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par

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Étude des paramètres influençant la migration de contaminants organiques et inorganiques de l’emballage métallique vers les denrées alimentaires consommées au Liban

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List of Abbreviations

AAS	Atomic Absorption Spectrometry
ACC	American Chemistry Council
ACN	Acetonitrile
AES	Atomic Emission Spectroscopy
AFS	Atomic Fluorescence Spectrometry
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
APCI	Atmospheric Pressure Chemical Ionization
ASE	Accelerated Solvent Extraction
AVS	Anodic Stripping Voltammetry
BADGE	Bisphenol A Diglycidyl Ether
BFDGE	Bisphenol F Diglycidyl Ether
BHT	Butylated hydroxytoluene
BPA	Bisphenol A
BPAF	Bisphenol AF
BPAP	Bisphenol AP
BPF	Bisphenol F
BPS	Bisphenol S
BPZ	Bisphenol Z
BSTFA	N-O-Bis(trimethylsilyl) trifluoroacetamide
CCD	Central Composite Design
CRM	Certified Reference Material
CVAAS	Cold Vapor Atomic Absorption Spectrometer
DAD	Diode Array Detector
DART	Direct Analysis in Real Time
DGCCR	Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes
DOE	Design Of Experiments

DRD	Draw and Redraw
DSS	Direct Solid Sampling
DVB	Divinylbenzene
DWI	Drawn and Wall Ironed
EC	European Commission
ECCS	Electrolytic Chromium Coated Steel
ECD	Electron Capture Detector
ECNI	Electron Capture Negative Ion
ED	Electrochemical Detection
EFSA	European Food Safety Authority
ESI	Electrospray Ionization
ETP	Electrolytic Tinplate
EU	European Union
EVA	Ethylene-Vinyl Acetate
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agriculture Organization
FCM	Food Contact Material
FDA	Food and Drug Administration
FLD	Fluorescence Detector
GC	Gas Chromatography
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GML	Global Migration Limit
GMP	Good Manufacturing Practice
HCL	Hollow Cathode Lamp
HDPE	High Density Polyethylene
HGAAS	Hydride Generation Atomic Absorption Spectroscopy
HIPS	High Impact Polystyrene
HLB	Hydrophilic-Lipophilic Balance

HP	Heat Process
HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
ICP	Inductively Coupled Plasma
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
L	Large
LC	Liquid Chromatography
LDPE	Low-Density Polyethylene
LLE	Liquid-Liquid Extraction
LOD	Limit Of Detection
LOQ	Limit of Quantification
MAE	Microwave-Assisted Extraction
MDL	Method Detection Limit
MIP	Molecularly Imprinted Polymer
MPL	Maximum Permissible Limit
MQL	Method Quantification limit
MS	Mass Spectrometry
MSPD	Matrix Solid-Phase Dispersion
NA	Not Available/Not Analyzed
NAA	Neutron Activation Analysis
ND	Not Detected
NHP	No Heat Process
NIAS	Non Intentionally Added Substance
NOAEL	No Observable Adverse Effect Level
NOGE	Novolac Glycidyl Ether
OEHHA	Office of Environmental Health Hazard Assessment
OES	Optical Emission Spectroscopy

OML	Overall Migration Limit
PA	Polyamide
PCA	Principle Component Analysis
PEN	Polyethylene Naphthalate
PET	Polyethylene Terephthalate
PFP	Pentafluorophenyl
PLE	Pressurized Liquid Extraction
PMTDI	Provisional Maximum Tolerable Daily Intake
PP	Polypropylene
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PTWI	Provisional Tolerable Weekly Intake
PVC	Polyvinyl Chloride
RSD	Relative Standard Deviation
RSM	Response Surface Methodology
S	Small
SBSE	Stir Bar Sorptive Extraction
SD	Standard Deviation
SE	Solvent Extraction
SIM	Selected-Ion Monitoring
SML	Specific Migration Limit
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SRL	Specific Release Limit
SRM	Selected Reaction Monitoring
TDI	Tolerable Daily Intake
TDS	Total Diet Study
TFS	Tin-Free Steel

TMCS	Trimethylchlorosilane
TMS	Trimethylsilyl
TOF	Time Of Flight
UHPLC	Ultra-High Performance Liquid Chromatography
US	United States
UV	Ultra Violet
WHO	World Health Organization
XRF	X-Ray Fluorescence

List of Publications and Communications

This thesis work gave rise to the publications and communications referred to below.

- ***Publications***

S. Noureddine ElMoussawi, R. Karam, M. Cladière, H. Chébib, R. Ouaini & V. Camel (2018). Effect of sterilisation and storage conditions on the migration of bisphenol A from tinplate cans of the Lebanese market, *Food Additives & Contaminants: Part A*, 35:2, 377-386 (DOI: 10.1080/19440049.2017.1395521)

S. Noureddine El Moussawi, V. Camel, M. Cladière, N. Lebbos, H. Chébib & R. Ouaini (2018). Parameters influencing the migration of trace metals in uncoated fruit cans, *Journal of Food Processing and Preservation (*Submitted)

S. Noureddine El Moussawi, R. Ouaini, J. Matta, H. Chébib, M. Cladière & V. Camel (2018). Simultaneous migration of bisphenol compounds and trace metals in canned vegetable food, *Food Chemistry* (Submitted)

- ***Poster Communications***

R. Karam, **S. N. ElMoussawi**, M. Cladière, H. Chebib, R. Ouaini, V. Camel. Poster presentation «Évaluation de la migration du bisphénol A et F de boîtes métalliques fabriquées au Liban vers un simulant d'aliments». Les troisièmes journées franco-libanaises (JFL3), Lebanese University, Beirut-Lebanon, 29-30 October 2015.

S. N. ElMoussawi, M. Cladière, H. Chebib, R. Ouaini, V. Camel. Poster presentation «Effects of sterilization and storage conditions on contamination of food simulants from Lebanese can coatings». 6th International Symposium on Food Packaging: Scientific Developments Supporting Safety and Innovation. "ILSI Europe 2016", Barcelona-Spain, 16-18 November 2016.

S. N. ElMoussawi, M. Cladière, H. Chebib, R. Ouaini, V. Camel. Poster presentation «Migration of organic and inorganic contaminants from Lebanese coated tinplate cans: effect of sterilization and storage conditions». The 4rd Edition of MATBIM Packaging Material / Bioproduct Interactions. “MATBIM 2017”, Porto-Portugal, 26-28 April 2017.

S. N. ElMoussawi, R. Ouaini, M. Cladière, R. Karam, H. Chebib, V. Camel. Poster presentation «Migration of Bisphenol A (BPA), Bisphenol A Diglycidyl Ether (BADGE), and their derivatives in canned food consumed in Lebanon: Effect of sterilization and storage conditions». UL Forum 2017, Lebanese University, Hadath-Lebanon, 26-27 September 2017

S. N. ElMoussawi, M. Cladière, S. Besançon, H. Chebib, R. Ouaini, V. Camel. Poster presentation «Effects of sterilization, storage conditions and can denting on the migration of bisphenols and metal trace elements into canned fava beans and okra». 8th International Symposium on Recent Advances in Food Analysis. “RAFA 2017”, Prague-Czech Republic, 7-10 November 2017.

Introduction

Food packaging should prevent food spoilage by protecting the contents against atmospheric conditions, micro-organisms, light, air, insects and rodents. The packaging industry is a very important economic sector. The most widely used consumer packaging material is plastic (38%), followed by paper and cardboard (30%), metal (19%), and glass (8%) **[1]**.

Metal food cans, first developed two hundred years ago and based on canning process developed by Nicolas Appert **[2]**, are excellent form of food packaging material as the material offers excellent barrier properties and sterilization can preserve food for up to four years if sealed properly. Moreover, these food cans are well able to resist the wear and tear of storage and transportation. Metal food cans are used for a wide variety of foods and beverages including vegetables, fruits, meat, fish, carbonated soft drinks, teas and many others. After sealing, canned foods are sterilized at 121°C for 30 to 90 min. Heating up to 100°C is usually sufficient for highly acidic foods (pH value below 4.5) **[3]**.

Canned foods have become important part of the human diet during the past century. The changing consumer patterns in terms of food habits due to the influence of western culture have also fueled the demand for canned food in the Asia-Pacific and South American regions **[4]**. A 2013 survey of more than 1,000 Americans showed that greater than 60% of the respondents reported using canned foods at least once or twice each week **[5]**.

According to EU 1935/2004 recommendations, food contact materials (such as food packaging) must not release constituents in amounts that can endanger human health or bring about an unacceptable change in the composition of the food or a deterioration of its organoleptic characteristics. Yet, it is commonly known that food packaging acts as an important source of chemicals, when it comes in direct contact with foods. At this point it is important to mention that consumers' demand for safer products has enhanced the study of food contaminants, which can be either intentionally or accidentally found inside food packaging.

In case of tinplate food cans, organic polymer is often applied on the inner walls for the sake of limiting direct food/metal contact and metal corrosion. Yet, uncoated tinplate cans are still preferred for certain light-color foods due to the beneficial antioxidant behavior of tin that preserve the food color. Therefore, this multi-materials structure of tinplate cans represent important food contamination sources. More specifically, the organic coating may release endocrine disrupting chemicals such as bisphenol A, whereas metal trace elements can be released from the alloy substrate.

In this research project we intend to assess the migration of metal elements and bisphenol compounds from coated and uncoated food cans into different categories of foods and their simulants. These two classes of contaminants are of great interest since: (1) according to the Nordic guidance for authorities (2015) **[6]**, there is no detailed regulations for materials like metals and alloys, and (2) the toxicity of bisphenol compounds is still of great interest for the European Union where they very recently reduced the SML of BPA from 600 to 50 µg/kg **[7]**. Food contamination from metal cans has be essential topic to many countries. However, in Lebanon, there are insufficient regulations controlling the packaged foods and the database on packaging-food contamination is, therefore, limited. As a matter of fact, there is a need to analyze the Lebanese canned food market aiming to evaluate the quality of its production, especially that there is a fast growth of exports of Lebanese preserved foods to Europe, the USA and neighboring countries as a result of its gourmet quality cuisine **[3]**.

External parameters and properties of canned food products must be carefully considered as they are expected to have a great influence on migration phenomena. Such parameters mainly include heat treatment conditions, storage time and temperature **[1], [8]–[15]**, coating (nature and process) **[9], [16]** and chemical properties of foodstuffs **[10], [17]–[20]** (pH and fat content).

From this perspective, the principle objectives of this research project are to monitor the migration of metal trace elements and bisphenol compounds that are likely to be released from uncoated and coated tinplate food cans, as well as to investigate the main influencing parameters on the migration of these targeted contaminants. These objectives are demonstrated in the following six chapters. After a bibliographic review on this topic is provided in chapter 1, the experimental work and main findings of this thesis are presented in chapters 2 to 5 in the form of published or submitted papers to international peer-reviewed journals. In chapter 2, the influence of sterilization and storage conditions were first investigated on the migration of BPA from coated tinplate cans into aqueous food simulants, i.e. water. This objective was expanded in chapter 3 to address the effect of additional parameters concerning the type of food simulant and cans on the migration of BPA and BPF-related compounds. Chapter 4 investigates the potential release of metal elements as another type of food contaminants caused by direct contact with the uncoated metal of fruit cans, while still focusing on a number of significantly contributing parameters. Then the objective of chapter 5 comes to combine, for the first time, the migration of bisphenol compounds and metal trace elements from coated tinplate cans into highly consumed and exported Lebanese canned foods. In this chapter the effects of sterilization, storage conditions and correlation with type of food and tinplate cans are deeply discussed. A general discussion of the main thesis findings is described in chapter 6. Finally, the conclusion sums up the main results and gives helpful take home advices for food industries and customer to minimize the dietary intake of undesirable chemicals. This work is a basis for future studies as will be shown in the perspectives.

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Chapter 1:

Bibliographic review

The beginning of knowledge is the discovery of
Something we do not understand."
~Frank Herbert

This chapter gives background information that provide the reader with the essential context needed to understand our research problem and its significance. First, a general knowledge is provided on the canned food market and commercially available can types and coatings. Then, the main food contaminants arising from food-cans contact materials will be demonstrated within, along with the leading parameters affecting the migration phenomena and their corresponding authorized regulations. Afterwards, I will state the most available techniques used for the analysis of trace metals and bisphenol compounds in foods, as well as the main mathematical models used to predict the migration trends of food contaminants. Last but not least, a thesis overview is illustrated at the end of the chapter.

1. CANNED FOOD MARKET

1.1. Worldwide scale

About 100 billion cans are produced annually worldwide for packing perishable food [1]. In 2013, about US $30 billion and US $9 billion were globally earned with beverage and food cans, respectively. The global canned food market was estimated to be US $64 billion in 2014 and rise to US $75 billion in 2019 [2] to reach US $118 by 2023 [3].

Canned food market has been geographically segmented into North America, Europe, Asia-Pacific, South America and Africa. The market is currently dominated by Europe, followed by North America. The market in the Asia-Pacific region is also expected to experience a boom in the coming years. The slowly evolving and changing food habits along with growing urbanization play a vital role in driving the growth of the canned food industry in countries like India and China [4].

1.2. Lebanese market

In Lebanon, the domestic market is quite developed for canned food items such as the Middle Eastern food specialties or ready to eat meals. Canned hummus (chickpeas mixed with tahina), fava beans (foul muddammas) and babaghanouge (eggplant mixed with tahina) are among the leading products. However, there is a competition in these foods between long shelf life (canned food) and short shelf life (packed in plastic trays and bags) products, since the latter delivers a fresher taste. Nevertheless, canned products are still advantageous because of their long shelf life that can last for up to two years.

Exports of canned ready to eat meals started in the 1930s. The first exports were to introduce hummus in the U.S., Canadian and other markets. Indeed, Lebanese producers were pioneers in exporting Middle Eastern canned products, but they failed to keep up with new developments in the overseas markets for these products. Nonetheless, Lebanese canned food exports have a great opportunity for growth if processors are able to deliver the right products. Their chance for success is promising since Lebanese cuisine is highly appreciated regionally and recognized globally due to the opening of gourmet quality Lebanese restaurants in various international cities such as Paris, London and New York as well as in Gulf cities. In fact, foreign consumers also appreciate Lebanese canned products since they are not able to simulate them at home.

In order to deliver a good quality product aiming to increase the exportation rate, two critical parameters must be considered: (1) the importance of customizing products to suit the taste of local consumers, and (2) upgrading of Lebanese processors in terms of quality and food safety [5].

According to the trade statistics provided by the Lebanese customs [6], there is an important increase in the exportation of processed foods, mainly canned food, between 2014 and 2018 as shown in **Figure 1.1**. Therefore, the control of quality and safety of Lebanese products and food packaging should now gain a strong attention from the world leading countries.

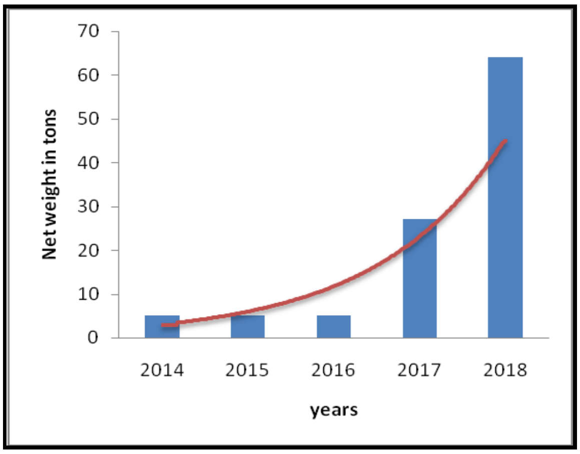


Figure 1. 1: Annual exportation of Lebanese processed packed food (mainly canned food) in the period 2014-2018 [6].

2. MIGRANTS FROM FOOD CANS

2.1. Can constituents and fabrication

2.1.1. Principal materials of cans

Three principal materials are used in food and beverage cans: tin-coated steel (tinplate), electrolytic chromium coated steel (ECCS), and aluminum [7], [8].

2.1.2. Tinplate cans

One third of total tin production goes into manufacturing of tin plates for food packaging [9]. In case of tinplate cans, approximately 1 µm of tin layer (1 to 15.1 g/m^2 in weight) is electrolytically applied (electrolytic tinplate (ETP)) on cold-rolled low carbon mild steel sheet or coil (usually less than 0.5 mm thick). In addition to its corrosion resistance power, tin acts as an efficient oxygen scavenger which undergoes oxidation in preference to canned product [10].

Uncoated tin cans are limited by the possible food/metal interactions and are therefore mainly used for, e.g. light fruits in brine or tomato-based products, where it is advantageous in this case to protect against color darkening and oxidation [2], [10].

2.1.3. Electrolytic chromium coated steel (ECCS)

A 0.2 mm electrolytically coated-chromium/chromium oxide steel (ECCS) contains a chromium layer in nanometer range. ECCS are also known as tin-free steel (TFS). The advantage of chromium coating is to prevent atmospheric oxidation or sulfur staining of the steel by foodstuffs, where sulfhydryl fractions resulting from protein can attack tin and iron layers to form black tin sulfide and white iron sulfide. Chromium layer also improves the lacquer adhesion which is always present in case of ECCS. This type of chromium-coated steel is normally used for the manufacture of can ends and lug closures, where welding is not required [10] .

2.1.4. Aluminum cans

Aluminum is widely used in food contact materials [11]. It is mainly used in canned beverages, which is light and ductile, but relatively weak with wall thickness of about 0.1 mm [2]. Aluminum is highly resistant to corrosion. When exposed to air, the metal develops a thin film of aluminum oxide (Al_2O_3) [10].

Aluminum cans are always coated with lacquers since at pH below 4.5 and above 8.5, the solubility of aluminum markedly increases, it is especially attacked by most dilute acids **[10]**.

2.1.5. Types of cans

The two main types of metal cans produced today are 2-piece cans and 3-piece cans. Three-piece cans are composed of a cylinder, a top and a bottom end, while two-piece cans have the wall and bottom form one piece and a separate top. The size of cans range from very small to catering pack sizes (typically for contents of few grams–10 kg) **[10]**.

2.1.5.1. Three-piece cans

Three-pieces cans are mainly used for food, but may be used for some non-carbonated beverages, particularly fruit juices. The different parts of three-piece cans are shown in **Figure 1.2**. ETP is only used for the body, in order to facilitate welding, whilst TFS or ETP can be used for the ends **[10]**.

Figure 1.2: Three-piece can terminology **[12]**

Before forming the body, sheets of tinplated steel are usually coated from interior and exterior with the protective lacquer (if used) (**Fig. 1.3a** and **3b**), However, welding margins should be left uncoated since the polymeric nature of the lacquer would influence the electric current passage through the metal. The lacquer is cured as sheets pass through an oven (**Fig. 1.3c**). The sheets are then unloaded from a stacker to a conveyor and transported to the slitter, which cuts the sheet into body blanks (**Fig. 1.3d**). The wall of the can is rolled to form a cylinder (**Fig. 1.3e**) and the joint is welded and coated with a “side seam stripe” to protect exposed metal along the seam (can be applied from the inside and outside), powder coating is usually applied electrostatically to the interior of the side seam (**Fig. 1.3f** and **3g**).

The side seam is then cured in an electric or gas-fired oven. Then the cans pass through a flanger, where the top and bottom of the can are flanged outwards to accept the ends (**Fig. 1.3h**). A bottom end (either a classic or easy- open end), which is fabricated separately, is then attached (**Fig.1.3i**), while the other end is attached after filling. Some cans pass through a beader that forms ridges on the can to provide additional axial and panel strength (**Fig.1.3j**). The finished cans are checked for leaks, and then are stacked on pallets for storage. Decorations may be printed on the can body or on paper labels that are glued **[12], [13]**.

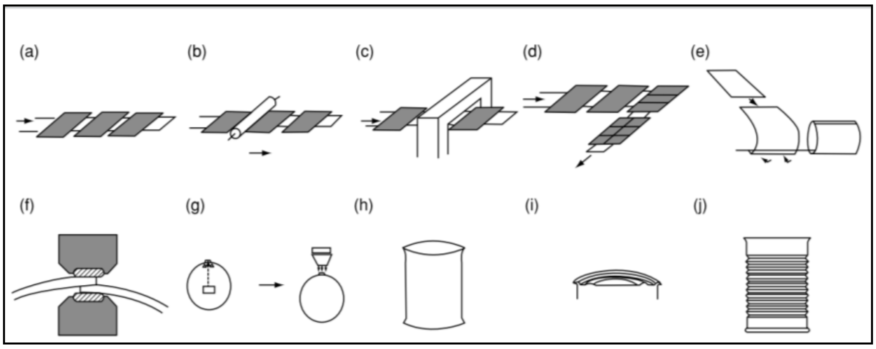


Figure 1.3: Three-piece can-manufacturing process: (a) starting metal sheets; (b) inner side coating with protective lacquer; (c) curing in oven ; (d) sheets are cut into blank bodies ; (e) the blank is fed into the forming roll; (f) cylinder edges are welded; (g) coating on side seam; (h) top and bottom of the can are flanged outwards; (i) seaming of one end; (j) beading **[12]**.

2.1.5.2. Two-piece cans

Both steel and aluminum substrates can be used to form two-piece cans. Although it is possibly used for packing food, the main application of two-piece cans is for beer and beverages (B&B). This type of cans is manufactured either by the draw and wall-ironed or the draw-redraw processes **[13]**.

2.1.5.2.1. Draw and wall-ironed cans (DWI)

During draw and wall-ironed process (**Fig. 1.4**), aluminum or steel coil is lubricated with oil and then fed into cupping press which stamps and draws the disks into cups. Each cup is rammed through a series of tungsten carbide rings for drawing and ironing, through which the cups are redrawn into smaller diameters and thinner walls. The irregular edges are then trimmed at a specified height. The trimmed bodies pass through washers (washing for several times) which serve to remove any lubricant from the wall ironing process. After drying, the cans are externally decorated. The next stage is the application of the internal lacquer, which is spray-applied and cured in an oven.

The cans are then passed through a necker/flanger, where the diameter of the body is reduced (necked in), and the tops of the cans are flanged outwards to accept the ends after the cans have been filled. Every can is tested at each stage of manufacture. In case cans are filled with beers and soft drinks at the filling plant, the cans are then pasteurized rather than sterilization, which involves heating to about 65°C for 20–30 min and then cooling **[13]**.

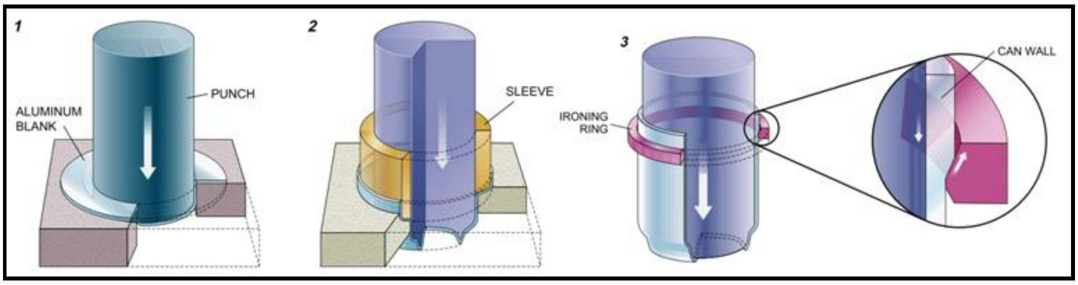


Figure 1.4: Draw and wall-ironed process of two-piece cans; (1) Initial draw transforms the blank into a small cup (2) the cup is transferred to a second punch, which redraws the can; the sleeve holds the can in place to prevent wrinkling (3) the punch pushes the can past ironing rings, which thin the walls **[14]**.

2.1.5.2.2. Draw and redraw cans (DRD)

As in the draw-and-iron process, aluminum or steel coil is continuously fed into a cupper that stamps shallow metal cups from the coil. Shallow cans may be stamped only once, or more in case of deeper cans **[13]**.

2.1.6. Coatings

The primary function of interior can coatings is to prevent any interaction between the can and its content, although some are used to improve the appearance of the pack. Exterior can coatings may be used to provide protection against environments, as well as for decoration and product labeling **[10]**.

2.1.6.1. Main components

Most coatings are applied as a wet film where the final dry film is obtained after thermal treatment (cure schedule or stoving). The major constituents in a can coating include:

- Resin(s)
- Cross-linking agents (almost always present)
- Additives
- Solvents (not always present)

The first three components are incorporated into the dry (final) film. In case a solvent is used, it evaporates during the cure schedule. Coatings form a very thin film between 1 and 10 µm, and in some cases, solvent-free coatings (e.g. powder side seam stripes) are used, albeit at higher film weights **[10]**.

Further chemical reactions normally occur during the cure schedule where most of coatings attain their final properties. Typically the resin(s) would react with one or more cross-linking agents (or resins), which join individual resin molecules together to form a three-dimensional cross-linked network. Nevertheless, it is this network with the density of cross-links in combination with the different molecules used in the resins that give the coating its corrosion resistance and flexibility, amongst other properties **[10]** .

Further additives could also be used to prevent foam formation during production and to improve the adhesion of the coating on the metal surface. Scavengers for hydrochloric acid are especially added to vinyl-based coatings **[2]**. Lubricants such as waxes, paraffins, fats and oils, partial acyl glycerols or fatty acid amides are used to enable the can forming process **[15]**.

Coatings obtain their final color in various ways. For instance, epoxy phenolic coatings normally generate a “gold” color when cured due to the chromophores in the phenolic resin. Indeed, epoxy phenolics are often referred to as “gold” lacquers and color can be used as an indication of degree of cure for a given system. In most cases, the white coatings are made from pigmentation with titanium dioxide, often in an epoxy anhydride coating or sometimes an organosol. Aluminum is added to a coating to give a grey “aluminumized” appearance to the final film **[10]**.

2.1.6.2. Applying and curing coatings

Internal coatings for metal packaging are typically applied by either roller coating or spraying before baking. In three-piece cans, coatings are usually applied before deformation of the metal to form the container or cap, in this case the coating has to withstand this severe mechanical deformations. In contrary, two-piece cans are coated after forming the object, but nearly always further deformation is required before the final object is obtained, e.g. necking the cans. Coatings can be applied more than once, after the first coating is dried. The curing conditions are determined by the industry depending on the materials used. Generally, the curing time ranges from few seconds to 12 min, while the temperature is in the range 195-

270°C. The typical weight of coating per area and per can is, respectively, in the range of 5–15 g/m^2 and 110–180 mg/330 mL can **[10]**.

2.1.6.3. Commercially available coatings

Internal lacquer must be inert, must provide a good barrier, and must also have good mechanical resistance, as well as thermal resistance especially that almost all canned products undergo thermal processing.

These coatings must be approved for food contact, i.e. only substances that had successful migration or extraction tests and that do not impart any flavor to the contents, can be used. Therefore, there are limited of different chemical functionalities available for food contact coating, resulting in a limited number of different types of resins that can be used for coatings for metal packaging**[12]**. **Table 1.1** shows the properties of most used internal can coatings.

Table 1.1: Types and properties of resins used in internal can coatings **[7], [10]**

Coating	Flexibility	Corrosion resistance	Adhesion	Universality	Organoleptic properties
Epoxy phenolic	Good	Very good	Good	Very good	Very good
Vinyl coating	Excellent	Poor	Poor	Good	Good
Vinyl organosol	Excellent	Good	Good	Good	Good
Phenolic	Poor	Excellent	Poor	Good	Change food organoleptics
Acrylic	Poor	Good	Very good	Poor	Change food organoleptics
Polyester	Material dependent	Poor	Good	Good	Good
Polyolefins	Good	Good	Good	Good	Good
Oleoresin	Good	Limited	Poor	Poor	Change food organoleptics

- ***Epoxy resins***

Epoxy resins, based upon bisphenol A (BPA) and epichlorohydrin, have been used in coatings for light metal packaging since the 1950s and are the single most widely used for metal packaging **[10]**. Epoxy phenolic coatings, which is formed either by straight blending of solid epoxy resins and phenolic resins or through condensation of a mixture of two resins in appropriate solvent, found the largest application. Due to its linear three-dimensional structure these coatings possessed high flexibility **[12]**. In 2013, their market share was estimated to be 95% of used coatings. The lowest molecular weight epoxy resin used is BADGE (bisphenol A diglycidyl ether) **[10]**. Only a very small percentage of epoxy resins do not use BPA as the starting monomer (< 0.1% of epoxy resins are based on bisphenol F and are used only as thermal stabilisers for polyvinyl chloride [PVC]) **[7]**.

- ***Vinyl***

Vinyl coatings are synthesized using the monomers vinyl chloride and vinyl acetate. The addition of stabilizers and plasticizers is generally needed and vinyl coatings are often blended with other resins to optimize their properties. Vinyl coatings do not withstand high temperatures that make them suitable for cans which are not sterilized after filling (e.g. soft drinks) **[2]**. Vinyl resins are often blended with alkyl, epoxy and phenolic resins to improve their performance. Unblended vinyl resins are applied over underlying coat due to their poor adhesion property on metal substrate **[7]**.

Vinyl organosols are prepared from dispersion of high molecular weight PVC resin in a hydrocarbon solvent. Organosols offer improved fabrication and application properties (**Table 1.1**) **[2]**. Novolac glycidyl ethers (NOGE) are used as both plasticisers and HCl scavengers to prevent potential corrosion of the metal due to potential dehydrochlorination of the PVC **[10]**.

- ***Phenolic resins***

Phenolic resins are based upon the reaction products of phenolic monomers such as phenol, cresols, xylenols or mixtures of these with one or more aldehydes **[10]**. Their prime use in coatings is as a cross-linking resin to improve resistance to sulfur staining (for example, with meats and fish) and enhance corrosion resistance for very aggressive foods **[7]**. Their limited flexibility is due to the presence of the bulge phenolic ring **[12]**.

- ***Acrylic***

Ethylacrylate is the most commonly used monomer to synthesize acrylic coatings. Acrylic resins display corrosion and sulfide stain resistance. Since acrylics are too brittle to withstand can fabrication, they are used as a spray. This coating may change the organoleptic properties of food because of its extremely low threshold for odor detection (2×10^{-4} parts per million), it’s commonly used as external coatings **[7]**. However, acrylics and their blends are currently under investigation as replacements for BPA-based epoxy coatings **[2]**.

- ***Polyesters***

Polyester resins are produced by condensing an acid with one or more alcohols or epoxides followed by copolymerization with one or more cross-linking agents (phenolic resins, amino resins, or poly-isocyanates), typically through their hydroxyl functionality **[7]**. Polyester-based coatings can be thermoplastic, as used in side seam stripes [10]. Polyester resins fail with aggressive or acidic foods due to hydrolytic attack of the ester bond under low pH conditions. Depending on the resins with which they are blended, polyester resins range from extremely hard to extremely flexible [7]. The laminate material polyethylene terephthalate (PET), polyester produced by reacting ethylene glycol with dimethyl terephthalate, was discovered in Japan since the 1990 as a safer alternative to epoxy coatings and labeled "BPA-reduced cans" [2].

- ***Polyolefins***

Polyolefin coatings have recently entered the market as a new technology which allows the dispersion of high molecular polyolefins in aqueous systems without the addition of surfactants or emulsifiers [2].

- ***Oleoresinous-based coatings***

Oleoresins are derived from fusing natural gums and rosins and then blending them with drying oils (e.g. linseed or tung oil) [7]. These coatings find little general usage today, only specific applications. Being based on natural substances, they tend to cause issues with organoleptic quality when used for some foodstuffs [10]. However, oleoresins were rediscovered as BPA-free alternatives [2].

- ***Alternatives to epoxy coatings***

Food companies have started to replace BPA-based epoxy coatings by alternatives in response to toxicological evidence, public discussions, and recent regulatory decisions. Already in 2013, patent filings and regulatory approvals by paint and chemical firms showed that many new coatings were under development [2]. For example, biobased, BPA-free epoxy coatings are under development but will require additional research to improve thermo-mechanical properties. Acrylic and polyester coatings are currently used and the alternatives. More recently, polyolefins have been developed with the aim to replace traditional epoxy coatings [16], [17]. Other inventions developed to reduce BPA migration include BPA capturing systems [18] and top coatings [19]. However, some food manufacturers preferred to change completely to other types of packaging (e.g. from cans to plastic bottles or composite cartons) instead of replacing epoxy coatings [2].

Nevertheless, none of the above alternatives fulfill all the mentioned requirements of an 'ideal' can coating. Most of them are more expensive than epoxy coatings and have certain limitations. Furthermore, their use may reduce the storage time of foods because the stability is not sufficient or it has not been adequately tested before bringing onto the market **[2]**.

2.1.6.4. Coatings for different foodstuffs

2.1.6.4.1. Coatings for beverage cans

Corrosion due to extremely acidic soft drinks, being based upon phosphoric acid, represents a serious problem; therefore, it is vital that internal coatings provide complete protection. Since soft drinks are more aggressive than beer, higher film weights must be used. On the other hand, beers are susceptible to flavor contamination by metallic traces present particularly by the slightest trace of contamination with iron or tin which requires higher film weights in case of steel beverage cans compared to aluminum cans **[10]**.

DWI cans are sprayed with coating after fabrication, usually with two coats of lacquers. These cans are mostly coated with a water-based epoxy acrylic system, normally with an amino resin for cross-linking **[10]**.

2.1.6.4.2. Coatings for food cans

The choice of lacquer mainly depends on the nature of the food and the can to be used. The most demanding foods are meat, fish, high-sulfur vegetables (peas and sweet corn), highly acidic foods, and highly colored foods (fruits). Accordingly, the lacquers must resist the contents of the can during processing and any produced by-products in food, such as hydrogen sulfide. The bulk of the food can coatings are based upon epoxy resins, primarily epoxy phenolics (gold lacquer) and to a lesser extent epoxy anhydride (white lacquer), with PVC-based organosols being the next most-used category **[10]**.

High-sulfur foods usually cause sulfur staining. Solid packs including ham, luncheon meat or solid fish such as tuna, which are not in a covering liquid, requires a lacquer that presents a barrier to the sulfur products in the pack and will therefore prevent sulfur blackening of the tinplate. In this case aluminum pigmentation on the lacquer is used to obscure the brown-violet tin-sulfide formed **[10]**. Titanium dioxide also provides a clean white appearance of the coating and masking sulfide stains because of its good hiding power **[2]**.

Packs for liquid foods (or foods covered with liquid) that are rich in proteins (e.g. peas or beans, shellfish and some soups) are dealt with in a different way. During sterilization, the sulfur products that are formed in the pack (particularly hydrogen sulfide) can move freely through the liquid and are mainly concentrated in the headspace of the can. If a barrier-type coating is used, the volatile sulfur products in the headspace may produce an unpleasant smell when the can is opened. For this reason, it is important to absorb and neutralize the sulfur compounds that are liberated in the pack. This is done using lacquer pigmented with sufficient amount of zinc oxide to absorb odorous sulfides where during sterilization zinc sulfide is produced, which is white in color and harmless. For products with high sulfur content such as sweet corn, it is normal to use pigmented lacquer over the whole of the can. Acidic products should not be permitted to come into contact with lacquers containing zinc oxide because the reaction may produce zinc salts that could destroy the continuity of the film. Another solution is simply to leave the body of the can unlacquered where the sulfur products are removed by being absorbed on the surface of the tinplate, which consequently becomes blackened while the ends are sometimes lacquered, to give a clean and hygienic look **[10]**.

The incorporation of waxes and silicones helps in meat sliding from the pack and so called "meat release agent" which is widely used for luncheon meat, pâté and fish roe **[10]**.

Cans do not always need an internal coating. Uncoated ETP cans are used for specific food types, including tomatoes and other tomato-based products, white fruits and some vegetables (e.g. mushrooms, asparagus). For many acidic products, the presence of tin is desirable because it eliminates oxygen, which would otherwise cause discoloration of the contents. Additionally, for many tomato-based products, bare tin surface leads to protection of the natural flavor and appearance of the food, through oxidation of the tin surface in preference to oxidative degradation of the food. In some cases, uncoated cans are preferred to lacquered cans in situations where a small lacquer discontinuity (e.g. scratch) would result in a concentrated attack of the base steel resulting in a quick disappear of tin area which causes microbiological contamination due to pin-hole formation **[10]**.

In the case of colored fruits that contain anthocyanin pigments, such as strawberries, raspberries, blackberries, black cherries and red plums, the fruit must be packed in a totally lacquered can because the juice will be quickly discolored by contact with tin or iron. For these packs, it is necessary to apply two coats of lacquer, where the second coat effectively covers any imperfections in the first coat. Epoxy-phenolic lacquers are normally used.

Double-lacquered cans are also used for other strongly acidic foods such as pickles, gherkins, cucumbers and concentrated tomato paste [10].

2.1.7. Main migrants

Packaging material is rarely inert and there are occasions when packages and materials that come in direct contact with foods can act as a source of chemicals and elements. This phenomenon is termed "migration". It mainly occurs through diffusion, where mass transfer due to random movement of molecules from regions of high concentration to regions of low concentration until equilibrium is reached [20].

In case of light metal cans, which is the case of our work, the migration of chemical substances can occur through two sources: (1) the diffusion of un-reacted free monomers or additives from the protective polymeric lacquer; (2) the release of trace metals from the underlying tin and steel substrates.

2.1.7.1. Migration of organic contaminants

Many migrants from all different can coatings belong to the group of non-intentionally added substances (NIAS), which may be structurally and toxicologically characterized or even completely unknown [21]. In case of epoxy-based lacquers, which are epoxy phenolic resins, the lacquer is synthesized from bisphenol A (BPA) and epichlorohydrin, forming bisphenol A-diglycidyl ether (BADGE) epoxy resins [22]. Numerous studies from all over the world demonstrated that the occurrence of BPA in epoxy can coatings and its migration into food and beverages are common (e.g. [19], [23]–[26]). Migration of BPA mainly occurred during can processing and sterilization [25].

Moreover, BADGE, which is the main monomer of epoxy coatings, has also commonly been added to organosol coatings as scavenger for hydrochloric acid which is formed as unwanted by-product after exposure to heat [27]–[29]. Depending on the intended function of BADGE and the production and storage conditions of the can, different reaction products are formed [30]. The epoxy groups of BADGE can hydrolyze in the presence of water to BADGE·H_2O and BADGE·$2H_2O$. When BADGE is used as scavenger for hydrochloric acid or in the presence of salty food, BADGE·HCl, BADGE·HCl·H_2O and BADGE·2HCl are formed. Furthermore, a cyclic product (cyclo-diBPA) is a common by-product from BPA and BADGE during the production of epoxy resins [31], [32]. In general, the total migration of BADGE and its derivatives was higher from organosols than from epoxy coatings because of its different functions in the two materials [2]. In 2010, more complex reaction products of BADGE with food ingredients such as sugars and peptides were identified [33].

Similarly, NOGE has commonly been used as scavenger for hydrochloric acid in organosols; in the EU, it has replaced BADGE for certain years until regulatory action banned the use of NOGE in can coatings [28]. NOGE is a complex mixture of epoxidized molecules based on the three isomers of bisphenol F (p,p-BPF, o,p-BPF, o,o-BPF) and its 3- to 8-ring derivatives [28], [34], it typically contains 30-40% BFDGE [2].

Other bisphenol analogues, including bisphenol S (BPS), bisphenol AF (BPAF), and bisphenol B (BPB), have been gradually developed as substitutes for BPA in the production of epoxy resins [35].

The presence of this group of compounds has received attention lately owing to its suspected toxicity. BPA has been identified as an endocrine disruptor due to its potential to elicit developmental and reproductive toxicity [36], [37]. Acute toxicity, genotoxicity and estrogenic activity of BPS, BPB and BPF have been reported [38]–[40]. One study showed that exposure to BPAF considerably reduced testosterone levels in adult male rats [41]. A few other studies have shown that BPF and BPS are more resistant to degradation in the environment than is BPA [42], [43]. In 2004, the toxicity of BADGE was reviewed and it was concluded that it neither affects reproduction and developmental endpoints nor acts as endocrine toxicant [44]. In the same year, EFSA concluded in a scientific opinion that BADGE and its derivatives do not raise concern for genotoxicity and carcinogenicity in vivo [45]. However, more recent studies showed effects of BADGE e.g. on the testes of rats [46], on adipocytes in vitro [47], and on the development of amphibians [48]. The lack of toxicity data for NOGE and BFDGE led to the prohibition of their use and presence in food contact materials (FCMs) in Europe [2].

Finally, the variety of monomers used in coatings makes the prediction, analysis and quantification of oligomers very challenging and analytical standards are generally not available yet [49].

2.1.7.2. Migration of metal trace elements

Metals are also common migrants from non-coated cans; yet these elements can still migrate in the presence of coatings. In fact, the release of metals occurs due to corrosion of the metal material. As this mechanism differs from diffusion the correct term concerning metal materials is release instead of diffusion **[50]**.

In the case of coated tinplate cans, corrosion can occur in two possible occasions:

(1) Corrosion under the varnish film: the tin undergoes corrosion. A black area of corrosion may develop under the varnish. Although, in this case, the life of the cans is not threatened, there is still a high risk of metallic contamination of the product.

(2) Perforation of the can: the iron undergoes corrosion. The preserved food can be enriched in iron and the perforation of the cans is the ultimate evolution. Iron has an adverse effect on the taste and color of certain food products **[51]**.

As a result, tin (Sn) and iron (Fe) are released in the food products. Other metals can also be intentionally or non-intentionally present in the alloy; including: nickel (Ni) that enhances the corrosion resistance, zinc (Zn) that is used in galvanized iron to protect iron from rusting due to its stronger reducing ability, copper (Cu) which hardens the cans, chromium (Cr) (usually present in the Cr(III) form) is used in TFS lids that has better corrosion resistance than Sn, and finally cadmium (Cd) and lead (Pb) that are expected to have higher potential toxicity than other elements and are present as contaminants rather than basic constituents **[50]**.

Although metals generally play important roles in our life functioning in wide spectrum, these metals can become toxic when consumed excessively (e.g. very high doses of iron indicates acute damage of gastrointestinal, hepatic, pancreatic and cardiovascular structures**[50]**). Other metals, such as Cd and Pb, are toxic even at low doses and usually imitate the action of an essential element in the body, interfering with the metabolic processes to cause illnesses **[50]**.

At this point it is important to mention that consumers' demand for safer products has enhanced the study of food/packaging interaction, which can lead to the migration of either intentionally or accidentally contaminants from packaging material.

2.1.8. Regulation relative to cans

According to the European Framework Regulation EC 1935/2004 **[52]** on food contact materials (FCMs), the can coatings generally have to comply with Article 3 in compliance with good manufacturing practice (GMP), so that they do not transfer chemical substances to food in quantities which could harm the consumer or affect the composition of the food or deteriorate the organoleptic characteristics of the food (i.e. affect the smell and the taste).

The regulation (EU) No 10/2011 **[53]** consists of a consolidation of existing Directive 2002/72/EC **[54]**. These Directives set the migration tests conditions and appropriate food simulants used for specific measures of migrants from plastic materials and articles.

As the use of food simulants greatly simplifies the compliance testing, six food simulants are stated in the regulation (EU) No 10/2011 for testing migration from FCMs, which cover the whole range of foods as shown in **Table 1.2** **[53]**. One amendment over the Directive 2002/72/EC **[54]** is the introduction of new food simulants, for example water was replaced by 10% ethanol to simulate aqueous food. In fact, water is considered now as a food and not as a food simulant. However testing can be performed into water only for plastic materials intended to come into contact with water.

Table 1.2: List of food simulants stated by the regulation (EU) No 10/2011 **[53]**.

Ref.	Food Simulant	Food Types
A	10% ethanol	Aqueous food
B	3% acetic acid	Acidic foods with a pH below 4.5
C	20% ethanol	Alcoholic foods with an alcohol content of up to 20% and those foods which contain a relevant amount of organic ingredients that render the food more lipophilic
D1	50% ethanol	Semi-fatty food
D2	Vegetable oil	Fatty food
E	poly(2,6-diphenyl-p-phenylene oxide)*	Dry food

**particle size 60-80 mesh, pore size 200 nm*

In contrary to the old Directive, the new regulation introduces separate sets of standardized testing conditions for overall migration limit (OML) and for specific migration limit (SML) **[53]**.

2.1.8.1. Overall migration testing

The overall migration (the total amount of the material migrating into food) is performed through matrix evaporation for the gravimetric determination of the residue **[55]**.

The test conditions (contact times and temperatures) for overall migrations are set out in chapter 3 of the annex V of the regulation (EU) No 10/2011 as described in **Table 1.3** **[53]**.

Table 1.3: Standardized testing conditions of overall migration (OM) set by regulation (EU) No 10/2011[53].

Test number	Contact time in days [d] or hours [h] at Contact temperature in [°C]	Intended food contact conditions
OM1	10 d at 20°C	Any food contact at frozen and refrigerated conditions
OM2	10 d at 40°C	Any long term storage at room temperature or below, including heating up to 70°C for up to 2 h, or heating up to 100°C for up to 15 min.
OM3	2 h at 70°C	Any contact conditions that include heating up to 70°C for up to 2 h, or up to 100°C for up to 15 min, which are not followed by long term room or refrigerated temperature storage.
OM4	1 h at 100°C	High temperature applications for all food simulants at temperature up to 100°C.
OM5	2 h at 100°C or at reflux or alternatively 1 h at 121°C	High temperature applications up to 120°C.
OM6	4 h at 100°C or at reflux	Any food contact conditions with food simulants A, B or C, at temperature exceeding 40°C.
OM7	2 h at 175°C	High temperature applications with fatty foods exceeding the conditions of OM5.

Additionally, the OML test results are expressed in mg/dm², except of infant food where the units remain mg/kg food or food simulant, which is more stringent for small packs with higher surface to volume ratio [53].

2.1.8.2. *Specific migration testing*

The specific migration test is assessing the quantity of one specific substance that migrates. The test conditions (contact time and temperature) for the specific migrations are set out in chapters 1 and 2 of the annex V of the regulation (EU) No 10/2011 as described in **Tables 1.4** and **1.5 [53]**.

Table 1.4: Contact time for specific migration testing as described in the regulation (EU) No 10/2011 [53]

Contact time in worst foreseeable use	Test time
$t \leq 5$ min	5 min
5 min $< t \leq 0{,}5$ h	0.5 h
0,5 h $< t \leq 1$ h	1 h
1 h $< t \leq 2$ h	2 h
2 h $< t \leq 6$ h	6 h
6 h $< t \leq 24$ h	24 h
1 day $< t \leq 3$ days	3 days
3 days $< t \leq 30$ days	10 days

Table 1.5: Contact temperature for specific migration testing as described in the regulation (EU) No 10/2011 [53]

Contact of contact in worst foreseeable use Contact temperature	**Test conditions** Test temperature
$T \leq 5$ °C	5°C
5°C < T ≤ 20°C	20°C
20°C < T ≤ 40°C	40°C
40°C < T ≤ 70°C	70°C
70°C < T ≤ 100°C	100°C or reflux temperature
100°C < T ≤ 121°C	121°C (*)
121°C < T ≤ 130°C	130°C (*)
130°C < T ≤ 150°C	150°C (*)
150°C < T < 175°C	175°C (*)
T > 175°C	Adjust the temperature to the real temperature at the interface with the food (*)

(*) This temperature shall be used only for food simulants D2 and E. For applications heated under pressure migration testing under pressure at the relevant temperature may be performed. For food simulants A, B, C or D1 the test may be replaced by a test at 100°C or at reflux temperature for duration of four times the time selected according to the conditions in Table 4

In case the contact time was above 30 days at room temperature and below the specimen shall be tested in an accelerated test for 10 days at 60°C. In fact, testing time and temperature conditions is based on the following equation:

Equation 1:

$$t_2 = t_1 * \exp((-E_a/R) * (1/(T_1 - 1/T_2))) \quad (1)$$

where:

- E_a is the worst case activation energy 80kJ/mol
- R is the gas constant of 8.31 J/Kelvin/mol
- t_1 is the contact time
- t_2 is the testing time
- T_1 is the contact temperature in Kelvin. For room temperature storage this is set at 298 K (25°C). For refrigerated and frozen conditions it is set at 278 K (5°C).
- T_2 is the testing temperature in Kelvin.

According to this formula the European Union specified the following specific migration testing condition in its regulation (EU) 10/2011:

- “Testing for 10 days at 20°C shall cover all storage times at frozen condition.”

- “Testing for 10 days at 40°C shall cover all storage times at refrigerated and frozen conditions including heating up to 70°C for up to 2 hours, or heating up to 100°C for up to 15 minutes.”
- “Testing for 10 days at 50°C shall cover all storage time at refrigerated and frozen conditions including heating up to 70°C for up to 2 hours, or heating up to 100°C for up to 15 minutes and storage times of up to 6 months at room temperature.”
- “Testing for 10 days at 60°C shall cover long term storage above 6 months at room temperature and below including heating up to 70°C for up to 2 hours, or heating up to 100°C for up to 15 minutes.”
- “For storage at room temperature testing time can be reduced to 10 days at 40°C if there is scientific evidence that migration of the respective substance in the polymer has reached equilibration under this test condition.”

Moreover the regulation indicated that “…at the test temperature the test specimen should not undergo any physical changes” **[53]**.

2.2. Trace metals

Metals are chemical elements naturally present in the earth’s crust, so that they are found in all foods, most of the time at trace levels. Some of these elements (such as iron, nickel, copper, chromium and zinc) are considered essential when speaking of human diet, at least within certain specific tolerances. On the opposite, other metals (such as lead and cadmium) are considered at risk for human health because of their toxicity and their known bioaccumulation as well. As a consequence, it was deemed necessary to establish guidelines to regulate metal contamination in foodstuffs **[56]**.

2.2.1. Regulation relative to trace metals in foods

The presence of trace metals in foods is regulated through two series of laws relating to the unprocessed raw food on the one part, and to packaging materials (containers) on the other side **[56]**.

2.2.1.1. Dietary exposure and maximum permitted levels of trace metals in foods

The dietary exposure to trace metals can be either essential or associated with toxic and adverse health effects. However, all trace metals, including the essential ones, may be toxic when taken in excessive amounts. In fact: “All things are toxic and there is nothing without toxic properties. It is only the dose which makes something a poison” **[57]**.

Iron, chromium(III), nickel, zinc and copper are essential micronutrients consumed in adequate amounts to maintain certain physiological functions, while researchers are still unsure of whether tin is essential to human health **[58]**. On the other hand, trace metals like lead, cadmium and aluminum are non-essential to human body and are of particular concern in relation to harmful effects on health.

- ***Iron***

Iron is the fourth most abundant element (5%) in the earth's crust **[59]**. It is present in most foods and beverages. In general, liver, kidney, beef, ham, egg yolk, and soybeans have iron concentrations to the order of 30-150 mg/kg **[60]**.

Iron is essential for the synthesis of blood pigments and iron deficiency is a generally acknowledged problem; it should be noted that the body's iron requirements are variable and depend on age, gender, physiological state. However, accidental poisoning with iron indicates acute damage of gastrointestinal, hepatic, pancreatic and cardiovascular structures after ingestion of very high doses **[50]**. Also, presence of iron in foods at concentrations above 20 mg/kg causes alterations in the organoleptic properties of the latter **[61]**.

