de comprendre l'origine de la perception des isolats protéiques de pois à partir de leur composition (en molécules volatiles et non volatiles), puis d'étudier le rôle de la composition d'aliments formulés avec ces ingrédients protéiques sur les propriétés physicochimiques et les perceptions au cours du processus oral. Pour cela, une stratégie originale et multidisciplinaire a été mise en place combinant analyse sensorielle, physico-chimie et statistique. Un plan de mélange a été construit pour développer une gamme de 23 solutions formulées à partir de 6 fractions issues d'isolats protéiques de pois commerciaux. Un profil a permis de caractériser les propriétés sensorielles de ces solutions. En parallèle, leurs compositions en composés volatiles, peptides et composés phytochimiques (acides phénoliques, flavonoïdes et terpénoïdes) ont été caractérisées. Les relations entre les profils sensoriels et compositions chimiques ont été analysées à l'aide de corrélations de Pearson et de modèles linéaires (Partial-Least-Square) et non linéaires (Artificial Neural Network). A partir de ces solutions de pois, 79 molécules volatiles ont pu être identifiées, dont 34 corrélées aux notes « beany » ;

3500 peptides dont 14 corrélés à l'amertume ; et 48 molécules phytochimiques dont 29 corrélés à l'amertume et l'astringence. Puis, des modèles PLS multi-blocs ont été réalisés pour identifier le rôle relatif de ces blocs de composés de nature différente sur les perceptions « beany », amères et astringente. La seconde partie du travail s'est intéressée à comprendre comment la formulation d'une boisson à base de pois peut moduler les perceptions. Une large gamme de boissons végétales a alors été développée en faisant varier le type de protéines utilisées, la teneur en matière grasse, en épaississant et en sel. Les propriétés sensorielles temporelles ont été évaluées lors de la consommation d'une bouchée, mais aussi d'une portion entière d'aliments, ainsi que les propriétés rhéologiques et les composés volatiles. Les résultats ont montré que les notes beany et d'amertume dépendent principalement du type de protéine et de la teneur en gellane. L'astringence, fortement persistante, peut être limitée par la formulation. De plus, les propriétés rhéologiques permettent une prédiction fiable des perceptions d'astringence. Enfin, les profils en molécules volatiles, fortement affectés par la présence de salive, ont permis de montrer le rôle clés du processus oral sur ces perceptions. Au-delà des approches méthodologiques originales et des connaissances nouvelles sur les produits, ces travaux ouvrent sur des pistes pour revisiter les procédés de fractionnement du pois et aider à la mise en œuvre des ingrédients protéiques végétaux dans des aliments, afin de diminuer leurs défauts sensoriels.

Title : Pea protein based foods for the future: From chemical composition to statistical models to understand the mechanisms behind perceptions of beany, bitterness and astringency

Keywords : aroma compounds, phenolics, peptidomics, perceptions, formulation, modelling

Abstract : With population growth and the coming climate crisis, how can we offer a diet with higher levels of plant proteins? The use of pulses such as peas in foods would contribute to the evolution of this offer. However, their bitterness, their "beany" aromatic notes and their persistence are obstacles to their use. The objective of this PhD was to understand the role of their composition (volatile and non-volatile compounds) on perceptions. The role of food formulation was also studied on their physicochemical properties and perceptions during the oral process. For this, an original and multidisciplinary strategy combining sensory analysis, physico-chemistry and statistics was set up. A mixing plan was built to create a range of 23 solutions formulated from 6 fractions derived from commercial pea protein isolates. A profile was used to characterize the perceptions of these solutions. In parallel, their compositions in volatile compounds, peptides and phytochemical compounds (phenolic acids, flavonoids and terpenoids) were characterized. The links between sensory and chemical profiles were analysed using Pearson correlations, linear (Partial-Least-Square) and non-linear (Artificial Neural Network) models. From these pea solutions, 79 volatile compounds were identified, including 34 correlated to beany notes; 3500 peptides including 14 correlated to bitterness;

and 48 phytochemical compounds, including 29 correlated to bitterness and astringency. Then, multi-block PLS models were carried out to identify the relative role of these blocks of chemical compounds on beany, bitter and astringent perceptions. The second part of the work focused on understanding how the formulation of a pea-based beverages can modulate perceptions. A wide range of plant beverages was then developed by varying the type of proteins and the fat, texturizing and salt contents. The temporal sensory properties were evaluated when consuming a spoon, but also a whole portion of food; rheological properties and volatile compound profiles were determined. The results showed that the beany and bitterness notes depend mainly on the type of protein and gellan content. Astringency, which is highly persistent, can be limited by the formulation. In addition, rheological properties allow a reliable prediction of astringency perceptions. Finally, volatile molecule profiles, strongly affected by the presence of saliva, have shown the key role of the oral process on these perceptions. Beyond the original methodological approaches and new knowledge on products, this work opens up avenues to revisit pea fractionation processes and help the formulation of plant protein in foods, in order to reduce their off-notes.