According to the World Health Organization (WHO 2003), iron needs are between 10 and 50 mg/day. But a daily dose of iron around 200-250 mg/kg of body weight (bw) can cause death **[62]**. The recommended daily intakes of iron are 7-9 mg/day for adult men and 10-13 years old children and 9-19 mg/day for adult women (Nordic Council of Ministers 2014) **[63]**, whereas the provisionally maximum tolerable daily intake (PMTDI) is 0.8 mg/kg bw (Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1983) **[64]**.

For canned food the maximum permissible limit (MPL) of Fe is 15 mg/kg (General Directorate of Protection and Control 2002, Republic of Turkey) **[65]** and 50 mg/kg of preserved foods (Health Ministry of Italian Republic) **[66]**.

- ***Tin***

Tin occurs in the earth's crust mainly as cassiterite or tinstone (SnO_2), which is the main source of tin production **[59]**. It is present in the diet only in small quantities of complex bound Sn(II)-ions **[67]**. Levels of tin are generally less than 1 mg/kg in unprocessed foodstuffs **[68], [69]**. In fact, the major source of tin in the diet is food contact materials, especially the release from tin cans (tinplate cans) to acidic foodstuffs **[67]**. Hence, tin concentrations in foodstuffs from unlacquered cans may exceed 100 mg/kg, while foodstuffs stored in lacquered cans have tin levels generally below 25 mg/kg [68]. Canned vegetables and fruits in unlacquered cans make up only a small percentage by weight of total food intake, while they may contribute 85% of the total intake of tin [70].

Tin compounds act as an irritant for the gastrointestinal tract mucosa, causing nausea, vomiting, diarrhea, fatigue and headache [68]. The number of cases of tin poisoning in humans is limited; they were reported following the consumption of canned fruit juices, tomato juice, cherries, asparagus, herrings and apricots [70]. The provisional tolerable weekly intake (PTWI) of tin is 14 mg/kg bw, which is equivalent to a daily intake of 2 mg/kg bw (JECFA 1989) [71].

The EU regulation 1881/2006 set maximum limits for tin (inorganic tin) at 200 mg/kg for canned food other than beverages, 100 mg/kg for canned beverages and 50 mg/kg for canned baby food [72]. According to the Lebanese Standard Institution (Libnor) [74]–[79], Codex [68] and JECFA [64] the maximum limit for tin is 250 mg/kg in canned solid foods. Besides, the Codex stated a maximum level of 200 mg/kg for liquid foods in cans [68], while JECFA stated the maximum level of 150 mg/kg in canned beverages [64].

- ***Chromium***

Chromium is found in the environment mainly in the trivalent form Cr(III), which is the most prevalent form present in the diet [67]. Hexavalent chromium Cr(VI), or chromate, may also be found in very small amounts, arising usually from industrial contamination [59]. Most foodstuffs contain less than 0.1 mg/kg of Cr [50]. The most contaminated food are cereals, meat, vegetables and unrefined sugar, while fish, vegetable oil and fruits contain smaller amounts of Cr [67]. Canned foodstuffs in non-lacquered cans and other processed foodstuffs, particularly acidic foodstuffs such as fruit juices, may be significantly higher in chromium than fresh foodstuffs [70].

The speciation of chromium is of great importance since Cr(III) is considered as an essential element for humans, whereas Cr(VI) is highly toxic, including effects such as genotoxicity. The tolerable daily intake (TDI) for Cr (III) is established at 300 µg/kg bw/day (EFSA 2014) [78]. The EU drinking water directive (EU 98/83/CE) specifies a limit of 50 µg/L.

- ***Nickel***

Nickel, combined with other elements, occurs naturally in the earth's crust. There has been a growing interest in possible effects of nickel in foodstuffs, i.e. a possible worsening of nickel-related dermatitis **[70]**. It is found in small quantities in many foodstuffs (0.001-0.01 mg/kg) and in higher concentrations in foodstuffs such as grains, nuts, cocoa products and seeds (up to 0.8 mg/kg). In the diet it is found as complex bound Ni^{2+} ions **[67]**.

Nickel intake from food causes problems to approximately 10–15% of the population, mainly women with nickel allergy **[67]**. These consumers get flare-up eczema from oral ingestion of even small amounts of nickel. The main targets for the toxicity of orally ingested nickel salts are kidneys, spleen, lungs and the myeloid system **[50]**.

EFSA (2005) could not derive a tolerable upper intake level for nickel in the evaluation of safety of fortified foods and food supplements **[50]**. In 2008, the French agency AFSSA set the TDI at 22 µg/kg bw/day **[79]**, and more recently WHO (2011) has derived a TDI of 12 µg/kg bw/day **[80]**.

- ***Zinc***

Zinc is one of the most ubiquitous of the essential trace metals. It occurs in most foodstuffs and beverages. The main food contributors to zinc intake are meats, especially organ meats, whole grain cereals and milk products including cheese**[81]**.

The absorption of ingested zinc is highly variable (10-90%) **[82]**. Zinc deficiency or excessively high levels of zinc may enhance susceptibility to carcinogenesis **[59]**. Nevertheless, cases of zinc poisoning have been reported – e.g. from galvanized iron containers holding acidic drinks. Symptoms of acute effects include nausea, vomiting, epigastric pain, abdominal cramps and diarrhea **[50]**.

In 1982, the JECFA has established a PMTDI of 0.3-1 mg/kg bw/day, while the required daily intake for adults is about 15 mg/day (varying with age) **[83]**. In 2006 the EFSA set the TDI of Zn at 25 mg/adult person/day **[84]**. By referring to Libnor 2004 **[85]**, the maximum level of Zn in canned plums is 150 mg/kg.

- ***Copper***

Copper exists in two oxidation states: Cu(I) (cuprous) and Cu(II) (cupric) **[86]**. It is naturally present in most foodstuffs in the form of copper ions or copper salts **[67]**. Generally, the concentration of copper in foodstuffs is about 2 mg/kg or less, the main sources being meat, offal, fish, pecans, milk chocolate and green vegetables **[86]**.

Acute toxicity due to ingestion of copper is infrequent in humans. However, when it occurs it is usually a consequence of the migration of copper into beverages. Symptoms include vomiting, epigastric pain, nausea, and diarrhea **[50]**. The JECFA (1982) has established a PMTDI of 0.5 mg/kg bw/day, while the daily requirement is established at 0.05 mg/kg bw weight/day **[83]**. According to EFSA 2006 the TDI is 5 mg/person/day **[84]**. Libnor 2004 **[85]** set the maximum level of Cu in canned plums as 10 mg/kg.

- ***Lead***

Lead is found as a contaminant in air, waters and soils **[67]**. It is present in the environment in the form of metallic lead, inorganic ions and salts, as well as organo-metallic compounds **[70]**. Plant contamination is to a large extent governed by air-borne lead contamination, making leaves and leafy vegetables more vulnerable to the air-borne deposition **[70]**. Canned foodstuffs might contain higher lead levels than fresh foodstuffs due to lead release from food cans as a consequence to its presence as an impurity in tin **[50]**.

Lead influences the nervous system, especially in children as their developing nervous system is sensitive **[50]**. Short-term exposure to high levels of lead can cause brain damage, paralysis and gastrointestinal symptoms, while longer-term exposure can cause damage to the kidneys, reproductive and immune systems **[87]**.

According to EU 1881/2006 **[72]** the MPL of lead in food is in the range 0.02-1.5 mg/kg, while Libnor 1999 **[73]–[77]** and Codex **[67]** specify a maximum level in canned food of 1 mg/kg. More recently, Libnor 2004 set maximum level in canned plums to 0.5 mg/kg **[85]** and the Codex Commission set Pb lower limits for processed fruits and vegetables (0.1 mg/kg for pulses, 0.4 mg/kg for jams, jellies and marmalades, and 0.05 mg/kg for preserved tomatoes) **[88]**. According to the European Commission Regulation (2006) **[72]** lead MPL in vegetables and fruits is 0.10 mg/kg. Finally, the U.S. Food and Drug Administration (FDA) refers to the maximum limits set by the Codex Alimentarius to make decision on the obtained levels of lead in food **[89]**.

- ***Cadmium***

Cadmium is one of the metallic elements of most concern in the food and environment of man **[67]**. It occurs in all soils and rocks **[90]**; also zinc ores contain cadmium, which is emitted during the melting of zinc **[91]**. Cadmium is found in most foodstuffs in the range of 0.005-0.1 mg/kg **[91]**. Certain foodstuffs (e.g. mushrooms, kidney and oysters) may contain much higher concentrations **[91]**. The lowest levels of cadmium are found in fruits and beverages **[70]**.

The principal toxic effect of cadmium is its toxicity to the kidney, although it has also been associated with lung damage and skeletal changes **[87]**. The PTWI for Cd is 2.5 µg/kg bw (EFSA, 2009) **[92]**. According to the EU 1881/2006 **[72]** and Codex (1995) **[67]** the MPL of Cd is 0.05 mg/kg in canned food. As for Pb, the U.S. FDA refers to the maximum limits set by the Codex Alimentarius to make decision on the obtained levels of Cd in food **[89]**.

- ***Aluminum***

Aluminum does not occur in nature in a free element state because of its reactive nature **[59]**. Many of its natural-occurring compounds are insoluble at neutral pH and thus concentrations of the element in water are usually low (<0.1 mg/L). Inorganic compounds of aluminum normally contain Al(III) **[70]**. The main source of aluminum is the natural occurring content in foodstuff. Unprocessed foodstuff can contain between about 0.1 to 20 mg/kg of aluminum **[70]**.

Once ingested, aluminum is excreted by the kidneys, and only a small amount is absorbed. The established TWI by JEFCA (2006) **[93]** and EFSA (2008) **[94]**, based on effects on the developing brain, neuro- and embryotoxicity, is 1 mg/kg bw/ week.

2.2.1.2. Regulation relative to migration of trace metals from food cans

In general the release of metals occurs due to corrosion of the metal material. As this mechanism differs from migration, the correct term concerning metal materials is release instead of migration. The inner walls of food and beverage cans are often covered by a surface coating, which reduces the migration/release of trace metals in foodstuffs. However, their migration/release can still occur through the coating pores.

At the regulatory level, two terms are used: global migration and specific migration. The latter concerns plastics and varnishes, but can be extended to cover the migration/release of metallic trace elements [52].

The migration/release level of the metals into the foodstuffs should be determined in the worst foreseeable conditions, taking into account the metal content of the foodstuffs itself. Food simulants can be used for estimating migration/release, but sometimes food simulants will not correctly estimate the release of metal ions from FCM to food. For example, in case of acidic foodstuffs the analysis should be carried directly on the foods and not on acetic acid, otherwise the phenomenon of corrosion may be produced. Therefore, results from measurements in food will prevail over results from measurements in food simulants [50].

In December 2013, a Resolution of Council of Europe on metals and alloys used in food contact materials and articles (CM/RES (2013)9 [95]) was adopted by Council of Europe member states, with the aim of overcoming the lack of specific regulations materials in the EU [96]. Thus the recommended specific release limits (SRLs) metals from metals and alloys FCMs are provided in the Council of Europe technical guide as shown in **Table 1.6**.

Table 1.6: Specific release limit (SRL) of metals from metals and alloys food contact materials according to the Council of Europe 2013 [96].

Main components	
Metal element	SRL (mg/kg)
Fe	40
Sn	100
Cr	0.25
Ni	0.14
Cu	4
Zn	5
Al	5
Impurities	
Metal element	SRL (mg/kg)
Cd	0.005
Pb	0.01

2.2.2. Trace metals in canned food

As mentioned previously, corrosion is the major cause of the release of metal trace elements from the inner wall of the packaging to the preserved foodstuffs. In the presence of moisture and oxygen, the metal undergoes an electrochemical reaction with components of the surrounding medium. In the simple case of uniform corrosion, this reaction results in the formation of compounds of the metal (e.g. hydroxides) on the surface of the metal. Some metals (e.g. chromium, aluminum and nickel) are less susceptible to corrosion than other metals (e.g. iron, copper and zinc) **[70]**. The actual rate of corrosion is dependent on a number of factors.

2.2.2.1. Parameters influencing the migration of trace metals

The corrosion process of tinplate cans is very complex, depending on a large number of parameters including can material (tin coated steel or tin free steel), nature of the contacting medium (aqueous or fatty foodstuffs), composition of the contained product (acid foods, sulfur and/or salt containing foods, etc.), and properties of protective lacquer **[97]**.

2.2.2.1.1. Nature of food

Food products often have low pH values or contain corrosive ingredients as salt and corrosion accelerators, like sulphur dioxide and nitrate **[50]**. The presence of oxidizing agents or depolarizers that corrode tin by direct chemical attack without evolution of hydrogen is probably most significant. Nitrates, possibly originating from fertilizers in the ingredients of the food, are the most frequently found oxidizing agents. When sufficient concentrations of nitrates are present, detinning may proceed at a rapid rate. Another chemical group, anthocyanins, has also been suggested to accelerate the rate of dissolution of tin **[98]**.

The rate at which corrosion proceeds is highly dependent on the pH of the food. At $pH < 4.5$, metals like Sn, Cd, Cr(III), Cu, Zn and Al are easily released in the canned food. These are unreactive in neutral solutions, thus light metal cans holding acidic drinks such as orange juice or alcoholic beverages have resulted in a number of reports on metal release **[59], [99]**.

2.2.2.1.2. Level of oxygen

The presence of oxygen in the head free space of food tinplate cans mainly causes oxidation of the tinplate followed by unavoidable migration of the tin ions formed into the foodstuff **[70]**. According to a study made by Parkar et al. **[100]**, they showed that the oxygen dissolved within the juice and entrapped in the headspace of the metal can readily reacts with tin to form stannous ions yielding to accelerated corrosion of tin layer.

Moreover, storing foodstuffs in opened unlacquered cans results in substantial increases in the tin concentration of foodstuffs **[68]**. It is also highly recommended not to store food in open cans, even in the presence of lacquer, because opening the can will break the lacquer sealing. This causes direct contact between food and metals in the can. Additionally, leakage of air through poor sealing and/or inadequate sterilization during packaging highly contribute to the release of Fe **[100]**. Other metals like cadmium and zinc are also rapidly corroded in the presence of air **[50]**.

2.2.2.1.3. The time of contact

The expiry date of tinplate cans primarily depends on its microbiological status. However, the evolution of metal contents should also be taken into account. The migration of most metal elements starts fast at the early storage time then tends to stabilize after a while, enriching the food with metals such as iron, tin, aluminum **[61], [101]**. In fact the magnitude of leaching of elements from packaging material (e.g. Sn and Fe) is highly dependent on storage time **[67], [100]**.

In general, the expiry date is usually set between two and four years from the date of manufacture. This duration varies mainly according to the foodstuffs, but it can also be different from one manufacturer to another **[7]**.

2.2.2.1.4. The temperature of contact

Temperature is a primary kinetic factor in any chemical reaction, including the corrosion reaction. Hence theoretically, an increase in the storage temperature of cans should initiate the transfer of metal trace elements. This finding is demonstrated in published scientific studies; for example, one study showed that at high temperature and in the presence of a small amount of dissolved oxygen in the juice of canned mango, rapid Sn leaching of the can took place **[100]**. Similar influence was also observed in a study made on aluminum migration as a function of storage temperature **[102]**. In another study of canned US military rations, the tin content of five types of fruit in unlacquered cans after 20 months was 12-fold higher at 37°C than at 1°C (420 and 34 mg/kg, respectively) **[98]**.

2.2.2.1.5. Influence of can defects

The FDA regulation **[103]** prescribes that no damaged packaging may be offered for sale. Indeed, the inappropriate handling of cans at the supermarket and at home expose food cans to several deformations. This can damage the inner protective film and increase the contact area between the metal and the corrosive matrix. One study has shown that higher concentrations of aluminum were detected in deformed cans **[104]**.

Moreover, in case of lacquered tinplate cans, any defects in the protective lacquer (such as scratches, tearing and discontinuity) would result in a concentrated attack of the base steel, resulting in a quick disappear of tin area **[10]**.

2.2.2.1.6. Lacquer characteristics

The application of protective organic coating prevent reactions between the can's metals and the food. Yet, in case of lack of continuity of the coating or through the lacquer pores, the migration/release of trace metals can still occur.

The lacquer thickness and the quality of tinplated can greatly affect the migration of tin **[70]**. Also, a study showed that the migration of Cd, Pb, Cu, Zn and Sn into food was significantly influenced by the coating type **[97]**. In the same study, all key metals were significantly influenced by the porosity of the coating (positive correlation) **[97]**.

Interestingly, one reference mentioned that: "…the risk of finding high concentrations of Fe in canned foods is greater when cans are internally lacquered"**[56]**.

2.2.2.1.7. Heat treatment (sterilization)

To our best knowledge the effect of sterilization on the release of metal elements is rarely discussed in literature. In case of sterilization of unlacquered cans, the oxidation of tinplate is followed by the release of tin ions into the foodstuff by a "sacrificial anode effect" that protects the can from possible perforation and protects the contents from degradation (changes in color and flavor) **[96]**.

2.2.2.2. Levels of trace metals reported in food

The level of trace metals in food generally, and canned food mainly, have been reported intensively in literature.

- ***Iron***

According to a study made on the Lebanese population diet, the average dietary intake of Fe is 13 mg/day and about 1.6% from canned food. In this study, Fe ranged between 0.16 mg/kg in juices and 38.57 mg/kg in meat and poultry **[57]**. Korfali et al. **[9]** also made a survey on canned food present in the Lebanese market and Fe was found within the range from 3 mg/kg in canned fish to 25 mg/kg in canned liver paste. In these studies Fe was obtained at lower concentrations in canned vegetables and fruits in comparison to meats **[9], [57]**. On the other hand, a similar study carried on trace metals in the diet of French population **[105]** revealed the range of Fe between 0.60 mg/kg in dairy products and 45.5 mg/kg in ice cream.

In other studies, Fe was within the range of 19–42.89 mg/kg in canned scallops **[106]**, which was close to the range of 20.2–38.7 mg/kg found in canned tuna **[107]**. The mean concentration of Fe was found to be 50.71 and 6.98 mg/kg in canned anchovies and canned rainbow trouts, respectively **[108]**. According to Raptopoulou et al., Fe was within the range from 9.75 mg/kg in tomato paste packed in aseptic paper to 50.9 mg/kg in double concentrated tomato paste packed coated cans **[109]**, while Waheed et al. **[110]** reported a mean concentration of Fe at 6.03 mg/kg in canned tomato paste. In the latter study, Fe mean concentration was high (126 mg/kg) in canned apple jam **[110]**. Canned mushroom, as well, can contribute to high levels of Fe, average found at 79.6 mg/kg **[111]**.

The migration of Fe from tinplate cans was also discussed in different papers. For instance, Fe increased from 5 to about 25 mg/kg over 3 months storage of canned chickpeas in coated cans, which were collected from the Lebanese market **[101]**. Iron also increased from 11 to 15 mg/kg over 3 years in canned pork in coated cans and from 140 to 170 mg/kg in canned liver pate in coated cans according to Buculei et al. **[112]**. Finally, it was shown that Fe reached 2308.6 mg/kg in uncoated cans of mango pulp after 6 months at 48°C, and 549.1 mg/kg, under same storage conditions in baked beans in tomato paste packed in coated cans **[100]**.

- ***Tin***

Very low concentrations of Sn were reported in canned food from coated cans: < 1 mg/kg in canned pork **[112]**, tomato **[112]**, fish **[9], [108]**, tuna **[107], [113]**, chickpeas **[101]**, beverages and energy drinks **[114]**. Higher average concentration (179 mg/kg) of Sn was obtained by Korfali et al. in canned vegetables, probably due to the fact that some of these products were stored in uncoated cans **[9]**. Additionally, Sn was in the range between 0.005 mg/kg (cereals and cereal products) and 8.55 mg/kg (fruits and vegetables) according to analyses performed on pooled samples during the second French Total Diet Study (TDS) **[105]**. Upon comparing the migration of Sn in food in coated and uncoated cans, important disparity should be highlighted. For example, Sn reached only 1.2 mg/kg after 3 years in coated pork cans, while it reached 22.5 mg/kg in coated tomato cans under same duration **[112]**. However, much higher concentrations were obtained after only 6 months of storage of uncoated cans (up to 361.2 mg/kg) **[100]**.

- ***Chromium***

Chromium was in the range of 0.01–1.63 mg/kg in canned seafood **[9], [106]**, 0.08–0.19 mg/kg in canned vegetables and legumes **[9], [111]**, and 0.041–0.623 mg/kg in canned tomato and tomato paste **[109], [111]**.

- ***Cadmium***

The reported ranges of Cd were 0.0146–4 mg/kg in canned fruits, vegetables and legumes **[9], [101], [110]**, 0.001–0.022 mg/kg in canned seafood **[107], [108], [113]**, 0.011–0.097 mg/kg in canned tomato paste **[109]**, 0.003–0.081 mg/L for canned beverages and 0.006–0.071 mg/L for non-canned beverages **[115]**, and 0.00019–0.138 mg/kg in canned meat and 0.0000245-0.0071 mg/kg in processed meat present in the Lebanese market **[116]**. Furthermore, the Lebanese dietary intake of Cd was calculated as 15.82 µg/person/day by Nasreddine et al. **[57]**.

- ***Lead***

Lead quantities in canned food were found to lie within the following ranges: 0.001–2.04 mg/kg in canned fruits and vegetables **[9], [110]** and 0.036–0.45 mg/kg in canned seafood **[107], [108], [113]**. Lead was in the range 0.002–0.0073 and 0.001–0.092 mg/L for the non-canned beverages according to Maduabuchi et al. **[115]**, and in the ranges 0.00020–0.816 mg/kg in canned meat and 0.00025–0.0613 mg/kg in processed meat collected from the Lebanese market **[116]**. Additionally, the Lebanese dietary intake of Pb, calculated by Nasereddine et al., was 8.26 µg/person/day **[57]**.

In a study carried by Raptopoulou et al. on tomato paste, the concentration of Pb in coated cans was much higher (0.138 mg/kg) than that obtained in tomato paste packed aseptic paper (0.0044 mg/kg) **[109]**. Similarly, another study made on chickpeas in coated cans showed that Pb rapidly increased from about 0.05 to about 0.6 mg/kg during the first 3 months of storage **[101]**.

- ***Nickel, copper and zinc***

Data collected on canned vegetables and fruit products showed a range of Cu of 0.003–8.88 mg/kg **[9], [110]** and a range of Zn within 0.19–22.8 mg/kg **[9], [110]**. On the other hand, canned seafood contained Cu in the range 0.48–1.14 mg/kg **[9], [107], [108]** and Zn in the range 8.20–22.47 mg/kg **[107], [108]**. The highest concentrations of these three elements were found in canned mushrooms (0.75 mg Ni/kg food and 21.9 mg Zn/kg food) and canned beans (7.77 mg Cu/kg food) according to Tuzen et al. **[111]**. Raptopoulou et al. **[109]** showed that the concentration of Ni was higher in tomato paste packed in coated cans (1.05 mg/kg) than those packed in aseptic paper (0.1 mg/kg), yet it was not the same for Cu (3.05–518 mg/kg in coated cans and 3.38–3.48 mg/kg in aseptic paper). Nasereddine et al. **[57]** calculated the Lebanese dietary intake for Ni, Cu and Zn of 0.126, 1.104 and 10.97 mg/ person/day, respectively.

- ***Aluminum***

Aluminum cans are usually used to pack beverages and soft drinks, however, traces of aluminum were detected in canned food as well. Aluminum ranged between 0.09 and 3.17 mg/kg in canned vegetables, and between 0.93 and 7.5 mg/kg in canned meat and fish **[9], [111]**. On the other hand, canned beverages contained higher range of Al that is 9.76-387.5 mg/L according to Nicholas et al. **[114]**. In canned orange juice, Al increased during 12 months from 4.4 to 21.59 mg/kg when cans were stored at 28°C, and from 5.21 to 26.71 mg/kg when cans were stored at 38°C **[100]**.

2.2.3. Summary

From the references cited herein, there appears to be evidence on the migration/release of metal elements from light metal cans. Most of these metals are essential for human health to maintain certain physiological functions, but must be consumed within safety limits, otherwise high levels of Sn and Fe, for example, can cause gastrointestinal problems, in addition to possible deteriorate of food organoleptic properties. Tin, iron, and chromium are the basic constituents of tinplate and tin-free steel cans, while other essential metals like Ni, Zn and Cu are used to enhance the corrosion resistance and strength of the cans. In contrary, metals like Pb and Cd are considered to possess potential toxicity, such as lung and kidney damages, and are present as contaminants in the alloy.

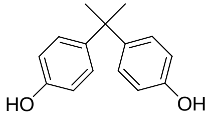
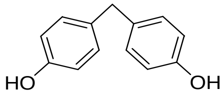
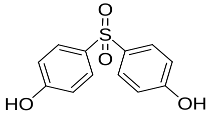
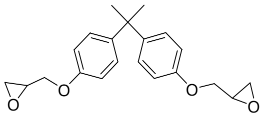
In fact the release of metals from food and beverage cans is related to the corrosion of the container, which is in turn affected by different parameters. Some of these parameters are: nature of food (e.g. acidic or fatty) and its constituents (e.g. the presence of nitrates accelerates the dissolution of tin), level of oxygen, time and temperature of contact, defects in the cans, lacquer characteristics (in case present) and heat treatment or sterilization.

In order to protect the consumer, regulatory authorities (e.g. EU, JECFA, Codex Alimentarius, Libnor, etc.) fix and control maximum permissible limits in order to ensure that consumers are exposed below maximum daily intake of metals. The Council of Europe in 2013 provided the specific release limits of metals from metal food contact materials. To our best knowledge, migration test of metals from food and beverage cans in literature are directly carried on food rather than food simulants. In most cases, and under ambient storage conditions, the detected concentrations of metals originally present in foods and those migrating from cans comply with the standardized limits.

2.3. Bisphenol compounds

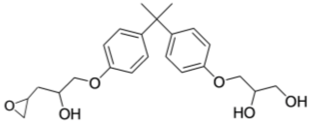
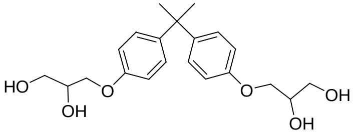
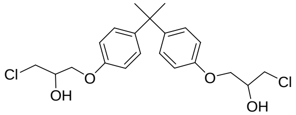
At present, the predominant protective coatings for the interior of metal food cans are epoxy-resin based. Epoxy resins are commonly synthesized from the condensation of BPA with epichlorhydrin, yielding to BADGE. Bisphenol analogues such as BPF and BPS are also used as alternatives to BPA. Other popular coatings, like PVC organosol resins, require the use of additives like BADGE and BFDGE to act as hydrochloric acid scavengers during the curing process. This can result in the formation of many chlorinated compounds such as BADGE·HCl, BADGE·2HCl, BFDGE·HCl and BFDGE·2HCl. The remaining epoxy groups can be hydrolyzed via contact with aqueous and acidic foods resulting in the formation of mono- and di-hydrolyzed products such as BADGE·H_2O, BADGE·$2H_2O$, BADGE·HCl·H_2O, BFDGE·H_2O, BFDGE·$2H_2O$, and BFDGE·HCl·H_2O. Due to incomplete polymerization, residuals of these compounds can migrate into foods that are in contact with the coating **[117]**. The chemical structure, physicochemical properties and toxicity of predominant bisphenol compounds are shown in **Table 1.7**.

Table 1.7: The chemical structure, physicochemical properties and toxicity of predominant bisphenol compounds

Compound	Chemical name (IUPAC)	Chemical structure	Molecular weight (g/mol)	Solubility (mg/L) in water at 25 °C	Boiling point (°C at 760 mm Hg)	Toxicity
BPA [118]	4-[2-(4-hydroxyphenyl)propan-2-yl]phenol		228.291	300	360.5	• Skin sensitization and photosensitization • Effects on the upper respiratory tract, liver and kidneys. • Toxic effects on reproduction
BPF [119]	4-[(4-hydroxyphenyl)methyl]phenol		200.237	NA	NA	• No endocrine mediated effects • Toxic effect on liver
BPS [120]	4-(4-hydroxyphenyl)sulfonylphenol		250.268	Insoluble in water	NA	• Similar estrogenic activity as BPA
BADGE [121]	2-[[4-[2-[4-(oxiran-2-ylmethoxy)phenyl]propan-2-yl]phenoxy]methyl]oxirane		340.419	0.7	Not pertinent (decomposes)	• Dizziness, drowsiness, dry skin, eye redness, no evident data on its carcinogenetic effect

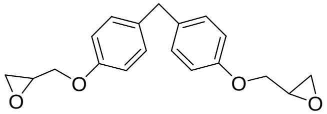
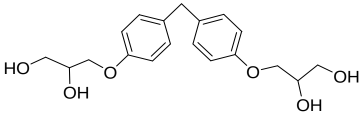
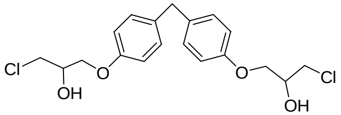
NA: not available

Table 1.7 (Continued): The chemical structure, physicochemical properties and toxicity of predominant bisphenol compounds

Compound	Chemical name (IUPAC)	Chemical structure	Molecular weight (g/mol)	Solubility mg/L water at 25°C	Boiling point (°C at 760 mm Hg)	Toxicity
BADGE.H_2O **[122]**	3-[4-[2-[4-(oxiran-2-ylmethoxy)phenyl]propan-2-yl]phenoxy]propane-1,2-diol		358.434	NA	NA	• The clastogenic and mutagenic potential of BADGE is lost after hydrolysis of the epoxide rings **[123]** • The mono-diol BADGE may be genotoxic but not the bis-diol BADGE **[122]**
BADGE.$2H_2O$ **[124]**	3-[4-[2-[4-(2,3-dihydroxypropoxy)phenyl]propan-2-yl]phenoxy]propane-1,2-diol		376.449	NA	NA	
BADGE.HCl.H_2O **[125]**	3-[4-[2-[4-(3-chloro-2-hydroxypropoxy)phenyl]propan-2-yl]phenoxy]propane-1,2-diol		394.892	NA	NA	
BADGE.HCl **[126]**	1-chloro-3-[4-[2-[4-(oxiran-2-ylmethoxy)phenyl]propan-2-yl]phenoxy]propan-2-ol		376.877	NA	NA	• Potential estrogenic activity due to the presence of halogen atoms in its chemical structure **[127]**
BADGE.2HCl **[128]**	1-chloro-3-[4-[2-[4-(3-chloro-2-hydroxypropoxy)phenyl]propan-2-yl]phenoxy]propan-2-ol		413.335	NA	NA	

NA: not available

Table 1.7 (Continued): The chemical structure, physicochemical properties and toxicity of predominant bisphenol compounds

Compound	Chemical name (IUPAC)	Chemical structure	Molecular weight (g/mol)	Solubility mg/L water at 25°C	Boiling point (°C at 760 mm Hg)	Toxicity
BFDGE [129]	2-[[4-[[4-(oxiran-2-ylmethoxy)phenyl]methyl]phenoxy] methyl]oxirane		312.365	NA	Not pertinent (decomposes)	• The allergenic effect of BFDGE is stronger than that of BADGE, but toxicological profiles of BFDGE and its derivatives have been lacking [130]
BFDGE.2H$_2$O [131]	3-[4-[[4-(2,3-dihydroxypropoxy)phenyl]methyl]phenoxy] propane-1,2-diol		348.395	NA	NA	
BFDGE.2HCl [132]	2-chloro-3-[4-[[4-(2-chloro-3-hydroxypropoxy)phenyl]methyl]phenoxy] propan-1-ol		385.281	NA	NA	

NA: not available

2.3.1. Regulation relative to bisphenol compounds

Bisphenol compounds are known to migrate from food contact materials, especially can coatings, and do not naturally occur in foodstuffs. Due to their potential health hazard, these compounds have been regulated by food safety authorities all over the globe. Based on TDI assessed for human (generally estimated upon the No Observed Adverse Effect Level (NOAEL) in animal testing), Specific and Global Migration limits (SML and GML, respectively) from can coating have been set to protect the consumer.

2.3.1.1. Dietary exposure and maximum permitted levels of bisphenol compounds in foods

In 2015, the EFSA issued risk assessments for consumers and revised its BPA temporary - TDI to 4 instead of 50 µg/kg bw/day **[133]**. The same EFSA set the TDI of BADGE and its hydrolysis products to 0.15 mg/kg bw/day **[134]**.

The U.S. FDA has estimated that BPA exposure from use in food contact materials is 2.42 and 0.185 µg/kg bw/day in infants and adults, respectively **[135]**. Thus, these values are near or above the TDI set by the EFSA, meaning that risk may not be excluded for these populations. Similarly, the French Anses **[136]** and the Danish Food **[137]** also consider that the risk associated with the presence of BPA in diet, especially due to canned food, cannot be excluded. In contrast to previous regulatory authorities, Health Canada's Food Directorate **[138]** has concluded that the current dietary exposure to BPA through food packaging is not expected to pose a health risk to its general population, including newborns and infants. Therefore, there are no Canadian regulations or maximum levels established for BPA in food.

2.3.1.2. Regulation relative to migration of bisphenol compounds from can coatings

Can coatings are not regulated by a wide legislation, but specific measures exist in several countries. In the beginning of 2016, the European Commission (EC) published a draft regulation on the use of BPA in varnishes and coatings as well as an amendment of the plastics regulation (Commission Regulation EU 10/2011) **[53]**. According to this Directive the stated SML of BPA is 600 µg/kg food from plastic materials and articles intended to come in contact with foodstuffs, and the SML of BPS is set to 50 µg/kg food. Recent regulation has revised the SML value for BPA down to 50 µg/kg food or food simulant **[139]**.

Specific migration limits for BADGE and its derivatives are defined in Commission Regulation EC 1895/2005 **[34]**; the sum of BADGE and its hydrolysis products

($BADGE \cdot H_2O$ and $BADGE \cdot 2H_2O$) shall not exceed a total SML of 9 mg/kg food or food simulant, while a total SML of 1 mg/kg food or food simulant was assigned to the chlorohydrins of BADGE ($BADGE \cdot HCl$, $BADGE \cdot 2HCl$ and $BADGE.H_2O.HCl$). This Directive also banned the use of BFDGE and NOGE in FCMs due to the lack of toxicological data, except in the coating of large containers intended for repeated use; for such applications, no migration limits were set.

In January 2015, France banned the use of BPA in FCMs including all packaging, containers and utensils intended to come into direct contact with food (LOI n° 2012-1442) **[140]**. However, in September 2015, the French Constitutional Council decided to partially lift the ban on the manufacture and export of BPA-containing FCMs, while the ban remains valid at national level **[2]**. Spain has recently updated its coatings legislation with the enactment of Royal Decree 847/2011**[141]**: this legislation allows the use of substances listed in the European Regulation (EU) No 10/2011 for plastic articles and materials, with the same restrictions there mentioned for BPA.

In July, 2012, the U.S. FDA amended its regulation to no longer provide for the use of BPA-based polycarbonate resins in baby bottles, this action was in response to a food additive petition filed by the American Chemistry Council (ACC) **[142]**. Afterward, in July 2013, the U.S. FDA amended its regulations to no longer provide for the use of BPA-based epoxy resins as coatings in packaging for infant formula in response to a food additive petition filed by Congressman Edward Markey **[142]**. A specific legal measure concerning can coatings exists also in California **[143]**, since in May 2015, the Californian Office of Environmental Health Hazard Assessment (OEHHA) added BPA to the list of chemicals known to cause reproductive harm under Proposition 65. As a consequence, manufacturers, distributors and retailers had to inform the consumers of BPA-containing products with a clear and reasonable warning regarding the chemical hazards; in addition, by the end of 2017, products containing BPA were required to be directly labeled.

Despite all these regulations, to our best knowledge, with respect to the developing countries (such as Lebanon), there are no specific regulations for bisphenol compounds in FCMs. Yet, the commercial products controlled should follow the limits set by regulatory authorities as the European Union and U.S. FDA.

2.3.2. Migration of bisphenol compounds in food simulants

Since foods are very complex matrices, which are not chemically uniform and the determination of migrants can be very complicated, food simulants (previously discussed) have been intensively used to monitor the migration of bisphenol compounds from can coating. Many studies tested the influence of different production and storage conditions on the migration phenomena.

2.3.2.1. Influence of processing parameters

During migration tests different parameters should be taken into account, such as heat treatment process, contact time-temperature conditions, nature of food/food simulant, coating characteristics and can damaging [144].

- ***Effect of heat treatment or sterilization***

Beside the fact that heat sterilization of canned foods is essential for the destruction of all contaminating bacteria including their spores, many studies reported that this process has an important influence on inducing the migration of residual compounds from can lining into foods [36], [145]–[147]. According to Goodson et al. [25] and López et al. [148], 80–100% of free BPA present in the can coating migrate during the sterilization stage (typically 30 min at 121°C) into 10% ethanol and fatty-food simulant, respectively. In a particular study [24], the authors showed that BPA release was continuous as a function of time (< 0.2 to 12.5 ± 1.3 μg/kg over 160 days) in unsterilized canned water as food simulant, while after sterilization water cans contained a constant level of BPA (80–90 μg/kg, more than 6 times higher than unsterilized water) over the same storage time. In another study [148], sterilization of fatty food simulant for 50 min at 121°C caused much less BPA to migrate than cans autoclaved for 135 min at 111°C, suggesting that sterilization time is more influencing than sterilization temperature. The same conclusion was drawn by Kang et al. [149] on fatty-food simulant, and by Takao et al. [150]. In the latter study, after heating empty cans for 30 min at 80°C, BPA concentrations were up to 6 times (average 3 times) less than in cans heated for 30 min at 100°C. On the other hand Simoneau et al. [151] reported the insignificant difference on the migration of BADGE into sunflower oil between short (30 min) and long (60 min) sterilization at 115°C.

- ***Contact time-temperature conditions***

The significant effect of storage time and temperature on the migration of bisphenol compounds was highlighted on food simulants before sterilization. Furthermore, many studies showed that storage temperature and time are insignificant on triggering further migration of free monomers after sterilization process **[25], [36], [145]**. Hence, Goodson et al. **[25]** indicated that BPA migration occurs mainly during can processing step (i.e. sterilization), while its level did not increase in 10% ethanol during 9 months of storage and showed insignificant influence toward storage temperature (5, 20 and 40°C) **[25]**. López et al. obtained a similar result, where storage time and temperature had minor effect after sterilization on the migration of BPA into fatty food simulants, even with samples stored at 40°C for 10 days which simulates long-term storage under ambient temperature **[148]**.

In another study on canned oil **[151]**, BADGE levels in non-heat processed cans exhibited insignificant increase over 13 months of storage, except for storage at 60°C where migration was observed. Thus, BADGE seems to be more affected by storage temperature than storage time since diffusion process could be faster at a higher storage temperature **[152]**.

- ***Nature of food/food simulant***

The main food simulants (with reference to amended European regulation (EU) 10/2011 **[53]**) used for testing the specific migration of bisphenol compounds are: 10% ethanol (after amendment) or water (before the amendment) (simulant A mimics aqueous food), 3% acetic acid (simulant B mimics acidic food with pH < 4.5), as well as 50% ethanol and vegetable oil (simulants D1 and D2 which simulate food of lipophilic character).

The migration of bisphenol compounds is dependent on the type of food simulant, where this is related to the partitioning of the migrant between the simulant and the coating that is based on the migrant solubility in the simulant tested **[144]**. For instance, Schecter et al. **[153]** reported that BPA levels could be higher in foods of pH 5 compared to more acidic foods, which also matches with another study **[23]**; this result was expected since BPA has greater solubility at alkaline pH values due to its dissociation constants (pKa 9.6 to 10.2) **[154]**. BPA concentrations were lower in olive oil than in 10% ethanol **[155]**; low BPA levels in fatty food simulants could be due to its antioxidant behavior in response to the possible oxidation of oil during heat treatment **[148]**.

BADGE and BFDGE tend to hydrolyze in 3% acetic acid and distilled water food simulants after 10 h of storage **[156]**. In fact, BADGE is highly affected by acidic medium, where its half-life in water is 3.5 times longer than that in acetic acid **[157]**. As expected, the hydrolysis of BADGE and BFDGE is not common in case of fatty foodstuffs **[30]**. The hydrolysis of BADGE can be significant in 50% ethanol, possibly due to the increased solubility of oligomers in presence of ethanol, resulting in increased migration and more potential for hydrolysis products **[158] [159]**.

- ***Coating properties***

The type and properties of the applied lacquer are essential parameters for defining the fate of migration of bisphenol compounds. Some of these properties are: coating formulation (e.g. the glass transition temperature (Tg) has an important role in the mass transfer of migrants, since at a working temperature below Tg, the diffusion coefficient of migrants appears to slow down) **[152]**, initial concentration of free compounds, polymer density and thickness **[144]**, and the addition of slipping agent (mainly in cans used for meat) **[160]**.

- ***Effect of can damaging***

To our best knowledge, only few studies investigated the effect of can damaging on bisphenol compounds migration **[25], [161]**. In particular, Goodson et. al **[25]** showed that damaging of canned 10% ethanol did not have any significant effect on BPA migration, even for cans heated up to 15 min. On the opposite, Errico et al. **[161]** exhibited a significant increase in the migration of BPA when tomato cans were damaged by denting.

2.3.2.2. Levels observed in food simulants

Wide variations in the concentrations of bisphenol compounds have been reported in food simulants, since their concentrations greatly depend on the processing parameters indicated above (especially type of coating, origin of the cans and the simulant studied).

For example, Lopéz et al. obtained 3.62 ± 1.67 µg of BPA/kg acidic food simulant, and higher levels (11.3-138.2 µg/kg) in fatty food simulant using epoxy-resin coated cans **[23]**, **[148]**. On the other hand, BPA concentration was below quantification limit in cans coated with organosol coating. Goodson et al. **[25]** also studied the migration of BPA from can coating into 10% ethanol; they obtained an average concentration of 68.3 ± 9 µg/kg, thereby exceeding the recent revised SML for BPA in Europe (i.e. 50 µg/kg food or food simulant) **[139]**.

The migration from two types of coating (epoxy resin and acrylic phenolic) was monitored in water and 50% ethanol food simulants **[152]**. In the case of epoxy resin, higher migration were observed in 50% ethanol (BPA: 13 ± 0.01 µg/dm^2, BADGE.2H_2O: 23 ± 0.5 µg/dm^2, other BADGE derivatives: from 22 ± 1 to 325 ± 7 µg/dm^2) as compared to water (BPA: 5.5 ± 0.3 µg/dm^2, BADGE.2H_2O: 14 ± 0.4 µg/dm^2, other BADGE derivatives: 18 ± 9 µg/dm^2). The migration of these compounds into 50% ethanol was further studied on two other epoxy resins, and quite significantly different levels were obtained (yellow epoxy resin: 3.2 µg BPA/dm^2; 38 µg BADGE.2H_2O/dm^2; 376 µg cyclo-di-BADGE/dm^2; white epoxy resin: 2.1 BPA/dm^2; 56 µg BADGE.2H_2O/dm^2; 406 µg cyclo-di-BADGE/dm^2). In comparison, lower levels migrated in acrylic-phenolic coating: 0.15 ± 0.01 and 0.6 ± 0.02 µg/dm^2 of BPA and BADGE.2H_2O in water simulant, while the migration in 50% ethanol were respectively 2 ± 0.5, 0.7 ± 0.1 and 12 ± 0.7 µg/dm^2. In all cases, bisphenol concentrations remained below the SMLs set by the European Union.

Limited studies compared the concentrations obtained in food simulants and real food for similar cans **[23]**, **[25]**. They suggest an overestimated migration of BPA using food simulants, by a 2.5 factor with the acid simulant **[23]**. The presence of ethanol (even at 10%) can interact with the coating to release more free BPA than could be extracted with foods **[25]**.

2.3.3. Bisphenol compounds in canned foods

The main contribution in dietary exposure to bisphenol compounds is canned food **[162]**. However, these compounds (especially BPA) can also be present in foods before canning or packing, possibly due to food contamination during the production process if equipments or containers with epoxy coating or plastic parts have been used. Additionally, bisphenol compounds may be present in the ingredients used to prepare the ready-to-eat food or in the brine used for canned food **[36]**.

2.3.3.1. Influence of processing parameters

The influence of processing parameters on the migration of bisphenol compounds have been mainly investigated on food simulants, yet, some studies were also carried on real food. The influence of heat treatment (or sterilization) as well as storage conditions (time and temperature) was generally common between food simulant and real food. As obtained for food simulant, heat treatment or sterilization is the main contributing factor for the migration of unreacted free epoxy-phenol monomers **[25], [161]**.

Both papers published by Lopéz et al. [23] and Goodson et al. [25] monitored the effect of storage time on the migration of BPA into both food simulants and real food. After heat treatment, the authors of the two studies noted important increase of BPA in canned jalapeno peppers (heat-treated at 100°C for 9 min) and in or four other types of sterilized canned food (minced beef, milk, carrots, soup), however, no significant variation in the migration levels was observed in these foods over the extended storage time. Similarly, no BADGE migration was reported over 18 months of storage of canned seafood [145], while migration was evidenced for BFDGE with levels increasing significantly after 6 months of storage. In another study, Errico et al. [161] revealed a significant effect of storage time and temperature on the migration of BPA in sterilized canned peeled tomato (e.g. at 37 and 45°C, the concentrations of BPA were, respectively, about three and ten times those measured at 25°C), perhaps due to the aggravating acidity of peeled tomato.

Some studies highlighted the correlation between fat food content and the migration of compounds such as BPA, BADGE and BFDGE [145], [163]. In the case of BPA, it has been argued that it seems to have a preference for a lipophilic environment rather than an aqueous phase due to its higher partitioning to solid portion than liquid portion of canned food [147]. On the other hand, correlation between pH of the food and BPA migration could not be established [146], [147], [164]. Yet, levels of BPA in canned beverages were almost 40 times lower than in canned food [147], which matches with the previously mentioned correlation of BPA content and acidic pH [153]. Also, BADGE and BFDGE migration into food products with high fat content and high pH was less affected by high storage temperature [145]. In addition, some studies reported reaction between BADGE or BFDGE and food proteins [33], [163] leading to their disappearance and the formation of adducts with unknown toxicity.

Other parameters, such as can damage and heating food in cans, were also discussed on real food. For instance, Errico et al. [161] showed that accidental or careless exposure of tomato products to heat (e.g. sunlight) and/or can damage during storage and transportation are an aggravating factor in the migration process. This study contradicts previous results obtained on different canned food [25], probably due to the high acidity of canned tomato.

Finally, the significant variation in the levels of bisphenol compounds between brands of same canned product could be due to the differences in can coatings (type, amount, thickness, etc.), sterilization conditions (temperature and duration) used by the different companies, and the coating to food ratio [147], [161], [165].

2.3.3.2. Levels observed in canned foods

Due to the growing public health concern of oral intake of bisphenol compounds, many surveys were carried on canned and non-canned food present in the markets of different countries. Among bisphenol compounds, BPA was intensively studied. For instance, up to 730 µg BPA/kg was obtained in highly consumed canned food in the U.S **[164]**, exceeding the European SML of 600 µg/kg **[53]**. In contrast, lower BPA concentrations were revealed in another study on food still consumed in the U.S. **[153]**, where BPA levels ranged from 0.23 to 65.0 µg/kg whatever the type of food or packaging. Additional studies on canned food present in the Japanese **[166]**, Canadian **[167]** and Belgian **[147]** markets reveal BPA contents in the range ND (not detected)–842 µg/kg. Overall lower BPA concentrations were obtained in food packed in plastic and paper containers (ND–14 and ND–1 µg/kg, respectively) **[166]**.

Between food products consumed in Canada, Heath Canada **[168]** reported that canned tuna products had the highest BPA levels, in general, with average and maximum BPA levels of 137 and 534 µg/kg, respectively, below the (EU) SML at that time. Canned soup products had the next highest BPA levels, with average and maximum BPA levels of 52 and 94 µg/kg in condensed soups compared to 15 and 34 µg/kg for ready-to-serve soups. Interestingly, BPA levels in canned tomato paste products were considerably lower (the average and maximum BPA levels for tomato paste products were 1.1 and 2.1 µg/kg, respectively). Errico et al. **[161]** also found lower BPA levels in canned tomato consumed in Italy than in other canned food products (e.g. fruit, vegetables, meat, etc.).

Other studies investigated the levels of other bisphenol compounds, such as BADGE and BFDGE and their derivatives. Liao and Kennan **[162]** measured the levels of bisphenol analogues in 289 food samples collected from nine cities in China: BPA and BPF were found widely in foodstuffs at concentrations ranging from below the limit of quantitation (LOQ) to 299 µg/kg (mean = 4.9 µg/kg) and from below the LOQ to 623 µg/kg (mean = 2.5 µg/kg), respectively. The highest total concentrations of bisphenols ((∑BPs: sum of eight bisphenols, BPA, BPAF, BPAP, BPF, BPP, BPS, BPZ, BPB) were found in the category of vegetables that included canned products (mean = 27.0 µg/kg), followed by fish and seafood (16.5 µg/kg) and beverages (15.6 µg/kg). Concentrations in milk and milk products, cooking oils and eggs were low (mean = 2–3 µg/kg). Food samples sold in metallic cans contained higher mean ∑BP concentrations (56.9 µg/kg) in comparison with those packaged in glass (0.43 µg/kg), paper (11.9 µg/kg) or plastic (6.40 µg/kg). The daily dietary intakes of bisphenols were estimated, based on the mean concentrations measured and daily consumption rates of foods: 646 and 664 ng/kg bw/day for men and women, respectively. Among bisphenol analogues, BPA and BPF were prevalent in foods, accounting for 64% and 10%, respectively, of the total bisphenol concentrations. Moreover, close ranges of BPA (20.5-115.3 µg/kg) and BPB (27.1 to 55.9 µg/kg) were also observed in canned peeled tomato collected in Italy **[169]**.

The results obtained by Cabado et al. **[145]** on canned seafood collected from Spanish market did not show any BADGE migration in most food products; on the opposite, BFDGE migration occurred in all foods. Main migration takes place in mackerel in red pepper sauce, reaching the highest levels (740 µg BFDGE/kg and 340 µg BADGE/kg net product). All foodstuffs did comply with the European total SML for BADGE and its hydrolysis derivatives (9000 µg/kg **[53]**), but not for BFDGE since this compound is not allowed by the European legislation. In another study on canned food from the Spanish market, Gallart-Ayala et al. **[127]** obtained concentrations in the ranges of 2.7–675 µg BADGE.$2H_2O$/kg, 35–53 µg BADGE.H_2O/kg, 3.4–274 µg BADGE.HCl.H_2O/kg and 0.9–2.8 µg BADGE.2HCl/kg, whereas BADGE was not found in any sample.

The concentration of BPA, BADGE and its derivatives in canned food collected from the Singaporien market **[170]** were as follows: ND-160 µg BPA/kg (highest in canned sliced mango), ND–400 µg BADGE.$2H_2O$/kg (highest in canned young corn in brine), ND–50 µg BADGE.H_2O/kg (highest in canned spiced pork cubes), ND-200 µg BADGE.H_2O.HCl/kg (highest in canned pineapple slices), ND-810 µg BADGE.2HCl/kg (highest in canned spiced pork cubes), ND–440 µg BADGE/kg (highest in canned sandwich tuna), while BADGE.HCl was not detected. BPA and BADGE.$2H_2O$ were the most abundant elements. Likewise, Yonekubo et al. **[146]** analysed 38 canned foods sold in Japan and obtained close ranges of bisphenol compounds: BPA (ND–235 µg/kg), BADGE (ND–3.4 µg/kg), BADGE·$2H_2O$ (ND –247 µg/kg), BADGE·HCl·H_2O (0.2–196 µg/kg), BADGE·HCl (ND–3 µg/kg), and BADGE·2HCl (ND–25.7 µg/kg).

2.3.4. Summary

The above sections provide a detailed overview of the main bisphenol compound that might be present in preserved food, parameters affecting the migration of these compounds and regulations that are set to protect the consumer from health risks due to dietary exposure.

Epoxy phenolic coating of food and beverage cans is well-known to be the main contributor to the dietary source of bisphenol compounds, including BPA, BPF, BADGE and its hydrolysis and chlorinated derivatives, as well as BFDGE and its hydrolysis and chlorinated derivatives. It appears that BPA and BADGE.2H_2O are the dominant bisphenol compounds found in canned food.

The prime cause behind the migration of unreacted free-monomers from can coating is the high heat treatment of canned food or sterilization (up to 80% of free-monomers are released). After sterilization, storage time and temperature have a minor effect on the migration of bisphenols. For the other possibly influent parameters (nature of food/food simulant, coating type and characteristics, can damage), results reported from literature may be contradictory, so that their effect is still not clear.

Many studies were conducted on food simulants due to the simplicity of such matrices for further bisphenolic compounds analysis (less sample treatment is required). However, since food simulants may overestimate the migration of bisphenolic compounds as compared to real foods, it is necessary to complement migration studies in simulants with experiments on real canned foods.

2.4. Global summary

After deep research in literature it is noticed that intensive work have been carried on migrants, either from the coating or from the underlying metal substrates. However, to our best knowledge, migration tests that do simultaneously consider both bisphenol compounds and trace metals for the same canned food or simulant are rare. It is extremely interesting to analyze both bisphenols and trace metals in same canned products as, unlike other packaging, food cans are common source of both types of contaminants. Besides, such a study could reveal a probable correlation between the quantity of organic and metal migrants.

3. ANALYTICAL METHODS FOR DETERMINING MIGRANTS FROM PACKAGING

3.1. Trace metals

3.1.1. Sample treatments

To our best knowledge, the quantification of trace metals and migration tests in literature are directly carried on real food rather than food simulant due to the possibility of total destroy of food matrices, enabling quite simple trace metal determinations.

Sample preparation of food matrices (solid samples) for metal elemental analysis consists of several stages prior to instrumental analysis. In most cases these stages include homogenization (mixing, crushing, etc.) followed by a sub-sampling, mineralization, and dissolution of a subsample. The obtained solution is finally filled-up to a fixed volume. Some of these steps performed in the laboratory may be sources of contamination due to the type of vessels, purity of reagents and water used. Thus it is highly recommended to use analytical grade chemicals and ensure a clean environment in the laboratory **[171]**.

The most frequent methods for solid food preparation is mineralization, which results in a complete destruction of organic matter and total recover of metal elements. Extraction methods are also available in literature, however their use is less often. These methods can lead to incomplete recovery of targeted metals, yet, recently, their use has been attractive due to the offered advantages that are propelled by the reduced consuming time and contamination **[172]**.

3.1.1.1. Complete mineralization methods

3.1.1.1.1. Dry ashing

Dry ashing is usually performed by placing 0.1–1 g of the sample in an open crucible, and the organic matter is removed from samples through thermal decomposition at typically 450-550°C in muffle furnace. Ash residues are obtained after 4 to 16 h, and then dissolved in an appropriate acid **[172]**.

The loss of analytes by volatilization is a limiting factor and depends on the applied temperature, the form in which the analyte is present in the sample, and the chemical environment in the ashing stage. Volatilization of analytes can be overcome by using oxidizing reagents (such as high-purity magnesium nitrate and magnesium oxide), that also speed up the ashing process. However, the addition of an ashing aid can increase the content of inorganic salts significantly, which might be a problem for the subsequent determination of trace elements. In addition it might also contribute to contamination, necessitating careful blank control **[171]**.

The advantages of dry ashing methods are: (1) simple approaches, (2) large quantities of food samples may be treated at the same time that permits the pre-concentration of trace elements in the final solution, and (3) the ash is completely free of organic matter, which is a prerequisite for some analytical techniques **[172]**.

3.1.1.1.2. Wet ashing

Wet digestion is used for the decomposition of organic food matrices by dissolution of the trace metal elements. Wet digestion is performed by the means of concentrated acids (or their mixtures) in open vessels (like tubes and beakers) on a hot plate or in a heating block, or in closed systems at elevated pressure (digestion bombs), using different forms of energy (thermal or ultrasonic) and radiant (infrared, ultraviolet and microwave) **[171], [173]**.

Unlike dry ashing, there are wide varieties of wet digestion methods which mainly concern the reagents used and the devices. Moreover, other parameters are also considered when developing the wet digestion procedure including: sample nature, concentration and strength of the acid, acid oxidizing power and boiling point, as well as safety and purity of the reagent. Generally, HNO_3, HCl, H_2SO_4, H_3PO_4, $HClO_4$, HF and H_2O_2 are used for food samples, where concentrated HNO_3 is the most favorable oxidant for destruction of the organic matter. However, due to relatively low oxidation potential of HNO_3, it may lead to incomplete digestion of materials with organic-rich matrices (such as fats, proteins and amino acids). In this case the addition of stronger H_2SO_4 or $HClO_4$ is required. At present, the mixture of HNO_3, H_2SO_4 and H_2O_2 is a very efficient medium for different wet digestion procedures. Furthermore, aqua regia (HCl with HNO_3 (3:1)) can be used for wet digestion of food matrices, although it is widely used to dissolve soils, sediments and sludges **[174]**.

With regard to the applicability of the used reagents on the analytical instruments, it is commonly known that in all atomic spectrometric techniques nitric acid is the most desirable reagent (up to 10% or sometimes higher). Hydrogen peroxide, added in most mineralization procedures, is also rarely responsible for analytical problems **[175]**. On the other hand, the presence of hydrochloric acid is prohibited in graphite furnace atomic absorption spectrometry (GFAAS) analysis because of the possible formation of volatile and difficult-to-dissociate analyte chlorides that could cause vapor phase and/or spectral interferences **[176]**. Sulfuric acid is usually avoided, in spite of its efficiency in digestion of organic matrices, due to its high viscosity; thus it is particularly undesirable in analytical techniques where the sample introduction is by nebulization (e.g. flame atomic absorption spectrometry (FAAS) and inductively coupled plasma mass spectrometry (ICP-MS)) **[172]**.

3.1.1.1.3. Microwave assisted digestion

Microwave-assisted digestion is usually accomplished by adding strong acid to the sample in a closed vessel while raising both the pressure and temperature through microwave irradiation. Under high temperature and pressure and with low pH medium, food samples are thermally decomposed and heavy metals are released in solution**[177]**.

This technique is an attractive method, especially for small samples. Microwave digestion is widely used with nitric or hydrochloric acid, with or without the addition of hydrogen peroxide for the dissolution of food samples. This technique provide key advantages over heating on hot plate such as much shorter digestion times and reduced need for aggressive reagents to obtain complete digestion **[172]**. Yet, extreme care should be taken upon using sealed pressure vessels since vessels rupturing can occur during microwave digestion. Moreover, the applicability of this technique is strictly dependent on the type of food: for instance carbohydrates are easily mineralized with nitric acid at 180°C, while fats, proteins, and amino acids cause incomplete digestion due to the relatively low oxidation potential of nitric acid at 200°C. In case of incomplete digestion, sulfuric and/or perchloric acid is required with all the problems related to their use at high temperature and pressure **[172]**.

There are two different systems available for microwave digestion, pressurized closed-vessel systems and open focused-microwave systems that work under atmospheric pressure. Microwave-assisted digestion in closed vessels minimizes the risk of sample contamination and loss of volatile elements that are limiting factors open-vessel digestion. One of the limitations of pressurized closed-vessel systems is the time required for cooling before the vessels can be opened, which may take hours. The main advantages of focused-microwave radiation are safety, versatility, control of microwave energy released to the sample, and the possibility for programmed addition of solutions during the digestion **[173], [175]**.

3.1.1.2. Extraction methods

3.1.1.2.1. Ultrasound-assisted extraction

Ultrasound-assisted procedures are considered as other alternatives for solid sample pre-treatments. Extraction is usually performed in ultrasonic baths or with sonoprobes, which are commonly employed for extraction of organic compounds. Chemical effects of ultrasound extraction are attributed to acoustic cavitation, that is, bubble formation and subsequent disruptive action through the production of microjets at the collapse of cavitation bubble. This disturbance leads to high local temperature (5000 K) and pressure (10 GPa) gradients and to mechanical action between solid and liquid interfaces, which help in sample preparation. In ultrasound-assisted procedures diluted acid media are normally used for leaching element ions from powdered materials, thus, decreasing blank values and reagent consumption. Additionally, smaller sample amounts can be used as well **[172], [174]**.

The application of ultrasound to assist sample preparation points to some singularities that align to the feature of facilitating and accelerating sample preparation steps such as dissolution, fusion and leaching **[174]**. This expeditious preparation has been employed for sample preparation in order to improve analytical throughput **[178]**.

However, a rigorous experimental control is strongly recommended to avoid losses of precision and accuracy. Uncontrolled ultrasound-assisted extraction can lead to a strong local temperature increase and free radical production **[172]**, which could provoke analyte loss and gross analytical errors **[179]–[182]**.

3.1.1.2.2. Slurry Sample Preparation

Slurry sample preparation and direct solid sampling are aimed for direct analysis of complex matrices by AAS **[174]**. Slurry sampling was considered to have certain advantages over direct solid sampling, since it is possible to change the slurry concentration by simple dilution, hence combining the advantages of solid and liquid sampling. Another advantage that has been claimed is that aqueous standards may be used for calibration **[172]**.

Slurry sampling means preparation of a suspension of solid powdered particles of a sample in a liquid phase. Usually, after grinding the solid sample, the slurry is formed in water or in diluted acid (mainly HNO_3) in order to partially or totally extract analytes to the aqueous phase.

Main advantages of the slurry sampling procedure are: elimination of time-consuming step of sample dissolution, avoid the use of concentrated reagents and dilutions introducing contaminants, safety and simplification of operation, minimize losses of analytes (especially volatile) and possibility of use of smaller amounts of samples (1-100 mg). Nevertheless, several disadvantages affecting accuracy and precision of measurements and such variables as: stabilization of the slurry, its homogeneity, sample particle size and sedimentation must be carefully considered. To avoid these disadvantages slurried samples must be stirred periodically by magnetic stirring which helps to avoid sedimentation of sample particles and settling of solid particles. Moreover, the presence of larger particles is most critical factor in analysis **[174]**.

3.1.1.2.3. Direct solid sampling analysis

Another good alternative to wet digestion procedures used in elemental analysis is direct solid sampling (DSS). In fact, this technique is a widely used technique in metallurgical laboratories. DSS technique can be combined with AAS, ICP-OES or ICP-MS. Nowadays, direct analysis of solid samples can be performed using GFAAS because of the absence of a nebulizer system, which simplifies the introduction of the solid material into the atomizer. In most cases aqueous standards can be used for calibration.

Main attributes of this method are: low detection limits, minimal sample manipulation, operational simplicity, short time required to obtain results, higher accuracy as errors due to analyte loss and/or contamination are dramatically reduced, higher sensitivity due to the lack of any sample dilution and absence of any corrosive or toxic waste. The drawbacks of DSS approach are associated with: quite short linear working ranges in AAS, which limits analysis to determination of low concentrations and thus low sample weights are recommended, the natural samples inhomogeneity results in precision of order of 10% and enhanced interferences as compared to analysis of dissolved samples, where matrix is simplified upon mineralization. The later can therefore lead to overestimation or underestimation of final results **[183]**.

3.1.2. Analytical techniques

Many instrumental analytical methods may be employed to measure the concentration level of heavy metals in food. The most predominant techniques are atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS).

3.1.2.1. Atomic Absorption Spectrometry (AAS)

AAS is a quantitative method of metal analysis suitable for determining up to 70 elements. This method measures the concentration of the element from the reduction in the amount of light intensity of specific wavelength after passing through cloud of atoms from a sample. Atoms will absorb light emitted by a radiation source of a particular element known as hollow cathode lamp (HCL).

A typical AA spectrometer consists of energy (light) source, sample compartment (atomizer), monochromator, detector and a data process system. Different atomizers are used in various AAS techniques such as flame and graphite furnace **[184]**.

3.1.2.1.1. Flame Atomic Absorption Spectrometer (FAAS)

FAAS is a suitable technique for determining metals at part per million (ppm) concentration levels with good precision. FAAS atomizers are air-acetylene and/or nitrous oxide flame. Samples are introduced into the atomizer as an aerosol by the nebulizer. FAAS technique provides fast analysis of 10-15 s per sample, with very good precision (repeatability), moderate interferences that can be easily corrected, and relatively low cost **[185]**.

However, the analyte can form complexes with other elements in the sample solution, producing refractory compounds. This can be prevented by adding an excess of another element which will combine with the interferent in preference to the analyte. This will remove the interference. For example lanthanum or strontium nitrate can be added to solutions containing phosphorous, which allows atomization in air-acetylene flame without phosphate interferences.

Another signal suppressor on FAAS is the ionization of the analyte element in high temperature flames such as nitrous oxide/acetylene. In this case the addition of alkali and alkaline-earth metals can be more susceptible to ionization than the transition elements. The addition of readily ionized elements such as sodium, potassium and cesium at concentrations between 2000 and 5000 µg/mL creates an excess of electrons in the flame and effectively suppresses ionization of the analyte **[186]**.

3.1.2.1.2. Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)

GFAAS is an appropriate atomization technique used to determine analytes concentrations in samples with an acceptable precision of detection limit at the parts per billion (ppb) levels. In this technique, samples are dispensed into a graphite tube which is programmed to be heated in series of steps, including drying, ashing and atomizing to remove the solvent and matrix components and to atomize the remaining sample. The atomized sample carried by an inert gas (argon) and retained within the tube and the light path for a prolonged period of time, which leads to an improvement in sensitivity **[187]**.

Analysis of some metals in GFAAS requires the use of matrix modifiers that are added to the sample before injection or injected to the atomizer together with the sample. These compounds affect the thermal processes taking place in the atomizer to minimize losses of analyte during pyrolysis, enable more effective matrix components removal, and minimize chemical interferences. Some modifiers change the sample matrix to evaporate the matrix components at lower temperature; other type of modifiers work as an analyte stabilizer.

Various salts and metals have been utilized as matrix modifiers, such as sulfates or phosphates that form less volatile salts with analyte and prevent the formation of volatile salts such as halides. Modifiers liked NH_4NO_3 convert a non-volatile matrix NaCl to a volatile compound **[188]**. Ascorbic acid is also added to enhance the reduction and distribution of the analyte metals into small drops. A further example on matrix modifiers is the addition of metals such as Pd, Ni and Pd-Mg that increase retention times and keep the analyte element at the ground state level during the atomization process thus enhancing the absorbance **[189]**. Different modifiers were reported in literature, for example $Mg(NO_3)_2$ was used in the analysis of Al in alcoholic beverages **[190]**, while H_3PO_4 or $NH_4H_2PO_4$ are efficient in analysis of Pb and Cd that permits a higher ashing temperature and stabilizes the signal **[191]**.

GFAAS has a very good detection limits for majority of elements, as a small sample size of 20 µl is required for analysis. However, the technique has some disadvantages like limited working range, slow analysis, and high cost **[184]**.

Background absorption in AAS is an attenuation of the analytical line radiation, which is not due to absorption by free atoms of the element. This is mainly caused by:

- Light scattering on solid particles of dry aerosol or particles of carbon resulting from the incomplete burning of acetylene in reducing flame. In GFAAS the non-volatile components of the sample matrix cause this effect during atomization if they were not removed during pyrolysis.

- Absorption of the radiation by molecules and molecular fragments (alkali halogenides) or fine structure rotational and vibrational transitions of radicals, molecules or molecular ions and fragments **[188]**.

There are several ways to control the background interferences due to light scattering by particles in the flame, including two-line background correction, continuum source or deuterium lamp background correction, Zeeman background correction, pulsed lamp background correction, and wavelength-modulation correction methods. Both deuterium lamp and Zeeman background correction are widely used **[192]**.

Background correction by continuum source is performed through measuring two signals: the total absorption (measured by HCl) and the non-specific absorption (measured with the continuum source of either D2 lamp (most effective up to 350 nm) or halogen bulb with tungsten filament (in the region of visible light)). Almost all FAAS are equipped with deuterium background correction. On the other hand Zeeman background correction is based on splitting the absorption lines in magnetic field. Zeeman background correction is mainly used for GFAAS **[188]**.

3.1.2.2. *Inductively Coupled Plasma Mass spectrometry (ICP-MS)*

ICP-MS is a quantitative multi-element measuring system that offers wide detection range of elements. ICP-MS uses an argon plasma source to dissociate the sample into its basic atoms or ions. The liquid sample is pumped from a vial into the plasma torch via a peristaltic pump were high number of ions are produced. The released ions pass from the plasma into the mass spectrometer where they are isolated according to their atomic mass-to-charge ratio by a quadrupole or magnetic sector analyzer. Accordingly, metal ions are detected rather than the light that they emit. ICP-MS provides good detection limits for most elements, normally in the parts-per trillion (ppt) ranges **[193]**.

3.1.2.3. *Other analytical techniques*

Other analytical techniques include atomic emission/ fluorescence spectrometry (AES/AFS), hydride generation atomic absorption spectroscopy (HGAAS), cold vapor atomic absorption spectrometer (CVAAS) (especially used for analysis of mercury at room temperature), inductively coupled plasma optical emission spectrometry (ICP-OES), neutron activation analysis (NAA), X-ray fluorescence (XRF), and striping voltammetry (AVS) **[184]**.

3.1.3. *Overall method performances*

Wide varieties of method preparation and analysis techniques are available in literature for the determination of metal elements in foodstuffs (see **Table 1.8**), including vegetables and fruits **[57], [100], [101], [111], [194]–[197]**, fish **[9], [107], [113], [198], [199]**, meats **[9], [116]** and others **[57], [197], [200], [201]**. Some methods aimed to totally decompose the organic food matter while others developed extraction methods to isolate trace metals from food matrices. Many authors used microwave assisted digestion for samples mineralization **[57], [100], [101], [107], [111], [194], [197], [200]**. Indeed microwave digestion is preffered over open system wet digestion on hot plate and dry ashing **[197], [200], [202]** since it offers good precision (%RSD <10), better recovery (mostly 95-100%), shorter time (about 30 min for microwave digestion, 4 h for wet digestion and 8-16 h dry ashing), wet and microwave digestions, respectively), prevent the loss of volatile elements and present less interferences **[197], [202]**. Nevertheless, wet digestion and dry ashing are still efficient and applicable methods for extraction/release of metals and trace elements, from various food matrices **[9],**

[113], [195], [198], with good performance as shown in **Table 1.8**. On the other hand, in view of reducing the time consumed during mineralization step and contamination problems arising during sample preparation, some studies aimed to develop extraction preparation methods including ultrasound-assisted extraction **[179]**, direct solid sampling **[201]** and slurry sampling **[199], [203]**, showing that they can be good alternatives for microwave digestion, yet still representing some limitations such as essential homogeneity of particles and homogenous distribution of the elements in the particles to obtain high precision and accuracy. In general, methods developed in previous studies were validated using certified reference materials including NIST-SRM 1515 apple leaves **[200]**, NIST SRM 1573a tomato leaves **[197]**, NIST-SRM 8418 wheat gluten **[202]**, NIST 1566b oyster tissue **[203]**, etc.

According to **Table 1.8**, AAS is the most used technique for the determination of metal elements. As previously discussed GFAAS provides better sensitivity and detection limits than FAAS (10 to 1000 times lower LOD). Although ICP-MS provides better sensitivity to metal elements (up to 10000 times lower LOD compared to FAAS and up to 100 times compared to GFAAS) and multi-elemental analysis, yet it is less often used compared to AAS.

Table 1.8: Method performance for metals determination in food samples found in literature

Samples	**Elements**	**Preparation Method**	**Analysis Technique**	**Detection limit**	**%RSD**	**Recovery (%)**	**Linearity regression**	**Ref.**
Canned chickpeas	Fe, Sn, Pb, Cd	Microwave assisted digestion with HNO_3 (65%) and H_2O_2(30%)	FAAS: Fe, Sn GFAAS: Cd, Pb	Fe: 2 Sn: 8.5 Pb: 0.059 Cd: 0.003 (mg/kg)	<10	80-102	0.9977-0.9994	[101]
10 different canned foods (mostly vegetables)	Cu, Zn, Mg, Fe, Se, Al, Cr, Ni, Co	Microwave assisted digestion with HNO_3 (65%) and H_2O_2(30%)	FAAS: Cu, Zn, Fe, Mn GFAAS: Se, Al, Cr, Ni, Co	Cu: 0.013 Zn: 0.019 Fe: 0.011 Mn: 0.010 (mg/L) Elements analyzed by GFAAS had much lower detection limits	<10	95-99	NA	[111]
Vegetables	Ni, Cr, Cd, Pb, Cu, As	Microwave assisted digestion with HNO_3 (65%)	GFAAS	Ni: 0.045 Cr: 0.010 Pb: 0.012 (mg/kg)	<20	81.1-94.1	NA	[194]
Canned fruits	Sn, Cr, Cd, Pb	Wet digestion by heating with HNO3 and HCl until complete decomposition	Sn: ICP-OES Cr, Cd, Pb: ICP-MS	Sn: 0.024 Cr: 0.002 Cd: 0.004 Pb: 0.003 (mg/kg)	6-11	94-102	NA	[195]
Canned orange juice, mango pulp, baked beans, sweet corn cream layer	Al, Fe, Mg, Si, Sn	Microwave assisted digestion with HNO_3 (65%) and H_2O_2(30%)	ICP-AES	NA	NA	> 95	NA	[100]
Fruit Juice and soft drinks	Cu, Fe, Pb, Zn	Dry ashing in muffle furnace at 550°C for 8 h	FAAS	Cu: 0.02 Fe: 0.03 Pb: 0.1 Zn: 0.005 (mg/L)	NA	94-97	>0.99	[196]
Wide variety of foods (81 food items)	Co, Cu, Fe, Mn, Ni, Zn	Sample drying in oven at 105°C + Microwave assisted digestion with HNO_3 (65%) and H_2O_2(30%)	ICP-MS	Fe: 0.1 Other elements: 0.0005 (mg/kg)	< 20	82.1-120	NA	[57]

NA: not available

Table 1.8 (Continued): Method performance metals determination in food samples found in literature

Samples	Elements	Preparation Method	Analysis Technique	Detection limit	%RSD	Recovery (%)	Linearity regression	Ref.
Wide variety of foods	Fe, Cu, Mn, Zn, Al, Se	Dry ashing in muffle furnace at 450-500°C for 16 h Wet digestion by heating with 2:1 HNO3:H2O2 for 4h Microwave assisted digestion with 2:1 HNO3:H2O2	Fe, Cu, Mn, Zn: FAAS Al and Se: GFAAS	Cu: 0.013 Zn: 0.019 Fe: 0.011 for Fe, Mn: 0.010 (mg/L)	<10	Dry ashing:30-96 Wet ashing: 70-96 Microwave assisted digestion: 96-99	NA	[197]
Wide variety of foods	Fe, Zn, Cu, Al, Sn, Cr, Pb, Cd, Hg	Wet digestion by heating with HNO_3	Cd, Cr, Cu, Fe, Pb, Zn: GFAAS Al, Hg, Sn: ICP-MS	NA	NA	94-102	NA	[9]
Canned tuna	Fe, Sn, Cd, Pb, Zn, Cu, Hg	Microwave assisted digestion with HNO_3 (65%) and H_2O_2(30%)	ICP-MS	Fe: 0.1 Sn: 0.0005 Cd: 0.0001 Pb: 0.0001 Zn: 0.00015 Cu: 0.0001 Hg: 0.00015 (m/kg)	NA	Average of 102.9	NA	[107]
Canned tuna	Hg, As, Sn, Cd, Pb	Hg: Wet digestion by heating with HNO_3 + H_2SO_4 As: Wet digestion by heating with HNO_3 + dry ashing Cd and Pb: Wet digestion by heating with HNO_3 Sn: Wet digestion by heating with HNO_3 + HCl	Hg and As: Hydride generation Cd and Pb: GFAAS Sn: FAAS	NA	NA	91.7-99.3	NA	[113]

NA: not available

Table 1.8 (Continued): Method performance metals determination in food samples found in literature

Samples	**Elements**	**Preparation Method**	**Analysis Technique**	**Detection limit**	**%RSD**	**Recovery (%)**	**Linearity regression**	**Ref.**
Fish	Pb, Cd, Fe, Cu, Mn, Zn	Wet digestion by heating with concentrated HNO_3 in a high pressure digestion bombs	GFAAS	Cu: 0.36 Mn: 0.23 Zn: 0.25 Fe: 0.42 Pb: 0.98 Cd: 0.065 (mg/L)	< 7	95	NA	**[198]**
Fish	Ca, Cu, Fe, Mg, Zn	Slurry sampling with HCl, HNO3, NaOH, and disodium ethylenediaminetetraacetate	FAAS	Ca: 22.8 Cu: 0.884 Fe: 5.07 Mg:35.5 Zn: 1.17 (mg/kg)	1.3-11.1	95.5-111	NA	**[199]**
Fish and mussel	Cu, Zn	30 min sonication with 1:1:1HNO_3(4 M):HCl(4 M):H_2O_2(0.5 M)	FAAS	Cu: 0.13 Zn: 0.63 (mg/kg)	13-19	80.9-87.2	0.998-0.999	**[179]**
Meat products	Cd, Pb	Sample drying at 70°C overnight + Microwave assisted digestion with HNO_3 (65%) and H_2O_2(30%)	GFAAS	NA	NA	80-120	0.9954-0.9998	**[116]**
Honey	Cd, Pb, Fe, Mn, Cu, Ni, Cr, Zn, Al, and Se	Dry ashing in muffle furnace at 450°C for 8 h Wet digestion by heating with 2:1 HNO3:H2O2 for 4h Microwave assisted digestion (HNO3 (65%)+H2O2(30%))	Fe and Zn : FAAS Other metals : GFAAS	Zn: 0.009 Fe: 0.005 (mg/ L) lower LOD for Pb, Cd, Cu, Cr, Ni, Al, Se and Mn	NA	Dry ashing:40-94 Wet ashing: 80-97 Microwave assisted digestion: 96-102	NA	**[200]**
Green coffee	Co, Cu, Mg	Direct solid sampling	GFAAS	Co: 0.012 Cu: 0.006 Mg: 0.004 (mg/kg)	<10	>95	NA	**[201]**

NA: not available

3.2. Bisphenol compounds

3.2.1. *Sample treatment*

The determination of bisphenol compounds in foods and food simulants often requires extensive sample preparation prior to instrumental analysis. The typical preparation steps include pre-treatment, extraction, clean-up, concentration and sometimes derivatization. Moreover, special treatments can be required depending on the matrix composition, for example, protein precipitation and freeze drying. In case of canned foods, both liquid and solid portion can be mixed and analyzed as one sample, or can be analyzed separately after filtration **[204]**.

As mentioned in previous sections, several migration tests have been carried on food simulants due to their great advantage of being easy to handle and analyze **[27], [152], [157], [205]**. However, many authors preferred to work directly on real food to give more reliable results. Working with either foods or simulants requires distinct sample treatment and extraction methods as shown in **Table 1.9**.

Table 1.9: Analytical extraction methodologies of bisphenol compounds between solid foods and food simulants

Sample analyzed	Solid foods	Food simulants and liquid foods
Pre-treatment	Homogenization Freeze drying	Centrifugation Filtration
Extraction	SE, MAE, PLE, MSPD	LLE, SPE, SPME, SBSE

SE, solvent extraction; MAE, microwave-assisted extraction; PLE, pressurized liquid extraction; MSPD, matrix solid-phase dispersion; LLE, liquid–liquid extraction; SPE, solid-phase extraction; SPME, solid-phase microextraction; SBSE, stir bar sorptive extraction **[204]**.

The obtained extracts from both foods or simulants are purified in the same way mainly through liquid-liquid extraction (LLE) or solid phase extraction (SPE) **[204]**.

3.2.1.1. *Solvent extraction (SE) and liquid–liquid extraction (LLE)*

Through allowing the soluble components to be removed from the solid sample by the aid of an appropriate solvent, solvent extraction (SE) approach is the most common technique used to isolate bisphenol compounds from solid foodstuffs. In a similar way, liquid–liquid extraction (LLE) is the simplest technique for treatment of liquid foods, through the ability of the solute to distribute itself between the aqueous solution and the immiscible organic phase **[204]**.

The typical sample size required is in the ranges 0.5–30 g (with 5 g being the most frequent **[148], [206], [207]**) and 10–50 mL **[155], [208]**. Nevertheless, higher sample sizes have also been reported, e.g. 120 g for canned jalapeno peppers **[23]** and 500 mL for mineral water **[209]**.

Among extraction solvents, acetonitrile is usually preferred in case of solid foods **[155], [207], [208]** although others like acetone **[207]**, methanol **[23], [148]** and ethanol **[210]** may also extract bisphenol compounds efficiently. Aqueous liquid foods are commonly extracted with ethyl acetate, chloroform **[204]** or dichloromethane **[211]**.

As a general rule, repeated extractions are usually necessary to ensure complete extraction. The typical overall solvent consumption is between 40 and 300 mL, while extraction times range from 10 to 120 min. Indeed, extraction by a solvent requires mechanical stirring or sonication to favor equilibrium partition. Anhydrous sodium sulphate is frequently added to the sample **[155], [207], [208], [212]** in order to remove undesirable trace amounts of water in the organic layer.

Solvent-based extractions have a limited selectivity due to the complexity of food matrices which, therefore, need an extensive clean-up prior to instrumental analysis. For instance lipids, originating from foods like fish meats, can significantly reduce the analytical performance on liquid and gas chromatography. The removal of lipids from the extract can be achieved by liquid–liquid extraction with n-heptane **[155]**, trimethylpentane **[208]** and n-hexane **[148], [209], [210], [212]** or by freezing the lipids in the extract at −24°C for 40 min followed by filtration **[204]**.

3.2.1.2. Microwave-assisted extraction (MAE)

MAE constitutes a good alternative to the solvent extraction of solid food samples. This technique is based on the application of microwave energy to the sample during extraction, which is consequently agitated and heated quickly. Therefore, good extraction efficiencies can be achieved using less solvent and shorter extraction times.

The use of MAE as extraction method was limited in literature **[204]**, but Hong Zhang et al. **[213]** proved that MAE approach for extraction of BPA, NOGE and BADGE derivatives from canned foods has 20-50% improved efficiency compared to LLE.

3.2.1.3. Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), involves the use of liquid solvents at elevated temperatures (40–200° C) and pressures (1000–2500 psi). Under high temperature and pressure the extraction power of the solvent is enhanced **[214], [215]**.

The use of ASE for extraction of bisphenol compounds is rare **[204]**, yet its efficiency for the extraction of BPA in matrices of animal origin like meat by dichloromethane **[216]**, or plant like cereals by methanol has been proven **[217]**.

Despite being reliable techniques for the extraction of BPA from foods, SE, LLE, MAE and ASE requires a subsequent purification step. Indeed, the use of SPE for clean-up of the extracts becomes inevitable in most of sample treatment procedures **[207], [212]**.

3.2.1.4. Solid-phase extraction (SPE)

During SPE, the bisphenol compound in a sample solution is either retained on appropriate stationary phase or eluted in the percolate. In case it is retained a subsequent washing and elution by organic phase is performed.

SPE is by far the most used technique for both the extraction of bisphenols-containing liquid foodstuffs and the clean-up of crude extracts after solvent extraction. The careful selection of a suitable sorbent for bisphenol compounds is mainly dependent on the compounds properties (bisphenol compounds, such as BPA, have moderately polar character and presence of hydrogen acceptor/donor groups) and the type of food matrix **[204]**.

3.2.1.4.1. Non-specific sorbents

The OASIS HLB sorbent has been the most reported phase for the extraction of bisphenol compounds **[212], [218], [219]**. This sorbent is a divinylbenzene/N-vinylpyrrolidone copolymer, where the hydrophilic N-vinylpyrrolidone polymer acts as a hydrogen acceptor and the hydrophobic divinylbenzene polymer provides reversed-phase retention for target compounds. OASIS HLB sorbent offers advantages over the classical silica sorbents such as the high specific area, possibility to dry out during the extraction procedure without reducing its ability to retain bisphenol compounds, and stability over the entire pH range **[220]**. The samples size ranges between 1 and 50 mL **[212], [219]** and 10 g **[204]**.

A second clean-up step with a normal-phase SPE sorbent (Florisil, a synthetic magnesium silicate) is also necessary in some applications (fish, fruit and vegetable samples **[207], [212]**). Bisphenol compounds are retained on Florisil sorbent mainly through adsorption, and the application on this sorbent is carried out from sample extracts that was previously evaporated and redissolved in a non-polar organic solvent such as n-hexane. High recoveries (above 80%) are usually obtained with SPE Florisil sorbent.

Other good-recovery methods were proposed using chemically bonded reversed-phase silica (C18) and polystyrene-divinylbenzene (PS-DVB) sorbents. Such sorbents were reported on the isolation of BPA from mineral water and wines **[221]**, canned condensed and powdered milk **[222]**, infant formula and canned vegetables and fruit juices **[223], [224]**.

3.2.1.4.2. Specific sorbents

A number of specific SPE sorbent materials have been developed for the determination of bisphenol compounds in such complex samples as foods. Some of these specific sorbents are immunoaffinity columns (IACs) and molecularly imprinted polymers (MIPs).

IACs are made by covalently bonding antibodies onto an appropriate support, which provide unique selectivity on the basis of molecular recognition. Several studies reported their use for purification of liquid foods or crude extracts for subsequent determination of BPA by LC–fluorescence detection. Such approach was applicable for BPA extraction from many foodstuffs including canned beverages, fruits, vegetables **[225], [226]**, fat- containing foodstuffs (e.g. tuna, cream, potato soup) **[226]** and wine **[227]**. The main drawbacks of IACs are the cost to produce the antibodies and the short-life of columns **[204]**.

On the other hand, MIPs, which are synthetic polymers that also have molecular recognition ability for a target analyte, offer some advantages over IACs such as stability against organic solvents, strong acids and bases and heating **[204]**. A number of approaches have been used to prepare BPA imprinted polymers, but for food applications, MIPs have been rarely proposed **[204]**. One study aimed to develop MIP-based extraction method of BPA from canned peas **[228]**.

3.2.1.5. Less common techniques

Other extraction techniques such as solid-phase micro-extraction (SPME), stir bar sorptive extraction (SBSE), and matrix solid-phase dispersion (MSPD) are capable of improving the isolation and clean-up of organic contaminants from food. These techniques have advantages in terms of solvent consumption, automation, sample handling reduction, and simplifying the extraction of solid samples. However their application to the extraction of bisphenol compounds from food is still limited **[204]**.

3.2.2. Analysis of bisphenolic compounds

The determination of bisphenol compounds in foodstuffs requires the use of highly sensitive and selective techniques due to the trace levels. The determination of these compounds is mainly carried out by liquid chromatography (LC) coupled to fluorescence detector (LC-FLD) or mass spectrometry (LC-MS), and by gas chromatography coupled to mass spectrometry (GC-MS). Liquid chromatography offers the advantage of simplicity over GC for which derivatization step is necessary, while the latter provides higher peak resolution. Other techniques like LC–electrochemical detection (LC–ED) and immunoassays have been used in a lesser extent **[204]**.

3.2.2.1. Liquid Chromatography

The use of ultra high performance liquid chromatography (UHPLC) corresponds to the use of stationary phases consisting of small porous particles with particle sizes of sub-2 µm that significantly improve the resolution per unit time and the analytical run-time. Therefore, the available columns are now reduced in lengths with stationary phases that can withstand very high pressures (up to 1000 bar) **[229]**.

The separation and determination of bisphenol compounds are usually carried out in reversed-phase C18-silica columns **[204]**. However, the use of C18-PentaFluoroPhenyl (PFP) phase provides selectivity for challenging applications: this stationary phase combines a C18 chain with integral PFP functionality, resulting in a phase that maintains the hydrophobic stability and low bleed characteristics of C18-silica phases, yet providing the multiple retention mechanisms of a PFP phase (hydrophobic, π-π interaction, dipole-dipole, hydrogen bonding and shape selectivity) that are responsible for the unique selectivity of the C18 – PFP stationary phase **[230]**.

Mobile phases vary according to the detector coupled to LC. Water and acetonitrile are the most common binary solvents when fluorescence detection is used, while water and methanol are preferred for electrospray ionization (ESI)-MS. LC is usually performed at room temperature but temperatures up to 40°C are sometimes recommended to reduce analysis time and increase the reproducibility **[204]**.

3.2.2.1.1. Fluorescence detection (FLD)

Most bisphenol compounds show native fluorescence with excitation and emission wavelengths around 275 and 305 nm, respectively, which keep constant in the more frequently used solvents in LC mobile phases (i.e. water, acetonitrile and methanol).

The fluorescence intensity of bisphenol compounds is, in fact, much higher in organic media and thus the sensitivity in LC will be dependent on the mobile phase composition. The identification of these compounds in complex sample matrices is only based on retention times, therefore, the possibility of interference from other fluorescent food migrants from can coatings, e.g. other non-targeted bisphenol compounds or NOGE, should always be considered since they may produce false-positives. Indeed, confirmation by LC-MS after quantification by LC–fluorescence detection is essential **[204]**.

3.2.2.1.2. Electrochemical detection

The electrochemical detection (ED) of bisphenol compounds is based on the electroactivity of the phenolic groups present in the molecules. The pH and electrolyte content of the mobile phase influence the electron transfer rate constants, so they have to be optimized in order to maximize the sensitivity. In fact, a main drawback of ED is the recommended isocratic elution **[209]**; otherwise large equilibrium times will be required for measurements. LC-ED has been used for the analysis of BPA in food simulants (water, acidified water and water:ethanol) **[231]**, yet it was rarely applied to common complex food matrices **[204]**.

3.2.2.1.3. Mass spectrometry detection

Mass spectrometry (MS) provides both qualitative (structure) and quantitative (molecular mass or concentration) information on analyte molecules after their conversion to ions **[232]**. The use of mass spectrometry can reduce sample preparation; yet, clean sample extracts are preferred because they prolong the life of the column, reduce the maintenance of the apparatus, enhance ionization efficiency and reduce the background noise **[204]**. Triple quadrupole (QqQ) mass analyzers are the most popular instruments, because of their higher sensitivity and selectivity when operated in selected-reaction monitoring (SRM) mode.

The ionization in LC-MS is carried out using atmospheric pressure ionization interfaces, namely electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). ESI is more often used than APCI since it offers better sensitivity for the determination of bisphenol compounds (e.g. reported instrumental quantitation limits for BPA were of 5 µg/L using ESI(-) [65] and 20.7 µg/L using APCI(-) [53]). On the other hand, ion-trap instruments have not been used frequently for the analysis of BPA in food samples, but ESI(-) ion-trap MS was at least six times more sensitive than APCI for determination of BPA in environmental samples **[204]**. Moreover, response in ESI is strongly dependent on the composition of the mobile phase. For example, methanol-water mobile phases give higher response for BPA standard solutions than those consisting of acetonitrile–water due to the lower boiling point of the former, which favor the desolvation of the electrospray droplets. Addition of 0.01% of ammonia or 0.01% acetic acid modifiers to acetonitrile–water results in 3 to 40 enhancement in the response. On the other hand, in some cases (not always), the addition of 0.1% ammonia to methanol-water can increase the response for bisphenol compounds **[204]**.

In order to solve the problem of matrix interference, improving sample-treatment procedures and/or resolution of the chromatographic separation (i.e. using smaller particle-size columns) or modifying the gradient elution can be helpful **[233]**. The use of high resolution MS (HRMS) is also an alternative. In the meantime, the addition of an internal standard is always needed to overcome sample preparation losses and matrix effects (suppression or enhancement of the signal), which lead to low method absolute recoveries; the most used internal standards for bisphenol compounds have been 4-nonylphenol or stable isotope labelled $^{13}C_{12}$-BPA or $^{2}H_{16}$-BPA (also written BPA-d16) **[204]**.

The negative ionization mode gives the best sensitivity for the detection of BPA and other bisphenols (e.g., BPE, BPB, BPF and BPS) that is dominated by the deprotonated molecule, $[M-H]^-$. Regardless of the type of analyzer and ionization source, the most abundant ion in the BPA mass spectrum is $[M-H]^-$ *m/z* 227. Further, *m/z* 211 and 212 fragmentations are also obtained in quadrupole mass spectrum that are formed by the additional loss of oxygen, $[M-H-O]^-$, and a methyl radical, $[M-H-CH_3]^{\bullet-}$, respectively. Indeed, the *m/z* 212 was the most prominent product ion obtained by LC–MS/MS, so it is often used for confirmation and/or quantification of BPA. Using mobile phases of acetonitrile + 0.01%NH_3 in water, an ion with *m/z* 113 can be detected in the BPA mass spectrum, which is related to the loss of both acidic protons $[M-2H]^{2-}$. Other fragments of lower relative abundance were reported in the MS^2 ion-trap spectrum, namely the ion $[M-H-C_6H_5OH]^-$ *m/z* 133, resulting from the cleavage of the hydroxybenzyl group, and the ion $[M-H-C_9H_{10}O]^-$ *m/z* 93, formed by the loss of hydroxyphenyl propyl **[204]**.

BADGEs and BFDGEs show a high response in ESI+ mode, where they tend to form clusters of $[M+Na]^+$, $[M+K]^+$, $[M+NH_4]^+$ and $[M+ACN]^+$ ions. Yet, some of these cluster ions, such as $[M+Na]^+$, are very stable and don't undergo further fragmentation in MS/MS, whereas, efficient fragmentation occurs for ammonium adducts with a stable signal under MS/MS. In this case, formic acid/ammonium formate buffer is generally used as an additive in the mobile phase in ESI+ to enable the formation of ammonium adducts and ensure signal reproducibility for the analysis of BADGEs and BFDGEs compounds **[234]**. The fragmentation of $[M+NH_4]^+$ for BADGEs starts with the cleavage of the phenyl-alkyl bond, which is followed by the α-cleavage of the ether group to generate the characteristic product ions at *m/z* 135, $[C_9H_{11}O]^+$,and *m/z* 107, $[C_7H_7O]^+$. The *m/z* 107 is also a common product ion for the loss of one phenyl-glycidyl ether of BFDGEs **[234]**.

3.2.2.2. *Gas chromatography*

GC–MS is the most sensitive technique for the determination of BPA in foods; it provides higher resolution and lower detection limits than LC–MS. However, the main drawback for GC-based methods is the need for derivatization step that introduces new sources of errors, mainly due to contamination. Additionally, the presence of lipids can significantly reduce the analytical performance of GC **[235], [236]**, therefore, extensive clean-up is required for fatty foods, such as fish **[148]** Silylation and acetylation have been by far the most used derivatization procedures, which are usually carried out by adding 100–200 µL of the corresponding reagent to the dried extract and allowing the mixture to stand for 30–60 min under room temperature or at 65–80°C. Silylation of the active hydrogens of BPA is mainly made using N-O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) **[210], [237]**, while acetylation of the hydroxyl groups of BPA with acetic anhydride **[155], [208]** or trifluoroacetic anhydride **[211]** is frequent procedure for BPA derivatization.

GC–MS with electron impact (EI) ionization has widely been used for the confirmation of BPA in food analysis **[148], [224], [231]**. Like in LC-MS methods, the use of an internal standard is common (usually BPA-d16 and BPA-d14).

3.2.3. Overall method performance

Several research groups have developed various suitable analytical methodologies for the determination of bisphenol analytes in various types of canned foods as well as in food simulants. The reversed-phase high performance liquid chromatography technique using fluorescence detection (LC-FLD) was a common analytical tool **[23], [30], [145], [161], [169], [238], [239]**. In order to measure bisphenol. compounds (mainly BPA) without the effects of interfering food components, GC-MS was also utilized after chemical derivatization of the analytes (e.g. with acetic anhydride) to improve peak shapes and robustness of the method **[23], [147], [153], [240]–[243]**. Mass spectrometry was frequently used in these study for confirmation of the detected compounds **[23], [35], [127], [147], [152], [153], [161], [164], [166], [169], [238], [240]–[243]**, while other detectors such as UV **[169], [206]**, DAD **[152], [161]**, and ECD **[166]** are also available in literature.

In some studies the preliminary sample treatment step is freeze drying **[35], [153]** which facilitates the extraction of bisphenol compounds and allows storing the food samples for a long time without spoilage. Then the first step in food samples preparation is solvent-assisted extraction that is usually performed using solvents such as acetonitrile (ACN) **[23], [35], [147], [153], [164], [166], [169], [238]**, methanol **[23]**, ethyl acetate **[30], [35], [127]**, etc., or a mixture of two solvents **[30], [161]**. In most studies, solvent extraction is followed by SPE where purification on HLB cartridges is most operated **[127], [147], [166], [206], [242]**. Reversed-phase silica (C18) has also been proposed as a SPE sorbent **[169]**, yet HLB cartridges are more efficient **[206]**. Further purification on Florisil cartridges is sometimes required. For fat removal, lipid freezing **[242]** and washing with hexane **[30], [153]** are proved to be efficient. The results of the developed methods are summarized in **Table 1.10**.

Table 1.10: Method performance of determination of bisphenolic compounds in food samples found in literature

Samples	**Bisphenol compounds**	**Preparation method**	**Analysis technique**	**Detection or Quantification limit**	**Recovery (%)**	**RSD (%)**	**Linearity regression**	Ref.
Canned vegetables and fruits	BPA	SPE with OASIS-HLB cartridge Clean up on Florisil cartridges	HPLC-UV at 228 nm	LOQ : 5- 10 µg/kg	87.1-90.1	<5	0.997	[206]
Canned vegetables	BPA, BADGE, BADGE.2H_2O, BADGE.2HCl, BFDGE, and BFDGE.2H_2O	SPME with microextraction with (CW) microfibers	HPLC-FLD at 275 and 30 nm excitation and emission wavelengths, respectively.	LOD: 0.7-2.4 µg/L	27-90	14-32	0.9106-0.9991	[239]
Canned peeled tomatoes	BPA and BPB	Solvent extraction with ACN SPE with C18 Strata E cartridge followed by Florisil cartridge	HPLC- UV/FLD LC-MS ([M - H]-): BPA: 227 BPB: 241	HPLC- UV: BPA: 20 BPB: 15.4 (µg/kg) HPLC-FLD: BPA: 1.1 BPB: 0.7 (µg/kg)	NA	HPLC- UV: 0.04-2.2 HPLC-FLD: 0.04-2.82	HPLC- UV: BPA: 0.9994 BPB: 0.9996 HPLC-FLD: BPA: 0.9966 BPB: 0.9974	[169]

NA: not available

Table 1.10 (Continued): Method performance of determination of bisphenolic compounds in food samples found in literature

Samples	**Bisphenol compounds**	**Preparation method**	**Analysis technique**	**Detection or Quantification limit**	**Recovery (%)**	**RSD (%)**	**Linearity regression**	Ref.
Canned tomatoes	BPA	Solvent extraction with n-hexane: acetone (97:3) SPE with Florisil cartridges	HPLC-FLD at 230 nm and 315 nm excitation and emission wavelengths, respectively HPLC-DAD at 230 and 254 nm HPLC-MS/MS	LOD: 0.09 µg/kg	90.8-93.1	3.4-4.2	HPLC-DAD: 0.9975 HPLC-FLD: 0.9968	**[161]**
Canned sea food	BADGE BFDGE	Solvent extraction with n-heptane LLE with 90% acetonitrile	UHPLC-FLD at 230 nm and 305nm excitation and emission wavelengths, respectively.	BFDGE: 2.4 µg/kg: BADGE: 3.4 µg/kg:	BFDGE: 84-92 BADGE: 89-100	BFDGE: 1.8-4.9 BADGE: 1.6-2.3	NA	**[145]**
Canned tuna	BADGE and NOGE	Ultrasonication-assisted solvent extraction with ACN Fat removal with hexane from oil part	HPLC-FLD APCI(+) – MS	LOD: 5 µg/kg	81-125	<15	0.9994-0.9999	**[238]**

NA: not available

Table 1.10 (Continued): Method performance of determination of bisphenolic compounds in food samples found in literature

Samples	Bisphenol compounds	Preparation method	Analysis technique	Detection or Quantification limit	Recovery (%)	RSD (%)	Linearity regression	Ref.
Fish	BPA	Ultrasonication Freezing-lipid filtration Silyl-derivatization, with N,N'-methyl-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) SPE with HLB and Florisil cartridges	GC-MS with SIM[a] mode with monitored m/z: 357 and 441	LOD: 0.41 µg/kg	105-120	5-17	0.994	[242]
Variety of canned food (vegetables, fruits, meat and fish)	BPA and BADGE	Solvent extraction with acetonitrile SPE with OASIS-HLB column	HPLC-ECD LC-MS LC/MS/MS: (SRM)[b] BPA: ESI-precursor ion (m/z) of 227 as $[M–H]^-$ and a product ion of 212 BADGE: ESI+ precursor ion of 358 as $[M+NH4]^+$ and a product ion of 191	LOD (µg/L) BPA: HPLC-ECD: 0.2 LC/MS: 0.1 LC/MS/MS: 0.1 BADGE: LC/MS/MS: 0.05	BPA: HPLC-ECD: 71.6-137.6 LC-MS: 58.2-129.4 BADGE: LC/MS/MS: 97.4	BPA: HPLC-ECD: 2.9 LC/MS:3.2 LC/MS/MS: 1.2 BADGE: LC/MS/MS: 3.55	NA	[166]

NA: not available
[a] *Selected Ion Monitoring Mode ;*
[b] *Selected Reaction Monitoring mode ;*

Table 1.10 (Continued): Method performance of determination of bisphenolic compounds in food samples found in literature

Samples	**Bisphenol compounds**	**Preparation method**	**Analysis technique**	**Detection or Quantification limit**	**Recovery (%)**	**RSD (%)**	**Linearity regression**	Ref.
Variety of canned food (vegetables, fruits, meat and fish)	BADGE and its derivatives	Ultrasonication-assisted solvent extraction with ACN:hexane LLE with hexane for fat removal LLE with ethyl acetate	HPLC-FLD with 225 and 295 nm as excitation and emission wavelengths.	LOD: 0.04-0.2 µg/kg	77-103	< 3	1.000	**[30]**
Canned beverages and foods	BPA	❖ Beverages: Degassing by ultrasonication SPE with OASIS-HLB cartridges Derivatization with pentafluorobenzoylchloride SPE with acidified silica cartridges ❖ Food: Solvent extraction with ACN Derivatization with pentafluorobenzoylchloride SPE with acidified silica cartridges	GC/MS in (ECNI)[c] mode	LOQ in µg/kg: Beverages: 0.02 Foods: 0.1	85-109	<6	NA	**[147]**

NA: not available, [c] Electron Capture Negative Ion ;

Table 1.10 (Continued): Method performance of determination of bisphenolic compounds in food samples found in literature

Samples	Bisphenol compounds	Preparation method	Analysis technique	Detection or Quantification limit	Recovery (%)	RSD (%)	Linearity regression	Ref.
Canned beverages and foods	BADGE, BFDGE and their derivatives	❖ Beverages: SPE with OASIS-HLB cartridges ❖ Foods: Solvent extraction with ethyl acetate	LC-MS/MS: ESI+ as $[M+NH_4]^+$	LOD : 0.15-8 µg/kg	60-95	<10	>0.999	[127]
Canned soft drinks	BPA	SPE (C18-silica) Derivatization with acetic anhydride	GC-MS (SIM)[a] BPA selected ions (m/z): 213, 228, 270, 312	LOD : 0.045 µg/L	99.9-101	1.3-6.6	NA	[240]
Fresh, canned, and plastic wrapped foods	BPA	Freeze drying Solvent extraction with ACN by ultrasonication LLE by hexane Purification on ENVI-Carb Derivatization by BSTFA (BPA-TMS-derivate) Purification on silica column	GC-MS with SIM[a] mode with monitored m/z: 357.2 and 372.2	LOD : 0.2 µg/kg	65-112	NA	NA	[153]

NA: not available
[a] *Selected Ion Monitoring Mode ;*

Table 1.10 (Continued): Method performance of determination of bisphenolic compounds in food samples found in literature

Samples	**Bisphenol compounds**	**Preparation method**	**Analysis technique**	**Detection or Quantification limit**	**Recovery (%)**	**RSD (%)**	**Linearity regression**	**Ref.**
Canned and frozen foods	BPA	Solvent extraction with ACN	LC-MS/MS (ESI): [M - H]-: 227.1	LOD: 2 µg/kg	94-110	≤10	>0.99	[164]
Preserved foods	BPA, BPF, BPS, BPAF, BPB	❖ Beverages: LLE with ethyl acetate SPE with Strata NH_2 cartridge ❖ Foods: Freeze drying Solvent extraction with ACN SPE with Strata NH_2 cartridge	LC-MS/MS: ESI- (MRM)[d] mode	LOQ : 0.01-0.05 µg/kg	62-107	NA	>0.99	[35]
Milk products	BPA and BADGE	SPE with C-18 cartridges SPE with Florisil cartridges	GC-MS with monitored m/z of BPA: 213 , 119, 228 BADGE: 325, 340, 269, 213	LOD: BPA: 0.15 µg/kg BADGE: 0.36 µg/kg	81-119	5-9	>0.99	[241]
Infant formula	BPA	Derivatization with K_2CO_3 and acetic anhydride SPE with C18 cartridge	GC-MS with SIM[a] mode with monitored m/z: 213, 224, 270, and 312	LOD : 0.1 µg/kg	85-94	2.7-3.9	>0.99	[243]

NA: not available

[a] Selected Reaction Monitoring mode ; [d] Multiple Reaction Monitoring

3.3. Global summary

The determination of bisphenol compounds and metal elements in foods/food simulants is a requirement to support the enforcement of legislation and assess the risk related to human dietary exposure to these migrants.

Atomic spectroscopic techniques, after appropriate sample preparation, are most frequently used in order to quantify metals in foods, particularly at trace levels. Recently, the application of ICP-MS in this field is gaining most interest, due to the offered advantage of higher sensitivity and less time required for analysis. The digestion or dissolution of a sample still remains an integral and important part in the determination of metals in a wide range of various samples. The integrity of chemical information is strongly dependent on the prior analytical steps and an adequate selection of sample preparation procedure is of capital importance. Microwave-assisted digestion is a preferred technique for sample dissolution due to the advantages it offers over dry and wet ashing such as: less consumed time, avoid loss of volatile elements, and minimized contamination.

LC–fluorescence detection is still frequently used and gives satisfactory quantitative results for the determination of bisphenol analogues and NOGE derivatives, but further identification of these compounds by LC-MS is crucial to confirm their existence. The +analysis by GC–MS is also attractive, but the required derivatization step can be time-consuming.

Sample preparation still constitutes the key-step for the determination of bisphenol compounds in food and it is the origin of the main drawbacks in the available methodologies. Solvent extraction and SPE are by far the most used extraction techniques for both isolation of bisphenol compounds and clean-up of matrix components. Further purification may be required to remove matrix components in order to avoid errors and maintain satisfactory long-term chromatographic system performance.

Finally, preparation of food samples will continue to be an area of interest as the need to determine many different contaminants from food contact materials continues to grow.

4. MIGRATION PREDICTIVE MODELING

Migration of low-molecular weight compounds is one of the most important problems of food-contact materials, namely plastic packaging. In order to reduce the time-consuming and expensive experimental control measurements as much as possible, theoretical predictions of migrations values can be used if they are based on scientific evidence. In fact, modeling of potential migration is already used in the U.S. and European Union as an additional tool to help regulatory decisions, aiming in the protection of consumers against toxic migrating substances **[244]**. The verification of the compliance of food packaging materials with the existing regulations can be done by comparing the SML of a migrant with the values predicted by ‘‘generally recognized migration models” **[245]**.

During the last three decades, many scientific studies have been performed to collect knowledge concerning the migration process and its behavior on FCMs. Most of the related research developments have been carried out in support of the international and European food-contact materials legislations and guidelines.

Mathematical models can follow different approaches as: deterministic, empirical, stochastic or probabilistic. The deterministic models are based on a theory describing a physic-chemical phenomenon, where a considerable amount of studies were dedicated to develop and assist of deterministic models based on Fick’s law of diffusion. Empirical models are purely mathematical and are based on equations that yield a good fit with experimental data regardless of any physico-chemical mechanism. On the other hand, stochastic models are represented by functions of probability distributions, such as the normal distribution; this approach yields to most probable value of migration occurring in a certain food/packaging combination for a given time and temperature, instead of predicting the migration level observed after a given storage time. Probabilistic models are similar to stochastic models but they further take into account the variability and uncertainty of the variables and the probability of their occurrence: the observed values of migration are subjected to variability due to heterogeneity in the composition and structure of both the packaging system and food product, lack of knowledge concerning the distribution chain (e.g. temperature), time of contact, and other non-systematic error sources that would contribute to uncertainty in the values of the variables **[246]**, **[247]**.

Various predictive models have been developed and reported for a given migrant through the years [248]–[252], with the Piringer‘s model [251] and the Limm and Hollifield model [250] being the two main ones [244].

4.1. Deterministic models based on Ficks's law

Most of the reported studies in this area employ a deterministic approach, based on the assumption that the mass transfer from the packaging material into the food is a diffusion process that can be described by Fick's 2nd law (one dimensional) (Equation 2).

Equation 2

$$\frac{\partial C_A^P}{\partial t} = D_A^P \frac{\partial^2 C_A^P}{\partial x^2} \tag{2}$$

With:

C_A^P: the concentration of the migrating species A in the packaging material P

t: the time

x: the linear dimension of migration

D_A^P: the diffusivity of A in the packaging material P

Accordingly data must be collected for the diffusion coefficient that measures of "how fast" the migrant travels in the matrix, which is influenced by several factors related to the polymer (such as molecular weight distribution, polymer glass transitions) and to migrant (such as molecular size and shape) [246]. Moreover, the behavior at the packaging/food interface also depends on whether the partition effects must be considered (the explanation of empirical relations with partitioning coefficient K_p are discussed elsewhere [246]). K_p describes the relation between the concentration in the packaging material and in the food, at equilibrium, or "how much" migrant is transferred to the food [246]. Indeed, K_P also depends on the specific properties of the migrant (like chemical structure and molecular size), the food (like the pH and fat content) and the nature of the packaging material and its storage temperature properties.

Since migrants will be present in low amounts in the food, it is assumed that there is a linear equilibrium relationship, that is a constant partition coefficient. In absence of specific data, in order to model worst case scenarios, the partition coefficient of the migrant between polymer

P and food F should be taken as $K_{P,F} = 1$, which means that the substance is well soluble in F. This assumption leads to the highest migration values at equilibrium and commonly used in models employed in the assessment of compliance with migration limits established by legislation. If the migration limits are not exceeded then the safety is assured and no further experimental work is required. For all other cases, that is for which the migrant is only sparingly soluble in F, the partition constant should be set at $K_{P,F} = 1000$, e.g. for lipophilic substances when the polymeric material is in contact with aqueous food/simulant **[144]**.

Most of mathematical migration models are based on Crank's analytical solutions **[253]** of Fick's Law. Two main models can be pointed out: the "Piringer model" and the "FDA model". By far, the "Piringer's" model is the most widely used. This approach describes the diffusion coefficients in gases and condensed phases, including the plastic materials **[247], [251]** (model described elsewhere **[246]**). Piringer (1994) **[251]** obtained solutions of Fick's law according to two extreme cases: (i) when partitioning $K_p < 1$, as with hydrophobic migrants migrating into fatty foods, where it can be assumed that the whole amount of A migrates ("worst case"), and (ii) when $K_p > 1$, as in aqueous foods, where only part of the initial amount in the packaging material migrates into the food **[246]**. When it is assumed that there is complete migration or migration is over short time, a simplified equation is derived that is called the FDA model (model described elsewhere **[246]**).

On the other hand, the diffusion coefficient may be determined from migration values, thus the determination of model coefficients can be as time consuming as the actual migration experiments. For this reason, an empirical relationship between the diffusion coefficient and the molecular weight of the migrant and the temperature was obtained by Piringer **[251]**:

$Dp = f(A_p', \tau, M_r, T)$

where A_p' is the polymer specific constant, τ is the polymer specific temperature constant, M_r is the relative molecular weight and T is the temperature.

The specific parameters and diffusion constant were first established for LDPE, HDPE, and PP based in published results and later improved and extended to other plastics **[246]**, as summarized in **Table 1.11**

Table 1. 11: specific parameters and diffusion constant of different polymers available in literature [246].

Polymer	**Ap'**	**T**	**T (°C)**
LDPE/LLDPE	11.5	0	<90
HDPE	14.5	1577	<100
PP (homo and random)	13.1	1577	<120
PP (rubber)	11.5	0	<100
PS	0.0	0	<70
HIPS	1.0	0	<70
PET	6.0	1577	<175
PEN	5.0	1577	<175
PA (6,6)	2.0	0	<100
Constants	C_1	C_2	C_3
	0.135	0.003	10454

4.2. Other models

4.2.1. *Limm and Hollified*

Limm and Hollified (1996) developed a semi-empirical model for the determination of diffusion coefficients using experimental migration data from data bank [246], [250]. This model uses Arrhenius-type equation for quantifying the temperature dependence for various additives' diffusion, given a specific polymer. As a first step, the model was evaluated on the prediction of additives' migration in polyolefins (POs). Their approach used the relationships between molecular diameters and activation energies as shown in equation 3, which have been established for relatively small molecules (≤100 daltons) [246].

Equation 3

$$D = D' e^{\left(\frac{-E_D}{RT}\right)} \qquad (3)$$

where D is the diffusion coefficient, D' is a pre-exponential factor, E_D is the activation energy, R is the gas constant (8.314 J/mol K) and T is the temperature.

4.2.2. *Helmroth*

Helmroth et al. (2002) [244] presented a stochastic model to predict the probability distribution of the diffusion coefficient from the molecular mass of the migrant, assuming a normal distribution. This allows the calculation of the probability of predicted migration values and, for example, the probability that a given migration limit is exceeded. The detailed background of this model is treated in their work [244] and is only briefly discussed here. For calculating a mean diffusion coefficient, the following equation was proposed:

$$D = a\exp(-M^{b})$$

where M is the molecular weight (g/mol), a and b are parameters specific for each polymer type and temperature.

Parameters a and b along with the probability distribution of the diffusion coefficient were derived from a dataset of experimental diffusion coefficient values in polyolefins at 23°C **[244]**.

4.2.3. Fauconier

Fauconier et al. (2001) **[254]** proposed a pure empirical model, where the migrated concentration was described as a polynomial function of temperature and time. The influence of time and temperature on the additives was modeled by adopting the “response surfaces methodology (RSM)” (Box and Wilson, 1951) **[255]**, a well-known statistical method, where the following polynomial equation was used:

Equation 4

$$z = A + Bx + Cy + Dx^2 + Ey^2 + Fxy \qquad (4)$$

where x and y are temperature and time respectively, z is the migrated quantity (mg/g) and A to F are coefficients calculated for all migrants in each migration liquid.

In this context, sufficient data must be collected from real experiments at first.

4.3. Discussion

Most migration models assume that migration is a deterministic process **[244]**. As mentioned previously, the “Piringer’s” model is the most used **[256]**. It was evaluated for different experimental validation conditions, such as the migration of Irganox 1010, 1076, Irgafos 168 and BHT from PP, LDPE, HDPE and EVA plastic materials to olive oil, HB307, corn oil and ethanol food simulants under storage conditions of 10 days at 40°C **[246]**. This model is indeed very useful for getting rough estimations of the migration of polymeric compounds **[244]**.

In Europe, the Piringer model has been evaluated and a comparison of model predicted values with experimental results has shown that, in 95% of the cases, the model overestimates the migration values [257], [258]. This means that the model is useful to support and verify compliance with the regulations, since if the overestimated migration didn't exceed the recommended limits, then no work should be further conducted. However, there is a need for models to be able to produce estimations close to the real values, reducing the overestimations [258] and reducing the risk of rejecting safe packaging systems [246].

O'Brien et al. (1999) [257] compared the prediction of both Piringer's and FDA models for the migration of a number of additives from HDPE into olive oil, and found that the FDA model was more accurate in most situations, but underestimated the results more frequently [246]. Haitao et al. [259] tested the FDA model prediction on the long migration of BHT from HDPE into 95% ethanol. The authors similarly obtained that FDA migration model data is far lower than the actual value of the test data [259].

Helmorth et al. showed that their stochastic model can be used for migration prediction in polyolefins at 23°C. Their model calculates the most likely migration values from a given packaging/food combination [244]. It has the advantage of giving a more realistic prediction in combination with a probability distribution, which enables a better risk evaluation. However, the use of any migration model must be accompanied by knowledge on the scientific background and application area of the model [246].

The Limm and Hollified's model is based on empirical constants obtained from actual migration experiments, therefore the model eventually provides a quite good correlation with experimental values, especially for migrants with high molecular masses into oils that come in contact with POs at high temperatures [247], [250]. This model has also been tested on a large number of diffusion coefficients found in literature, giving prediction deficiencies ranging from 2 times underestimation to 8 times overestimation [244], [250]. It was also validated for different experimental conditions, such as the migration of Irganox, BHT, n-C18, and n-C32 from PP, LDPE, and HDPE plastic materials to corn oil, ethanol, HB307, and tributyrin at 30-60°C [246].

Finally, the polynomial migration model built by Fauconier et al. [254], based on response surface methodology, was not based on physical principles but on the inter-relation of statistically important variables such as storage time and temperature. This model was actually built by the extrapolation of the statistical variables into regions where the results could not be acceptable, which results into false values. Therefore, to avoid such an incorrect approach it is quite important to remove some critical regions. In order to validate their method, the authors performed a series of experiments using HDPE and three aqueous food simulants (ethanol, lemon-origin terpenes and an emulsion of terpenes) at 4, 20 and 60°C until 7 weeks **[246]**. They obtained an accuracy of higher than 90% for all the phenolic migrants as well as for polymer oligomers migrating into the various simulants **[254]**.

The RSM designs is also useful to estimate interaction and even quadratic effects (in contrast to the often used "one-factor-at-a-time"-method) of statistical influencing parameters, and therefore gives us an idea of the shape of the response surface we are investigating **[260]**. Nevertheless, conducting an important series of experimental migration tests can be time consuming. To overcome this difficulty, the use of design of experiments (DOE) can help in minimizing and optimizing the number of experiments to be run, thus reducing the operating time and cost **[261]**.

As a conclusion, all of the presented models and the ones that are not discussed here can be applied to estimate the specific migration of compounds from packaging materials under specific physico-chemical properties or environmental parameters (e.g. storage time and temperature). Yet, these models represent certain limitation due to their uncertainty. Thus researchers are seeking for models to be able to produce estimations close to the real values. Additionally, it may be of particular importance to produce migration models that can be easily applied by the manufacturers as a tool to assess the interactions between the food and the packaging and assure food safety, as well as when a more quality-driven food packaging -system design is in focus.

5. THESIS OVERVIEW

According to the above literature review, there is a lack of knowledge on the migration of bisphenol compounds from cans produced in Lebanon, and suspicious levels of metals in canned food consumed in this country. So, there is a need to investigate the extent of migration of these contaminants into canned food consumed and produced in Lebanon in an effort to safeguard public health. The results of this research will also allow the Lebanese regulatory bodies to assess the background levels of contaminants in canned foods available in the local market and to compare with results obtained from other countries. In addition, although many contributing parameters have been previously reported to have significant effect on the migration of food contaminants from metallic cans, yet some published results are still under discussion and need additional data to support these findings. Therefore, this project aims at highlighting the conditions under which the migration phenomena is most significant. Finally, the results of this work can also be used to confirm or improve the quality of some Lebanese canned products that can allow for the removal of barriers to international trade as demonstrated at the beginning of the chapter 1.

This thesis will cover two broad areas of contaminants from food cans:

1. Determination of the migration of bisphenol compounds from can coatings into food simulants and real foods, and studying the effect of different parameters that can affect the migration of such compounds;

2. Determination of the content and release of trace metals in coated and uncoated food cans. As for bisphenol compounds, this work is also conducted to monitor different processing and storage parameters that are suspected to have an effect on the migration of metal elements from the cans metal substrate into foodstuffs.

As a first step the empty tinplate cans and canned food were collected from the biggest Lebanese factories and supermarkets. Then a number of experimental and analytical objectives must be fulfilled to obtain accurate and reliable concentration of target analytes. The followed methods of samples preparation and instrumental analysis were inspired from the most used techniques available in the above literature review:

1. Analysis of bisphenol compounds will require the:

    - Optimization of chromatographic conditions to obtain the best separation and detection of 8 bisphenol compounds on UHPLC-Fluorescence system and on ESI-TOF-MS operating in both negative and positive modes.

    - Development, optimization and validation (accuracy and precision) of the extraction method of bisphenol compounds: from food simulants using SPE on OASIS HLB cartridges, and from lyophilized canned food using solvent assisted extraction followed by SPE purification on OASIS HLB cartridges.

    - Testing the reproducibility and efficiency of the pilot sterilization process between cans.

2. Analysis of metal elements will require the :

- Validation of previously published method **[101]**, on chickpeas, for determination of the target metals in various vegetables, using microwave-assisted digestion for sample treatment followed by analysis on flame and graphite atomic absorption spectrometry.

The proposed research is divided into four sections, chapters 2-5. In chapter 2, a DOE is built to study the effect and interaction of sterilization, storage time and temperature on the migration of BPA from the coating of two different types of cans (one used for vegetables and fruits, while the other used for canning of meat) into water food simulant. This section will allow us to better understand the original conditions under which contamination from the coating occurs and provides the rationale for further exploratory studies in this area. The original work of this chapter has been published in 2017 in *Journal of Food Additives and Contaminants: part A* under title «*Effect of sterilization and storage conditions on the migration of bisphenol A in tinplate cans from the Lebanese market*» **[262]**.

In chapter 3, polynomial predictive models were developed and validated, on the basis of RSM methodology, to predict the concentrations of 8 analytes (BPA, BPF, BPS, BADGE, BADGE.2H_2O, BADGE.2HCl, BFDGE.2H_2O and BFDGE.2HCl) in canned food due to migration from the inner coating. The proposed models were built after data collection by the aid of DOE that combines the following parameters: cans brand, food simulant type (aqueous, acidic and semi-fatty), sterilization time, storage temperature and time. This work is intended to be submitted to an international peer-review journal.

The objective of the chapter 4 is to determine the influence of storage time and temperature, can denting, and leaving opened cans in the fridge on the migration of 8 most available metals in foods (Fe, Sn, Cr, Pb, Cd, Cu, Ni, and Zn) from uncoated and partially coated tinplate cans into fruit cocktail, peach and pears marketed in Lebanon. The interest of analyzing canned fruits is their acidic medium (pH<4.5) and the direct contact with the tin layer (in most cases, fruits are canned in uncoated tinplate cans). This chapter is submitted to *Journal of Food Processing and Preservation*.

During the last experimental section, chapter 5, for the very first time a combined analysis on both the 8 bisphenol compounds and the 8 metals are carried on highly consumed and exported traditional Lebanese canned vegetables (legumes mainly) including fava beans, red beans, chickpeas, chickpeas with sesame paste and okra. Similar to the previous three section, the effect of sterilization, storage time and temperature, can denting, brand, packaging (cans vs. jars) and heating the product in the cans, are studied on the migration of both the organic and inorganic analytes from coated food cans into the same food products. Principal component analysis (PCA) is performed here in order to examine the correlation between the content of metals and bisphenol compounds and the studied parameters. This chapter is also submitted to *Food Chemistry*.

Finally in chapter 6, the main findings and conclusions of all of the published or submitted papers will be linked and discussed to answer the main research questions, and to valorize the outcome of this thesis to directly benefit the consumer and canned food industries.

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Chapter 2:

Effect of sterilization and storage conditions on the migration of bisphenol A from tinplate cans of the Lebanese market

Introduction

The first step in answering my research question is about the parameters that mainly influence bisphenol compounds migration from can coatings. For that purpose, a set of experiments was conducted, focusing on the sole monitoring of bisphenol A (BPA) since it is considered as the most potentially toxic and abundant bisphenol. Tinplate cans dedicated for this study were collected from a well known Lebanese food cans industry.

In this article are detailed the effect of sterilization and storage (time and temperature) on the migration of BPA into aqueous food simulant. We chose food simulant for preliminary migration tests aiming at overcoming the difficulties of extracting BPA from complex food matrices. Since our target canned foods are mainly aqueous foods, water simulant was chosen here.

Hereafter are first introduced the main uses of BPA and its authorized limits in canned foods. Then we will argue about the concentrations of BPA obtained in canned food from different countries, and highlight the missing data in Lebanon and the urgent need to survey the Lebanese market. Then we describe the methodology used in the research work, as it was inspired from the techniques available in the previous bibliographic review chapter. Finally, our results will provide a clear answer to the impact of sterilization and storage conditions, while the obtained levels of BPA will help to evaluate the quality of Lebanese food cans production.

Effect of sterilization and storage conditions on the migration of bisphenol A from tinplate cans of the Lebanese market

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Highlights

- First report of BPA detection in tinplate cans manufactured in Lebanon
- BPA levels in aqueous simulant from sterilized cans ranged between 80 and 150 µg/kg
- Sterilization process accelerated BPA migration (around 10.5 µg/dm^2)

Abstract

The use of bisphenol A (BPA) in lacquer coating of food cans has been restricted by different authorities in many countries, such as in Europe. However, such regulation does not exist in many countries as Lebanon. Due to the lack of data on the quality of Lebanese can production, this study investigates the migration of BPA from two types of tinplate cans manufactured in Lebanon, before and after sterilization. Cans were analyzed under different storage conditions (time and temperature) and filled with an aqueous simulant. The determination of BPA was carried out using UPLC with fluorescence detection, and further confirmed by MS detection. After sterilization BPA levels drastically increased from an average of 0.15 to 109 µg/kg, giving a BPA migration around 10.5 µg/dm^2 for both types of cans. Storage temperature and time had no significant influence on BPA levels in sterilized cans (p-value > 0.05); on the opposite, these factors significantly affected BPA levels in non-sterilized cans.

Keywords: bisphenol A, tinplate cans, aqueous food simulant, sterilization, storage conditions.

1. Introduction

Bisphenol A (BPA) or 2,2-bis(4-hydroxyphenyl)propane is a chemical used as a monomer in the manufacturing of resins and plastics in order to prevent direct contact between the metal wall of the can and the food/ beverage as well as to protect the inside wall of the can from rusting and corrosion. As BPA was discovered to have estrogenic activity [1], it gained a great concern in the past few years since BPA free monomers can migrate in residual amounts into food or beverages once packed in materials containing the substance, so that BPA may further interrupt our endocrine system once ingested [2], [3]. Moreover, many studies have reported possible association between BPA ingestion and various health hazards such as heart disease including heart attacks, coronary heart disease, and angina as well as decreased sexual desire, erectile, and ejaculatory problems [4], [5]. Due to health concern, the European Commission has set the specific migration limit of 600 µg/kg for BPA in plastic materials and articles intended to come in contact with foodstuffs (EU Regulation 10/2011) [6]. Very recently, on January 2015, France even banned the use of BPA in all food contact materials [7]. Still in 2015, the EFSA (European Food Safety Authority) issued risk assessments for consumers and revised its BPA temporary Tolerable Daily Intake (t-

TDI) to 4 instead of 50 µg/kg bw per day **[8]**. Upon assessment of external exposure to BPA from all sources, diet was found the main source for all population groups, specifically due to canned food **[8]**. Even if dietary exposure is not at risk according to the EFSA, conclusions from other agencies widely differed. In particular, the French Anses and the Danish Food Institute still consider that risk associated with the presence of BPA in diet, especially due to canned food, may not be excluded **[9], [10]**. Hence, the EFSA continues to work on this topic in 2017, especially by reviewing all the scientific evidence published after 2012 and relevant for BPA hazard characterization (including immunotoxicity) **[11]**.

Many studies showed migration of BPA from packaging into food. Most of them focused on epoxy lining in tinplate cans which is expected to present the highest possible migration among other food packaging. According to a study on Belgian market, BPA ranged between 1 and 40.3 µg/kg in canned food **[12]**. Similar values (average of 1.1 µg/kg) were reported in baby food packed in glass jars with metal lids coated with epoxy lining present in the Canadian market **[13]**. Up to 730 µg/kg has been even reported in highly consumed canned food on the U.S. market **[14]**. The migration of BPA in canned food in nine cities of China was reported to be higher than in food preserved with other types of packaging, with an average BPA concentration around 20 µg/kg **[15]**. This issue was highly discussed in many other countries, but to our best knowledge there is a lack of data in Lebanon, where tinplate cans are manufactured and used in the food industry. In addition, since Lebanese cuisine is highly appreciated regionally and recognized globally, considerable amounts of canned food are being exported annually to Europe, the US, and neighboring countries (according to the Lebanese customs on national trades, the average of annual exportation over the 2013-2016 period is about 29,000 tons of canned food) **[16]**. Consequently, it is of prime importance to insure the validity of this production sector.

Therefore, the aim of the present work was to study the effect of storage time and temperature as well as the sterilization process on the migration of BPA into aqueous food simulants packed in two different types of tinplate cans manufactured in Lebanon and dedicated to non-acidic aqueous foods. The experiments were planned using a full factorial design and the significance of the effect of each parameter was established using the p-value approach. BPA was quantified by UPLC/Fluorescence and confirmed using UPLC/MS. Our results give a preview of BPA levels that shall migrate from Lebanese tinplate cans into food, which is of concern with respect to exposure assessment of both the Lebanese population (for which there is a lack of data until now, despite its high consumption of canned food) and the European population since Lebanese canned foods are widely exported to Europe.

2. Materials and method

2.1. Reagents

Bisphenol A (BPA, 99.9% purity, 228.29 g/mol) was purchased from Fluka Analytical (France). Ethanol, methanol and acetonitrile (HPLC-plus gradient grade) were obtained from Carlo Erba (France). Ultrapure, Milli-Q, water (18.2 MΩ/cm at 25°C) was produced by an Integral 3 water purification system from Millipore®.

2.2. Sample collection

Tinplate cans were manufactured in one of the biggest canning factories in Lebanon. According to the manufacturer, the raw tinplate sheets were imported from France while the liquid epoxy coating was coming from Italy. In the plant, the tinplate sheets had been coated with wet epoxy resins, cross linked with phenolic binders, and cured in an oven for 20 min at 200°C. The sheets were then cut and shaped into cylinders by electric welding. Additional epoxy-phenolic powder was applied on the welding side and subjected to another curing. Two types of cans were collected; the cans of each type were from the same batch, while both types of cans were produced within the same period. Cans of the first type were intended for packing vegetables (fava beans, okra) and fruits; the epoxy-phenolic resin was pigmented with titanium oxide to give a white appearance (18 cans: 10.1 cm height, 7.3 cm diameter, 400 g capacity, food contact surface area of 3.15 dm^2). The cans of the second category were of smaller size and used to pack processed meat; the epoxy-phenolic resin was pigmented with carbon black, giving a gray appearance, and covered with wax layer to facilitate meat sliding from the can (17 cans: 5.3 cm height, 7.3 cm diameter, and 140 g capacity, food contact surface area of 2.05 dm^2). Large and small cans are referred to as (L) and (S) respectively. All cans were three-piece cans with easy open ends.

2.3. Analytical instruments and conditions

2.3.1. UPLC/Fluorescence

A UPLC Agilent® 1260 Infinity Series system equipped with a multi-wave fluorescence detector was used for quantification of BPA. The system was accompanied with an autosampler; the injected volume was set at 20 µl. Pentafluorophenyl grafted octadecyl silica column (C18-PFP, 150*2.1 mm ID, 2µm particle size, ACE®) was used for the separation of targeted compound at constant temperature of 25°C. The mobile phase, consisting of water (A) and acetonitrile (B), was pumped at a flow rate of 0.4 ml/min and the following binary gradient was applied: 0 to 5 min - 30% B, 8 to 12 min - 40% B, 14 to 17 min - 100% B, and 17 to 20 min - back to 30% B. For detection of BPA, excitation and emission wavelengths were set at 230 nm and 316 nm respectively. Data were exported using ChemStation (Agilent, LC1260).

2.3.2. UPLC/MS

The confirmation of BPA peak was carried out on a UPLC XEVO G2S® (Waters, France) system equipped with quantitative time-of-flight mass spectrometry detector (QuanTof™ MS) incorporating StepWave™ ion optics, using the same chromatographic column as previously. Mass spectrometer was equipped with electrospray ionization mode switching (ESCi) source, operating in negative mode (ESI^-). The mobile phase was composed of water plus 10 mM of ammonium formate (A) and methanol (B).The flow rate of mobile phase was 0.3 ml/min with gradient: 0 to 5 min - 50% B, 8 to 12 min - 60% B, 14 to 17 min - 100% B and 17 to 20 min - back to 50% B. The injection was automatic with 10 µL injection volume. For each analysis a full scan spectrum (mass range 190-600 *m/z*, scan time 0.5 s) was acquired for identification purposes under the following conditions: capillary voltage 1 kV; sample cone and source offset 65 and 45 a.u, respectively. Desolvation and cone gas flow were respectively 600 and 20 L/min, while desolvation and source temperature were set at 500 and 130°C. Data acquisition was performed on MassLynx™ version 4.

2.4. Sample preparation

Since the majority of canned foodstuffs concerned by the studied tinplate cans here are aqueous (estimated range 60-95% of water / net weight), we initially chose 10% ethanol as food simulant according to EU Regulation 10/2011[6]. However, most of the cans containing 10% ethanol failed to support sterilization. Since BPA levels in the few cans filled with 10% ethanol that passed the sterilization process were similar to the levels found using water for the same type of cans, water was finally retained as the simulant in all our experiments. Other authors have also used water as simulant since it was recommended in previous European regulation. In the case of BPA, water could be more appropriate than 10% ethanol since the latter was reported to overestimate BPA migration as compared to foods **[17]**.

(L) and (S) cans were filled with 350 and 175 ml of water respectively, leading to 111 and 85 mL/dm^2 as values of the ratio between simulant volume and food contact surface area for (L) and (S) cans respectively. Cans were manually sealed and sterilized in two different batches (15 cans per batch, randomly distributed in the pilot) in an ACB autoclave for 30 min at 121°C. Some cans were sealed without sterilization in order to investigate the effect of sterilization. Sterilized and non-sterilized samples are referred to as (HP) and (NHP) respectively (HP meaning heat process).

Aliquots from (HP) samples were diluted ten times with 30% acetonitrile prior to UPLC analysis due to the presence of high concentrations of BPA after sterilization. On the other hand, (NHP) samples were found to have non-detectable levels of BPA when directly analyzed; therefore, the latter required pre-concentration by SPE before analysis. For that purpose, Supelco-HLB (60 mg/3 mL) cartridges (SPE manual Visiprep^{TM} system used) were conditioned and equilibrated with 5 mL of methanol followed by 5 mL of ultrapure water. After percolation of the sample (10 mL) onto the SPE cartridge and washing with 5 mL of water/methanol 95:5 (v/v), each extract was eluted with 5 mL of methanol. After subsequent evaporation to dryness under nitrogen flow at 40°C, residues were recovered with 1 mL water/acetonitrile 70/30 (v/v) and analyzed. Background contamination arising from lab ware was avoided by rising glassware with methanol before use. Blank samples were also performed to investigate possible BPA contamination coming from the SPE cartridges or the simulant.

2.5. Storage conditions

Heat processing as well as storage time and temperature are expected to be the primary influencing parameters on the migration of BPA into food simulant **[18]**. In this study, (HP) and (NHP) cans filled with the simulant were stored from one to fifteen days at 5, 22.5 and 40°C. Since most of studies investigated the short term migration of BPA, we tested the effect of long term storage of cans at room temperature through accelerated aging under 60°C for 10 days (equivalent to 6 months at room temperature) according to EU Regulation 10/2011 **[6]**.

2.6. Procedure used in the experimental design

Plackett–Burman designs constitute a variation of saturated fractional designs, allowing the evaluation of the weight of each factor on the outcomes as well as the effect of interaction between these factors [19]. For evaluating the effect of two factors (storage time and temperature for HP samples) as well as their possible interactions, a Central Composite Design (CCD) with ten experiments has been built. This full factorial design 3^2 contains center points in addition to one repetition of one experiment. Three levels per factor were chosen as low (-), middle (0) and high (+) levels representing 1, 8, 15 days of storage time and 5, 22.5, 40°C of storage temperature, respectively. The CCD matrix was performed on both (L) and (S) cans. All the experimental data were processed using JMP 10 Design of Experiments and Microsoft Excel 2007. In order to establish statistical differences between the means of BPA concentration in food stimulants stored under different conditions, a multi-factor analysis of variance, with least significant difference (LSD) at significance level 0.05, was calculated by Fisher's test.

3. Results and discussion

3.1. Quality assurance

3.1.1. Analytical performance

Quantification was performed with external calibration of integrated peak areas of ten points in the range between 0.25 and 40 µg/L by diluting the stock solution with 70:30 (v/v) of water/ acetonitrile mixture. Linearity with R^2 value 0.999 was observed for BPA's calibration over the range 0.25-40 µg/L (**Table 2.1**). Precision under repeatability and reproducibility conditions were also estimated from standard deviation of five calibration curves. The deviation between different calibration curves was < 5%. Intra-day and inter-day precision of instrument were determined through injecting three different standards (0.1, 20 and 40 µg/L) and one (HP) sample within the same day or on three different days (n=5), respectively. The instrumental within-day repeatability and between-day reproducibility are acceptable (< 5 and 10% respectively) as indicated in **Table 2.1**.

3.1.2. Detection and quantification limits

Instrumental LOD and LOQ were determined by preparing 5 replicates of 10 calibration standards, ranging between 0.1 and 1 µg/L, each replicate being analyzed twice. The repeatability (expressed as % RSD) at each level was calculated based on these 10 values per level. LOD and LOQ were then estimated as the BPA concentrations leading to RSD of 30 and 10%, respectively.

Table 2.1: Method performance and accuracy

	BPA concentration (µg/L)	RSD (%)	Calibration equation	Regression coefficient [b]
Linearity	0.25– 40		y=18.25(±1.5)x-4.83(±3.5)	R^2 = 0.999
LOD [a]’	0.1			
MDL’	0.01			
LOQ [a]’	0.25			
MQL	0.025			
Within-day repeatability [c]	0.1	4.39		
	20	0.33		
	40	0.24		
	(HP) sample [d]	0.51		
Between-day reproducibility [e]	20	6.52		
	40	9.47		
Repeatability between cans [f]	87	9.4		
Recovery [g, h]	0.08	110 ± 1.5%		
	1	98 ± 2.6%		

[a] n = 10; [b] mean regression coefficient (n=5)
[c] n=5 injections of one solution per level
[d] (HP) samples around 100 µg/L, diluted 10 times before injection
[e] n=5 injections per day over 3 days of one solution per level
[f] 12 cans - 3 different batches
[g] n=3
[h] mean recovery ± RSD

LOQ was then experimentally checked when assessing the calibration curve. Method detection and quantification limits (MDL and MQL) were estimated by dividing respectively LOD and LOQ by the method pre-concentration factor (f_c = 10). The results are shown in **Table 2.1**.

In order to investigate method selectivity and the absence of any interferences of BPA coming from the analytical method, 10 sample blanks (i.e. ultrapure water) were allowed to run through SPE cartridges and through the rest of the method. After the analysis of these sample blanks on UPLC system, no interferences appeared to elute at the retention time of BPA.

3.1.3. BPA pre-concentration recovery

Two levels of BPA (0.08 and 1 µg/L) were fortified into water samples of 10 mL (n=3) and SPE extraction was conducted under the same conditions as previously detailed for (NHP) samples. The mean recovery ranged between 98±2.6% and 110±1.5% as indicated in **Table 2.1**.

3.1.4. Sterilization repeatability

Repeatability in sterilization between different batches as well as the homogenous distribution of temperature in the pilot was tested. Fifteen cans of (L) type, each containing 350 mL of water, were sterilized in three different batches. The samples were distributed randomly in the autoclave. Three cans (one per batch) were dedicated to temperature controlling samples in order to follow the temperature variation inside the cans during the sterilization duration, and the other 12 cans were used for testing between-batch repeatability of BPA migration during sterilization. The process was highly repeatable with a variation coefficient not exceeding 10% as indicated in **Table 2.1.**

3.2. Peak confirmation by UPLC/MS analysis

Chromatograms obtained with UPLC/Fluorescence were very close for sterilized or non-sterilized samples, with only variations in BPA peak intensities depending on storage conditions. Therefore, only a few samples were chosen to be analyzed by UPLC/MS to ensure BPA confirmation (about 15% of total number of sterilized samples were analyzed by UPLC/MS). The most intense ion for BPA is [M-H]- at m/z = 227.108. The confirmation was done with both m/z ± 0.01 and retention time ± 0.05 min (**Fig. 2.1**). Two other chromatographic peaks merged with the same m/z (227.108), but at different retention times. Further investigation is required to identify the chemical structures corresponding to these peaks. The presence of other bisphenol analogues (such as BPF and BPS) was also examined; however, their presence could not be confirmed yet.

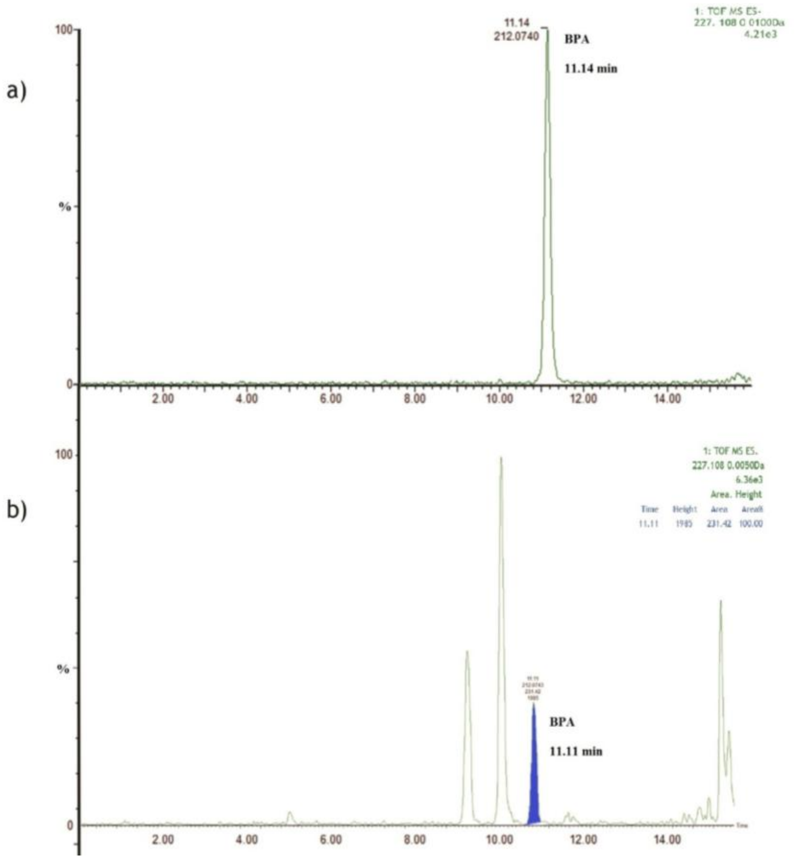

Figure 2.1: Example of UPLC/MS chromatograms obtained upon extracting m/z = 227.108, characteristic of BPA. (a) BPA standard (100 µg/L); (b) BPA in simulant from (HP) can.

3.3. Effect of heat processing

All canned food is subjected to sterilization and it is well known that leaching of BPA is highly related to sterilization procedure. It is therefore interesting to study the coating resistance to sterilization and the level of BPA that can migrate before and after heat treatment. To our best knowledge only few studies considered the effect of sterilization of food cans on the migration of BPA **[17], [18], [20]–[22]**. In our work, (HP) and (NHP) cans stored at room temperature (22.5°C) were analyzed at day 1 and day 15. Results are presented in **Figure 2.2**. BPA levels in aqueous simulant from (NHP) cans at day 1 were very low (0.1-0.2 µg/kg) whatever the type of cans; after 15 days of storage at room temperature, BPA concentrations increased significantly in aqueous simulant from (NHP) cans, reaching around 0.8 µg/kg. Much higher levels of BPA were found in simulant from (HP) cans on day 1 (around 110 µg/kg), but no significant change was noted after 15 days of storage. These results indicate that, even though free BPA monomer can migrate from the inner coating layer into the food stimulant in the case of non-sterilized cans stored under ambient temperature, heat processing is by far the most contributing factor on accelerating the migration of BPA. Most of free BPA monomer shall migrate into the canned food during the sterilization step, in agreement with data reported by other authors **[17], [21], [23]**.

In particular, similar experiments using cans filled with water as an aqueous food simulant also showed a moderate BPA level increase in (NHP) cans (from < 0.2 to 12.5 ± 1.3 µg/kg) after 160 days of storage, while after heat-processing migration of BPA remained constant around 80-90 µg/kg whatever the duration of storage is (0, 40, 70 and 160 days) **[18]**.Yet, the same authors obtained different results upon analyzing sterilized and non-sterilized canned fatty food simulant (sunflower oil), where most of the samples contained BPA below LOQ even in sterilized cans at 121°C for 50 min **[24]**.This may be due to other contributing factors such as the nature of food simulant, the coating type (they tested samples packed in cans coated with both organosol and epoxy resins) and the sterilization conditions. Their results showed that sterilization of fatty food simulant for 50 min at 121°C caused much less BPA to migrate (most < LOQ) than cans autoclaved for 135 min at 111°C (54.3–64.8 µg/kg); therefore, it can be concluded that the sterilization time is more influencing than sterilization temperature **[24]**. The same conclusion was reported for fatty-food simulant packed in epoxy-coated cans **[25]**. Then, for a given sterilization time, migration is enhanced with elevated temperature as reported: BPA concentrations in empty cans heated for 30 min at 80°C were up to 6.1 times (average 3 times) less than in those heated 30 min at 100°C **[21]**.

So, the sterilization conditions do affect the level of BPA leaching into food although it’s not very clear and requires further investigation to compromise between minimizing BPA leaching while still ensuring control of bacterial hazards.

In our study, the level of BPA in water simulant from sterilized cans ranged between 80 and 150 µg/kg. These values are higher than the migration levels reported by most recent studies from different countries dealing with real foodstuff **[12], [14], [15], [22], [26]**, but still lower than the specific migration level (600 µg/kg) authorized by the European regulation **[6]**. They are also much lower than previous reported BPA levels (around 800 µg/kg) in water simulant from tuna cans **[27]**.

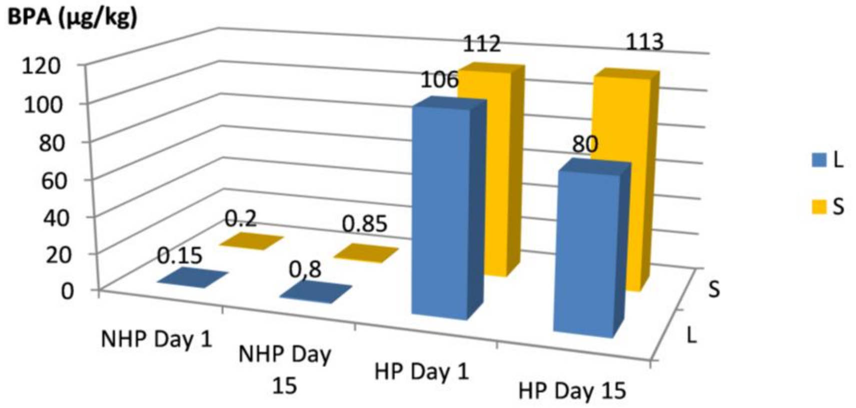

Figure 2.2: Migration level of BPA (µg/kg of food simulant) in large (L) and small (S) cans either sterilized (HP) or non-sterilized (NHP) and stored at room temperature for 1 or 15 days.

3.4. Effect of storage time and temperature

Considerable amount of BPA migrated during sterilization, and the BPA levels found in sterilized cans showed only very small variations upon storage. The response surface of experimental design as well as the effect significance of storage time and temperature interaction is shown in **Figure 2.3** and **Table 2.2**, respectively. As the results show, there was no significant effect of storage temperature and time, neither their interaction ($p\text{-value} > 0.05$), on the migration of BPA into aqueous food simulant. These results agree with previous work reporting 80-100% of free BPA present in the can coating to migrate into food during the sterilization stage (typically 90 min at 121°C) **[17], [24]**. Hence, for (HP) cans, storage time and temperature were found to have a minor effect on the migration of BPA into foodstuffs and food simulants, even with samples stored at 40°C for three months that simulates up to 3 years storage under ambient temperature **[17], [24], [26]**.

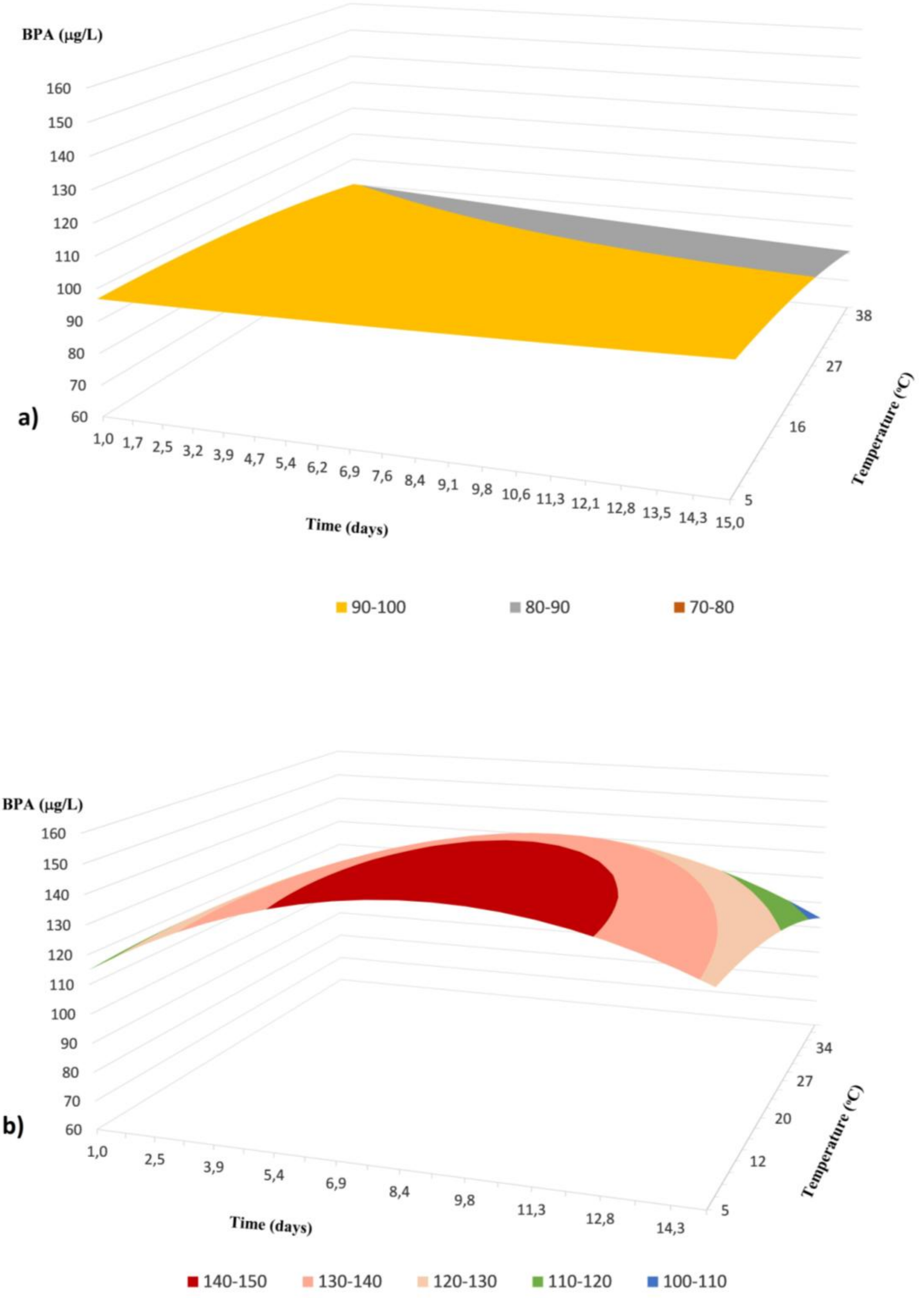

Figure 2.3: Surface plot of BPA migration level (µg/kg of food simulant) in sterilized cans as a function of storage time and temperature, (a) large (L) cans, (b) small (S) cans.

Table 2. 2: Response and effect significance of each experiment in the (JMP) experimental design for (HP) cans.

Experiment		**Storage temperature(°C)**	**Storage time(days)**	**BPA concentration (µg/kg)**		
				(L) type cans	**(S) type cans**	**p – value [a]**
1	aa	-1	-1	94	118	> 0.05
2	aA	-1	1	108	133	> 0.05
3	Aa	1	-1	83	110	> 0.05
4	AA	1	1	85	107	> 0.05
5	a0	-1	0	92	138	> 0.05
6	A0	1	0	88	126	> 0.05
7	0a	0	-1	106	112	> 0.05
8	0A	0	1	80	113	> 0.05
9	0	0	0	84	134	> 0.05
10	0	0	0	106	150	> 0.05

[a] *probability of significant effect at 95% confidence level ($p<0.05$)*

3.5. Types of cans

BPA migration may be affected by both the nature of the inner coating but also the geometry of the can. In our study, (L) and (S) cans differed in both aspects, since the former were coated with epoxy resin containing titanium oxide while the latter contained epoxy resin with carbon black and wax. As storage conditions had no effect on BPA levels in simulant for (HP) cans, we decided to consider these cans as two groups: all (L) and (S) type cans (n=8 per type of cans). A Student test clearly indicated (p-value <0.0001) that BPA mean concentration in simulant from (S) cans (122.9 µg/L) was significantly higher than in simulant from (L) cans (92.3 µg/L). Interestingly, taking into account the food surface contact area, there is no more evidence of difference between (L) and (S) cans since BPA migration is around 10.5 µg/dm^2 for both types of cans. This value is in the same range as BPA migration data (around 8 µg/dm^2) recently reported for sterilized (127°C, 24 min) epoxy-coated cans using also water as the aqueous simulant **[28]**.

3.6. Estimation of long term migration of BPA

A few (NHP) and (HP) of (L) and (S) cans were stored at 60°C for 10 days. As mentioned in European Regulation 10/2011, this accelerated test shall mimic long term storage (above 6 months) at room temperature (or below) **[6]**. BPA concentration in simulant from (NHP) cans after 10 days increased from 0.1 to 45 µg/kg and from 0.2 to 23 µg/kg, in (L) and (S) samples respectively (**Fig. 2.4**). On the other hand, the level of

BPA in simulant from (HP) cans remained constant after 10 days in (L) cans (near 105µg/kg). Again this confirms the previous conclusion that migration of free BPA in the coating is enhanced during sterilization and that there is no further significant migration after storage in (HP) cans. Surprisingly, in sterilized (S) cans, the BPA level in aqueous simulant significantly decreased after 10 days at 60°C. This unexpected result could possibly be attributed to BPA repartitioning in the coating under this temperature. As a matter of fact, microcrystalline wax (E 905) was used in the small cans to facilitate the meat sliding from the can, so that absorption of free BPA released in the simulant could have occurred in the wax over storage of (S) cans.

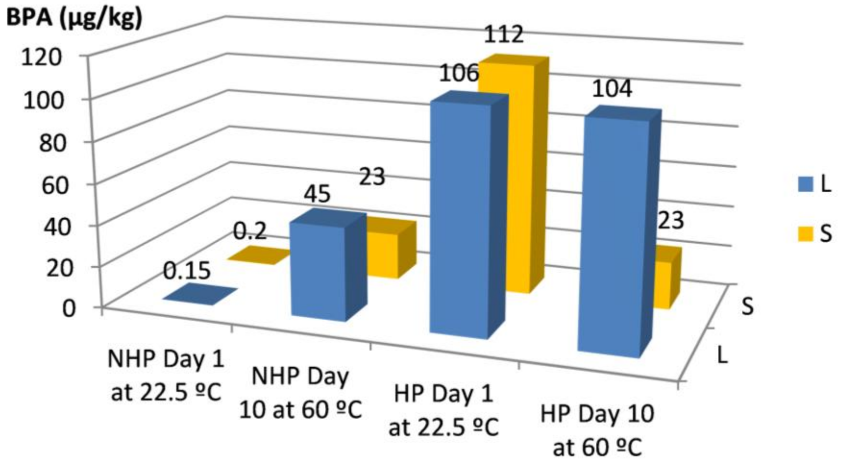

Figure 2.4: Difference in migration level of BPA (µg/kg of food simulant) in sterilized (HP) and unsterilized (NHP) (L) and (S) cans, stored for 1 day at 22.5ºC or 10 days at 60ºC.

4. Conclusion

Our results show that sterilization, at 121ºC for 30 min, greatly increases the release of BPA into aqueous food simulant. Nevertheless, the average migration levels reached (92 and 125 µg/kg in large and small cans respectively) were well below the EU regulation (600 µg/kg), which suggests that the BPA migration in Lebanese production of tinplate cans should be within the permitted limit. Interestingly, according to the computation done on JMP design of experiments software, there was no significant effect of short and long storage durations, under different temperatures, on sterilized cans. On the opposite, unsterilized cans were significantly affected by storage time and temperature, but BPA migration levels remained below those observed in sterilized cans. Hence, our results give clear evidence of the key role of the sterilization step on BPA migration level from tinplate cans. Consequently, the sterilization step should be carefully monitored in order to minimize the risk of releasing free monomers from the inner coating. Moreover, since our results show a constant level of BPA after sterilization, regardless of the storage duration and temperature, monitoring of can-varnishing could be helpful in minimizing, or even eliminating, these residual quantities of BPA free monomer. Finally, this study was based on aqueous food simulant and it is important to further investigate the migration of BPA into a wide variety of real canned foods, where many other factors might affect the long term diffusion process, such as pH and amount of water or fat. Finally, BPA is not the only compound susceptible to migrate from inner coatings, and related compounds (such as bisphenol A diglycidyl ether and its derivatives) should be investigated along with BPA to better understand migration mechanisms or competitions.

5. References

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Conclusion

The results presented in this article clearly show that sterilization at 121°C for 30 min is the main contributing parameter on the migration of BPA into aqueous food simulant. In the meantime, according to the statistical computations obtained using JMP design of experiments software, it was evident that there is no significant effect of subsequent storage time and temperature after sterilization. One explanation is that majority of BPA residues have migrated during heat processing. Interestingly, released BPA levels were overall the same between cans dedicated for vegetables and those used for meats (coating modified with carbon black pigmentation and wax slipping agent) probably since these cans were from the same brand. Therefore, the work should be continued to study other brands as well as different food simulants. Indeed the fluorescence detection revealed the presence of many peaks corresponding to non-targeted compounds, probably other bisphenol related compounds (e.g. BADGE), that must be also considered in the future work.

Chapter 3:

Building empirical models to predict the effect of sterilization and storage on bisphenols migration from metallic can coating into food simulants

Introduction

From the previous chapter we showed that sterilization for 30 min causes the release of the majority of BPA residues, where storage time and temperature would no longer have significant effect on its migration after sterilization. Consequently, the average content of BPA increased to around 100 µg/kg in aqueous food simulant, which complied with the European regulation (EU) n°10/2011. However, very recently, the SML of BPA was reduced from 600 µg/kg to only 50 µg/kg in the amended regulation (EU) n°2018/213 (with application on September 6th 2018). Therefore, a larger survey should be carried on Lebanese market of tinplate cans to evaluate whether the production quality comply with this new regulation.

The content of this chapter is in fact a sequel to the previous one. In order to reduce the effort of migration tests we intended to build empirical prediction models based on response surface methodology. Our aim was also to develop models capable of predicting migration of compounds at an early stage in the development process (e.g. during sterilization) with no need for previous knowledge on packaging physicochemical properties (since such information are not always available by the can manufacturers).

This work was carried out to assess the migration of most expected bisphenol compounds in epoxy-phenolic coatings, including BPA, BPF, BPS, BADGE and BFDGE as well as their hydrolysis and chlorinated derivatives. Based on the perspectives of the previous chapter, several factors were designated as models input variables, namely: four of most consumed Lebanese brands of tinplate cans (instead of only one brand), four different food simulants (aqueous, acidic, semi fatty simulants), expanded sterilization time between 0 and 90 min, storage temperature (as previous in chapter 2) and lastly storage time over 2 months (rather than 15 days previously).

Models were developed on a development data set, and further validated on another dedicated data set. They are presented and discussed hereafter, with a view of proposing a quite simple and efficient methodology that help industries to take precautions or provide advices with a view to minimizing concentrations of bisphenol compounds (specifically BPA).

Building empirical models to predict the effect of sterilization and storage on bisphenols migration from metallic can coating into food simulants

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Highlights

- Empirical models based on response surface methodology
- Models validated for BPA and BADGE migration from tinplate cans
- Prediction of usage conditions to comply with regulation

Abstract

Based on response surface methodology, custom design models were built to predict the influence of can processing (heat treatment) and storage conditions (time and temperature) on the migration of bisphenol compounds from the inner lacquer of tinplate cans (4 brands) into different food simulants. Analysis using liquid chromatography revealed the presence of BADGE.2H_2O and BPA in all samples; therefore, the empirical models were built specifically to predict the influence of the studied parameters on these two compounds migration. Models were greatly significant in fitting the level of migrants depending on the input variables with high adjusted coefficients of determination. Their prediction performance was validated through running new data set and comparing the observed with the predicted output responses. Finally, these models were used to determine the better parameters values to ensure migration compliance with the regulation. So, the proposed approach could be rapidly transposed and used as a convenient tool to control the conformity of cans before food processing.

Keywords: bisphenols, food simulants, migration, response surface methodology, tinplate cans

1. Introduction

Migrants from food packaging were, for a long time, of great concern by food safety authorities all over the world and among them, bisphenol A (BPA) and its substitutes (BPF, BPS) gained a special attention. BPA (or BPF) has been widely used as a monomer in polycarbonate plastic or epoxy-phenolic resins made of bisphenol A diglycidyl ether (BADGE) (or bisphenol F diglycidyl ether (BFDGE)). These coating resins are commonly applied on the inside metallic walls of food and beverage cans in order to prevent metal corrosion occurring with aggressive food ingredients (such as low-pH food).

Many studies have already proved that heat treatments generally applied during canned food processes lead to migration of important quantity of free BADGE (or BFDGE) and BPA (or BPF), resulting in food contamination about hundreds of µg/kg [1], [2]. Additionally, the instability of BADGE (or BFDGE) epoxide groups can easily produce hydrolyzed derivatives (such as BADGE·2H_2O or BADGE·H_2O) in contact with aqueous and acidic foodstuffs, while use of hydrochloric acid may form chlorinated derivatives such as BADGE.HCl. Finally, all these compounds can be released, along with oligomers and derivatives, into the canned foods [3]. Due to their possible endocrine disrupting effects, bisphenol compounds have been regulated with specific migration limits (SMLs) established for materials and articles intended to come in contact with foods, especially plastics and inner resins used by the can industries (e.g. for BPA, the European Regulation 10/2011 fixed a SML value of 600 µg/kg, this value being recently reduced to 50 µg/kg by European Regulation 2018/213 with application on September 6th 2018) **[4], [5]**. Testing the material before use with food simulants is required, to ensure migration levels below these SMLs values.

Due to such restrictions, canning industries are searching for epoxy coating alternatives, but it remains a long process to change package materials or develop new ones **[6]**. In the meanwhile, such industries can still control parameters affecting the migration of bisphenol compounds, such as sterilization conditions, brand of coating, type of packed food, and storage conditions. In such a context, predicting compounds migration from packaging into food is a key issue for canning and food industries. Numerous models have been developed, mostly deterministic based on theoretical diffusion equations and compounds partitioning between packaging and food simulants (aqueous and fatty food) **[7]–[10]**. Such predictive models fail when more than two parameters are unknown (e.g. identity of substances, concentration in the materials, diffusion and partition coefficients, external mass transfer resistance), which is common case in practice, with overestimation of migration values. Moreover, these migration models focus on packaging physicochemical properties that remain frequently unknown for several canning industries that buy from other companies their raw materials (we have experienced a lack of information relative to raw material composition in such industries in Lebanon). In addition, such models might ignore the effect of heat processing (sterilization) and storage. So, there is a need for developing simple models capable of predicting migration of compounds at an early stage in the development process (e.g. during sterilization).

This study aims at showing the capability of empirical models (based on surface response methodology - RSM) for predicting migration levels of bisphenols from cans. Models were built from collected data of experimental designs that combine can brand, food simulant type, sterilization time, storage temperature and storage time. The influence of all these parameters as well as their combination on the predicted values of bisphenols concentrations (here BPA and BADGE) are also discussed.

2. Materials and method

2.1. Choice of input variables

Five factors have been introduced as input variables for the models: type of tinplate cans (x_1), nature of food simulant (x_2), sterilization duration (x_3), storage temperature (x_4) and storage time (x_5). They can be divided into categorical and discrete variables as detailed in **Table 3.1**, also showing the tested levels.

Table 3.1: Symbols and levels of studied input variables.

Variables	**Symbols**	**Levels**			
Categorical		L1	L2	L3	L4
Tinplate can	x_1	C1	C2	C3	C4
Food simulant	x_2	S1: water	S2: 3% w/v acetic acid	S3: 10% v/v ethanol	S4: 50% v/v ethanol
Discrete		L1	L2	L3	
Sterilization time (min)	x_3	0	30	90	
Storage temperature (°C)	x_4	5	22.5	40	
Storage time (day)	x_5	1	15	60	

Four types of tinplate cans were investigated, in order to reflect different commercially available coating on the Lebanese market. Four simulants were also considered, that mimic aqueous (S1 and S3), acidic (S2) and semi-fatty foods (S4) according to the European regulation [5]. Sterilization at 121°C was considered here since it is the most common practice in food industries, being also recommended by European regulation 10/2011 to simulate the worst case for sterilization between 100 and 121°C; sterilization duration of 30 min was selected as the most common practice at this temperature, and 90 min was also considered as it is helpful for ensuring the complete cook of partially cooked food. The storage temperature was selected to simulate storage in a fridge (5°C), at room temperature (the most common: 22.5°C) and at room temperature in hot countries (40°C). Storage duration considered were 1, 15 and 60 days to mimic short and long storage.

As RSM model needs centered variables while experiments cannot always provide them. It is common to use rescaled variables for describing numerical independent variables in the experimental interval, coded as (-1, +1). So, variables x_3, x_4, and x_5 were replaced by the following terms:

$$x_3' = \frac{x_3 - 45}{45} \;\; ; \;\; x_4' = \frac{x_4 - 22.5}{17.5} \;\; ; \;\; x_5' = \frac{x_5 - 30.5}{29.5}$$

2.2. Building experimental designs

We used the JMP 13 software (SAS Institute. Cary, NC 1989-2007) to build our experimental designs on custom design based on RSM. This type of designs is general, flexible, and matches the experimenter requests (regarding the types of variables and the time available for experiments). In addition, it authorizes modeling in presence of categorical variables (i.e. can brand or food simulant type). First, second and interaction order of variables were all selected on software. Experimental designs were developed through running the minimum possible number of randomized experiments to create balanced models.

Preliminary experiments were conducted to establish the experimental domain. We faced problems with ethanol-based food simulants (S3 and S4), since most of cans filled with these simulants opened in the sterilization pot. Consequently, we decided to build two different experimental designs: Design I to study the effect of food simulant type on migration during storage of non-sterilized cans, and Design II to investigate the effect of sterilization with food simulants that comply with this process. As a matter of fact, Design I also aimed at investigating the possibility to further study the sterilization process on simulants S1 and S2 only, as representative of simulants S3 and S4. A total of 72 experimental scenarios (36 per design) were conducted to build the can processing and storage dependent migration models (see **Supplementary material - Table 3.S1**).

2.3. Building the models

Output responses are bisphenol levels in food simulants, so that different models were considered, each adapted to a bisphenol compound. Empirical models were built considering, as a first approach, a second order model to include both variable interactions and quadratic terms. Since x_1 and x_2 are categorical variables, any regressor associated with one or both of these parameters represents only a specific set of constants (for example $\beta_1 x_1$ represents only 4 constants depending on whether cans C1, C2, C3 or C4 were used). On the opposite, x'_3, x'_4 and x'_5 are numerical variables with infinite continuous input values: their corresponding linear, quadratic and interaction regression coefficients are constants multiplied with the variables themselves. Hence, general equations are the following for each experimental Design (since Design I does not include sterilization, x'_3 is absent as well as its corresponding interactions with other parameters):

General equation of Design I

$$\text{Migrant level} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_4 x'_4 + \beta_5 x'_5 + \beta_{12} x_1 x_2 + \beta_{14} x_1 x'_4 + \beta_{15} x_1 x'_5 + \beta_{24} x_2 x'_4 + \beta_{25} x_2 x'_5 + \beta_{45} x'_4 x'_5 + \beta_{44} {x'_4}^2 + \beta_{55} {x'_5}^2$$

General equation of Design II

$$\text{Migrant level} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x'_3 + \beta_4 x'_4 + \beta_5 x'_5 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x'_3 + \beta_{14} x_1 x'_4 + \beta_{15} x_1 x'_5 + \beta_{23} x_2 x'_3 + \beta_{24} x_2 x'_4 + \beta_{25} x_2 x'_5 + \beta_{34} x'_3 x'_4 + \beta_{35} x'_3 x'_5 + \beta_{45} x'_4 x'_5 + \beta_{33} {x'_3}^2 + \beta_{44} {x'_4}^2 + \beta_{55} {x'_5}^2$$

where coefficients β_0, β_i, β_{ij}, β_{ii} are the model intercept, effect parameter, interaction effect parameter and quadratic effect parameter, respectively.

The development data sets were used to build the models in order to properly fit our data. At first, in order to determine whether some variables could be discarded from the model, we tested hypotheses for the individual regression coefficients. The simple analysis starts with determining the main effect for each level of a factor, which is the difference between the average of output responses at the target level of a factor and the overall average of the output responses of all experiments. Thereafter, F-test is performed in order to identify the significance of the main factors, and the p-value approach was used (i.e. p-value < 0.05).

Because evaluating all possible regressions can be burdensome computationally, various methods have been developed for evaluating only a small number of subset regression models by either adding or deleting regressors one at a time **[11]**. In our case, backward elimination method was followed where we begin with a model that includes all candidate regressors. Then according to the p-value of each factor (i.e. significant or not), the factor is included or excluded (elimination is done in the order from most insignificant to least significant) until finding the suitable model. Some variables did not have a significant effect, but could not be deleted due to their significant interaction with other variables.

2.4. Models validation

Validation of models was conducted by an external validation thanks to new experiments performed (detailed in **Supplementary material - Table 3.S1**) in a different batch from development data set. Selection of these experiments was done on the basis of: 1) repetition of experiments with suspect output responses, 2) available conditions (remaining cans and available time within 15 days).

The validation process was achieved on three successive steps. Firstly we performed visual confirmation of attendance of validation experiment points within the confident interval of the linear model. Then, we calculated relative error (η) between the measured concentrations and the predicted concentrations with a fixed threshold about 0.2 [12]. Last but not least, the $R^2_{predicted}$ was calculated to assess the quality of model prediction for the new experiments (validation data set), according to the following equation:

$$R^2_{predicted} = 1 - \left(\frac{\sum_{i=1}^{n}(O_i - P_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2}\right)$$

where n is the number of validation experiments, O_i is the observed concentration for experiment i, P_i is the predicted value and $\bar{O}$ the mean of concentration from development data set.

If $R^2_{predicted}$ is higher or close to $R^2_{adjusted}$, the model correctly fits the new experiments, while if there is a significant decrease the model should not be used as predictive model.

2.5. Tinplate cans

All cans were three-pieces with easy open lids, collected from three main canning factories in Lebanon. According to the manufacturers, the can lacquers were imported from France, Germany or Turkey. Most of the cans (50 cans coded as C1, C2 and C4: 10.1 cm height, 7.3 cm diameter, 3.15 dm^2, 423 cm^3) were coated with epoxy-phenolic resin pigmented with titanium oxide (giving a white appearance) and were intended for packing vegetables and fruits. The cans C3 (12 cans: 5.3 cm height, 7.3 cm diameter, 2.05 dm^2, 222 cm^3), intended to pack processed meats, were coated with lacquer of same source as C1, but the coating was modified with microcrystalline wax (E 905) slipping agent in addition to aluminum pigmentation (gray appearance). This category allows studying the possible effect of slipping agent on food contamination.

Cans were filled either with 350 ml (C1, C2, C4) or 175 ml (C3) of food simulants. They were then sealed, without sterilization or once sterilized in an ACB autoclave pilot (maintained at 121°C). All filled sealed cans were then stored under the appropriate controlled conditions according to the experimental designs built.

2.6. Measuring bisphenols migration in food simulants

2.6.1. Standards and reagents

Acetonitrile (ACN) (HPLC plus Gradient grade and LC-MS grade), methanol (MeOH) (HPLC plus Gradient grade and LC-MS grade), ethanol (EtOH) (anhydrous absolute and HPLC plus Gradient grade), water (LC-MS grade), formic acid (FA) (LC-MS grade) and acetic acid (RPE glacial) were obtained from Carlo Erba (France). BPA (purity ≥99.9%), BPF (purity ≥98%), BPS (purity ≥98%), BADGE (purity ≥95%), BADGE.2H_2O (purity ≥97%), BADGE.2HCl (purity ≥95%), BFDGE (purity ≥95%,mixture of diastereoisomers), BFDGE.2H_2O (purity ≥95.0%), and BFDGE.2HCl (purity ≥90.0%, total assay of the three isomers) were obtained from Sigma Aldrich (France). Ultra-pure Milli-Q water (18.2 MΩ, 25.0°C) was produced by an Integral 3 from Merck-Millipore®.

2.6.2. Instrumentation

All bisphenols considered here (BPA, BPF, BPS, BADGE, BFDGE and their derivatives) were systematically investigated in the samples collected from the cans. For that purpose, the same UHPLC/Fluorescence and UHPLC/TOF-MS systems as described in our previous study [13] were used, but with different chromatographic gradients.

For UHPLC/Fluorescence, solvents A (water) and B (ACN) were pumped using the following gradient: 0 min - 43% B, 1 min ramp to 50% B (maintained for 4 min), 2 min ramp to 60% B (maintained for 5 min), 1 min ramp to 100% B (maintained for 2 min) and back to 43% B in 1 min (total duration 16 min). Quantification was performed with external calibration of integrated peak areas of ten points in the range 0.1 to 80 µg/L.

For UHPLC/TOF-MS, both ESI- and ESI+ ionization modes were operated. The ESI- operation parameters and mobile gradient were as previously described **[13]**, while ESI$^+$ conditions differed. ESI$^+$ operation parameters were: capillary voltage 1 kV; sample cone and source offset 60 and 100 a.u, respectively. Mobile phase composition for ESI$^+$ was: solvents A (water/FA 99.9/0.1 v/v) and B (ACN/FA99.9/0.1 v/v) pumped at 30°C with the same gradient used for UHPLC-Fluorescence system.

2.6.3. Sample treatment

All samples were either directly treated the same day after opening the cans, or stored in glass tubes at 5°C until their treatment (sample stability was checked under these conditions). Sterilized samples contained high amounts of organic contaminants, requiring ten times dilution with mobile phase (ACN/water 43/57 v/v) before their analysis. On the other hand, unsterilized cans contained only traces, thus requiring SPE pre-concentration step. For that purpose, 10 mL samples were loaded on Supelco-HLB (60 mg/3 mL) cartridges (SPE manual Visiprep^{TM}system used) previously conditioned with 5 mL of MeOH and equilibrated with 5 mL of blank food simulant. After a washing step with 5 mL MeOH/water (5/95 v/v), organic contaminants were eluted with 2 mL MeOH. Then the cartridges were dried under vacuum for 15 min, followed by another 2 mL of MeOH to elute any remaining residues. The two elution fractions were combined and gently evaporated to dryness under nitrogen stream at 35°C. The residues were then recovered with 1 ml mobile phase (ACN/water 43/57 v/v). The same protocol was used for treatment of samples from EtOH-based food simulants, except that the samples were water diluted to reach only 5% EtOH before SPE (to avoid bisphenols losses during sample percolation), and that the residues after evaporation were reconstituted in 0.5 mL mobile phase.

3. Results and discussion

Our approach being to build empirical models that best fit our experimental data, the first step was to investigate bisphenol compounds in all our samples. Then models were built to predict migration of those compounds depending on sterilization or storage conditions.

3.1. Migration of bisphenols in the simulants after can contact

The UHPLC/fluorescence analyses revealed several peaks in the simulant extracts, but only a few of them appearing at the expected retention times of target compounds (**Fig. 3.1).** However, due to the low levels of migrants in the extracts, only peaks corresponding to BADGE.2H_2O and BPA could be confirmed through the UHPLC/TOF-MS analyses. Consequently, the empirical models were built to fit the migration of these two bisphenol compounds.

The overall average migration of bisphenols measured in our sterilized cans are gathered in **Table 3.2;** since all conditions tested are considered, large standard deviations are observed. Concentrations measured are within the range of previous levels reported in canned food simulants **[1], [13]** and canned food **[14], [15]**. A logic correlation between the levels of BADGE.2H_2O and BPA can be noticed, where higher values of BPA were correlated to the higher values of BADGE.2H_2O and vice versa.

Observed BADGE.2H_2O release is higher than recently reported for other cans, while BPA migration is within the same range (7-8.4 and 14-16 µg/dm^2 for BPA and BADGE.2H_2O, respectively) **[16]**.

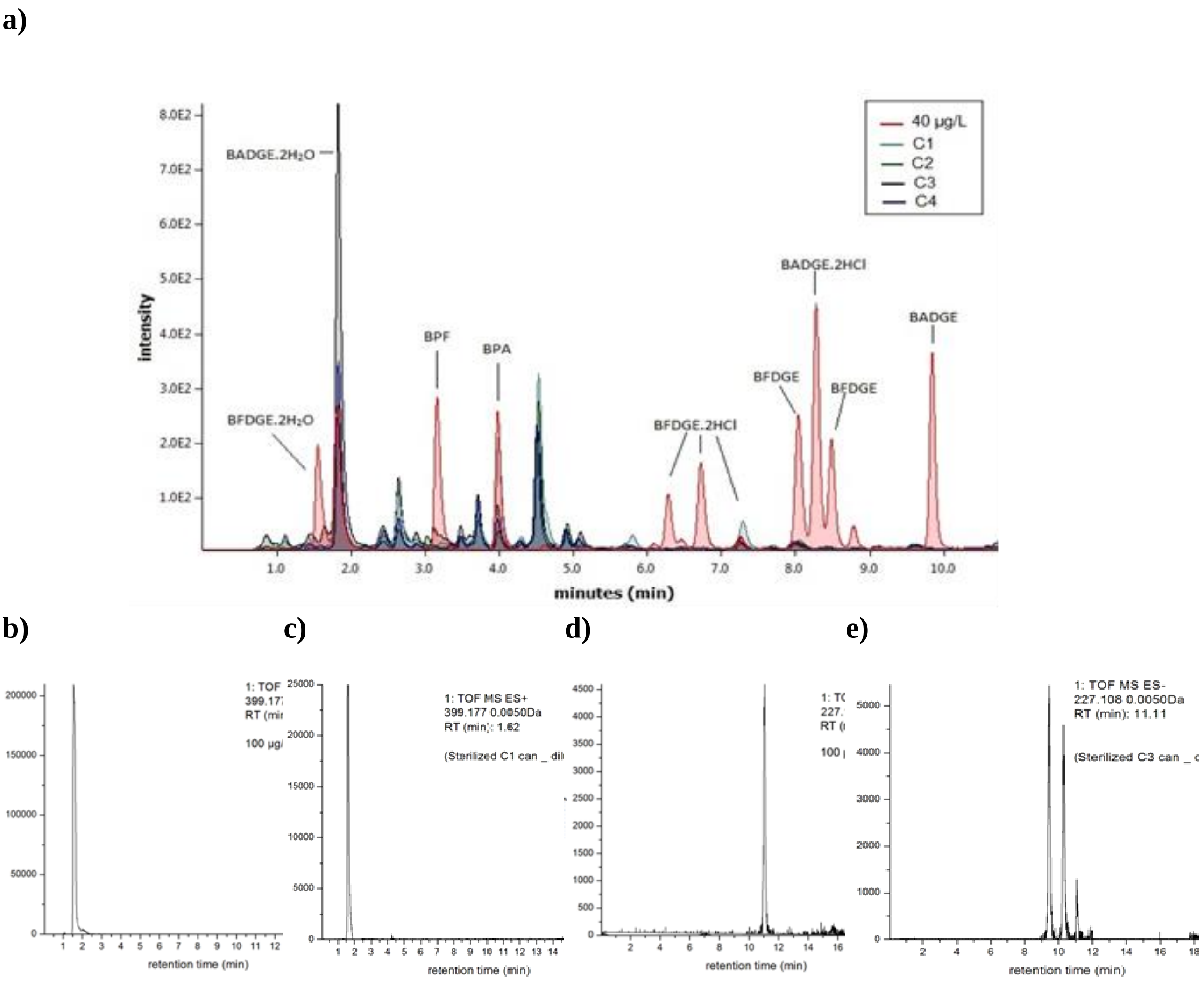


Figure 3.1: UHPLC-fluorescence chromatograms after analysis of four can categories and 40 µg/L standard (a), and UHPLC-TOF-MS chromatograms of detected compounds: (b) BADGE.2H_2O peak of 100 µg/L standard; (c) BADGE.2H_2O peak corresponding to a sterilized C1 can; (d) BPA peak of 100 µg/L standard; (e) BPA peak corresponding to a sterilized C3 can, with BADGE.2H_2O main m/z of 399.177 and BPA main m/z of 227.108.

All cans do comply with the current European regulation regarding migration of BPA as well as BADGE and its derivatives. Yet, as mentioned before, BPA regulation became more drastic since September 2018; only cans C2 may respect this new regulation. Indeed, cans C2 offer the minimum migration regarding both contaminants, closely followed by cans C4. Significant higher migration levels of BADGE.2H_2O were observed with C3, with also high BPA levels in cans C1 and C3. Both types of cans were from the same brand, being coated with lacquer of same source. However, the coating of C3 cans was modified with microcrystalline wax (E 905) slipping agent in addition to aluminum pigmentation (gray appearance).

Table 3. 2: Overall average migration measured for BPA and BADGE.2H_2O.

Type of can	**BADGE.2H_2O***				**BPA****			
	Average concentration (µg/kg)		Average migration (µg/dm^2)		Average concentration (µg/kg)		Average migration (µg/dm^2)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C1	520	116	57.8	18.4	197	85	21.8	9.4
C2	349	45	38.8	5.0	47	5.4	5.2	0.6
C3	1106	193	94.4	16.4	132	58	11.3	5.0
C4	355	87	39.4	9.7	65	12.7	7.2	1.4

* *European SML = 9,000 µg/kg (sum of BADGE and its hydrolyzed derivatives)*
***European current SML = 600 µg/kg, with new value of 50 µg/kg after 6th September 2018*

3.2. Migration modeling

3.2.1. *Effect of food simulant and storage with non-sterilized cans (Design I)*

After removal of non-significant parameters, empirical model equations obtained for BADGE.2$H_2$0 and BPA are:

$$[\mathrm{BADGE.2H_2O}] = \beta_0 + \beta_2\, x_2 + \beta_4\, x_4' + \beta_{24}\, x_2 x_4'$$

$$[\mathrm{BPA}] = \beta_0 + \beta_1\, x_1 + \beta_2\, x_2 + \beta_4\, x_4' + \beta_5\, x_5' + \beta_{12}\, x_1 x_2 + \beta_{14} x_1\, x_4' + +\beta_{15} x_1\, x_5' \\ + \beta_{24} x_2 x_4' + \beta_{25} x_2 x_5' + \beta_{45} x_4' x_5' + \beta_{44}\, x_4'^{2} + \beta_{55} x_5'^{2}$$

These equations suggest that the food simulant (term x_2) plays a role in the migration of both BADGE.2$H_2$0 and BPA, as confirmed by the p-values obtained after the significance test (0.027 and 0.0002, respectively – see **Table 3.3**). Yet, it is necessary to look deeper into the detailed results to explain how the food simulant affects bisphenols migration (estimated regression coefficients plus their standard error and significance based on p-value from a t-test assessing if the estimated values are statistically different from zero are given in **Supplementary material - Table 3.S2**).

Table 3.3: Effect test significance on the basis of p-value.

Effect test	**Variable symbol**	**Design I**				**Design II**			
		BADGE.2H$_2$O		**BPA**		**BADGE.2H$_2$O**		**BPA**	
Source		**F ratio**	**P-value**	**F ratio**	**P-value**	**F ratio**	**P-value**	**F ratio**	**P-value**
Type of can	x_1	2.4808	0.2376	81.8269	**0.0022***	63.5296	**<0.0001***	29.3157	**0.0002***
Type of simulant	x_2	14.6385	**0.0269***	406.8117	**0.0002***	13.9690	**0.0073***	0.2226	0.6514
Sterilization time	x'_3	NO	NO	NO	NO	700.2016	**<0.0001***	121.2632	**<0.0001***
Sterilization time*Sterilization time	$x_3'^2$	NO	NO	NO	NO	23.2471	**0.0019***	50.4977	**0.0002***
Storage temperature	x'_4	14.2800	**0.0325***	268.5944	**0.0005***	0.8298	0.3926	0.2755	0.6159
Storage temperature*Storage temperature	$x_4'^2$	1.1357	0.3647	292.6017	**0.0004***	1.4351	0.2699	19.0606	**0.0033***
Storage time	x'_5	3.0071	0.1813	318.5326	**0.0004***	0.0061	0.9398	21.4957	**0.0024***
Storage time*Storage time	$x_5'^2$	4.4966	0.1241	71.8117	**0.0035***	0.3666	0.5640	5.1982	0.0566
Type of can*type of simulant	$x_1 * x_2$	1.3822	0.4371	163.6626	**0.0007***	2.2700	0.1676	2.9407	0.1084
Type of can*Sterilization time	$x_1 * x'_3$	NO	NO	NO	NO	62.5342	**<0.0001***	8.2974	**0.0105***
Type of can*Storage temperature	$x_1 * x'_4$	1.4927	0.3750	57.2542	**0.0038***	0.1871	0.9019	2.1665	0.1801
Type of can*Storage time	$x_1 * x'_5$	1.4317	0.3876	110.1651	**0.0014***	0.9386	0.4714	11.7173	**0.0041***
Type of simulant*Sterilization time	$x_2 * x'_3$	NO	NO	NO	NO	7.8122	**0.0267***	2.0031	0.1999
Type of simulant*Storage temperature	$x_2 * x'_4$	10.8411	**0.0406***	15.7558	**0.0243***	2.8350	0.1361	0.0000	0.9955
Type of simulant*Storage time	$x_2 * x'_5$	3.1753	0.1840	132.6444	**0.0011***	0.3075	0.5965	0.0364	0.8542
Sterilization time*Storage temperature	$x'_3 * x'_4$	NO	NO	NO	NO	0.0161	0.9027	2.3360	0.1703
Sterilization time*Storage time	$x'_3 * x'_5$	NO	NO	NO	NO	0.0903	0.7725	5.0432	0.0596
Storage temperature*Storage time	$x'_4 * x'_5$	1.5134	0.3063	38.9732	**0.0083***	0.6908	0.4333	0.0235	0.8825

** Significant effect if $p<0.05$*

It appears that the effect of food simulant on BADGE.2H_2O migration is driven only by a significant effect of 50% ethanol (S4). For BPA the results are almost quite the same since a clear effect of S4 is shown. A slight effect of S3 is also suggested based on significance of a few estimated regression coefficients. However, considering the standard errors, the values of regression coefficients for S3 are not significantly different from regression coefficient for S1 and S2 (e.g. for $\beta_2 x_2$ the regression coefficient for S3 is in the range [-20.39; -10.87] while for S1 and S2 values are respectively in the ranges [-11.3;-1.14] and [-11.93; -1.77]). Only simulant S4 is significantly different from the three others(range [23.74; 33.71]). These results suggest that whether water (S1), 3% acetic acid (S2) or 10% ethanol (S3) are used, similar migration should be observed both for BPA and BADGE.2H_2O upon storage. This is in agreement with previous results from Biles et al. [17] showing, for polycarbonate plastic stored 10 days at 65°C with food simulant, that BPA migration was similar with water or 10% ethanol (≈ 1 µg/cm²) while it was greatly enhanced with 50% ethanol (5.9 µg/cm²). Also, Paseiro-Cerrato et al. [16] showed that BADGE.2H_2O continuously increased over 490 days of storage in non-sterilized cans filled with 50% ethanol, so that after 90 days BADGE.2H_2O levels in this food simulant were higher than in sterilized cans filled with water. The effect of 50% ethanol is possibly due to resistance to mass transfer for higher molecular weight oligomers, resulting elevated migration and more potential for hydrolysis products.

To conclude, results from experimental Design I are in favor of further studying the sterilization process on simulants S1 and S2 only, as representative of simulant S3 (i.e. Design II). That way, migration modeling mimics application to aqueous canned food.

3.2.2. Effect of process and storage conditions with sterilized cans (Design II)

After removal of non-significant parameters, the final models developed for this experimental design are the following (with estimated regression coefficients detailed in **Supplementary material - Table 3.S3**):

$$\mathrm{BADGE.2H_2O} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x'_3 + \beta_4 x'_4 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x'_3 + \beta_{23} x_2 x'_3 + \beta_{24} x_2 x'_4 + \beta_{33} {x'_3}^2$$

$$\mathrm{BPA} = \beta_0 + \beta_1 x_1 + \beta_3 x'_3 + \beta_4 x'_4 + \beta_5 x'_5 + \beta_{13} x_1 x'_3 + \beta_{15} x_1 x'_5 + \beta_{33} {x'_3}^2 + \beta_{44} {x'_4}^2$$

It is noticeable that storage time has no influence on BADGE.2H_2O migration since there is no occurrence of x_5 in the corresponding model. Similarly, the food simulant (S1 or S2) has no impact on BPA levels (no appearance of x_2). On the contrary, for both compounds, the type of can, sterilization time and storage temperature or their combinations are significant in the fit model.

Many studies reported that heat processing of cans has an important influence on inducing the migration of residual compounds from can lining into foods **[2]**. Sterilization time plays a major role on BADGE.2H_2O and BPA migration according to our model(p-values < 0.0001 for both compounds as shown in **Table 3.3**), possibly since time up to 90 min was considered as Yonekubo et al. **[18]** reported similar migration between 30 and 60 min sterilization at 115°C. Cabado et al. **[2]** also showed that BADGE migration was similar between sterilization at 115°C for 45 min and 121°C for 30 min.

Once the mass transfer from the can coating into stimulants has been achieved during sterilization, migration of BADGE.2H_2O remained stable whatever storage duration and temperature (p-value of 0.9398 and 0.3926, respectively – see **Table 3.3**). BPA migration was similarly insignificant over storage temperature (p-value = 0.6159) while storage time was significant on triggering its migration after sterilization (p-value = 0.0024), as already reported **[16]**. However, this significant effect of storage time was related to a specific case (cans C1) which requires further confirmation to clarify the conclusion since previous studies claimed stability of BPA levels over storage time **[1], [13], [19]**.

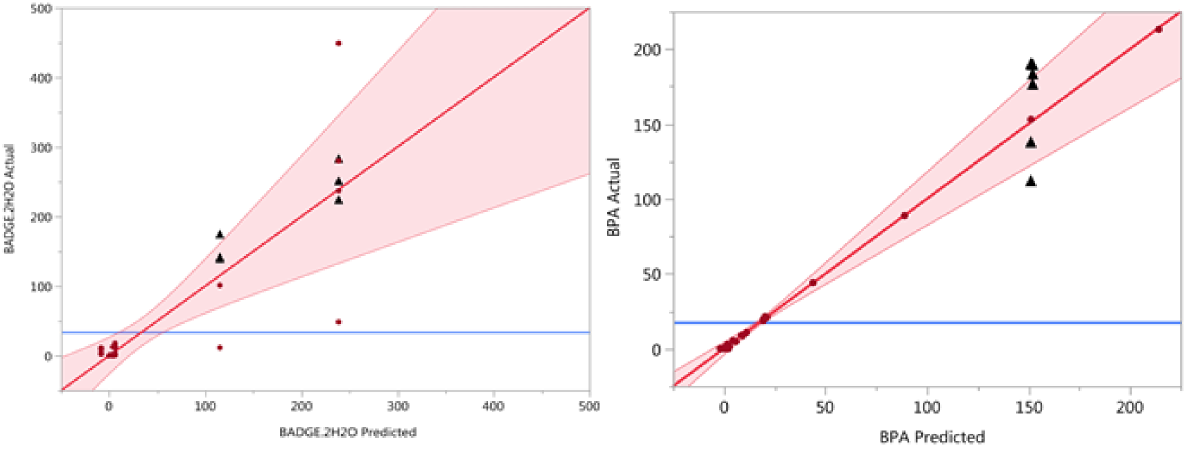
3.3. Testing models adequacy and validation

A comparison of predicted values given by the models and experimental values is given in **Fig. 3.2**. Most of the output responses of validation experiments lay within the confidence intervals (the red margins) of actual by predicted plots of BADGE.2H_2O and BPA, particularly for models based on Design II. So, it seems that our predictive models are quite satisfactory to predict BADGE and BPA migration in sterilized cans. This is confirmed by calculation of relative error (η) between the measured concentrations and the predicted concentrations are gathered in **Supplementary material - Table 3.S4**. Values are in the range 0.01-0.06 (mean = 0.04) for BADGE.2H_2O, and 0.004-0.34 (mean = 0.12) for BPA. Thus, except for extreme values observed for BPA, these relative errors are below the threshold and validate the models.

To test further the models adequacy, the analysis of variance, coefficient of determination, significance and lack of fit of the actual to predicted plot were carried out (see **Supplementary Material – Table 3.S5**). Adjusted coefficients of determination ($R^2_{adjusted}$) show good prediction of the models in the case of BPA for both experimental designs, and

BADGE,2H_2O for Design II. Interestingly, whatever the models, statistical F-ratios correspond to very small p-values, indicating that these migration models are significant to predict the migration of BPA and BADGE,2H_2O based on canned food processing and storage parameters. Also, the values of $R^2_{predicted}$calculated for Design II-based models are close to $R^2_{adjusted}$.

Design I



Design II

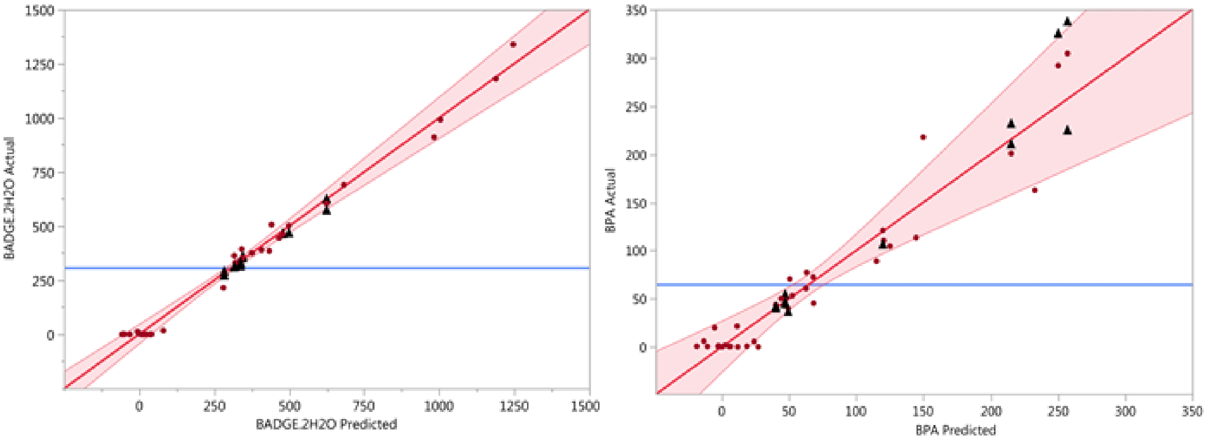


Figure 3. 2: Predictive capability of models built for both design of experiments (with red margins representing the confidence intervals): (●) data from development set, (▲) data from validation set.

In conclusion, the proposed model built on Design II enables good prediction concentration of BPA and BADGE.2H_2O migration for all can brands and process parameters considered.

3.4. Valuable models application for the industry

Experimental design modeling is a suitable tool to evaluate the impact of the different process factors on migrant concentrations, but also to determine the optimized combination of factors to comply with the regulation. Hence, it is possible with JMP software to change the desirability of model output (e.g. BPA concentration < 50 µg/kg) to obtain the best input combination to reach this maximum desirability (**Fig. 3.3**): the software evaluated all possible combinations between our studies parameters (i.e. type of can, sterilization time, storage temperature and storage time) in order to determine the best combination to achieve BPA levels lower than 50 µg/kg.

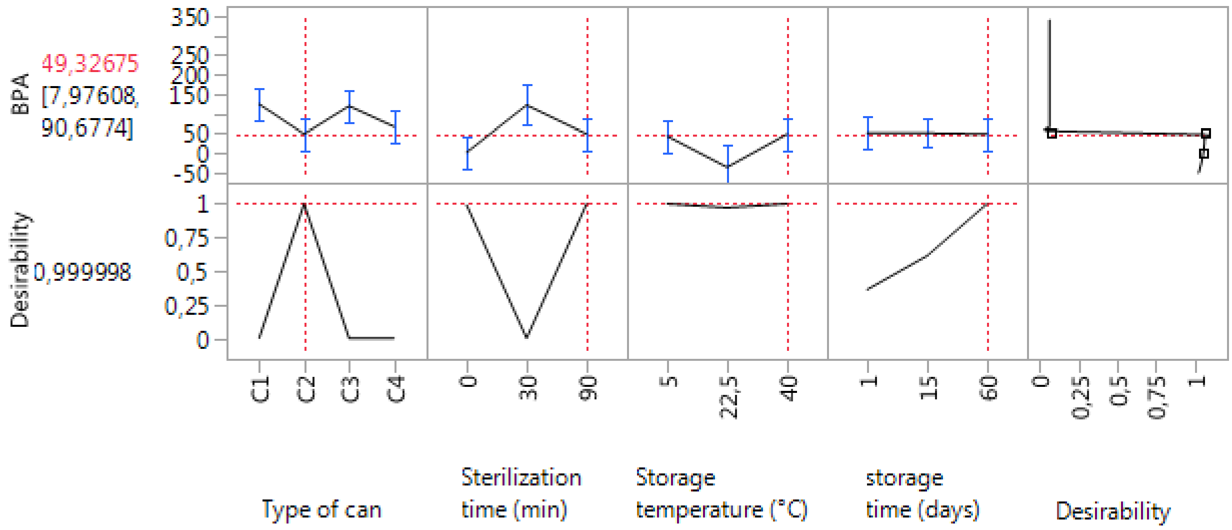


Figure 3.3: Desirability study for optimization of process parameters to comply European 2018/213.

In this illustrative application, the storage temperature has a very weak effect on desirability which is in accordance with the non-significance of that parameter in the model (p-value = 0.652). Other parameters play a key role on desirability. In particular, considering the types of can studied, only cans C2 are suitable to reach BPA migration in compliance with SML of 50 µg/kg, as previously discussed. For sterilization at 121°C, 90 min is more suitable than 30 min, which was unexpected as previously discussed. This could be explained either by BPA back retention in the coating over time, or by BPA disappearance due to reaction of phenolic groups at high temperature. Interestingly, in the case of storage time, despite a weak influence on BPA concentrations its effect on desirability is important. Thus, according to this desirability study, in our case the optimum combination to limit BPA levels lower than 50 µg/kg should be cans C2, 90 min sterilization, storage at room temperature for 60 days.

4. Conclusion

At the time where most migration models developed take into account the physicochemical properties of packaging, which is complex to control at the level of food canning industries, this paper provides interesting simple predictive models, based on response surface methodology. Such models could be very helpful for industries to take precautions or provide advices with a view to minimizing BPA concentration in order to comply with new European regulation 2018/213. Since our modeling methodology was limited to a small set of cans, it would be interesting to carry the same study on a wide variety of commercial cans and to follow the trend of migration of other intentionally and non-internationally added substances in the lacquer materials. Indeed, the proposed approach could be applied to the study of other migrants.

5. References

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Conclusion

Our main objective of this article was to build empirical models that are useful in predicting the migration of target bisphenol compounds from tinplate cans under different processing and storage conditions. The approach of the models was simple, and we think it could be valuable and helpful for industries to take precaution aiming at minimizing the release of bisphenol compounds (here BPA and BADGE.$2H_2O$). Our models were greatly significant in fitting the level of migrants depending on the input variables with high adjusted coefficients of determination. Nonetheless, the application of these models is still limited since our modeling methodology considered only small set of cans. Therefore, it is essential to carry the same study on a wide variety of commercial cans in the future.

In the meantime, there is another important source of food contamination from metal cans, that is the release of large family of metals present in the alloying layer. For instance, some trace metals represent serious hazards for human health, such as Pb and Cd. Accordingly, the release of trace metals from tinplate food cans into frequently consumed foods in Lebanon will be deeply discussed in the coming chapters.

Supplementary Material Chpt.3

Supplementary Material-**Table 3.S1**: Details of experimental Designs I and II (development and validation sets).

	Design I				Design II				
Experiment	Type of can	Food Simulant	Storage		Type of can	Food Simulant	Sterilization time (min)	Storage	
			T°C	Time (day)				T°C	Time (day)
Development									
1	C1	S1	5	60	C1	S1	90	5	60
2	C1	S1	40	1	C1	S1	90	40	1
3	C1	S2	40	1	C1	S2	0	5	60
4	C1	S2	5	60	C1	S1	0	22.5	15
5	C1	S3	22.5	1	C1	S1	0	40	60
6	C1	S3	40	60	C1	S2	0	22.5	1
7	C1	S4	40	15	C1	S2	90	40	60
8	C1	S4	5	1	C1	S2	90	5	15
9	C1	S4	22.5	60	C1	S2	30	40	15
10	C2	S1	5	1	C1	S1	30	5	1
11	C2	S1	40	60	C2	S1	90	40	60
12	C2	S2	40	60	C2	S2	90	5	60
13	C2	S2	5	1	C2	S2	30	22.5	15
14	C2	S3	40	1	C2	S1	0	40	1
15	C2	S3	5	15	C2	S2	0	40	60
16	C2	S3	22.5	60	C2	S2	0	5	1
17	C2	S4	22.5	1	C2	S1	90	5	1
18	C2	S4	5	60	C2	S2	90	40	1
19	C3	S1	40	1	C2	S1	0	5	60
20	C3	S1	5	60	C3	S2	90	5	60
21	C3	S2	5	1	C3	S1	0	5	60
22	C3	S2	40	60	C3	S1	90	40	60
23	C3	S3	40	1	C3	S1	0	40	1
24	C3	S3	5	60	C3	S2	0	40	60
25	C3	S4	40	60	C3	S1	90	5	1
26	C3	S4	5	1	C3	S2	0	5	1
27	C4	S1	40	60	C3	S2	90	40	1
28	C4	S1	5	1	C4	S2	90	5	1
29	C4	S2	5	60	C4	S2	90	40	60
30	C4	S2	40	1	C4	S1	0	40	60
31	C4	S3	5	1	C4	S1	90	5	60
32	C4	S3	40	15	C4	S1	90	40	15
33	C4	S3	5	60	C4	S1	30	22.5	1
34	C4	S4	40	60	C4	S2	0	40	1
35	C4	S4	40	1	C4	S2	0	5	60
36	C4	S4	5	15	C4	S1	0	5	15

Supplementary Material-**Table 3.S1 (Continued)**: Details of experimental Designs I and II (development and validation sets).

	Design I				**Design II**				
			Storage					Storage	
Experiment	Type of can	Food Simulant	T°C	Time (day)	Type of can	Food Simulant	Sterilization time (min)	T°C	Time (day)
Validation									
1	C1	S4	40	15	C1	S1	30	5	1
2	C1	S4	40	15	C1	S1	30	5	1
3	C1	S4	40	15	C1	S1	90	40	1
4	C1	S4	22.5	15	C1	S1	90	5	60
5	C1	S4	22.5	15	C1	S2	90	5	15
6	C1	S4	22.5	15	C1	S2	90	5	15
7					C2	S1	90	5	1
8					C2	S1	90	5	1
9					C2	S1	90	5	1
10					C2	S1	90	40	60
11					C2	S2	30	22.5	15
12					C2	S2	30	22.5	15

Supplementary Material-**Table 3.S2**: Selection of estimated regression coefficients with their standard error and significance* against zero for models based on Design I.

BADGE.2H_2O				
Term		Scaled Estimate	Std error	p-value scaled = 0
β_0		30.95	9.74	0.0036
β_2x_2	If [S1]	-28.93	17.48	0.190
	If [S2]	-27.64	17.48	0.125
	If [S3]	-27.73	16.23	0.0985
	If [S4]	84.30	16.23	**<0.0001**
β_4		32.85	10.26	**0.0034**
$\beta_{24}x_2$	If [S1]	-30.83	17.78	0.093
	If [S2]	-30.15	17.78	0.101
	If [S3]	-29.79	17.78	0.104
	If [S4]	90.7798	17.78	**< 0.0001**

BPA				
Term		Scaled Estimate	Std Error	p-value scaled = 0
β_0		53.16	10.16	**0.0004**
β_1x_1	If [C1]	10.16	5.13	0.075
	If [C2]	-16.82	4.90	**0.006**
	If [C3]	6.49	5.08	0.229
	If [C4]	0.16	4.81	0.974
β_2x_2	If [S1]	-6.22	5.08	0.248
	If [S2]	-6.85	5.08	0.207
	If [S3]	-15.63	4.76	**0.0082**
	If [S4]	28.71	5.03	**0.0002**
β_4		8.35	2.84	**0.003**
β_5		10.5	3.18	**0.025**
$\beta_{12}x_1x_2$	If [C1]* [S1]	-13.14	8.70	0.161
	If [C1]*[S2]	-12.15	8.70	0.192
	If [C1]* [S3]	-24.28	8.68	**0.0189**
	If [C1]*[S4]	49.58	8.68	**0.0002**
	If [C2]*[S1]	13.39	8.56	0.148
	If[C2]*[S2]	14.57	8.56	0.119
	If [C2]*[S3]	5.90	7.68	0.459
	If[C2]*[S4]	-33.87	8.63	**0.002**
	If [C3]*[S1]	-7.24	8.66	0.422
	If [C3]*[S2]	0.59	8.66	0.946
	If [C3]*[S3]	2.93	8.48	0.735
	If [C3]*[S4]	3.69	8.63	0.677
	If [C4]*[S1]	6.99	8.51	0.430
	If [C4]*[S2]	-3.01	8.51	0.730
	If [C4]*[S3]	15.43	7.76	0.074
	If [C4]*[S4]	-19.40	8.00	**0.035**

Supplementary Material-**Table 3.S2 (Continued)**: Selection of estimated regression coefficients with their standard error and significance* against zero for models based on Design I.

BADGE.2H$_2$O			
Term	Scaled Estimate	Std error	p-value scaled = 0

BPA				
Term		Scaled Estimate	Std Error	p-value scaled = 0
$\beta_{15}x_1$	If [C1]	16.65	5.02	**0.007**
	If [C2]	-14.41	5.00	**0.016**
	If [C3]	0.894	4.95	0.860
	If [C4]	-3.14	4.88	0.533
$\beta_{25}x_2$	If [S1]	-8.72	4.95	0.108
	If [S2]	-8.05	4.95	0.134
	If [S3]	-8.73	4.96	0.109
	If [S4]	25.51	5.01	**0.0005**
β_{55}		-43.20	11.05	0.0029

** based on p-value from a t-test assessing if the estimated values are statistically different from zero*

Supplementary Material **-Table 3.S3**: Estimated regression coefficients of the models built to fit data from Design II.

BADGE.2H_2O		
Term		Scaled estimate
β_0		494.10
$\beta_1 x_1$	If [C1]	-8.33
	If [C2]	-123.24
	If [C3]	249.25
	If [C4]	-117.68
$\beta_2 x_2$	If [S1]	-38.84
	If [S2]	38.84
β_3		300.79
β_4		9.25
$\beta_{12} x_1 x_2$	If [C1]*[S1]	-10.86
	If [C1]*[S2]	10.86
	If [C2]*[S1]	32.84
	If [C2]*[S2]	-32.84
	If [C3]*[S1]	-40.53
	If [C3]*[S2]	40.53
	If [C4]*[S1]	18.56
	If [C4]*[S2]	-18.56
$\beta_{13} x_1$	If [C1]	-27.25
	If [C2]	-117.37
	If [C3]	249.60
	If [C4]	-104.98
$\beta_{23} x_2$	If [S1]	-32.73
	If [S2]	32.73
$\beta_{24} x_2$	If [S1]	-19.76
	If [S2]	19.76
β_{33}		-187.99

BPA		
Term		Scaled estimate
β_0		97.38
$\beta_1 x_1$	If [C1]	53.93
	If [C2]	-37.09
	If [C3]	7.73
	If [C4]	-24.57
β_3		45.98
β_4		2.64
β_5		-19.75
$\beta_{13} x_1$	If [C1]	23.23
	If [C2]	-22.48
	If [C3]	16.93
	If [C4]	-17.67
$\beta_{15} x_1$	If [C1]	-42.80
	If [C2]	18.24
	If [C3]	5.04
	If [C4]	19.51
β_{33}		-117.62
β_{44}		82.02

Supplementary Material-**Table 3.S4**: Comparison between observed and predicted output responses for models based on Design I and Design II.

Can type	Food simulant	Sterilization time (min)	Storage temperature (°C)	Storage time (min)	**BADGE.2H2O (µg/kg)**			**BPA (µg/kg)**		
					Observed	predicted	errorη	observed	predicted	errorη
Design I										
C1	S4		40	15	283	229	0.19	138	151	0.10
C1	S4		40	15	224	229	0.02	112	151	0.35
C1	S4		40	15	251	229	0.09	190	151	0.,20
C1	S4		22.5	15	142	109	0.23	177	152	0.14
C1	S4		22.5	15	139	109	0.22	190	152	0.20
C1	S4		22.5	15	175	109	0.38	183	152	0.17
Design II										
C1	S1	30	5	1	354	343	0.03	225	257	0.14
C1	S1	30	5	1	363	343	0.05	338	257	0.24
C1	S1	90	40	1	467	483	0.03	325	250	0.23
C1	S1	90	5	60	471	489	0.03	45	47	0.05
C1	S2	90	5	15	574	611	0.06	55	47	0.14
C1	S2	90	5	15	623	611	0.01	47	47	0.01
C2	S1	90	5	1	317	323	0.02	232	215	0.07
C2	S1	90	5	1	334	323	0.03	211	215	0.02
C2	S1	90	5	1	326	323	0.01	107	120	0.12
C2	S1	90	40	60	312	294	0.06	37	49	0.34
C2	S2	30	22.5	15	274	289	0.06	42	40	0.05
C2	S2	30	22.5	15	292	289	0.01	40	40	0.004

Supplementary Material-**Table 3.S5:** Adequacy and significance tests of regression models.

Model Fit

	Design I		Design II	
	$BADGE.2H_2O$	BPA	$BADGE.2H_2O$	BPA
$R^{2\,a}$	0.693	0.999	0.989	0.912
$R^2_{adjusted}{}^b$	0.617	0.994	0.981	0.854
$R^2_{predicted}$	0.948	0.956	0.977	0.937

Analysis of variance

	Design I						Design II					
	$BADGE.2H_2O$			BPA			$BADGE.2H_2O$			BPA		
	Model	Error	Total	Model	Error	Total	Model	Error	Total	Model	Error	Total
Degree of freedom (*df*)^c	7	28	35	32	3	35	15	20	35	14	21	35
Sum of Squares (SS)^d	213651	94384	308035	6924	34.9	69280	4492456	49790	4542246	212182	20444	232626
Mean Square (MS)^e	30522	3371		2164	11.6		299497	2489		15156	973	
F-ratio^f	9.05			186			120.3			15.57		
P-value^g	<0.0001*			0.0006*			<0.0001*			<0.0001*		

** Significant effect if* $p<0.05$

a: coefficient of determination defined as $R^2 = 1 - SS_{(error)}/ SS_{(Total)}$ $(0 \leq R^2 \leq 1)$

b: adjusted coefficient of determination defined as $R^2_{adjusted} = 1 - (SS_{(error)}/ n\text{-}p)/ (SS_{(Total)}/n\text{-}1)$, *where (n) is the number of observations and (p) is the number of regression coefficients*

c: number of free units of information

d: calculated by summing the squared factor effect for each run

e: $MS = SS/df$

f: $F\text{-ratio} = MS_{(model)}/ MS_{(error)}$

g: looked up in the F table

Chapter 4:

Parameters influencing the migration of trace metals in uncoated fruit cans

Introduction

In the present article, we propose a separate focus and angle on the research topic than the previous two chapters. Our target now is to carry a series of assays on the potential migration of metal trace elements into foods, which may result from the direct contact with metal walls of cans in the absence of an internal protective organic coating. Uncoated tinplate cans are often used for acidic and light-color fruits. Since food processors in the Lebanese market are not producers of canned fruits, the designated canned fruit samples for this study are well known important brands.

Following the overall goals of this report, the bulk of this chapter is to investigate the influence of different parameters on the migration on 8 metal elements. Parameters related to storage conditions and canned fruit properties, as well as can denting and keeping opened canned fruits in the refrigerator, are deeply studied and discussed. Appropriate sample treatment and elemental analysis techniques were validated in an attempt for answering the research questions.

The main established findings will successfully serve to advice the consumers and canned food industries to take precautions in an effort to minimize the exposure to undesirable levels of potentially toxic metals from uncoated food cans.

At the end of the chapter a complementary section is provided to illustrate a new approach of data treatment (not available in the article submitted for publication). Accordingly, multivariate principle component analysis was performed to overview the impact of studied parameters on the evolution of all target metal elements at the same time.

Parameters influencing the migration of trace metals in uncoated fruit cans

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Graphical abstract

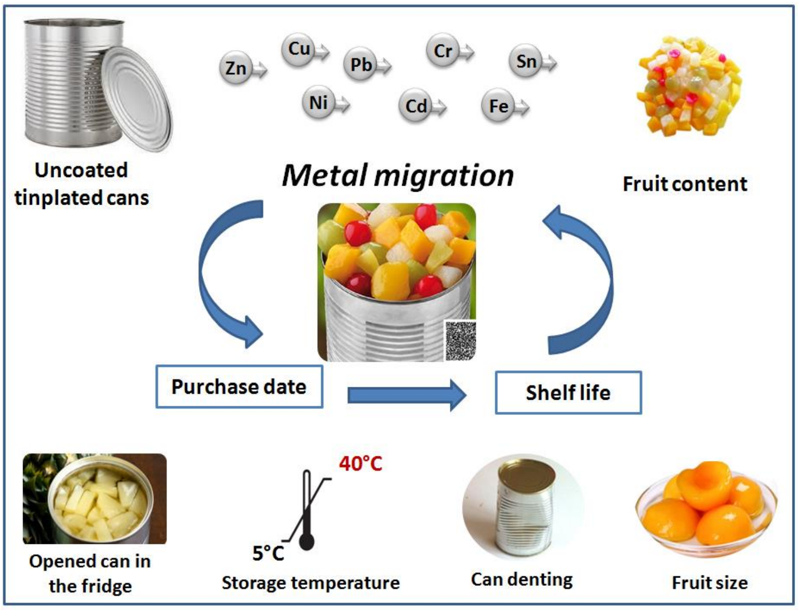

Figure 4.1: Graphical abstract

Abstract

The migration of 8 trace elements (Fe, Sn, Cr, Cd, Pb, Ni, Cu and Zn) was monitored in uncoated fruit cans as a function of storage time and temperature, until the shelf life. The effects of can denting and leaving opened cans in refrigerator were investigated. Product properties like fruit size and coating on side seam were considered. As a result, most of these elements significantly increased as a function of time and were affected by storage temperature. Particularly, storage at 40°C caused threatening release of Sn and Fe. Denting enhanced the release of most of metals. Moreover, Fe and Sn levels drastically increased in cans kept opened in fridge. Small pieces of fruits contained higher level of metals than larger ones. The side seam coating lowered the migration of trace elements. Finally, consumers are advised to purchase newly produced and undamaged cans, and to keep them at low temperature.

Keywords: trace metals, canned fruits, uncoated cans, storage conditions, migration

1. Introduction

Food packaging, namely food cans, are proven to be a significant source of potentially toxic metals that can migrate into food [1]. Typical tinplate cans are formed of steel alloys coated with a tin layer, thus iron (Fe) and tin (Sn) are their main constituents. Uncoated cans are frequently used for food types including white fruits and vegetables, but more often, an additional internal organic coating is applied to limit the direct contact between food and metallic layers. Nickel (Ni) and zinc (Zn) are sometimes intentionally added to enhance cans resistance to rusting and corrosion, while copper (Cu) could be present to provide a hardening advantage [2]. In some cases, Sn is replaced by corrosion-resistant chromium layer, mainly in the lids of cans (Tin free steel (TFS)). Other metals like cadmium (Cd) or lead (Pb) are likely present as impurities [2].

Food may contain trace metals which have recognized nutritional importance such as Fe, Ni, Zn and Cu, while high concentrations in food may still have adverse effects on health or organoleptic properties of food. On the other hand, Cd and Pb are recognized as neurotoxic compounds even at low concentrations [3]. Consequently, the levels of Cd and Pb in tinplate utensils and packaging are controlled with a maximum level of 0.010% [4]. Some other reports suggested a correlation between high content of Sn and gastrointestinal symptoms, diarrhea, and headache [5]. At last, the speciation of Cr is of great importance for toxicology since Cr(III), the most stable state, is an essential element for humans while Cr(VI) is highly toxic. Cans are expected to release Cr(III) form into food [2].

In order to prevent heath concern, the Council of Europe have set in 2013 specific release limits (SRLs) for all trace metals involved in food packaging. Hence, SRL for Fe is set to 40 mg/kg while for canned food a maximum permissible limit (MPL) was fixed at 15 mg/kg [6]. For other metals, the SRLs were set to 100 mg/kg for Sn, 0.25 mg/kg for Cr, 4 mg/kg for Cu, 0.14 mg/kg for Ni, 5 mg/kg for Zn, 0.01 mg/kg for Pb and 0.005 mg/kg for Cd [7].

Several parameters can affect the migration phenomenon. Yet, the studies discussing these parameters are few. For instance, significant increase of metal elements was observed as a function of time and temperature. [8], [9]) showed the importance of chemical composition of the coating and its properties (e.g. porosity) on the migration phenomenon. Kassouf et al. [10] indicated the significant effect of can denting on the migration of Fe and Pb, as well as the significant variation in metal content between brands of canned chickpeas. Likewise, the acidity of the food content plays an important role in leaching of Fe and enhancing the dissolution of Sn in canned food [11]. Sometimes people tend to store food in cans after opening, yet some studies showed that the increased concentration of oxygen upon opening accelerates corrosion of food cans [1], [11], [12]. More studies presented the levels of trace metals in canned or fresh vegetables and fruits regardless of the storage time and conditions [3], [13]–[18]. As most of studies were made on coated food cans, interesting to monitor the effect of storage parameters on the migration of trace metals from uncoated where there is direct contact between foods and tin layer. Such cans are frequently used for fruits that are often consumed all over the world [15]. Since canned fruits are acidic media (pH < 4.5), they are expected to present higher release of metal trace elements than other less acidic food canned in coated cans. Therefore, the aim of the present study is to investigate the influence of storage time and temperature, can denting, and leaving opened cans in the fridge on the migration of Fe, Sn, Cr, Pb, Cd, Cu, Ni and Zn from uncoated tinplate cans into fruit cocktails, peaches and pears The migration of metal elements was also compared between different brands of totally uncoated or partially coated (on side seam) tinplate cans of fruit cocktail products.

2. Materials and Method

2.1. Reagents

All reagents used were of analytical grade. Standard solutions were freshly prepared through dilution of stock solutions with ultrapure water. Fe, Sn, Cd, Pb and Zn standard solutions were prepared from 1000 ± 4 mg/L stock solutions from Fluka Analytical (Buchs, Switzerland), and those of Ni, Cu, and Cr were prepared from 1000 ± 10 mg/L stock solutions from HACH (Dusseldorf, Germany). Microwave digestion was performed with nitric acid (69%; AnalaR, England) and hydrogen peroxide (30-31%; Sigma Aldrich, USA). All glassware was soaked in nitric acid (1%) overnight and then rinsed with ultrapure water.

2.2. Sample collection and storage conditions

A total of 69 cans of fruit cocktail, peach halves and pears halves were purchased from the local market of Lebanon. The main properties of the studied samples and the experimental design are detailed in **Table 4.1.** Note that three cans were considered for each experiment. Fruit cocktail cans were from different brands (A, B and C). Cans of same fruit product had the same production batch number. After purchase, fruit cocktail samples from brands A and B were divided into three groups that were stored at different temperatures i.e. in refrigerator (5 ± 1ºC), at room temperature (22 ± 4ºC) and in the oven (40 ± 2ºC). The remaining fruit products were stored at room temperature. Moreover, another group of fruit cocktail samples from brand A were willingly dented and stored at room temperature. All samples were analyzed at different time intervals between purchase date and until their shelf life (2 years) as indicated in **Table 4.1**. Finally the effect of keeping food in their cans in the refrigerator was investigated through opening three cans of fruit cocktail (brand A) and keeping them in the refrigerator for 10 days.

2.3. Sample treatment

The total content (syrup and fruits) of three cans from each sample were homogenized in a stainless steel blender. Three replicates of 2 g from each mixture were accurately weighed into a modified polytetrafluoro-ethylene (PTFE-TFM) microwave bomb vessel. Then 7 mL of nitric acid and 1 mL of hydrogen peroxide were added. The digestion was performed in a high-performance microwave digestion system (Anton Paar, Multiwave 3000, Graz, Austria) according to a program previously described **[10]**. The final clear digest was then completed to 25 mL with ultrapure water and filtered with 4 µm PTFE filters for subsequent analysis by Atomic Absorption Spectrometry (AAS). During the analysis of some elements, the samples were further diluted to remain within the linearity range of the AAS. Method quantification (MQL) and detection limits (MDL) were determined by analyzing 10 method blanks, which were prepared following the previously described analytical method.

2.4. Instrumental conditions

All elements were analyzed by a Thermo Electron Corporation Atomic Absorption Spectrometer with data acquisition carried on Thermo SOLAAR (version 10.11) software. Fe, Sn and Zn were analyzed on a flame atomic absorption spectrometer (FAAS). The analysis of Fe and Zn were respectively performed at λ = 248.3 and 213.9 nm, with an air–acetylene flame while Sn absorption was analyzed at λ = 286.3 nm using a nitrous oxide–acetylene flame. Graphite furnace atomic absorption spectrometer (GFAAS) was used with argon as inert gas for determining Pb (λ = 217 nm), Cd (λ = 228.8 nm), Cu (λ = 324.8 nm), Ni (λ = 323 nm) and Cr (λ = 357.9 nm). The GFAAS instrument was equipped with a D2 lamp for background correction. Calibration stock standards were appropriately diluted to calibrate the spectrometer within the ranges shown in **Table 4.2**.

Table 4.1: Samples properties and storage conditions

	Brand	**Purchase time (months)**	**pH ± SD***	**Fruits size**	**Can coating**	**Free space (cm)**	**Can aspect**	**Storage temperature (°C± SD)**	**Storage time (months)**
Fruit cocktail	A	7	4 ± 0.05	Small pieces	Uncoated	0.5	Normal	Refrigerator (5 ± 1) Room temperature (22 ± 4) Oven (40 ± 2)	9, 19, 24 7, 9, 12, 19, 24 9
							Dented	Room temperature (22 ± 4)	9, 19, 24
							Stored opened	Refrigerator (5 ± 1)	1/3 (i.e. 10 days)
	B	7	3.49 ± 0.10	Small pieces	Coated on side seam	0	Normal	Refrigerator (5 ± 1) Room temperature (22 ± 4) Oven (40 ± 2)	24 7, 24 12
	C	9	3.83 ± 0.05	Small pieces	Uncoated	0	Normal	Room temperature (22 ± 4)	9, 24
Peach halves	A	10	3.54 ± 0.04	Fruit halves	Uncoated	0.7	Normal	Room temperature (22 ± 4)	10, 24
Pears halves	A	12	3.71 ± 0.10	Fruit halves	Uncoated	0.5	Normal	Room temperature (22 ± 4)	12, 24

n= 3 cans per experiment
** SD of pH was determined on 3 cans per fruit product*

Table 4.2: Performance of analytical instrument and method

Metal	Instrument	Linearity Range	R^2	MDL	MQL
		mg/L		mg/kg	mg/kg
Fe	FAAS	0.5-5	0.9998	0.65	1.7
Sn	FAAS	5-50	0.9998	9.8	25.4
Zn	FAAS	0.2-1.2	0.9997	0.33	1.1
		µg/L		µg/kg	µg/kg
Pb	GFAAS	5-70	0.9976	9.3	30.1
Cd	GFAAS	1.25-7.5	0.9915	1.7	2.09
Cu	GFAAS	6-40	0.9910	10.4	28.8
Ni	GFAAS	5-50	0.9930	17.1	61.0
Cr	GFAAS	0.9-4.5	0.9930	2.8	9.0

A buffer solution (0.2% w/v KCl of 1 g/L) was added to both the Sn standard and sample solutions to reduce its ionization in the nitrous oxide–acetylene flame (temperature around 2800°C). For Fe and Zn analysis, strontium releasing agent (1 g/L) was used in order to overcome most common interferences in air-acetylene flame. In the case of GFAAS, matrix modifier (4% v/v mixture of 1 g/kg magnesium nitrate and 1 g/kg of ammonium phosphate solution) was used to trap Cd and Pb during matrix ashing in order to overcome the matrix effect.

2.5. Method accuracy

For validating the used methodology, white cabbage certified reference material (BCR® -679, Sigma Aldrich, Geel, Belgium) was analyzed using the same analytical method. This reference material contains all targeted elements, except Pb and Sn. So, the latter two elements were spiked into fruit cocktail samples, in the homogenized mixture prior to microwave digestion, at three concentration levels covering the working ranges of Pb and Sn (**Table 4.3**).

2.6. Statistical analysis

The obtained data was analyzed with the XLStat and Microsoft Excel software (2007). Testing for significance of mean effects and interactions on all variables was calculated using ANOVA analysis of variance. Statistical significance was set at $p \leq 0.05$.

Table 4.3: Recoveries of trace metals using CRM and spiked samples

Metal	**Validation sample**	**Known Concentration (mg/kg)**	**Found Concentration (mg/kg)**	**Recovery (%)**
Fe	CRM (BCR® -679)	55.0 ± 2.5	59.7 ± 2.0	108.5 ± 3.6
Cr		0.60 ± 0.10	0.60 ± 0.04	100.0 ± 6.6
Cd		1.66 ± 0.07	1.51 ± 0.05	90.9 ± 3.0
Ni		27.0 ± 0.8	26.7 ± 3.0	98.8 ± 11.1
Cu		2.89 ± 0.12	2.65 ± 0.14	91.7 ± 4.8
Zn		79.7 ± 2.7	74.8 ± 3.3	93.8 ± 4.1
Sn	Spiked samples	25.0	25.3 ± 0.96	101.2 ± 3.8
		187.5	196.3 ± 6.7	104.7 ± 3.6
		375.0	341.8 ± 9.9	91.2 ± 2.6
Pb		0.050	0.054 ± 0.006	108.0 ± 13.3
		0.100	0.091 ± 0.006	90.8 ± 5.8
		0.200	0.170 ± 0.015	82.8 ± 7.5

3. Results and discussion

3.1. Method performance

Quantification was performed with external calibration using five concentration level. R^2 values of linearity were in the range of 0.9910-0.9998 (**Table 4.2**). MDL and MQL in **Table 4.2** were calculated as the blank signal, plus 3 and 10 times, respectively, its standard deviation (SD) **[19]**. Method recoveries were found satisfactory, ranging from 90 to 110% (**Table 4.3**).

3.2. Metal content in canned fruits

Metal elements are natural contents of foodstuffs. However, the contact with food packaging materials, uncoated cans in our case, contribute to increasing levels of metals in foods. In this study, metal elements were found in the ranges: 3.14-8.54 mg/kg for Fe, 28.2-138 mg/kg for Sn, 0.19-0.45 mg/kg for Cr, 2.22-24.07 µg/kg for Cd, 0.46-1.24 mg/kg for Ni, 0.03-4.00 mg/kg for Cu and 1.15-3.68 mg/kg for Zn. Levels of Fe, Sn, Cd and Cu are in agreement with the ranges reported in canned foods from the Lebanese market **[13], [14]**. These authors also found that the levels of Fe were lower in canned fruits than in vegetables and meats. On the contrary, Zn was observed at lower concentrations compared to Korfali et al. (2-12 mg/kg) **[14]**. The obtained concentrations of Sn, Cr, Cd and Ni were also within the ranges present in canned fruits from previous studies **[11], [15], [16], [18]**. Interestingly, Pb was not detected in any sample stored at room temperature, which does not match previously published values in fruits (0.0007-3 mg/kg) **[15], [16], [18]**.

3.3. Effect of storage time at room temperature

Since Fe and Sn are the basic components of tinplate cans, it is expected that the highest release of metals is related to these two elements. In fact, the migration of Fe was mainly insignificant ($p > 0.05$) over the studied time interval. Nevertheless, a significant increase was noticed in the case of fruit cocktail and peach halves from brand A (**Table 4.4**). These released levels were much lower than those reported in coated cans of meats, vegetables **[8]** and chickpeas, where Fe increased by around 20 mg/kg **[10]**. This is surprising, since the acidic medium of canned fruits (pH<4) and the absence of a protective coating should favour the corrosion and the subsequent release of Fe **[11]**. Therefore, this result is an indication of the good protection of the tin layer in our tinplated cans.

On the other hand, the evolution of Sn with storage time was significant ($p < 0.05$) in all the studied samples. According to **Table 4.4** and **Figure 4.2a**, Sn levels increased by a minimum value of 53% in fruit cocktail of brand A and a maximum value of 208% in peach halves of brand A. According to literature, Sn is present in foodstuffs only in small quantities, generally less than 1 mg/kg in unprocessed foodstuffs, but higher concentrations are found in canned foodstuffs **[2], [20]**. Consequently, most of the Sn present in our canned fruits potentially originate from the container. Moreover, higher migration levels are detected in our cans compared to those in coated cans **[8]–[10]** proving the efficiency of the organic coating in lowering the migration of Sn.

Further, the content of Fe and Sn along with the other targeted metal elements was significantly dependent ($p < 0.05$) on the brand of canned fruits (A to C) and the type of fruits (cocktail, peach and pears). At purchase, the lowest levels of Sn, Cr, Ni and Cu were detected in fruit cocktail of brand B (**Fig. 4.2a** and **Table 4.4)**. This result, more specifically for Sn, is correlated with the presence of protective coating on the side seam of band B cans. Moreover, brand A contained at purchase about one fourth the Cd concentration present in the two other brands. On the opposite, the highest concentrations of Cr, Ni and Cu were found in brand C. Nonetheless, it is difficult to estimate whether the food content or the cans stand behind this disparity in the metal content between brands. Therefore, a deeper investigation was carried on the migration trends of key metals between purchase date and shelf life, which to our best knowledge is rarely considered before.

Table 4.4: Evolution of trace metals in canned fruits (stored at room temperature) as a function of storage time

Product	Brand	Time (months)	Fe (mg/kg)	Sn (mg/kg)	Cr (mg/kg)	Cd (µg/kg)	Pb (µg/kg)	Ni (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Fruit cocktail	A	7	3.14 ± 0.27^{a}	75.7 ± 0.13^{a}	0.26 ± 0.007^{a}	2.22 ± 0.003^{a}	ND	0.46 ± 0.037^{a}	0.27 ± 0.021^{a}	2.58 ± 0.004^{a}
		9	4.63 ± 0.24^{b}	75.6 ± 0.02^{a}	0.33 ± 0.005^{b}	2.22 ± 0.0004^{a}	ND	0.62 ± 0.016^{b}	0.30 ± 0.026^{a}	2.87 ± 0.0001^{b}
		12	4.20 ± 0.50^{b}	80.7 ± 0.38^{b}	0.27 ± 0.034^{a}	4.41 ± 0.004^{b}	ND	0.70 ± 0.024^{c}	0.27 ± 0.021^{a}	3.24 ± 0.16^{c}
		19	-	123 ± 1.33^{c}	0.38 ± 0.015^{c}	4.21 ± 0.18^{b}	ND	0.87 ± 0.0001^{d}	0.38 ± 0.010^{b}	3.68 ± 0.26^{d}
		24	4.59 ± 0.25^{b}	116 ± 1.42^{d}	0.42 ± 0.038^{c}	3.81 ± 0.29^{b}	ND	0.94 ± 0.069^{d}	0.36 ± 0.009^{b}	3.15 ± 0.24^{c}
	% evolution (7-24 months)		**46.2 %**	**53.2 %**	**61.5 %**	**71.6 %**	**-**	**104.3 %**	**33.3 %**	**22.1 %**
	B	7	8.54 ± 0.23^{a}	36.9 ± 2.10^{a}	0.20 ± 0.020^{a}	8.71 ± 0.20^{a}	ND	0.29 ± 0.020^{a}	0.25 ± 0.030^{a}	2.19 ± 0.26^{a}
		24	8.41 ± 0.18^{a}	85.7 ± 6.90^{b}	0.24 ± 0.030^{a}	24.07 ± 0.96^{b}	ND	0.40 ± 0.070^{b}	0.82 ± 0.050^{b}	3.19 ± 0.31^{b}
	% evolution (7-24 months)		**Insignificant**	**132.2 %**	**Insignificant**	**176.3 %**	**-**	**37.9 %**	**228.0 %**	**45.6 %**
	C	9	6.86 ± 0.69^{a}	63.2 ± 2.30^{a}	0.45 ± 0.030^{a}	7.45 ± 0.70^{a}	ND	0.94 ± 0.110^{a}	0.35 ± 0.010^{a}	1.35 ± 0.09^{a}
		24	6.08 ± 0.52^{a}	138 ± 9.40^{b}	0.43 ± 0.010^{a}	9.73 ± 0.32^{b}	ND	1.24 ± 0.100^{b}	0.65 ± 0.060^{b}	2.74 ± 0.23^{b}
	% evolution (9-24 months)		**Insignificant**	**118.3 %**	**Insignificant**	**30.6 %**	**-**	**31.9 %**	**85.7 %**	**102.9 %**
Peach halves	A	10	4.79 ± 0.49^{a}	28.2 ± 2.10^{a}	0.19 ± 0.004^{a}	5.16 ± 0.40^{a}	ND	0.28 ± 0.002^{a}	0.29 ± 0.030^{a}	1.15 ± 0.17^{a}
		24	6.55 ± 0.26^{b}	86.9 ± 1.20^{b}	0.27 ± 0.004^{b}	19.81 ± 2.01^{b}	ND	0.48 ± 0.020^{b}	0.67 ± 0.040^{b}	1.36 ± 0.02^{a}
	% evolution (10-24 months)		**36.7 %**	**208.1 %**	**42.1 %**	**284 %**	**-**	**71.4 %**	**131 %**	**Insignificant**
Pears halves	A	12	4.72 ± 0.60^{a}	28.6 ± 1.80^{a}	0.19 ± 0.001^{a}	5.24 ± 0.09^{a}	ND	0.34 ± 0.007^{a}	0.27 ± 0.020^{a}	1.77 ± 0.05^{a}
		24	5.44 ± 0.46^{a}	79.6 ± 2.40^{b}	0.26 ± 0.012^{b}	21.25 ± 1.65^{b}	ND	0.43 ± 0.030^{b}	0.61 ± 0.030^{b}	1.87 ± 0.04^{a}
	% evolution (12-24 months)		**Insignificant**	**178.3 %**	**36.8 %**	**305 %**	**-**	**26.5 %**	**125.9 %**	**Insignificant**

* Within each element of each fruit product: a, b, c, and d correspond to significantly different concentrations of metals ($p<0.05$), whereas concentrations showing same letter are close

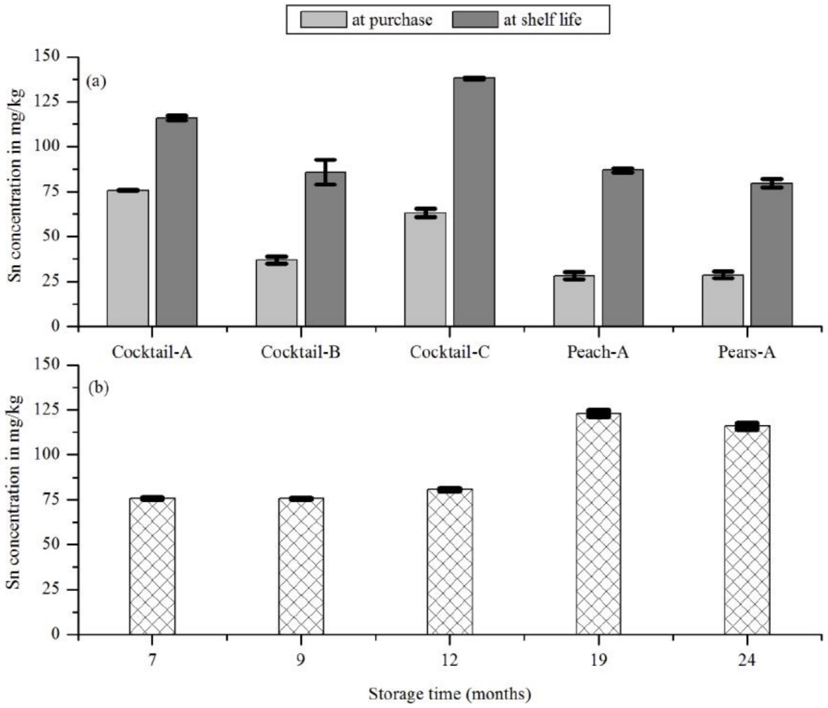

Figure 4. 2: Evolution of Sn between purchase date and shelf life: (a) in canned fruits from brands A, B and C and (b) as a function of time in fruit cocktail brand A.

The migration trends at different time intervals were surveyed in fruit cocktail of brand A. Interestingly, our results showed that each metal element has its unique behaviour, such that three migration trends can be highlighted (**Table 4.4** and **Fig. 4.2b)**; the early evolution of Fe that stabilizes at 9 months and is in agreement with previous finding **[9], [10]**, continuous evolution until shelf life that is the case of Sn, Ni and Zn, which tend to stabilize at 19 to 24 months, and finally the tendency to long-term slow migration that is observed by Cr, Cd and Cu.

In comparison between brands, the evolution of Fe and Cr were only significant in fruit cocktail of brand A, over the studied duration. This may be possible since Fe could have migrated at earlier storage time in the case of brands B and C, which is not available in our data. In case of Cr, the difference in the migration behaviour between brands is dependent on whether Cr is a constituent of the metal alloy or not. On the other hand, brand B showed the highest evolution of Sn (132%), Cd (176%) and Cu (228%), (**Fig. 4.2a** and **Table 4.4**). In fact, the comparably lower pH (3.49) of this brand could have reduced the protection efficiency of side seam coating resulting in a compensatory release of elements over long term storage time. The increase of the remaining element was highest in brands A (Ni (104%)

and C (Zn (103%)). No correlation between the content of metal elements and free head space was observed.

As a consequence of their migration, the release of Cd in brand B (+15.36 µg/kg) exceeded its SRL value of 5 µg/kg **[7]**, yet it maintained below its MPL of 50 µg/kg in foods **[21]**, while the release of Ni exceeded its SRL value of 0.14 mg/kg **[7]** in most designated samples except in brand B. The evolution of the rest of elements maintained within the recommended limits.

Another interesting finding is that, among canned fruits of brand A, most metals had close concentrations and migration rates in peach and pears halves, yet this observation greatly differ from fruit cocktail from same brand (A). In this case, the size of the fruits and their pH play an important role on the release of metal elements. For instance, canned peach and pears are larger fruit halves compared to the small pieces of the cocktail, therefore the former possess a smaller fruit-can contact surface. Consequently, Sn content in peach and pears halves at purchase time was less than half the concentration obtained in fruit cocktail (**Fig. 4.2a)**. On the other hand, according to **Table 4.1**, fruit halves have a comparably lower pH (around 3.60) than fruit cocktail (4.00). In this case, the lower pH of peach and pears halves was sufficient to accelerate the release of Sn up to 80-87 mg/kg at shelf life, but still lower than fruit cocktail (116 mg/kg).

Interestingly, Fe was found in close range between the three fruit products of brand A (4.59-6.55 at 24 months). This reveals that the content of Fe is more influenced by the constituent of the metallic can than the properties of food content. **Table 4.4** also shows that the migration of Cd and Cu was higher in peach and pears halves, while these fruits possess lower migration rates of Cr, Ni and Zn. In these samples, again, both Cd release in pears and Ni release in all samples except pears, exceeded their recommended SRL.

Briefly, although the results of Fe suggest that the variation in the migration phenomena between brands is higher than between fruit products, the evolution of other targeted metals revealed that the properties of both the cans and the food content have important contribution on metal release from uncoated tinplate cans.

3.4. Effect of storage temperature

A set of fruit cocktail cans from brands A and B were stored in the refrigerator (5°C) and in oven (40°C, simulating storage in hot areas). Surprisingly, at high temperature, the fruit cans were blown after few months (2 months for brand A and 5 months for brand B) due to important corrosion. In fact, under high storage temperature, the tin layer is more likely to be released leaving unprotected steel to corrode very rapidly, with vigorous evolution of hydrogen [5]. Consequently, Fe and Sn drastically increased in these samples (up to 118 times as indicated in **Table 4.5**), exceeding both their SRL value (40 and 100 mg/kg for Fe and Sn respectively) [7] and their maximum permitted levels in canned food (15 mg/kg of Fe [6] and 250 mg/kg of Sn [22]. The significant impact of 40°C was also noticed on the remaining metals, but to less extend than Fe and Sn.

Pb was not detected in fruit cocktail of brand A even at high temperature, yet it increased to 57.05 µg/kg in brand B, where it exceeded its SRL value of 10 µg/kg [7].

Lower storage temperature (5°C) was significant on slowing down the migration of most trace metals at earlier storage time (between 7 and 9 months in brand A). However, in most cases close concentrations were reached at 24 months between cans stored at 5 and 22°C.

Previous studies on coated cans highlighted the insignificant effect at high temperature (40-50°C) [9], [10], therefore, our study showed that in the absence of protective lacquer and in the presence of acidic medium, there is a significant effect of high storage temperature. This conclusion is in agreement with two studies made on unlacquered cans fruits [23] and mango pulp [11], where the content of Sn and Fe can increase by up to 12 fold when fruit cans are stored at 37 to 48°C

3.5. Effect of can denting

Upon denting of can walls, the dissolution of metal elements increased at early stage (2 months after denting). Levels of Fe, Sn and Cd were higher by 1.2- 1.9 times in dented cans (**Supplementary material - Table 4.S1**) than in the same undented cans. However, at shelf life, equal concentrations were reached in both cans. Thus, upon denting, the equilibrium of release of Fe, Sn and Cd is reached faster. On the other hand, the behaviour of Cu and Zn was different: accelerated at the beginning of storage and maintained higher until shelf life; at 24 months, dented cans were about 1.2 times richer in Cu (0.42 mg/kg) and Zn (3.89 mg/kg). The content of Cr and Ni was not affected by the denting.

Table 4.5: Evolution of trace metals in fruit cocktail (brands A and B) as a function of storage temperature

Brand	Storage time (months)	Storage Temp. (°C)	Fe (mg/kg)	Sn (mg/kg)	Cr (mg/kg)	Cd (µg/kg)	Pb (µg/kg)	Ni (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
A	7	22	3.14 ± 0.27	75.7 ± 0.13	0.26 ± 0.007	2.22 ± 0.003	ND	0.46 ± 0.037	0.27 ± 0.021	2.58 ± 0.004
	9	5	-	67.6 ± 0.02	0.20 ± 0.01	2.22 ± 0.0004	ND	0.38 ± 0.02	0.31 ± 0.002	2.49 ± 0.17
		22	4.63 ± 0.24	75.6 ± 0.02	0.33 ± 0.005	2.22 ± 0.0004	ND	0.62 ± 0.02	0.30 ± 0.03	2.87 ± 0.0001
		40	127 ± 3.5	345 ± 27	0.55 ± 0.02	4.3 ± 0.002	ND	0.74 ± 0.001	0.45 ±0.03	4.50 ± 0.25
	19	5	5.50 ± 0.27	106.4 ± 3.16	0.39 ± 0.003	3.50 ± 0.60	ND	0.81 ± 0.06	0.40 ± 0.002	3.11 ± 0.02
		22	6.06 ± 0.72	123 ± 1.3	0.38 ± 0.01	4.21 ± 0.18	ND	0.87 ± 0.0001	0.38 ± 0.01	3.68 ± 0.26
		40	Not analyzed*							
	24	5	4.96 ± 0.60	107 ± 10	0.42 ± 0.03	2.53 ± 0.09	ND	0.94 ± 0.03	0.35 ± 0.01	2.83 ± 0.01
		22	4.59 ± 0.25	116 ± 1.4	0.42 ± 0.04	3.81 ± 0.29	ND	0.94 ± 0.07	0.36 ± 0.01	3.15 ± 0.24
		40	Not analyzed*							
B	7	22	8.54 ± 0.23	36.9 ± 2.10	0.20 ± 0.02	8.71 ± 0.20	ND	0.29 ± 0.02	0.25± 0.030	2.19 ± 0.26
	12	40	1004 ± 35	538 ± 42	0.39 ± 0.03	21.12 ± 1.88	57.05 ± 1.56	0.39 ± 0.03	0.75± 0.04	2.67 ± 0.21
	24	5	8.53 ± 0.31	68.9 ± 4.3	0.25 ± 0.02	21.03 ± 0.41	ND	0.27 ± 0.03	0.76 ± 0.01	3.15 ± 0.12
		22	8.41 ± 0.18	85.7 ± 6.9	0.24 ± 0.03	24.1 ± 0.9	ND	0.40 ± 0.07	0.82 ± 0.05	3.19 ± 0.31
		40	Not analyzed*							

* The cans were blown and couldn't be analyzed

3.6. Effect of leaving opened cans in the fridge

Due to the atmospheric oxidation caused by increased air content in the opened cans, the tin layer was sloughed off. Consequently, the levels of Fe increased by 35 times to reach 108 mg/kg which greatly exceeded the recommended limits. About 105 mg/kg of Fe were released within 10 days (**Supplementary material - Table 4.S2**) exceeding its SRL **[7]**. Similarly, Sn increased by 3 times up to 223 mg/kg, exceeding its SRL of 100 mg/kg **[7]**, but yet remaining within its MPL in canned food of 250 mg/kg **[22]**. Copper was also affected by the increase of air-contact. In fact, according to a new study **[12]** metal release in canned food starts within only six hours after opening.

In contrast, our results showed that Ni, Zn and Cd were not affected by the severe corrosion in opened cans, probably requiring longer time to be released from the metal alloy. Interestingly, Cr showed a slightly significant decrease upon can opening (15.4%), there by indicating that Cr is only present in the can lids.

4. Conclusion

Taking in view that different parameters affect the migration of metal trace elements from tinplate cans into the packed food, elemental analysis revealed a significant release of almost all target metals into uncoated fruit cans. Under ambient conditions, Ni and Cd release exceeded its recommended SRL in 50% of designated fruit samples. In agreement with literature, the migration of Sn is significantly lowered upon applying protective coating, even if only on the side seem of the cans. Generally, the migration trend of metal elements was in relation with the size of fruits (smaller pieces = increased contact surface) and the pH of fruits content (lower pH was correlated to higher release of metals). Our study further proved that for fruits packed in uncoated tinplate cans, high storage temperature (40°C) caused a dramatic release of Fe and Sn. Refrigerating canned fruits (5°C) seems to be effective in lowering the migration of metals at early storage period. The effect of can denting was significant on accelerating the release of most target metals (except Ni, Cr and Pb) in fresh canned fruit cocktail. At shelf life, the effect of refrigerating and can denting became negligible on majority of metals. Finally, the increased air content upon the inappropriate storage of opened fruit cans in the refrigerator for few days caused a serious corrosion and threatening release of Fe and Sn.

Thereafter, aiming at minimizing the dietary intake of potentially toxic metals, this work advices the consumer to purchase recently produced canned foods that are stored at low temperature, especially when foods are packed in uncoated cans, as well as to avoid dented cans and leftovers in their cans, even if in the fridge.

5. References

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Complementary Analysis: Principle component analysis (PCA)

Further treatment of data obtained in this chapter was performed using multivariate principle component analysis (PCA) approach, which aids in visualizing the effect of studied parameters on the release of all key elements in the same time. Matlab R2016a was used here.

The studied matrix had 39 rows (samples) and 7 columns (metal concentrations). The first two principal components (PC1 and PC2) explained 80% of the total variance and were therefore chosen for the following discussions.

Differences between samples are examined in their projection on PC1 and PC2 score plot (**Fig. 4.3a** and **b**), where the presentation is made according to storage time, storage temperature, can denting and leaving opened cans in the refrigerator. Looking at the scores on PC1 (**Fig. 4.3a**) and upon comparing samples stored at room temperature (RT) and without denting (green dotted line), storage time seems to be at the origin of an increasing evolution of these samples' scores, until 19 months, tending to stabilize at 24 months. According to the corresponding loadings, all 7 trace metals contribute to this evolution. Thus, there is a potential evolution of all metal concentrations in fruit cocktail cans (brand A) as a function of storage time.

Samples, at same storage times, were noticeably distinguished with respect to their storage temperature. In fact, PC1 score plot (**Fig. 4.3a**) shows that samples at 40°C and 9 months have scores higher than all other samples. This indicates that the concentrations of the target metals in these cans are much higher than the rest of samples. Moreover, PC1 shows that samples stored in the refrigerator (5°C) at 9, 19 and 24 months (blue dotted line) had slightly lower scores than those stored at room temperature. Therefore, lower temperature seems to slightly minimize the release of metals from cans to food in contact.

The effect of can denting was also highlighted in PC1, where dented samples at 9, 19 and 24 months had slightly higher scores than undented samples at the same storage time and temperature. Finally, PC1 showed that samples stored opened in the refrigerator (O7 in **Fig. 4.3a**) are separated from sealed samples stored at room temperature over 7 months, where the former had higher score than the latest, thus the migration of target metals is potentially affected by the increase of air-content in the opened cans.

From these results, PC1 confirms the previous interpretation of the significant effect of the studied storage conditions on almost all studied metals.

PC2 (**Fig. 4.3b**) clearly shows the correlation between samples stored at 40°C and 9 months and the concentrations of Fe and Sn, therefore these two elements are the most affected by high storage temperature. In fact, as discussed previously, high storage temperatures enhances the corrosion of tinplate resulting in an important dissolution of Fe and Sn, as these two elements are the main constituents of can walls **[1]**. Moreover, PC2 provides a detailed correlation between "O7" samples and Fe, Sn and Cu. This result matches our previous discussion on this parameter. Here, PC2 showed that Cu was less affected by air-content in opened cans in refrigerator than Sn and Fe.

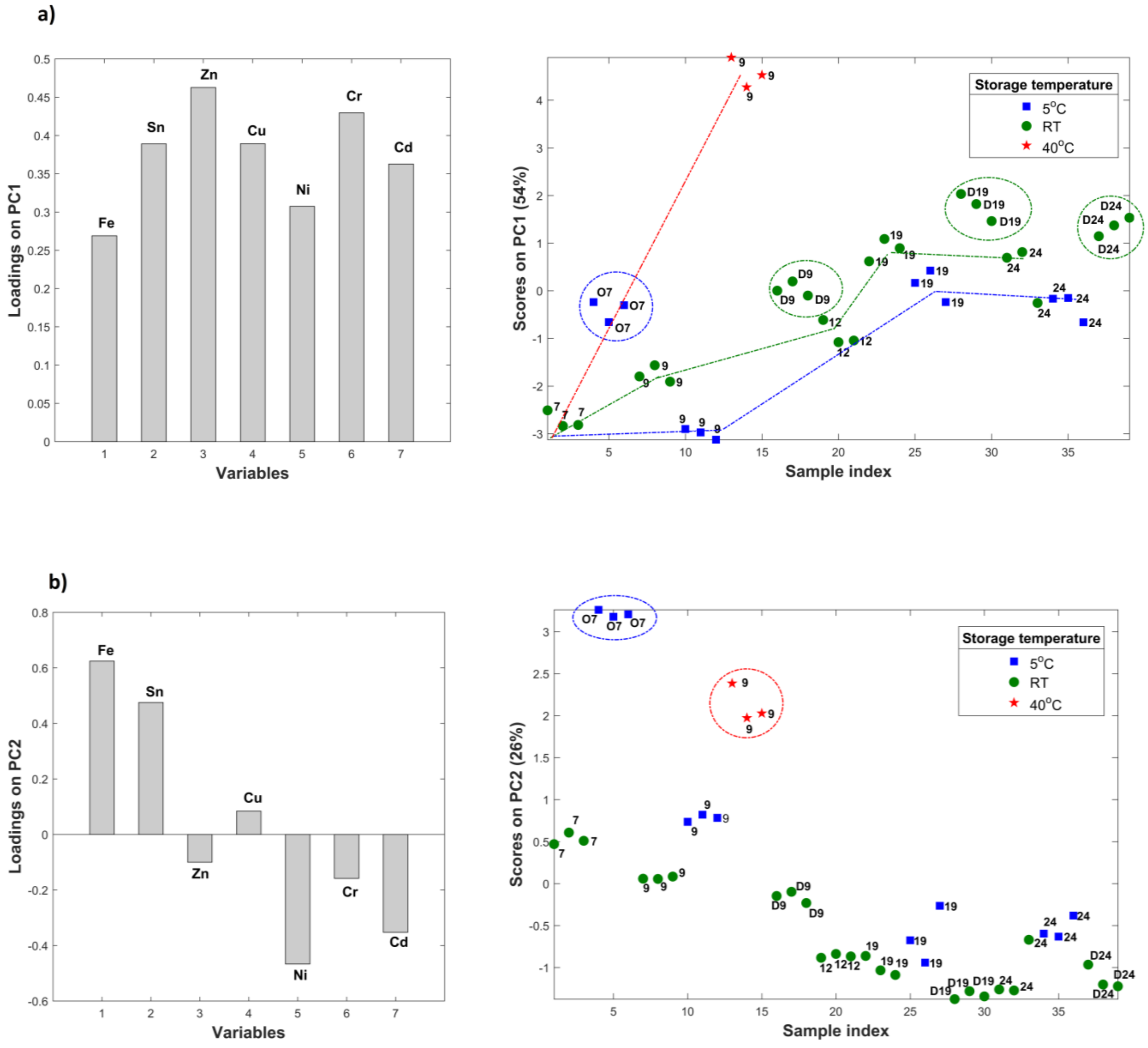

Figure 4.3: Scores and loadings on PC1 (a) and PC2 (b) resulting from PCA applied on 7 trace metals present in cans of fruit cocktail (brand A). Labels from 7 to 24 on the sample scores refer to storage time in months, whereas the letters (O) correspond to cans that were opened in the fridge and (D) to dented can.

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[1] J. Parkar and M. Rakesh, "Leaching of elements from packaging material into canned foods marketed in India," *Food Control*, vol. 40, pp. 177–184, 2014.

Conclusion

In this chapter we were interested in another source of food contamination than can coatings. We studied the migration of basic metal alloy constituents (e.g. Fe and Sn) and impurities (e.g. Pb and Cd) that are likely to be released from the direct contact with uncoated cans into fruits. We have established a significance of the tested parameters on the migration of key metals. High storage temperature for few months can lead to dangerous metals release (especially Fe and Sn), and causes severe corrosion to uncoated cans with acidic food content, like fruits. Most metals concentrations tend to increase as a function of time, but with different trends depending on the element. In this context some elements exceeded their maximum recommended release limits (e.g. Ni and Cd). Can denting seems to increase the migration phenomena, thus food cans showing similar defects should be avoided. Likewise, food should never be left in opened cans in the fridge due to excessive release of metals. Finally we showed that the properties of fruit content (e.g. fruit size) and metal can brands may have an effect on the resulted food contamination.

Moreover, part of data obtained in the article was further treated by PCA. The agreement between the classical interpretation and PC analysis, indicates that PCA is an efficient multivariate descriptive approach in our study. In this case, PCA allows to visualize and interpret the effect of all storage conditions on the migration of the target trace metals at the same time rather than one-factor and one-element at a time.

Here we have focused on uncoated cans, yet the majority of food cans on the market are often available with protective organic lacquer, so this packaging should be similarly tested. Moreover we didn’t consider in this chapter the effect of heat treatment as well as the initial concentration of metals in food. Indeed, we will overcome this shortfalls in the next chapter

Supplementary Material Chpt.4

Supplementary Material-**Table 4.S1:** Effect of can denting on the migration of target metals

Element	Time (months)	Normal	Dented
Fe (mg/kg)	9	4.63 ± 0.24	5.55 ± 0.02
	24	4.59 ± 0.25	4.74 ± 0.11
Sn (mg/kg)	9	75.6 ± 0.02	109.2 ± 2.7
	19	123 ± 1.33	124.8 ± 4.0
	24	116 ± 1.42	113.8 ± 0.99
Cd (µg/kg)	9	2.22 ± 0.0004	4.31 ± 0.002
	19	4.21 ± 0.18	4.50 ± 0.14
	24	3.81 ± 0.29	3.91 ± 0.07
Pb (µg/kg)	9	ND	ND
	19	ND	ND
	24	ND	ND
Cr (mg/kg)	9	0.33 ± 0.005	0.37 ± 0.005
	19	0.38 ± 0.015	0.36 ± 0.031
	24	0.42 ± 0.038	0.44 ± 0.008
Ni (mg/kg)	9	0.62 ± 0.016	0.50 ± 0.007
	19	0.87 ± 0.0001	1.07 ± 0.046
	24	0.94 ± 0.069	0.91 ± 0.041
Cu (mg/kg)	9	0.30 ± 0.026	0.42 ± 0.040
	19	0.38 ± 0.010	0.50 ± 0.003
	24	0.36 ± 0.009	0.42 ± 0.006
Zn (mg/kg)	9	2.87 ± 0.0001	3.14 ± 0.16
	19	3.68 ± 0.26	3.59 ± 0.33
	24	3.15 ± 0.24	3.89 ± 0.19

n= 3 replicates/experiment

Supplementary Material-**Table 4.S2:** Effect of leaving opened fruit cocktail cans for 10 days in refrigerator on the migration of target metals

Element	Before opening	After opening	p-value
Fe (mg/kg)	3.14 ± 0.27	108 ± 2.5	2.31E-07*
Sn (mg/kg)	75.7 ± 0.13	223 ± 4.7	6.94E-07*
Cr (mg/kg)	0.26 ± 0.007	0.22 ± 0.003	0.0020*
Cd (µg/kg)	2.22 ± 0.004	2.22 ± 0.001	0.327
Ni (mg/kg)	0.46 ± 0.04	0.51 ± 0.011	0.0925
Cu (mg/kg)	0.27 ± 0.021	0.44 ± 0.043	0.0037*
Zn (mg/kg)	2.58 ± 0.004	2.58 ± 0.002	0.608

n= 3 replicates/experiment

**: significantly different concentrations of metals ($p<0.05$)*

Chapter 5:

Simultaneous migration of bisphenol compounds and trace metals in canned vegetable food

Introduction

From undertaking deep research and investigations in previous chapters to assess the migration of metal elements and bisphenol compounds from tinplate food cans, it is noticed that intensive work is always carried on these migrants separately. To our best knowledge, migration tests that simultaneously consider both contaminants for the same canned food are rare. Therefore, the originality of this chapter is to obtain data that are the first one for the Lebanese and global market that couple metals and organic migration from tinplate cans.

The interest of this study lies within its multiple objectives. Previously we used food simulants to monitor the migration of bisphenol compounds, yet it is still a debate whether or not food simulants accurately estimate the real case scenario. It is, therefore, necessary to further carry the analysis on real foods. On the other hand, the migration study of metal elements was carried on uncoated cans, thus we expect to obtain different migration trends in coated cans. For this purpose, since our target is Lebanese market, we chose typical Lebanese vegetable food that are highly consumed worldwide (e.g. fava beans or foul muddammas, and okra) and packed in coated cans. Testing the contribution of processing and storage parameters on food contamination continues in this article as we study and discuss the effect of heat treatment temperature (sterilization and pasteurization), food type, can brand, storage conditions, can denting and cooking food directly in cans.

In the presence of organic coating it is expected that migration of metals will be limited, so that trace metals and bisphenol levels in food should be anti-correlated with respect to the studied parameters. Based on our results, we will represent here the probable correlations between the content metal elements and bisphenols in relation to the tested parameters. For this purpose principle component analysis (PCA) is a helpful multivariate analysis tool.

Simultaneous migration of bisphenol compounds and trace metals in canned vegetable food

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Highlights

- Sterilization favors bisphenol migration, with moderate effect on trace metals
- Storage conditions affect migration of both organic and inorganic contaminants
- Food type (pH, water content) has an effect on migration values
- BPA and Zn (and Pb to a lesser extent) show similar migration trends
- Most canned Lebanese food samples analyzed do respect European regulation

Abstract

For the very first time, this study investigates the simultaneous migration of several metals and bisphenol compounds from coated tinplate cans into vegetable food highly consumed worldwide. The role played by heat treatment, food type, can brand, storage conditions, can denting and cooking food directly in cans, is also assessed. Migration of bisphenol compounds (only BPA and BADGE.2H_2O detected) was mainly affected by sterilization, whereas metals release was greatly influenced by storage. Based on a principal component analysis different migration pattern groups were highlighted: BPA and Zn (and Pb to a lesser extent) showed similar migration trends, with dependence on food type, storage temperature and can brand. Cd, Ni and Cu were similarly influenced by food type and can brand. Fe has a particular trend, with clear influence of storage time, and to a lesser extent of food type. Overall Fe and BADGE.2H_2O migration were favored in acidic food.

Keywords: coated cans, heat treatment, food safety, food storage, migration, Principal Component Analysis (PCA).

1. Introduction

In recent years, changes in lifestyle have increased the need of ready-to-eat food and canned/packaged foods. Among the latter, canned food products offer several advantages, either for the producers or the consumers, namely ease of packaging, sterilizing, handling, transportation and preserving anaerobic environment of the food products [1]. In the meantime, the use of metal cans has to be carefully considered where it occasionally develops integrity problems due to corrosion which can lead to migration of metal ions [2]. To prevent corrosion and food contamination, metallic cans are commonly coated with a thin polymeric film on their inner surface. Yet, it is now well documented that polymerization of lacquer may not be fully complete and that a significant amount of unreacted compounds can be released in food packaged in coated cans [3]. Indeed, migration from packaging materials is of great concern due to possible adverse health effects [4].

Several trace metals are prone to migrate, among them tin, iron, lead, cadmium, chromium, nickel, zinc and copper being considered as the most common since they are often used in metal containers. In particular, low levels of tin are currently found in foods and beverages packaged in unlacquered or partially lacquered tinplate cans [5]. Also, iron is the basic element in the steel layer of food cans, while cadmium, nickel and copper may be found as alloying elements in steel. Furthermore, chromium treatment is widely applied to make the tin layer in tinplate cans less susceptible to oxidation damage and improve the enamel adherence [5]. Cr is also usually used in can lids. Likewise, zinc could be present to enhance tin and steel resistance to corrosion [6]. Finally, lead is often found in metal packages, so that it is one of the most important contaminants derived from packaging materials [7]. Accordingly, the resolution of Council of Europe on metals and alloys used in food contact materials and articles has reported the need for specific release limits (SRLs) for several trace metals to limit food contamination [8]. In addition, due to their toxicity, Sn, Pb and Cd are routinely determined in foodstuffs under the requirements of the European regulation n°1881/2006.

Besides trace metals, organic migrants are also of great concern. Common coating materials for food cans are epoxy resins, with possible migration of several bisphenol compounds. Indeed, bisphenol A diglycidyl ether (BADGE) is a key building block of epoxy resins (upon reaction of bisphenol A (BPA) with epichlorhydin). Yet, migration studies from epoxy-phenolic coated cans with aqueous foods or food simulants have shown that the migrant BADGE is rapidly hydrolysis to BADGE·H_2O and/or BADGE·$2H_2O$ [4]. Moreover when BADGE is used as a scavenger for hydrochloric acid or in the presence of salty food, BADGE·HCl, BADGE·HCl·H_2O and/or BADGE·2HCl are formed. Similar derivatives are obtained when bisphenol F diglycidyl ether (BFDGE) is used (being synthesized from bisphenol F (BPF) as an alternative to BPA). Due to their possible endocrine disrupting effects, bisphenol compounds have been regulated with specific migration limits (SMLs) established for plastics and coatings that are intended to come in contact with foods. For example, the SML of BPA was fixed to 600 µg/kg by European regulation (EU) n°10/2011, being recently reduced to 50 µg/kg according to amendment n°2018/213 (with application on September 6th 2018) [9].

Many factors affect migration from tinplate cans. In previous studies it was proved that there is a strong correlation between storage time and migration of several metals [1], [7], [10]–[12]. More specifically, there is significant migration of Sn when cans are not coated with plastic film layer [13]; in this case high contact temperature seems to accelerate the corrosion of unlacquered cans [1], [14]. On the other hand, sterilization was shown to have predominant effect on the migration of bisphenol compounds [4]. Other factors, including nature of food (pH [1], [3], [15] fat content, presence of oxidizing agent and nitrates [2], etc.), type of steel [14], tin coating thickness and surface defects [15], lacquer properties (type [12], [16], thickness [4], [10], porosity [4], [10], etc.) and volume of headspace [1], [2], [14], can significantly affect the migration of metals and/or bisphenol compounds. To our best knowledge, no study has ever combined the analysis of both types of contaminants from tinplate cans, probably due to different analytical methods required. Yet, organic and inorganic contaminants behave differently, so that conditions prevailing to their migration may differ. In addition, bisphenol and trace metals levels in food should be anti-correlated since trace metal migration is prevented by the presence of organic coating that may release bisphenols. Accordingly, the current work highlights the influence of several parameters (heat treatment, storage time and temperature, food content, can denting, brand, packaging and cooking food cans) on the migration of both inorganic (Sn, Fe, Cr, Cd, Pb, Ni, Cu and Zn) and organic contaminants (BPA, BPF, BPS, BADGE and BFDGE as well as their di-hydrolysis and di-chlorinated derivatives) from coated tinplate cans into food products. Several Lebanese vegetable food products frequently canned and consumed worldwide have been considered in this work.

2. Materials and methods

2.1. Reagents and standards

2.1.1. Analysis of bisphenol compounds

Acetonitrile (ACN) (HPLC plus gradient grade and LC-MS grade), water (LC-MS grade), methanol (LC-MS grade), ethanol (anhydrous absolute), acetic acid (RPE glacial), formic acid (FA) (LC-MS grade) and ammonium formate (LC-MS grade) were obtained from Carlo Erba (France). BPA (purity grade ≥99.9%), BPF (purity grade ≥98%), BPS (purity grade ≥98%), BADGE (purity grade ≥95%), BADGE.2H_2O (purity grade ≥97%), BADGE.2HCl (purity grade ≥95%), BFDGE (mixture of diastereomers, purity grade ≥95%), BFDGE.2H_2O (purity grade ≥95%) and BFDGE.2HCl (total assay of the three isomers, purity grade ≥90%) were all obtained from Sigma Aldrich (France). Ultra-pure Milli-Q water (18.2 MΩ, 25°C) was produced by an Integral 3 water purification system from Millipore®.

2.1.2. Analysis of metal elements

All reagents used were analytical grade. Individual standard solutions were freshly prepared through dilution of stock solutions with ultrapure water. Solutions of iron, tin, lead and zinc were prepared from 1,000 ± 4 mg/L stock solutions from Fluka Analytical (Buchs, Switzerland), cadmium solutions were from 1,000 mg/L stock solutions from Certipur (Darmstadt, Germany), copper solutions were from 1,000 mg/L stock solutions from Acros Organics (New Jersey, USA), chromium solutions were from 1,000 mg/L stock solutions from Alfa Aesar (Kandel, Germany) and finally nickel was 1,000 ± 10 mg/L stock solutions from HACH (Dusseldorf, Germany). KCl buffer was prepared from 99.5% pure KCl from

AnalaR (England) while strontium was purchased from Merck (Darmstadt, Germany) with purity >99%. Microwave digestion was performed with nitric acid (69%; AnalaR, England) and hydrogen peroxide (30-31%; Sigma Aldrich, USA). All glassware was soaked in nitric acid (1%) overnight and then rinsed with ultra-pure water before use.

2.2. Processing and storage conditions of food samples

A total of 177 food products, including canned “fava beans”, canned “red beans”, canned “chickpeas”, canned “okra” and glass jars of “okra”, were purchased from local industries and supermarkets in Lebanon. Their main characteristics along with their storage time and temperatures during this study are detailed in **Table 5.1**. Products of brands A and B were totally different Lebanese products, from fabrication of packaging to the filled food. Brand D chickpeas were filled in Lebanon but cans were imported, whereas brand C fava beans was imported product. Cans of the same product showed the same batch number. All cans had white coatings which were informed from industries to be epoxy-phenolic coating.

Canned products of brand A were analyzed from prior to heat treatment up to their shelf life of two years (730 days). Canned legumes (i.e. fava beans, red beans and chickpeas) were sterilized at 120°C for 30 min, and canned okra pasteurized at 100°C for 20 min. Non-heated cans were directly emptied after canning, so the contact time between food and can was very short in that case. However, as the cans were filled by boiling, we may expect a slight release of contaminants from can coating.

Okra glass jars (also pasteurized) were examined within the time interval 90 to 330 days. Likewise, legumes from other brands were analyzed between purchase date (i.e. 165 days after production) until 493 days. Furthermore, to test the influence of storage temperature, a set of canned fava beans and okra (both of brand A) were stored either in refrigerator (5 ± 1ºC), at room temperature (22 ± 4ºC) or in an oven (40 ± 2ºC). For each experiment, three cans were considered.

Another set of canned fava beans and okra (both of brand A) were willingly dented and stored at 22°C to simulate the effect of can damages during bad transport and storage in shops or in the house. All collected cans (dented and undamaged) had the same date of production and were stored under the same conditions.

Table 5.1: Detailed information of the studied food products

Product	Brand	Package	Production country	Net weight (g)	Can size (height* diameter) (cm^2)	Free space (cm)	pH[a]	Moisture (%)[b]	Fat (%w/w)[c]	Antioxidants [c]	Storage temperature (°C)	Storage time (days)
Fava beans	A*	Cans	Lebanon	400	10*7.5	0.5	5.9	79.8 ± 0.9	1	EDTA[e] (E385) Sodium metasulfite (E223)	5 ± 1 22 ± 4 40 ± 2	0 to 730
	B	Cans	Lebanon	600	11.5*8.5	0.4	5.9	80.4 ± 0.3	unknown	EDTA (E385) Citric acid (E330)	22 ± 4	purchase date + 493
	C	Cans	UAE**	400	10*7.5	0	5.6	72.7 [f]	0.8	EDTA (E385) Citric acid (E330)	22 ± 4	purchase date + 493
Chickpeas	A*	Cans	Lebanon	400	10*7.5	0	5.1	78.8 ± 6.9	2.5	EDTA (E385) Citric acid (E330)	22 ± 4	0 to 730
	D	Cans	Lebanon	400	10*7.5	0	5.2	79.0 [f]	2.4	EDTA (E385) Citric acid (E330)	22 ± 4	purchase date + 493
Red beans	A*	Cans	Lebanon	400	10*7.5	0.3	5.7	76.1 ± 0.8	0.5	EDTA (E385) Citric acid (E330)	22 ± 4	0 to 730
Okra	A*	Cans	Lebanon	820	11.6*10	0.2	3.7	93.8 ± 0.3[d]	0.3	Citric acid (E330)	5 ± 1 22 ± 4 40 ± 2	0 to 730
		Glass jars	Lebanon	660	11.5*8.5	0	3.9		0.3	Citric acid (E330)	22 ± 4	90 to 330

** Vacuum packed, ** United Arab Emirates, *** determined*

[a] *Determined by pH meter,* [b] *Determined from freeze drying where the difference in mass before and after drying is calculated*

[c] *According to product label,* [d] *For both cans and jars*

[e] *Ethylene diamine tetraacetic acid,* [b] *Determined from lyophilization*

Examination with the naked eye after emptying the can contents revealed no cracking or flaking of the inner coating in dented cans. Only a small amount of corrosion was noticed around the external side seam in dented okra cans at 493 days and beyond.

Finally three cans of fava beans (brand A), stored for 493 days at 22°C, were opened and heated close to boiling for 3 min, in order to mimic the practical food heating in the can during meal preparation. Canned fava beans were selected for this test since it was judged that they might be heated directly in the can by the consumer as a usual practice.

2.3. Sample preparation

The whole food content of three cans (or glass jars) was homogenized in a stainless steel blender. Two analytical procedures were then carried out, either for the extraction of bisphenol compounds or for metals dissolution from the food samples.

2.3.1. Bisphenol compounds extraction from food

About 100 g of the food mixture was freeze dried using a Labanconco freeze dryer. Three replicates of 0.2 g subsample were taken from each sample and vortexed with 4 mL of methanol **[17]** then shaken for 20 min and finally soaked for 12 h. After soaking, the samples were shaken again for 20 min. After ensuring the good separation between liquid and solid portions, 3 mL of liquid part were taken and another 3 mL of methanol were added. The mixture was shaken for final 20 min, then a second 3 mL were collected from the liquid phase. The total collected portions (i.e. 6 mL) of methanolic extracts were then evaporated to dryness under gentle nitrogen flow at 35°C. The dried residues were reconstituted with 10 mL of ACN/water (5/95 v/v). Further clean-up on Supelco-HLB (60 mg/3 mL) cartridges was performed on a SPE manual Visiprep^{TM} system. Conditioning was achieved successively with 5 ml of ACN and 5 ml of ACN/water (5/95 v/v). After sample percolation, washing was ensured with 4 mL of water and 5 mL of ACN/water (5/95 v/v). The target analytes were then eluted with 4 mL ACN. The final extract was evaporated to dryness under gentle nitrogen stream at 35°C. Then, the residues were recovered with 1 ml of chromatographic mobile phase. The final sample solution was diluted three folds (or more if necessary) and further filtrated using 0.2 µm PTFE syringe-less hand compressor filters (Whatman® Mini-UniPrep) before further analysis.

2.3.2. Bisphenol compounds extraction from empty tinplate cans

In order to assess the maximum extractable content of unreacted bisphenol compounds present in the can coating, Paseiro-Cerrato et al. **[18]** suggested that acetonitrile extraction could be performed. Here two cans from brand A were soaked with ACN for 24 and 48 h at room temperature. Since insignificant difference was observed between the two durations, solvent extraction of unreacted bisphenol compounds was carried through soaking for 24 h.

2.3.3. Dissolution of trace metals

The method was previously developed and validated on chickpeas **[11]**. Three replicates of 2 g fresh homogenized samples (no freeze drying was performed) were accurately weighed into a modified polytetrafluoroethylene (PTFE-TFM) microwave bomb vessel. After adding 7 mL of nitric acid and 1 mL of hydrogen peroxide, the sample digestion was performed in a high-performance microwave digestion system (Anton Paar, Multiwave 3000, Graz, Austria) according to the previously described program **[11]**. The final clear digest was then diluted to 50 mL with ultra-pure water and filtered with 4 µm PTFE filters for subsequent analysis. During the analysis of some elements, the samples were further diluted to remain within the linearity range.

2.4. Analysis of studied contaminants

2.4.1. Analytical instruments and conditions for bisphenol compounds

A UHPLC Agilent® 1260 Infinity Series system equipped with a multi-wave fluorescence detector was used for quantification of bisphenol compounds. The system was accompanied with an auto-sampler maintained at 10°C; the injected volume was set at 20 µL. Pentafluorophenyl grafted octadecyl silica column (C18-PFP, 150*2.1 mm ID, 2 µm particle size) was used for the separation of analytes at constant temperature of 20°C. The mobile phase, consisting of Milli-Q water (A) and HPLC plus gradient ACN (B), was pumped at a flow rate of 0.3 ml/min with the following binary gradient: 0 min - 43% B, 1 min ramp to 50% B (maintained for 4 min), 2 min ramp to 60% B (maintained for 5 min), 1 min ramp to 100% B (maintained for 2 min) and back to 43% B in 1 min (total duration 16 min). The excitation and emission wavelengths of fluorescence detection were set at 230 nm and 316 nm respectively. Calibration was performed using standard addition and aqueous solutions. The results obtained showed no difference between the two approaches indicating no matrix effect. Accordingly, quantification was performed with external calibrations of integrated peak areas of eight standard solutions in the range 0.5 to 250 µg/L. The instrumental repeatability was ensured through replicate injection of calibration standards: relative standard deviations (%RSDs) ranged from 0.45 to 5% for the inter-day precision tests (n=7), and from 0.15 to 6.2% for the intra-day precision (n=3) tests.

For bisphenol compound confirmation, a UHPLC XEVO G2S® (Waters) system equipped with a time-of-flight mass spectrometry detector (TOF-MS) was used. Both ESI- and ESI+ ionization modes were operated. The ESI- operation parameters and mobile gradient were as previously described **[19]**, while ESI+ conditions differed. ESI+ operation parameters were: capillary voltage 1 kV; sample cone and source offset 60 and 100 a.u, respectively. Mobile phase composition for ESI+ was: solvents A (LC-MS water/FA 99.9/0.1 v/v) and B (LC-MS ACN/FA 99.9/0.1 v/v) pumped with the same gradient used for UHPLC-fluorescence system at column temperature of 30°C. Details of major ions observed for confirmation of bisphenol compounds are indicated in **Supplementary material - Table 5.S1**.

2.4.2. Analytical instruments and conditions for trace metals

For analysis of trace metals, atomic absorption spectrometry (AAS) was used. Fe, Sn, Zn and Cu were analyzed on flame AAS (FAAS), while the analysis of Pb, Cd, Cr and Ni was carried on graphite furnace AAS (GFAAS). The FAAS system used was a Thermo Electron Corporation atomic absorption spectrometer with Thermo Solaar (version 10.11) data acquisition software, and the GFAAS system was a Shimadzu AA-6800 atomic absorption spectrometer with WizAArd data acquisition software. The instrumental conditions and matrix modifiers were inspired from the instrument supplementary cookbook and are shown in **Supplementary material - Table 5.S2**. Quantification was performed with external calibration of five points in ranges appropriate for each element (see **Supplementary material - Table 5.S3**).

2.5. Method validation

Due to the lack of available reference materials representative of the studied food matrices with the bisphenol compounds, our analytical method on extraction of these compounds from food was validated through recovery tests on spiked unsterilized fava beans and chickpeas. These selected samples were supposed to contain the lowest levels of analyzed compounds (as confirmed by blank analyses of such samples – see results presented below). About 0.2 g of samples were spiked with the target compounds at three levels (low: 100 µg/kg dried food; medium: 400 µg/kg dried food; and high: 1,200 µg/kg dried food), then these samples were vortexed and stored for one hour before extraction as the described method. Recoveries were found satisfactory, ranging from 70 to 120% (see **Supplementary material - Table 5.S4**).

In the case of trace metal analysis, white cabbage certified reference material (BCR® -679, Sigma Aldrich, Geel, Belgium) was used to control the accuracy of sample treatment and analysis methods. This reference material contained all targeted elements, except Pb and Sn. Overall, recoveries were in the range 94-111% for all considered trace metals (see **Supplementary material - Table 5.S5**). The accuracy of the used method for Pb and Sn was previously confirmed by Kassouf et al. on chickpeas samples **[11]**.

The overall methods limits of detection (MDL) and quantification (MQL) were estimated based on the average signals observed for blank solutions (i.e. blank reagents prepared following both sample preparation methods) plus three and ten times, respectively, the standard deviation of noise on analysis. For bisphenol compounds MDL and MQL values were in the range of 0.26-14.92 µg/kg and 0.31-30.6 µg/kg, respectively (see **Supplementary material - Table 5.S3**); hence our method is well appropriate for the analysis of such compounds in food samples since expected concentrations are generally in the 3-500 µg/kg range for most canned foods as already reported **[20]–[22]**. Performance of our method for trace metals was also satisfactory, with MDL and MQL values in agreement with levels expected in food samples (see **Supplementary material - Table 5.S3**) **[11], [23], [24]**. Method precision (RSD), estimated from the standard deviation of validation samples triplicates (spiked or reference material), was in general better than 15%.

2.6. Statistics

The obtained data were analyzed with the XLStat and Microsoft Excel software (2007). Testing for significance of mean effects and interactions on all variables was calculated using ANOVA analysis of variance. Statistical significance was set at $p \leq 0.05$.

The multivariate technique of Principal Component Analysis (PCA) was performed in order to examine the correlation between the food content of bisphenol compounds and metals with the studied parameters (type of food and brands, storage time and temperature). The analysis was carried on JMP 14 software. The studied matrix had 129 rows (samples) and 11 columns (variables).

3. Results and discussion

3.1. Occurrence of bisphenol compounds and metals in raw and canned food

Numerous peaks were observed in our UHPLC-fluorescence chromatograms (**Supplementary material - Fig. 5.S1**), some of them appearing at the expected retention times of our target compounds. Yet, only BPA and BADGE.$2H_2O$ could be confirmed with mass spectrometry, in agreement with previous studies reporting that BPA and BADGE.$2H_2O$ are most abundant bisphenol compounds in canned food **[25], [26]**. Interestingly, BADGE.$2H_2O$ was not detected in most foods (brand A) before heat treatment as shown in **Figure 5.1**, while BPA was present in most non-heated foods in the range ND-21.7 µg/kg. Non-heated food contamination by BPA may originate from processing on production line (prior to canning or during can filling), since initial contamination of raw food seems rather improbable considering our vegetable foods here.

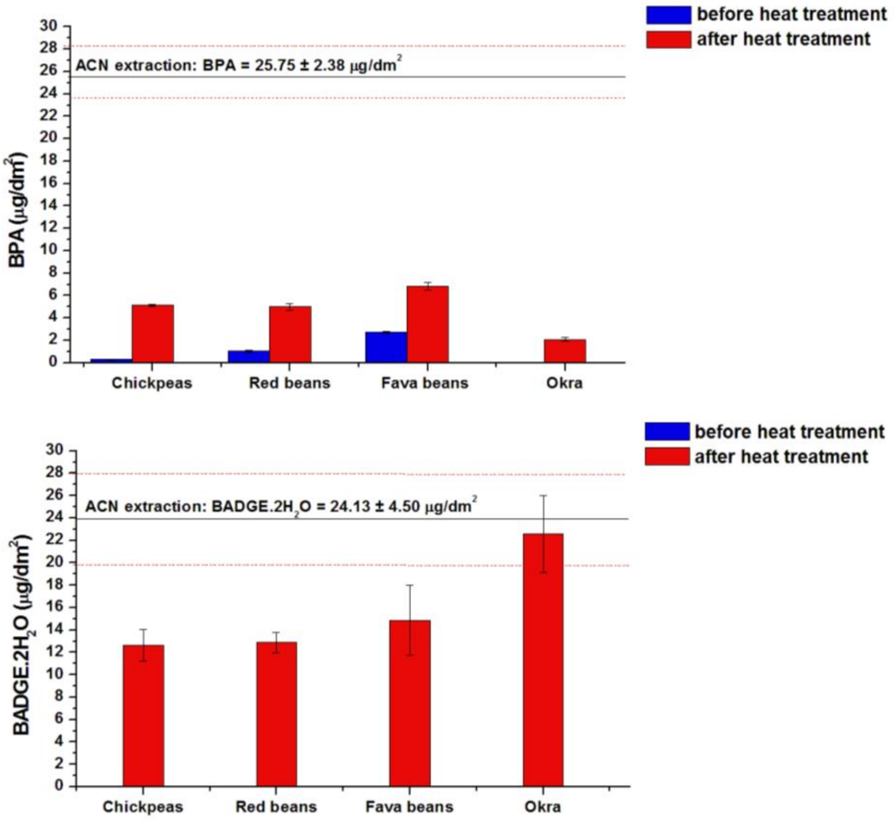

Figure 5.1: Impact of heat treatment on BPA and BADGE.$2H_2O$ migration (expressed in µg/dm^2) into canned food products from brand A. Levels observed after ACN extraction of empty tinplate cans are also indicated for comparison.

Previous studies reported BPA levels in canned food from Japanese (ND-235.4 µg/kg) **[25]** and US (2.6-730 µg/kg) **[27]** markets. According to **Table 5.2**, similar range is observed for heated canned food from Lebanese market (12.8-104.4 µg/kg). The highest BPA value was noted in fava beans from brand C imported in Lebanon. All observed levels comply with recommended European specific migration limit (i.e. 600 µg/kg), but some foods do not comply with the new European regulation (i.e. 50 µg/kg). In the same samples, BADGE.2H_2O was found in the range 88.62-210.89 µg/kg, well within the reported values by Yonekubo et al. (ND-247.2 µg/kg) **[25]** and Sun et al. (ND-400 µg/kg) **[26]**, and far below the specific migration limit fixed by the European regulation (EC) n°1895/2005 for the sum of BADGE and its hydrolysis derivatives (i.e. 9,000 µg/kg).

In the meantime, many metals are natural components of vegetables **[23]**. Consequently, most key metals were detected in non-heated food categories from brand A (see **Supplementary material - Table 5.S6**), with the following ranges: 6.26-13.00 mg/kg (Fe), 0.02-0.05 mg/kg (Pb), <MQL-0.04 mg/kg (Cd), 0.11-0.22 mg/kg (Cr), 0.06-0.46 mg/kg (Ni), 0.47-4.67 mg/kg (Cu) and 2.23-6.32 mg/kg (Zn). Only Sn remained non detected, which was expected since low Sn levels were reported in unprocessed foodstuffs (<1 mg/kg) **[6]**. Interestingly, Cu and Fe levels were the lowest in okra, while Ni, Cu and Cr levels were the highest in chickpeas. Fava beans and red beans had close concentrations of most metals. Overall our observed levels are in the ranges reported for unprocessed vegetables **[6], [28], [29]**, except for Cr with slightly higher values than reported for canned legumes (0.01-0.08 mg/kg) **[23]**.

Table 5. 2: Evolution of bisphenol compounds and metals in canned foods as a function of storage time and temperature

Food type	Storage time (days)	Storage temperature (°C)	Compound or element (conc. ± SD)								
			BPA	BADGE.2H$_2$O	Fe	Pb	Cd	Cr	Ni	Cu	Zn
			µg/kg		mg/kg						
Fava beans (brand A)	0*	22 ± 4	54.60 ± 2.62^{a}	119.37 ± 25.07^{a}	10.73 ± 0.17^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.12 ± 0.01^{a}	0.3 ± 0.02^{a}	1.62 ± 0.04^{a}	5.83 ± 0.07^{a}
	75 ± 18	22 ± 4	55.81 ± 6.98^{a}	168.87 ± 26.16^{b}	8.79 ± 1.01^{b}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.14 ± 0.01^{a}	0.31± 0.01^{a}	1.55 ± 0.11^{a}	7.32 ± 0.18^{b}
	330 ± 24	5 ± 1	46.67 ± 1.68^{b}	154.87 ± 16.41^{b}	10.92 ± 0.25^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.16 ± 0.00^{b}	0.36 ± 0.00^{a}	1.46 ± 0.10^{a}	7.30 ± 0.17^{b}
		22 ± 4	48.49 ± 1.50^{b}	152.34 ± 3.20^{b}	10.77 ± 0.19^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.16 ± 0.00^{b}	0.40 ± 0.01^{b}	1.78 ± 0.16^{a}	7.16 ± 0.28^{b}
		40 ± 2	63.63 ± 9.03^{c}	193.36 ± 5.42^{c}	13.80 ± 0.01^{c}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.16 ± 0.00^{b}	0.43 ± 0.01^{c}	1.65 ± 0.02^{a}	6.68 ± 0.67^{b}
	493 ± 38	5 ± 1	39.75 ± 2.47^{d}	112.12 ± 13.91^{a}	10.51 ± 0.37^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.14 ± 0.00^{a}	0.34± 0.03^{a}	1.84 ± 0.07^{a}	6.56 ± 0.37^{c}
		22 ± 4	50.53 ± 2.63$^{a, b}$	141.00 ± 11.60$^{a, b}$	13.06 ± 0.50^{d}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.15 ± 0.00^{b}	0.38 ± 0.01^{b}	1.75 ± 0.05^{a}	6.99 ± 0.18$^{b, c}$
		40 ± 2	47.63 ±1.90^{b}	149.14 ± 14.92$^{a, b}$	15.33 ± 0.08^{e}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.15 ± 0.01^{b}	0.38 ± 0.00^{b}	1.86 ± 0.06^{a}	6.72 ± 0.04^{c}
	730	5 ± 1	NA	NA	10.38 ±0.16^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.14 ± 0.02^{a}	0.37 ± 0.02^{b}	2.13 ± 0.07^{b}	6.48 ± 0.10^{c}
		22 ± 4	NA	NA	12.96 ± 0.01^{d}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.14 ± 0.01^{a}	0.35 ± 0.03^{a}	2.13 ± 0.07^{b}	6.65 ± 0.28^{c}
		40 ± 2	NA	NA	14.74 ± 0.24^{f}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.16 ± 0.01^{b}	0.38 ± 0.03^{b}	2.04 ± 0.09^{b}	6.89 ± 0.27^{b,c}
Fava beans (brand B)	0*	22 ± 4	24.61 ± 3.59^{a}	116.20 ± 19.78^{a}	5.59 ± 0.32^{a}	0.06 ± 0.01^{a}	0.01 ± 0.00^{a}	0.05 ± 0.00^{a}	0.11 ± 0.00^{a}	2.59 ± 0.04^{a}	4.29 ± 0.16^{a}
	75 ± 18	22 ± 4	19.61 ± 4.80^{a}	210.89 ± 15.18^{a}	8.82 ± 0.41^{b}	0.10 ± 0.01^{b}	0.01 ± 0.00^{a}	0.07 ± 0.00^{b}	0.14 ± 0.01^{b}	2.67 ± 0.06^{a}	4.43 ± 0.27^{a}
	493 ± 38	22 ± 4	NA	NA	11.58 ± 0.94^{c}	0.19 ± 0.01^{c}	0.02 ± 0.00^{b}	0.08 ± 0.00^{c}	0.24 ± 0.01^{c}	2.52 ± 0.18^{a}	4.41 ± 0.02^{a}
Fava beans (brand C)	165 ± 15	22 ± 4	104.42 ± 19.20	181.21 ± 16.30	7.44 ± 0.69^{a}	0.15 ± 0.00^{a}	0.02 ± 0.00^{a}	0.08 ± 0.00^{a}	0.41 ± 0.02^{a}	2.60 ± 0.16^{a}	4.34 ± 0.41^{a}
	493 ± 38	22 ± 4	NA	NA	10.16 ± 0.58^{b}	0.21 ± 0.02^{b}	0.03 ± 0.00^{b}	0.08 ± 0.01^{a}	0.50 ± 0.03^{b}	2.75 ± 0.17^{a}	4.41± 0.32^{a}

n=3
** after heat treatment*
NA: not analyzed
Within each contaminant of each food product: a to g correspond to significantly different concentrations (p<0.05), whereas concentrations showing same letter are not significantly different (p>0.05)

Table 5.2 (Continued): Evolution of bisphenol compounds and metals in canned foods as a function of storage time and temperature

Food type	**Storage time (days)**	**Storage temperature (°C)**	**Compound or element (conc. ± SD)**								
			BPA	**BADGE.2H2O**	**Fe**	**Pb**	**Cd**	**Cr**	**Ni**	**Cu**	**Zn**
			µg/kg		**mg/kg**						
Red beans (brand A)	0*	22 ± 4	39.95 ± 2.54^{a}	102.58 ± 7.22^{a}	14.04 ± 0.82^{a}	0.02± 0.00^{a}	0.01 ± 0.00^{a}	0.14 ± 0.00^{a}	0.29 ± 0.03^{a}	2.09 ± 0.15^{a}	3.67 ± 0.21^{a}
	75 ± 18	22 ± 4	42.34 ± 0.24^{a}	166.41± 18.19^{b}	17.70 ± 0.37^{b}	0.03 ± 0.00^{b}	0.01 + 0.00^{a}	0.14 ± 0.00^{a}	0.36 ± 0.00^{b}	2.09 ± 0.03^{a}	5.71 ± 0.20^{b}
	330 ± 24	22 ± 4	41.50 ± 1.88^{a}	182.99 ± 4.78^{b}	21.09± 1.02^{c}	0.03 ± 0.00^{b}	0.01 ± 0.00^{a}	0.16 ± 0.00^{b}	0.37 ± 0.01^{b}	2.26 ± 0.09^{b}	5.61 ± 0.08^{b}
	493 ± 38	22 ± 4	40.57 ± 9.30^{a}	182.36± 9.12^{b}	23.33 ± 2.30^{c}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.16 ± 0.01^{b}	0.31 ± 0.01^{a}	2.20 ± 0.07^{b}	5.92 ± 0.04^{c}
	730	22 ± 4	NA	NA	23.04± 2.44^{c}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.15 ± 0.01^{b}	0.36 ± 0.02^{b}	2.58 ± 0.12^{c}	6.03 ± 0.13^{c}
Chickpeas (brand A)	0*	22 ± 4	41.06 ± 0.84^{a}	100.96 ± 11.37^{a}	12.23 ± 0.38^{a}	0.02 ± 0.00^{a}	0.04 ± 0.00^{a}	0.22 ± 0.02^{a}	0.54 ± 0.03^{a}	4.42 ± 0.16^{a}	2.98 ± 0.05^{a}
	75 ± 18	22 ± 4	35.55± 0.46^{b}	156.95 ± 8.76^{b}	12.09 ± 0.39^{a}	0.02 ± 0.00^{a}	0.04 ± 0.00^{a}	0.24 ± 0.01^{a}	0.58 ± 0.01^{a}	5.09 ± 0.07^{b}	2.31 ± 0.00^{b}
	330 ± 24	22 ± 4	32.10 ± 0.21^{c}	163.37 ± 14.29^{b}	21.86 ± 0.24^{b}	0.02 ± 0.00^{a}	0.04 ± 0.00^{a}	0.25 ± 0.02^{a}	0.70 ± 0.04^{b}	5.68 ± 0.09^{c}	2.38 ± 0.13^{b}
	493 ± 38	22 ± 4	34.21 ± 4.70^{a,b,c}	155.78 ± 14.48^{b}	22.03 ± 1.04^{b}	0.02 ± 0.00^{a}	0.04 ± 0.00^{a}	0.24 ± 0.00^{a}	0.63 ± 0.06^{b}	6.13 ± 0.26^{d}	4.61 ± 0.07^{c}
	730	22 ± 4	NA	NA	23.37 ± 1.15^{b}	0.02 ± 0.00^{a}	0.04 ± 0.00^{a}	0.23 ± 0.00^{a}	0.67 ± 0.06^{b}	6.23 ± 0.14^{d}	5.04 ± 0.00^{d}
Chickpeas (brand D)	165 ± 15	22 ± 4	44.24 ± 1.36	88.62 ±4.58	9.08 ± 0.47^{a}	0.22 ± 0.02^{a}	0.02 ± 0.00^{a}	0.08 ± 0.01^{a}	0.77 ± 0.01^{a}	4.93 ± 0.11^{a}	6.35 ± 0.30^{a}
	493 ± 38	22 ± 4	NA	NA	12.20 ± 1.15^{b}	0.26 ± 0.02^{b}	0.03 ± 0.02^{b}	0.08 ± 0.00^{a}	1.11 ± 0.10^{b}	5.03 ± 0.18^{a}	7.69± 0.06^{b}
Okra Cans (brand A)	0*	22 ± 4	12.82 ± 1.09^{a}	146.20± 5.90^{a}	9.88 ± 0.65^{a}	0.05 ± 0.01^{a}	<MQL	0.13 ± 0.01^{a}	0.06 ± 0.01^{a}	0.55 ± 0.03^{a}	2.21 ± 0.27^{a}
	330 ± 24	22 ± 4	25.44 ± 1.36^{b}	122.07 ± 7.35^{b}	13.11 ± 1.24^{b}	0.05 ± 0.00^{a}	<MQL	0.19 ± 0.00^{b}	0.06 ± 0.00^{a}	0.48 ± 0.01^{b}	2.86 ± 0.19^{b}
	493 ± 38	5 ± 1	26.49 ± 1.95^{b}	169.53 ± 14.33^{a}	12.52 ± 2.02^{b}	0.05 ± 0.00^{a}	<MQL	0.21 ± 0.00^{c}	0.08 ± 0.01^{b}	0.56 ± 0.07^{a}	2.97± 0.21^{b}
		22 ± 4	30.28 ± 2.32^{c}	133.79 ± 1.39^{c}	23.21 ± 0.91^{c}	0.05 ± 0.00^{a}	<MQL	0.21 ± 0.00^{c}	0.07 ± 0.00^{b}	0.59± 0.03^{a}	3.30 ± 0.22^{c}
		40 ± 2	41.57 ± 1.67^{d}	330.36 ± 7.22^{d}	39.39 ±1.58^{d}	0.05 ± 0.00^{a}	<MQL	0.21 ± 0.00^{c}	0.10 ± 0.01^{c}	0.59± 0.02^{a}	4.23 ± 0.47^{d}
	730	5 ± 1	28.39 ± 1.21^{b,c}	134.49 ± 23.17$^{a, b, c}$	26.14 ± 0.97^{e}	0.05 ± 0.00^{a}	<MQL	0.22 ± 0.00^{c}	0.29 ±0.01^{d}	0.63± 0.04^{c}	2.68 ± 0.12^{b}
		22 ± 4	32.68 ± 0.18^{c}	130.88 ± 0.37^{c}	33.16 ± 1.76^{f}	0.06 ± 0.01^{a}	<MQL	0.21 ±0.01^{c}	0.30 ± 0.03^{d}	0.65± 0.04^{c}	3.25 ± 0.23^{c}
		40 ± 2	46.03 ± 0.98^{e}	500.21 ± 32.17^{e}	67.76 ± 2.50^{g}	0.06 ± 0.00^{a}	0.01 ± 0.00	0.21 ± 0.01^{c}	0.27 ± 0.02^{d}	0.86± 0.06^{d}	5.37 ± 0.13^{e}
Okra Jars (brand A)	90	22 ± 4	ND	ND	5.47 ± 0.40^{a}	0.04 ± 0.00^{a}	ND	0.16 ± 0.00^{a}	0.10 ± 0.00^{a}	0.52± 0.01^{a}	2.79 ± 0.04^{a}
	330	22 ± 4	ND	ND	6.86 ± 0.30^{b}	0.04 ± 0.00^{a}	ND	0.15 ± 0.00^{a}	0.09 ± 0.01^{a}	0.53± 0.01^{a}	3.34 ± 0.02^{b}

*n=3, * after heat treatment, NA: not analyzed, ND: not detected. Within each contaminant of each food product: a to g correspond to significantly different concentrations ($p<0.05$), whereas concentrations showing same letter are not significantly different ($p>0.05$)*

3.2. Influence of heat treatment

Figure 5.1 (above) clearly shows that heat treatment significantly enhanced migration of both BADGE.2H_2O and BPA into food, in agreement with previous studies **[30]–[32]**. Upon sterilization, BPA levels drastically increased in the range 151-391% (fava beans and red beans respectively). In the case of pasteurized okra, lower BPA levels were found (see also **Table 5.2**). Likewise, BADGE.2H_2O increased from non-detected levels to a range between 12.63-22.56 µg/dm^2 (100.96-146.20 µg/kg) after heat treatment, but here the highest level was detected in canned okra. Possibly, migration of BADGE.2H_2O was more influenced by properties of food content rather than heat treatment conditions, since okra possess the lowest pH (3.7) and highest water content (93.8%) that favor rapid BADGE hydrolysis. This assumption is also confirmed by the high percentage (52-94%) of extractable BADGE.2H_2O found with our food products, rich in water (>70%) **[33], [34]**. On the opposite, only part (<30%) of the extractable BPA was recovered in our canned food samples, which is far below previous observations reporting almost quantitative BPA extraction during sterilization (70-100%) **[18]**, **[30]**.

Considering trace metals migration (see **Supplementary material - Table 5.S6**), the effect of heat treatment was positively significant in some cases only, with moderate increase (up to 17-34%) for Cr, Ni, Cu and Zn. Fe levels were most affected for okra (58% increase), despite the mild pasteurization treatment, suggesting that high acidic nature of okra plays a major role on increasing Fe content in food during heat treatment **[1]**. Sn levels remained not detected in all studied food (both raw and canned), even at high storage temperature and long storage time. This could be explained by the pH working range (3.7-5.9) that is not in favor of Sn dissolution in the food **[15]** and the fully lacquered cans, since lacquering is expected to decrease Sn release by 99.5% **[2]**.

3.3. Migration as a function of storage time and temperature

The aspect of cans and food contents were carefully examined by naked eye each time the cans were emptied for analysis. Generally, the appearance of food and their cans was normal over the studied time and temperature intervals. The only worrying appearance was noticed in canned okra samples stored at 40°C for 730 days, with slight rusting appearing on the external walls of the cans and okra pieces showing darker color.

3.3.1. Migration of BPA and BADGE.2H_2O

Upon storage at 22°C, BPA content in canned fava beans (brand A and B) and red beans was not significantly influenced over time, in agreement with previous studies **[18], [19], [34]**. On the other hand, BPA varied significantly in canned chickpeas (brand A) and canned okra. In the latter, BPA rapidly increased between 0 and 330 days (from 12.82 to 25.44 µg/kg), then it continued to increase slowly up to 32.68 µg/kg at 730 days. Since okra cans were pasteurized, part of residual BPA could have remained on the coating after heat treatment and then mass transfer is completed during storage time. Similar results were reported for BPA upon pasteurization of canned jalapeno peppers **[34]**. For chickpeas (brand A), BPA level slightly decreased during the first 75 days (from 41.06 to 35.55 µg/kg), then remained almost constant, but this did not affect our migration results. Interestingly, BPA migration was found to be affected by storage temperature, with significant BPA level increases in canned fava beans and okra at higher storage temperatures (see **Table 5.2**).

The unclear fluctuation of BADGE.2H_2O content during storage of fava beans and okra at 5 and 22°C could be explained by possible interaction with food ingredients (for example with cysteine amino acid that is present in our food samples **[35]**) as discussed by Coulier et al. **[36]**. Yet, a significant increase of BADGE.2H_2O can be noticed at the beginning of storage (from 0 to 75 days) for canned fava beans (brand B), red beans and chickpeas (brand A) (total increase of 81, 62 and 55%, respectively), with further stability for longer storage. The impact of 40°C storage temperature was evident on the migration of BADGE.2H_2O in okra cans, where up to 500.2 µg/kg was reached after 730 days of storage.

Finally, in spite of some studies **[30]–[32]** suggesting that storage temperature and time are insignificant on triggering the migration of bisphenol compounds, our study clearly shows different cases of significant migration of BPA and BADGE.2H_2O attributed to storage conditions. In particular, higher migration was observed in acidic foods stored at high temperature. Our results also show that BADGE.2H_2O content of canned foods may drastically increase when combining high storage temperature, low pH and high water food content that promote the hydrolysis of BADGE.

3.3.2. Migration of iron

Just after food canning (day 0 of storage), Fe content varied between 5.59 and 14.04 mg/kg, in agreement with previous values reported for canned vegetable **[23]**. An increase is noted over storage time for all samples stored at 22°C (see **Table 5.2**). Yet, Fe migration does not follow the trend previously reported (fast at the early storage time, and then stabilization after a while) **[11]**. At 22°C, significant increase occurred between 330 and 493 days (21.5% increase) in fava beans (brand A). Fe migration was more marked in fava beans from brand B and C, possibly due to the presence of citric acid additive (E330) (see **Table 5.1**), which tends to solubilize iron **[37]**. Thus, in brand B fava beans, Fe release started from day 0 resulting in the highest increase (107% over 493 days) among fava beans brands. In red beans and chickpeas (brand A), Fe increase was significant between 75 and 330 days, where comparably high release was observed (64-91% increase) reaching around 22 mg/kg that is in turn about double the concentrations found in fava beans. Interestingly, Fe level in acidic canned okra, still at 22°C, greatly raised, where it gradually increased over time interval 0-730 days from 9.88 to 33.16 mg/kg. In opposite, glass jars were shown to be safer package, where minimal release of Fe was observed between 90 and 330 days (but Fe level still increased by 25%).

Interestingly, Fe levels were greatly affected by storage temperature. Hence, while constant concentration was noted in fava beans (brand A) stored at 5°C over 730 days, higher levels were found in the same food stored at 22°C and even 40°C (13 to 38% increase between storage at 22°C and 40°C). Same trend is observed with canned okra: 5°C was found to slow down the migration of Fe, while severe release of Fe was evident upon storage at 40°C.

Finally, our data reveals that Fe migration is directly dependent on storage time, storage temperature, food pH and container characteristics. Among all our tested conditions and food, Fe release remained below the recommended European SRL (i.e. 40 mg/kg) **[8]** except for canned okra stored for a long period at high temperature.

3.3.3. Migration of chromium

Cr concentrations in most of our food products were stable and not affected, neither by heat treatment nor storage. In exception, Cr level increased in fava beans (brand B) over 493 days, but this could be linked to can metal quality rather than migration. A slight increase in canned okra at 330 and 493 days is also noted, that could be attributed to the high acidity of the food that favors Cr dissolution.

3.3.4. Migration of lead and cadmium

Pb and Cd are considered as impurities in the metal substrate of cans. They are highly controlled in canned food due to their acute toxicity at very low levels **[11]**. Pb levels in our canned vegetable were in the range 0.02-0.26 mg/kg (see **Table 5.2**), the highest level being observed in chickpeas (brand D); they are in the range reported for canned legumes from Lebanese market (i.e. 0.05-0.60 mg/kg) **[11], [23]**. While levels remained constant over storage in most considered foods, they significantly increased in fava beans (brands B and C) and chickpeas (brand D), achieving after 493 days concentrations close to or higher than the European maximum permitted levels in legumes (i.e. 0.2 mg/kg) but still remaining below the maximum level allowed (i.e. 1 mg/kg) in canned beans according to the Codex Alimentarius standard (CODEX STAN 193-1995) and Libnor **[38]**. The corresponding Pb release values exceed the recommended SRL (i.e. 0.01 mg/kg) **[8]**. However, the increase in Pb levels was still lower than reported in canned chickpeas (from 0.05 to 0.6 mg/kg over 90 days) **[11]**. Interestingly, Pb levels remained unaffected by storage temperature.

Cd levels in canned legumes were comparably low (i.e. 0.01-0.04 mg/kg, the lowest values being noted for okra where majority of samples were below MQL), which was unexpected since average level of 0.197 mg/kg was previously reported for Lebanese marketed canned legumes **[23]**. Yet, our values are within those reported by WHO/FAO in vegetables for European and Asian countries (i.e. 0.006-0.10 mg/kg) **[39]**, thus complying with the European maximum permitted levels (i.e. 0.10 mg/kg) set by the Commission Regulation (EC) n°1881/2006. Our results suggest that Cd requires long storage time to be released from the cans, its migration becoming significant only at day 493 in fava beans (all brands) and chickpeas (brand D). After 493 days, a slight effect of temperature is noticed with fava beans (brand A); it was not observed at day 730, possibly since the maximum release of Cd was reached after that long storage. Cd in canned okra required more severe conditions (40°C and 730 days) to increase from <MQL to 0.01 mg/kg.

3.3.5. Migration of nickel, copper and zinc

Ni, Cu and Zn may be naturally present in the metal alloy of cans or intentionally added as additive to boost the durability of cans in terms of corrosion resistance and hardening **[6]**. To the best of our knowledge, data on the release of these metals from food cans is limited in literature. Insignificant increase of Ni and Cu levels were noted for okra jars over the studied period of time, whereas Zn was still increasing (by 20%) even in glass packaging. On the opposite, higher increase was noted in the levels of Ni, Cu and Zn for several canned products as discussed below.

Over the storage period, the concentration of Ni ranged between 0.06 and 1.11 mg/kg (with highest levels in canned chickpeas), in agreement with levels previously reported for Jordanian marketed canned green beans **[40]**. Significant Ni release was observed over storage for most considered canned products. As noted previously for Cd, our results suggest that Ni release requires long storage time. For example, in canned okra Ni levels increased by about 300% between day 493 and day 730 at 22°C, while Ni release was almost negligible between day 0 and day 493. Further, released quantities of Ni were above the recommended SRL value (i.e. 0.14 mg/kg) [8] for canned chickpeas (brand D) (i.e. 0.34 mg/kg) and okra (i.e. 0.24 mg/kg). Considering the storage temperature, a slight effect on Ni release was only noticed in okra cans when stored at 40°C for 493 days.

The highest Cu levels were found in chickpeas (4.42-6.23 mg/kg), while the lowest were observed in okra samples (0.48-0.86 mg/kg). Thus, unless, for okra samples, our Cu levels in canned legumes are higher than those previously reported for canned vegetable (average of 1.63 mg/kg) [23]. Interestingly, in our products, effect of storage was significant only for canned foods from brand A: Cu release was also characterized by slow long-term evolution (increase by about 23% in red beans, 31% in fava beans, 41% in chickpeas and 56% in okra after storage for 730 days at 22°C). As observed for Pb and Cr, Cu levels in canned fava beans (brand A) were not influenced by storage temperature, since only a slight effect was noticed for canned okra stored at 40°C for 730 days (32% increase as compared to cans stored at 5 and 22°C for the same period). In any case, Cu release remained below the recommended SRL (i.e. 4 mg/kg) [8].

Zinc occurs in most foodstuffs and beverages [41], with no maximum level regulated by European authorities. It was found at its highest concentrations (5.83 – 7.69 mg/kg) in fava beans (brand A) and chickpeas (brand D). Overall, our levels are in agreement with average level reported in canned vegetable (i.e. 7.42 mg/kg) [23], but lower than other reported mean values for canned legumes (i.e. 12.8 mg/kg) [24]. By referring to Libnor 2004 [42], the maximum level of Zn in canned plums is 150 mg/kg. Although, we studied different canned foods, yet, the obtained concentrations of Zn is much lower than the recommended level. Storage time was found significant on Zn release in most samples stored at 22°C (except fava beans, all brands). Yet, no clear trend can be drawn since variations were observed among food contents and brands. Interestingly, Zn level greatly increased in okra cans stored at 40°C for a long period (493 and 730 days); since this storage temperature did not promote Zn release in fava beans, such result suggests that storing acidic food for a long period under high storage temperature favors Zn migration.

3.4. Correlation of contaminants levels with food product, can brand and storage conditions

Principal components factor analysis identified three principal components with eigen values > 1 when the concentrations of BPA, BADGE.2H_2O, Fe, Cd, Pb, Cr, Ni, Cu, Zn, storage time and storage temperature are chosen as variables. To the best of our knowledge, this study is the first one to determine simultaneous migration of trace metals and bisphenol compounds enabling to point out common trends. The principal components (PC1, PC2 and PC3) explain 66% of data variance. The PCA scores and loadings are presented in **Figure 5.2**. From these results, PC1 is clearly related with food categories (classified as okra, red bean, fava beans and chickpeas), PC2 is correlated with storage conditions (mainly time, but also temperature), and PC3 is linked both to can brand and storage temperature. The highest dispersion of food sample for PC2 is observed for okra samples, suggesting that this type of food is more sensitive to storage conditions than other types of considered food here.

According to trace metals and bisphenol compounds, several groups can be distinguished. Clearly, Cd, Ni and Cu behave the same, with their levels linked to the food type (the highest being observed in chickpeas) and also to the can brand. Fe has a particular trend, with clear influence of storage time, and to a lesser extent also of food type; the can brand has no effect on its level since Fe is the basic constituent of metallic cans of the four designated brands. Then, BPA and Zn show similar behavior, their levels being mainly correlated with food type, storage temperature and also can brand; as a matter of fact, as BPA, Zn can be affected by the coating composition since zinc oxide is often added to the lacquer in order to absorb odorous sulphides liberated during and after heat treatment. Very similar is Pb, but its level is additionally influenced by storage time. Finally, BADGE.2H_2O and Cr have particular trends: the effect of storage temperature is highly marked for the former (with secondary effect of storage time and can brand), where the latter seems more influenced by can brand.

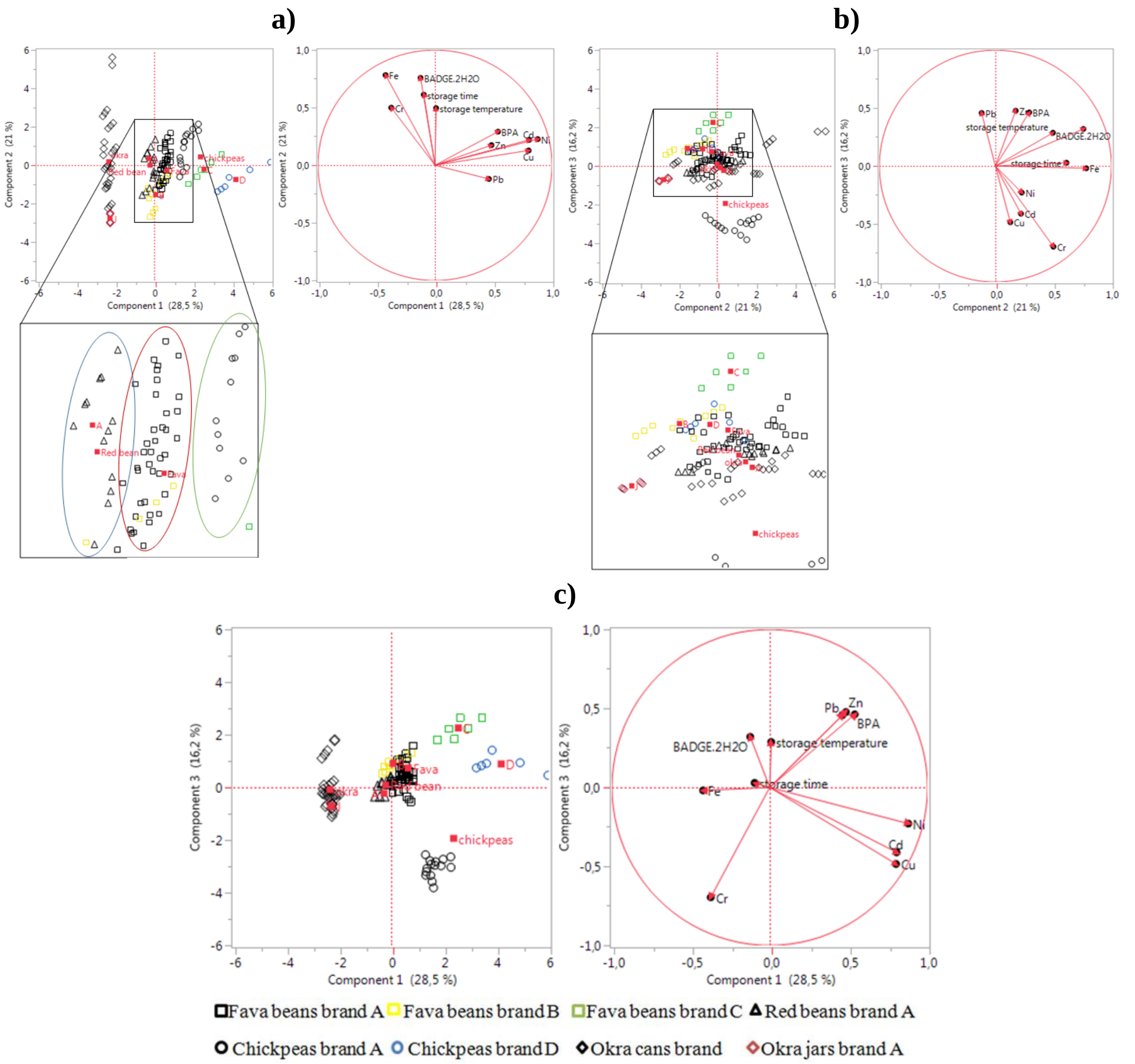

Figure 5. 2: Scores and loadings on the combination of PC1 and PC2 (a), PC2 and PC3 (b), PC1 and PC3 (c) resulting from PCA applied on all our data.

3.5. Effect of can denting

Damage to the can, in the form of denting, had no appreciable effect on the migration of BPA and BADGE.2H_2O in canned fava beans, which is in agreement with results reported for BPA migration in non-acidic canned food **[30]**. At certain times, elements like Fe, Ni, Cu and Zn showed a slight decrease upon denting (see **Table 5.3**). This unexpected decrease could be related to presence of antioxidants (such as EDTA) present in fava beans (brand A) that can form strong complexes with metal ions. In the case of acidic okra, we observed a significant release of BADGE.2H_2O, Fe, Cu and Cd at both storage durations, probably linked to corrosion resulting from can damage (the highest effect was observed on Fe where its release was enhanced by 86-90%, with subsequent Fe level of 61.91 mg/kg at 730 days). So, undamaged food cans are preferable over dented cans, especially in case of high acidic food.

3.6. Effect of heating food directly in the can

Overall, no significant effect of this practice was noticed on the considered contaminants levels in the canned fava beans (see **Table 5.4**). Similar conclusions were drawn for BPA in a previous study **[30]**, while to our best knowledge the effect of heating food directly in the cans has never been investigated for trace metals before. So, our results do confirm that, under the tested conditions, this practice is safe concerning the migration of potentially toxic metals and bisphenol compounds.

Table 5.3: Effect of denting fava beans and okra cans on the migration of bisphenol compounds and metal elements

Food	**Storage time (days)**	**Can aspect**	**Compound or element (conc. ± SD)**								
			BPA	**BADGE.2H_2O**	**Fe**	**Pb**	**Cd**	**Cr**	**Ni**	**Cu**	Zn
			µg/kg		**mg/kg**						
Fava beans (brand A)	330 ± 24	Undamaged	48.49 ± 1.50	152.34 ± 3.20	10.77 ± 0.19	0.03±0.00	0.01±0.0001	0.16±0.00	0.34±0.01	1.78±0.16	7.16±0.28
		Dented	47.16±6.61	153.76±13.38	11.20±0.54	0.03±0.00	0.01±0.0001	0.16±0.00	**0.36±0.01***	1.80±0.05	**6.71±0.31***
	493 ± 38	Undamaged	50.53 ± 2.63	141.00 ± 11.60	13.06 ± 0.50	0.03±0.00	0.02±0.00	0.16±0.01	0.38±0.01	1.75±0.05	6.99±0.18
		Dented	50.27±4.47	141.85±6.81	**10.60±0.23***	0.03±0.00	0.02±0.00	0.14±0.01	**0.35±0.02***	1.88±0.08	**6.03±0.23***
	720	Undamaged	NA	NA	12.96 ± 0.01	0.03±0.00	0.02±0.001	0.14±0.01	0.35±0.03	2.13±0.07	6.65±0.28
		Dented	NA	NA	**10.82±0.18***	0.03±0.00	0.02±0.003	0.13±0.01	0.35±0.01	**1.84±0.05***	**6.24±0.10***
Okra (brand A)	493 ± 38	Undamaged	30.28±2.32	133.79±1.39	23.21 ± 0.91	0.05±0.00	ND	0.21±0.00	0.07±0.00	0.59±0.03	3.30±0.22
		Dented	33.13±2.73	**145.96±0.20***	**44.01±4.23***	0.05±0.00	ND	0.21±0.00	**0.10±0.01***	**0.82±0.04***	**3.80±0.20***
	720	Undamaged	32.68±0.18	130.88±0.37	33.16 ± 1.76	0.06±0.01	ND	0.21±0.01	0.30±0.03	0.65±0.04	3.25±0.23
		Dented	28.57±2.18	**143.78±3.69***	**61.91±0.84***	0.06±0.00	**0.01±0.00***	0.21±0.01	0.27±0.03	**0.81±0.03***	**3.96±0.13***

n=3

NA: not analyzed, ND: not detected

** significant difference in concentration is noticed between dented and undamaged cans ($p<0.05$)*

Table 5.4: Effect of heating fava beans directly in the cans on the migration of bisphenol compounds and metal elements

Food		**Compound or element (conc. ± SD)**						
		BPA	**BADGE.2H_2O**	**Fe**	**Cd**	**Ni**	**Cu**	**Zn**
		µg/kg		**mg/kg**				
Fava beans (brand A)	Unheated	50.53 ± 2.63	141.00 ± 11.60	13.06 ± 0.50	0.02 ± 0.00	0.38 ± 0.01	1.75 ± 0.05	6.99 ± 0.18
	Heated	49.80 ±11.30	145.24 ± 9.00	12.40 ± 1.90	0.02 ± 0.00	**0.29 ± 0.06***	1.84 ± 0.05	7.08 ± 0.07

n=3

** significant difference in concentration is noticed between heated and unheated cans ($p<0.05$)*

4. Conclusion

This work highlights for the very first time the main factors that influence the simultaneous migration of trace metals and bisphenol compounds from coated tinplate cans into vegetable foods. Sn was undetected in all our samples, which may be attributed to the presence of lacquer as well as unsuitable food pH for its dissolution. All other considered metals were found, with some samples that did not comply with the European regulation, either due to levels above maximum levels authorized in food (e.g. Pb in canned chickpeas of brand D) or to release above the SRL value (e.g. Fe in canned okra when stored at 40°C and beyond 493 days, and when okra cans are dented). Among bisphenols, only BPA and BADGE.$2H_2O$ were confirmed in our samples; in a few samples (e.g. fava beans of brand C) BPA migration was found to exceed its new SML value recently set by the European regulation. Our study confirms that sterilization is the main contributing parameter on the migration of free bisphenol compounds (here release less than 80% of maximum free bisphenol compounds was observed); heat treatment in food glass jars should be preferred, as undetected levels of all bisphenol compounds were found in that case. Our PCA results suggest several trends of migration depending on the contaminants. Interestingly, the primary hypothesis that bisphenols and trace metals levels in food should be anti-correlated is invalid based on these results. Besides, the fact that BPA and Zn (and Pb to a lesser extent) have similar trends is interesting and shown for the first time. This suggests that their migration is affected by can composition and coating. In addition, food pH and water content seem to play a major role in favoring migration. Surprisingly, for non-acidic food, can denting as well as cooking food in cans showed no effect on the migration of inorganic or organic targeted migrants. On the other hand, in case of acidic food (namely okra), several recommendations can be drawn for industries and consumers: (i) to store cans under moderate temperature to limit excessive release of Fe and BADGE.$2H_2O$; (ii) to discard dented cans since high release of Fe is expected; (iii) to control the purity of metal substrate as well as the polymerization and formulation of polymeric coating, since our results confirm major differences in the packaging quality between brands.

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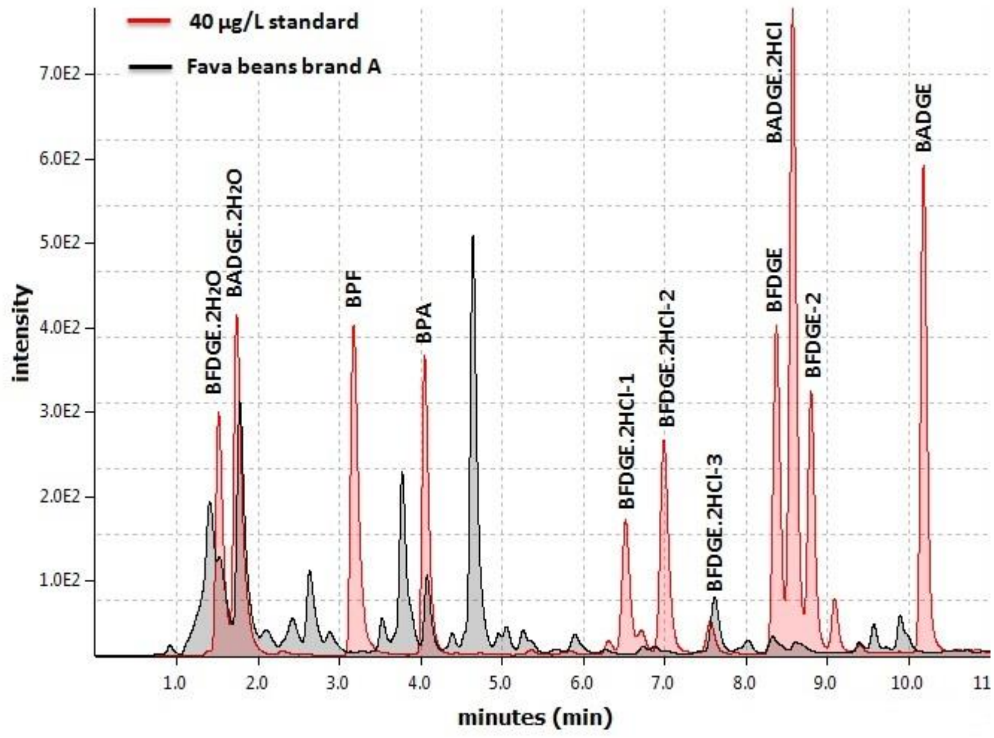
Conclusion

From the results of this article, we showed that bisphenol compounds and metal elements behave differently toward the tested parameters. For instance our previous conclusion on the highest contribution of heat treatment on the migration bisphenol compounds was confirmed, whereas metals release was greatly influenced by storage. Likewise, results from PCA applied on our data suggest that migration trends mainly depend on contaminant as well as food pH and water content. Indeed, BPA and Zn were most affected by coating composition and they were correlated. Thus our hypothesis on the anti-correlation between metals and bisphenols seems invalid. At the end of this chapter, useful advices were provided in order to limit diet exposure of potentially toxic food contaminants. The interpretation of main findings of this chapter will be continued in next chapter, aiming at highlighting the differences between bisphenol content in real foods and simulants, as well as between metal elements in coated and uncoated cans.

Supplementary Material Chpt.5



Supplementary material **Figure 5.S1:** UHPLC-fluorescence chromatograms of fava beans (brand A) extract and a standard (40 µg/L).

Supplementary material **Table 5.S1:** Ions used for confirmation of bisphenol compounds using TOF-MS detection

Compound	Ion	Elemental composition	*m/z* of precursor ion
BPA	$[M-H]^-$	$C_{15}H_{15}O_2$	227.108
BPF	$[M-H]^-$	$C_{13}H_{11}O_2$	199.076
BPS	$[M-H]^-$	$C_{12}H_9O_4S$	249.023
BADGE	$[M-Na]^+$	$C_{21}H_{24}O_4Na$	363.156
BADGE.2H$_2$O	$[M-Na]^+$	$C_{21}H_{28}O_6Na$	399.177
BADGE.2HCl	$[M-Na]^+$	$C_{21}H_{26}Cl_2O_4Na$	436.323
BFDGE	$[M-Na]^+$	$C_{19}H_{20}O_4Na$	335.125
BFDGE.2H$_2$O	$[M-Na]^+$	$C_{19}H_{24}O_6Na$	371.146
BFDGE.2HCl	$[M-Na]^+$	$C_{19}H_{22}Cl_2O_4Na$	408.271

Supplementary material **Table 5.S2:** Instrumental parameters of atomic absorption spectrometry

Element	**Instrument**	**Wavelength (nm)**	**Flame (FAAS)**	**Atomization temperature (GFAAS)**	**Modifier**
Fe	FAAS	248.3	Air-acetylene	-	1 g/L of strontium releasing agent to overcome most common interferences in air-acetylene flame
Sn		286.3	Nitrous oxide-acetylene	-	0.2% w/v KCl to reduce ionization in the nitrous oxide–acetylene flame
Zn		213.9	Air-acetylene	-	1 g/L of strontium
Cu		324.8	Air-acetylene	-	1 g/L of strontium
Pb	GFAAS	283.3	-	2400	4% v/v of 1 g/kg of magnesium nitrate and 1g/kg ammonium phosphate
Cd		228.8	-	2200	4% v/v of 1 g/kg of magnesium nitrate and 1g/kg ammonium phosphate
Cr		357.9	-	2300	-
Ni		232.0	-	2500	-

Supplementary material **Table 5.S3:** Performance of analytical instrument and method for determination bisphenol compounds and metal elements

Metal	**Instrument**	**Linearity Range**	**R^2**	**MDL**	**MQL**
		µg/L		**µg/kg**	**µg/kg**
BPA	UHPLC-FLD	0.5-250	0.999	0.63-2.40	1.22-4.71
BADGE			0.999	0.51-1.96	1.03-3.98
BADGE.2H$_2$O			0.998	1.29-4.99	3.11-11.97
BADGE.2HCl			0.999	0.47-1.80	1.00-3.86
BPF			0.999	0.93-3.56	1.95-7.49
BFDGE*			0.999	0.26-1.73	0.35-2.89
BFDGE.2H$_2$O			0.998	0.30-1.16	0.31-1.20
BFDGE.2HCl**			0.999	0.26-14.92	0.36-30.56
Fe	FAAS	150-5000	0.999	650	1670
Sn		1000-50000	0.999	9800	25400
Zn		50-1200	0.999	330	1090
Cu		50-1000	0.998	160	520
Pb	GFAAS	1-15	0.993	9.42	23.80
Cd		0.2-2	0.996	3.01	7.62
Ni		5-100	0.993	0.05	0.16
Cr		2-100	0.994	22.90	56.0

** Two isomers, ** Three isomers, Note: there is a range of MDL and MQL for bisphenol compounds since they depend on the type of food analyzed*

Supplementary material **Table 5.S4:** Recovery of bisphenol compounds from fava beans and chickpeas

	Spiked levels (µg/kg)	**Recoveries (%)**	
		Fava beans	**Chickpeas**
BPA	100	107.0 ± 17.3	94.8 ± 9.1
	400	109.0 ± 5.1	91.8 ± 8.6
	1,200	111.0 ± 2.4	96.3 ± 4.7
BADGE	100	80.0 ± 6.6	97.3 ± 4.0
	400	84.0 ± 1.0	102.0 ± 8.3
	1,200	92.0 ± 4.8	78.2 ± 3.0
BADGE.2H_2O	100	65.0 ± 1.0	86.4 ± 3.2
	400	76.0 ± 6.9	91.5 ± 9.1
	1,200	108.0 ± 14.1	104.0 ± 3.3
BADGE.2HCl	100	97.0 ± 9.2	88.8 ± 2.1
	400	95.0 ± 5.5	105.0 ± 4.6
	1,200	101.0 ± 5.7	97.6 ± 1.9
BPF	100	93.0 ± 6.1	79.4 ± 4.5
	400	105.0 ± 2.4	87.5 ± 8.6
	1,200	110.0 ± 1.6	97.5 ± 3.7
BFDGE	100	87.0 ± 6.0	82.0 ± 3.0
	400	86.0 ± 1.0	101.0 ± 3.0
	1,200	94.0 ± 5.0	83.0 ± 3.0
BFDGE.2H_2O	100	105.0 ± 0.4	116.0 ± 2.9
	400	101.0 ± 1.2	104.0 ± 1.8
	1,200	120.0 ± 32.5	110.0 ± 3.2
BFDGE.2HCl	100	100.0 ± 12.0	93.0 ± 3.0
	400	96.0 ± 9.0	100.0 ± 11.0
	1,200	100.0 ± 16.0	93.0 ± 2.0

Supplementary material **Table 5.S5:** Recoveries of trace metals from CRM (**BCR® -679)**

Metal	**Expected concentration (mg/kg)**	**Observed concentration (mg/kg)**	**Recovery (%)**
Fe	55.0 ± 2.5	58.0 ± 3.6	105.0 ± 6.5
Cr	0.6± 0.10	0.6 ± 0.04	100.0 ± 6.6
Cd	1.66 ± 0.07	1.59 ± 0.05	96.00 ± 3.01
Ni	27.0± 0.8	30.0 ± 2.1	111.0 ± 7.8
Cu	2.89 ± 0.12	2.73 ± 0.17	94.0 ± 5.8
Zn	79.7 ± 2.7	78.1 ± 2.7	98.0 ± 3.4

n=6

Supplementary material **Table 5.S6:** Influence of heat treatment on the migration of trace metals in several vegetable foods

Element (mg/kg)	Food							
	Fava beans		**Red beans**		**Chickpeas**		**Okra**	
	Before Sterilization	**After sterilization**	**Before sterilization**	**After sterilization**	**Before sterilization**	**After sterilization**	**Before pasteurization**	**After pasteurization**
Sn	ND	ND	ND	ND	ND	ND	ND	ND
Fe	**9.93 ± 0.05**	**10.73 ± 0.13***	13.00 ± 0.71	14.04 ± 0.82	12.80 ± 0.28	12.23 ± 0.38	**6.26 ± 0.15**	**9.88 ± 0.65***
Pb	0.03 ± 0.00	0.033 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.01	0.05 ± 0.01
Cd	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	<MQL	<MQL
Cr	0.11 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.14 ± 0.00	0.20 ± 0.02	0.22 ± 0.02	0.13± 0.01	0.13 ± 0.01
Ni	**0.29 ± 0.01**	**0.34 ± 0.02***	0.29 ± 0.00	0.29 ± 0.03	**0.46 ± 0.05**	**0.54 ± 0.03***	0.06± 0.01	0.06 ± 0.01
Cu	**1.40 ± 0.05**	**1.62 ± 0.04***	**1.73 ± 0.03**	**2.09 ± 0.15***	**4.67 ± 0.10**	**4.42 ± 0.16***	**0.47 ± 0.05**	**0.55 ± 0.03***
Zn	6.32 ± 0.40	5.83 ± 0.07	3.68 ± 0.19	3.67 ± 0.21	**2.23 ± 0.05**	**2.98 ± 0.05***	2.33 ± 0.07	2.21 ± 0.27

n=3
ND : not detected
** Significant increase after heat treatment with p-value < 0.05*

Chapter 6:

General discussion

1. Introduction

This chapter reflects on the main findings of the research in terms of its contributions to the key issues of the case study that is to determine the levels of bisphenol compounds and metal elements in canned food consumed in Lebanon along with the main parameters influencing their migration. The effects of heat treatment, storage time, food type, food packaging and brand, storage temperature, can denting and more others are clearly stated in this chapter. Many interesting conclusions are drawn between comparing the main results obtained between chapters, such as difference in the migration levels of bisphenol compounds between food simulants and real food, the difference in the release of metal elements between coated cans, uncoated cans and jars, etc. Finally, this chapter provides valuable advices and precautions for the industries and consumers to minimize dietary exposure of the targeted food contaminants.

2. Effect of heat treatment

Heat treatment of products is one of the main techniques in the food industry for food conservation, as it stops bacterial and enzyme activity, thus preventing a loss of quality and keeping food non-perishable. However severe heat treatment, such as sterilization, are known to induce release of chemicals from packaging materials into foods [1]–[4]. In this PhD work, effect of heat treatment on the migration of chemicals from food cans has been greatly considered. Two heating conditions were tested: sterilization (at 121°C for 30 min and 90 min) applied to food simulants (chapters 2 and 3) and canned legumes such as fava beans, red beans and chickpeas (chapter 5), and pasteurization (at 100°C for 20 min) in the case of canned okra (chapter 5).

2.1. Influence of heat treatment on the migration of bisphenol compounds

The migration models detailed in chapter 3 showed that BPA and BADGE.2H_2O levels in canned food simulants are significantly affected by sterilization conditions (time and temperature). This confirms the results obtained in chapter 2 on the high BPA content after sterilization. Accordingly, BPA and BADGE.2H_2O increased from almost non detected levels before sterilization to the ranges from 47 to 197 µg/kg for BPA and 349 to 1106 µg/kg for BADGE.2H_2O after sterilization. The same influence of heat treatment was noticed on real canned food, as discussed in chapter 5. In this case, BPA levels increased between 151 and 391% after sterilization, whereas BADGE.2H_2O increased from non detected levels to a range between 101 and 147 µg/kg. Therefore, our results show that during heat treatment the diffusion of free bisphenol compounds is significantly accelerated, which is in agreement with previous studies [1], [4], [5].

Interestingly, comparison between sterilization and pasteurization process on the same food (okra) showed contrasting results depending on the conditions as detailed in chapter 5. BPA level after pasteurization (12.3 µg/kg) was lower than in sterilized canned legumes (40-54.6 µg/kg), which is in agreement with results published by Takao et al. [6] on the significant effect of heat treatment temperature on BPA migration. In contrast, BADGE.$2H_2O$ level was higher in pasteurized okra (147.2 µg/kg) than in the sterilized legumes (101-119.4 µg/kg). This clearly show the influence of food composition: here, the acidic (pH of 3.7) and highly aqueous (93.8% water content) nature of okra may favor the migration and hydrolysis of BADGE [7].

2.1.1. Contents of bisphenol compounds in food simulants vs. real food

Same cans (C1, C2 and C4) were tested both with simulants (chapter 3) and real foods from brands A, B and D (chapter 5). Interestingly, in both cases only BPA and BADGE.$2H_2O$ were detected and confirmed as bisphenol compounds. Also, BADGE.$2H_2O$ became detectable in canned foods only after heat treatment (see chapter 5).

When water and 3% acetic acid come in contact with C1 cans for 1 day without sterilization, BPA level remained below 0.42 µg/kg. In contrast, unsterilized aqueous foods (such as unsterilized fava beans (A), red beans (A) and chickpeas (A)) contained BPA in the range between 2.2 - 21.7 µg/kg. This suggests that food contamination with BPA occurred before canning or packing, possibly during the production process if equipments or containers with epoxy coating or plastic parts have been used [4]. The addition of hot brine (at ≥ 90°C) before sterilization cycle in case of canned food may also explain these results, since this step is not performed when conducting migration test on food simulants.

A comparison of BPA and BADGE.$2H_2O$ levels found in sterilized food simulants (cans C1, C2 and C4) with those found in sterilized canned foods (from same can brand) is presented in **Table 6.1**. Clearly, food simulants overestimate bisphenol migration in real food, especially in the case of BADGE.$2H_2O$. Additional experiments were then performed, as detailed in **Table 6.2:** fava beans, red beans and chickpeas were considered as aqueous foods (so simulant S1 was supposed to mimic migration in such foods), while okra is considered acidic food ($pH<4.5$) (that case, simulant S2 is more appropriate). The migration models derived in chapter 3 using food simulants were then used to predict the bisphenol levels in sterilized foods. In practice, the values measured in sterilized foods were clearly lower than the values predicted by our models as shown in **Figure 6.1**. This confirm an overestimation of the migration of bisphenol compounds into our food simulants.

Table 6.1: Mean concentrations of BPA and BADGE.2H2O between real foods and their simulants

Cans/brands	Mean concentration ± SD* (µg/kg) BPA	BADGE.$2H_2O$
Simulants[a] in C1	197 ± 85	520 ± 116
Foods[b] in brand A	39.9 ± 11.2	170 ± 83.4
Simulants in C2	47 ± 5.4	394 ± 45
Foods in brand B	22.1 ± 3.5	164 ± 66.9
Simulants in C4	65.0 ± 12.7	355 ± 87
Foods in brand D	44.2 ± 1.4	88.6 ± 4.6

** large SD is obtained since the mean concentration consider all the studied conditions*

[a] *water (S1) and 3% w/v acetic acid (S2)*

[b] *legumes and vegetable (okra)*

Table 6. 2: Experimental conditions of food samples used to test the migration models derived on food simulants.

Food	Corresponding food simulant*	Brand of Cans	Sterilization time (min)	Storage time (days) at 22.5°C
Fava beans	S1	C1 (A)	30	0
Fava beans	S1	C1 (A)	30	60
Fava beans	S1	C2 (B)	30	0
Fava beans	S1	C2 (B)	30	90
Red beans	S1	C1 (A)	30	0
Red beans	S1	C1 (A)	30	90
Chickpeas	S1	C1 (A)	30	0
Chickpeas	S1	C1 (A)	30	45
Okra	S2	C2 (B)	30	0

** S1: water as aqueous food simulant*
S2: 3% w/v acetic acid as acidic food simulant

This overestimation was also observed in older studies on aqueous [5] and acidic [8] food simulants. According to the (EU) No 10/2011 [9], "…food simulant may in certain cases significantly overestimate migration into food. In these cases it should be foreseen that the result in food simulant is corrected by a reduction factor".

According to **Table 6.1**, the reduction factor of BPA and BADGE.$2H_2O$ is dependent on the brand, ranging between 1.5 and 5 for BPA and between 2.5 and 4 for BADGE.$2H_2O$. Therefore, more comparative tests should be done between foods and food simulants to determine precise reduction factors.

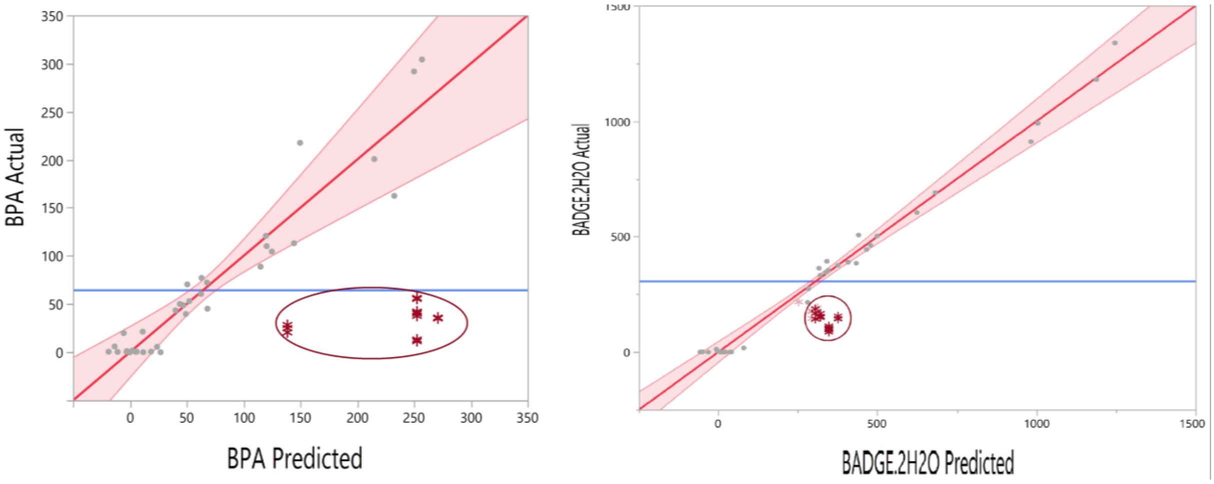


Figure 6.1: Prediction of BPA and BADGE.$2H_2O$ levels in sterilized canned legumes and vegetables based on the migration models built using food simulants. * corresponds to the values obtained in food samples.

2.1.2. Extent of bisphenols migration after sterilization

According to literature, up to 80–100% of BPA already present as free monomer in the coating had migrated into the food during sterilization [5].

Table 6.3: Migration of free BPA and BADGE.$2H_2O$ from can coatings into foods and food simulants

	BPA (µg/dm^2)	BADGE.$2H_2O$ (µg/dm^2)
ACN extraction [a]	25.7 ± 2.4	24.1 ± 4.5
Food simulants [b]	21.8 ± 9.4	57.8 ± 18.4
Average %migration[c]	85	240
Real foods [d]	5.6 ± 1.0	13.5 ± 1.2
Average %migration[c]	19	65

[a] *Solvent extraction through soaking C1 cans (n =3) for 24 h with ACN*

[b] *All conditions of sterilized food simulants in C1 cans, in chapter 3 , are considered*

[c] *%migration is calculated from the difference between the concentrations of bisphenol compounds in foods/food simulants and ACN extraction*

[c] *Legumes from brand A (without okra) studied in chapter 5*

In our work the percentage of migration after sterilization was determined by solvent extraction with ACN. This test was only carried on cans from brand A, or C1 cans. This allowed to assess the average migration of free BPA in our experiments with both foods and their simulants are presented in Table 6.3. With food simulants the average migration of free BPA reached 85%, whereas that of BADGE.$2H_2O$ was over 200%, probably due to its higher hydrolysis in aqueous food simulants compared to ACN [10]. On the other hand, the migration of BPA and BADGE.$2H_2O$ was lower in case of real food. The difference in the density between food simulants and real food can lead to differences in the diffusion of bisphenol compounds [11].

2.2. Influence of heat treatment on the migration of trace metals

To our best knowledge the effect of heat treatment on the release of trace metals from food cans was not studied in literature. This parameter was only monitored in chapter 5. The main finding was that, in contrary to bisphenol compounds, heat treatment had a minor effect on the release of metals. The significant effect was mainly noticed on Fe release in canned okra of acidic pH (3.7). The cans designated for this part of the study were coated cans, and this could have played a role on minimizing the release of metals during heat treatment. So, it would be interesting to investigate the effect of heat treatment on uncoated cans.

3. Effect of storage time

3.1. Influence of storage time on the migration of bisphenol compounds

Some studies [1], [4], [5] suggest that once the cans are sterilized, which is inescapable step, storage time is insignificant on triggering the migration of bisphenol compounds. This is in agreement with the conclusions drawn in chapters 2 and 3 for BPA and BADGE.2H_2O. Based on the data obtained from the experimental designs built in chapter 3, no significant correlation between storage time and migration of BPA and BADGE.2H_2O could be found (p-value > 0.05). On the other hand, the results obtained in chapter 5 on the migration of bisphenol compounds into foods, clearly show that there are some cases where BPA and BADGE.2H_2O increase as a function of storage time. For example, in canned okra, BPA rapidly increased from 12.3 to 25.4 µg/kg between 0 and 330 days, then it continued to increase slowly up to 32.7 µg/kg at 730 days. This is explained by the fact that okra is pasteurized and not sterilized, which means that the migration of BPA residues during pasteurization was not complete and still occurred with storage time. This behavior of BPA was also observed by Lopez et al. [8] upon pasteurization canned jalapeno peppers .

Levels of BADGE.2H_2O increased in almost all heat-treated foods (41-81% in fava beans, 62% in red beans and 55% chickpeas) upon storage, which was highlighted during the first 75 days of storage. This indicates that the migration of BADGE and its hydrolysis into BADGE.2H_2O in these foods may not be complete during sterilization. Contrariwise, BADGE.2H_2O did not increase in okra over storage at 22°C.

Although there is a large difference in the storage duration in the migration tests performed on food simulants (0 to 2 months) and real foods (0 to 2 years), our result do indicate that most of migration occurred during the first 2 to 3 months. Therefore, the difference in the effect of time on the migration of bisphenol compounds between real foods and simulants is in summary related to the degree of migration during heat treatment.

3.2. Influence of storage time on the migration of trace metals

The evolution of trace metals in foods as a function of storage time was discussed in chapters 4 and 5. The main finding was that almost all metals contents increased with time, yet this evolution was greatly dependent on the food type, brand and the packaging (i.e. uncoated cans, coated cans and glass jars). The detailed interpretation depending on these intervening factors will be demonstrated in the next paragraphs.

4. Effect of food/food simulant

It is known from previous studies that food properties such as pH, fat and water content, have a great influence on the migration of bisphenol compounds [1], [7], [11], [12] and metal elements [13], [14].

4.1. Contents of bisphenol compounds in canned aqueous, acidic and semi fatty foods/food simulants

In chapter 3, the content of bisphenol compounds was compared between different types of food simulants. In case sterilization was performed, the comparison was only carried between aqueous (water (S1)) and acidic (3% w/v acetic acid (S2)) food simulants. As shown in Figure 6.2 there is no significant difference in the level of BPA and BADGE.$2H_2O$ between S1 and S2 after sterilization.

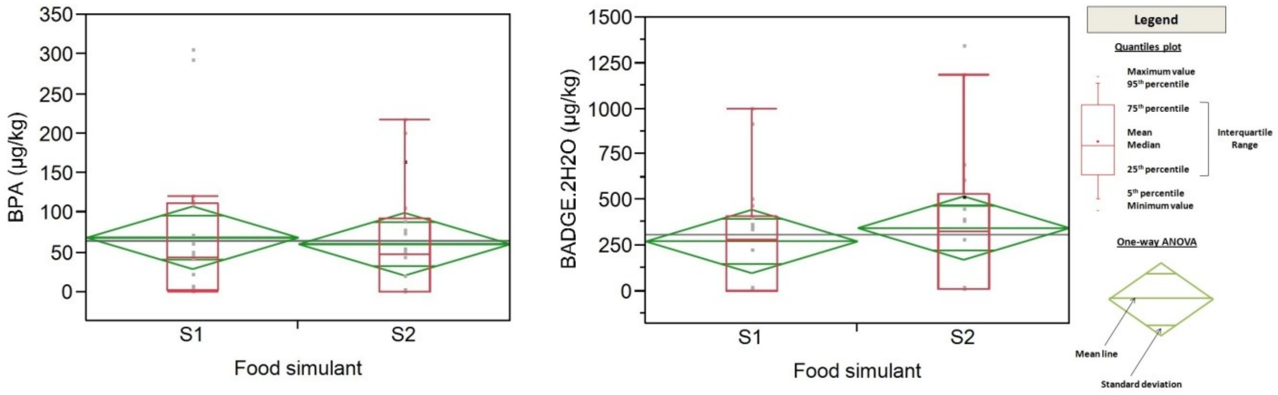


Figure 6. 2: One-way ANOVA analysis and quantiles plot of BPA and BADGE.$2H_2O$ levels by type of food simulant (S1 and S2) in sterilized cans

As discussed in the previous paragraphs, BPA and BADGE.2H_2O showed no significant evolution with storage time after sterilization of canned aqueous foods, where the obtained contents of BPA and BADGE.2H_2O obtained were in small ranges (BPA: 32.1-55.8 µg/kg and BADGE.2H_2O: of 101-183 µg/kg). The only difference was noticed in canned okra (acidic food), where BPA was lowest after heat treatment, but this behavior was related to mild conditions of heat treatment (pasteurization and not sterilization) as mentioned previously rather than to food type..

As BPA increased as a function of time in canned okra, still at 22°C, the maximum level reached at 730 days was very close to the BPA range found in aqueous foods (32.7 µg/kg). Similarly, levels of BADGE.2H_2O (122.1-147.2 µg/kg) found in canned okra were in the range observed in canned aqueous foods (legumes). Therefore, this finding confirms the conclusion that there is insignificant difference between aqueous and acidic foods on the content of BPA and BADGE.2H_2O. Generally, in literature, when the effect of foods/food simulants is discussed on content of bisphenol compounds, acidic and aqueous foods/food simulants are considered as close matrices [15]–[17]

Further comparison with semi-fatty foods was carried also in chapter 3, where 50% ethanol was used to simulate the influence of semi-fatty foods. In this case, the comparison of content of bisphenol compounds was carried between aqueous (water (S1) and 10% ethanol (S3)), acidic (3% w/v acetic acid (S2)) and semi-fatty food (50% ethanol (S4)), where heat treatment was applied. As shown in Figure 6.3, there is a major effect of 50% ethanol on the migration of both BPA and BADGE.2H_2O, whereas other simulants S1-S3 showed insignificant difference in their content of bisphenol compounds.

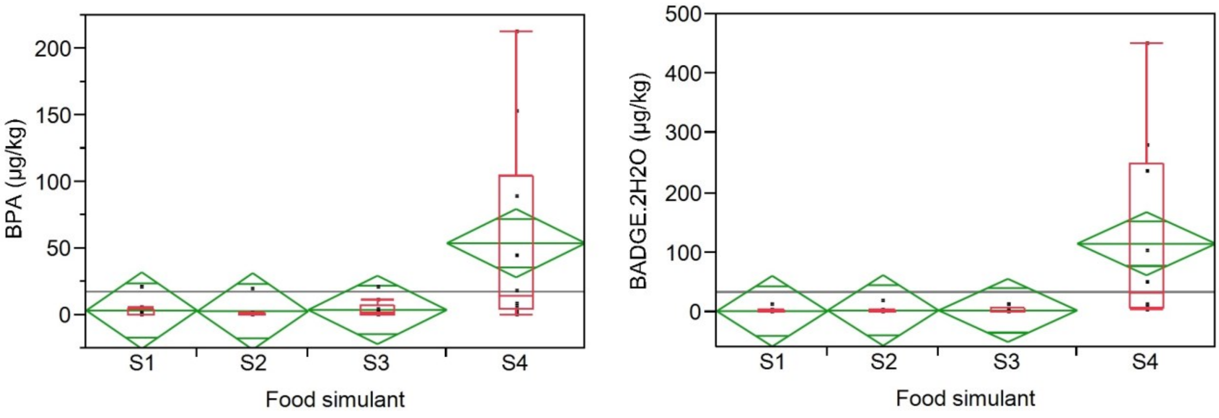


Figure 6.3: One-way ANOVA analysis and quantiles plot of BPA and BADGE.2H_2O levels by type of food simulant (S1, S2, S3 and S4) in unsterilized cans. NB: the legend for ANOVA and quantiles plots is the same as in Fig. 6.2.

The bisphenol contents in S1-S3 were too low due to absence of heat treatment. Despite the absence of sterilization, the levels of bisphenol compounds in S4 or 50% ethanol were still comparably high and close to those obtained in sterilized S1 and S2. Therefore we can assume that the effect of 50% ethanol could be amplified upon sterilization, thus semi-fatty food might favor the migration of bisphenol compounds. This is an agreement with the suggested lipophilic property of BPA **[3]**, yet this behavior of BPA is not evident according to Lopez et al. **[18]**. In the meantime BADGE could be greatly solubilized and hydrolyzed in 50% ethanol **[10]**.

4.2. Contents of metal elements in canned aqueous, acidic and semi fatty foods

Similar to bisphenol compounds, the metal contents between food categories was also investigated in chapter 5, where aqueous (fava beans, red beans and chickpeas) and acidic (okra) canned foods were analyzed. In order to compare the levels of metals with semi-fatty food, additional test was carried on canned chickpeas with sesame paste (hummus) which is rich in fat and has a thick creamy texture, thus presenting a greatly different food matrix. The 400 g hummus cans were from brand A, with same aspects as legumes cans from brand A. The analysis of hummus was carried from day 0 (after sterilization at 121°C for 50 min, where longer sterilization time is required to ensure the homogenous distribution of temperature inside the can) until 730 days (2 years, expiry date). The interest in this food is that it is Lebanese specialty, and is greatly appreciated worldwide **[19]**.

Upon comparing the migration trends between the five types of canned foods (fava beans, red beans, chickpeas, okra and hummus) over 2 years of storage, it is noticed that the highest evolution of total metal content was found in canned okra, where the content of six out of nine metals increased (Fe (236%), Cr (63%), Ni (407%), Cu (18%) and Zn (47%)). In this case, Fe, Cr and Ni showed the highest evolution in okra compared to other food types. On the other hand, only Fe (87%) and Ni (121%) significantly increased with storage time in canned hummus as shown in **Figure 6.4**.

The difference in the metal release between okra and hummus is related to the large difference in the food matrices: okra is acidic (pH = 3.9), highly aqueous (93.8% is water) and low in fat (0.3%), while hummus is slightly acidic (pH = 4.5), solid and creamy (65% of water) and rich in fat (10%). Therefore, the metal release seems to be easier in food matrices like okra rather than matrices similar to hummus. This was also shown in previous studies reported by Parkar et al. **[20]** and Buculei et al. **[21]**; the former authors obtained higher migration of Fe and Sn in canned mango than in canned sweet corn creamy layer, while the later authors showed that higher Sn release in canned tomato than in canned liver pate.

Nevertheless, Fe and Ni are still released in canned hummus, probably due to the presence of citric acid that tends to solubilize these elements [22], [23]. This also explains the weak release of Fe and insignificant release of Ni in fava beans from brand A that contains no added citric acid (see chapter 5-Table 5.2). Moreover, Ni migration was higher during the first 75 to 330 days in red beans and chickpeas, whereas it took longer time to migrate in hummus (between 493 and 730 days). On the other hand, the evolution of Fe in hummus was similar to that present in canned red beans and chickpeas.

The average concentrations of other targeted metals present in canned hummus are shown in Table 6.4. As in other coated food cans present in chapter 5, Sn is still not detected in canned hummus. Other metal elements such as Cd, Cr and Zn were highest in hummus compared to brand A canned foods.

We were informed from the industry (brand A) that the same chickpeas cooking is used to prepare canned chickpeas and hummus. Upon comparing the metal contents between canned hummus and chickpeas, it is noticed that probably part of several trace elements in canned hummus originates from Tahina (sesame paste).

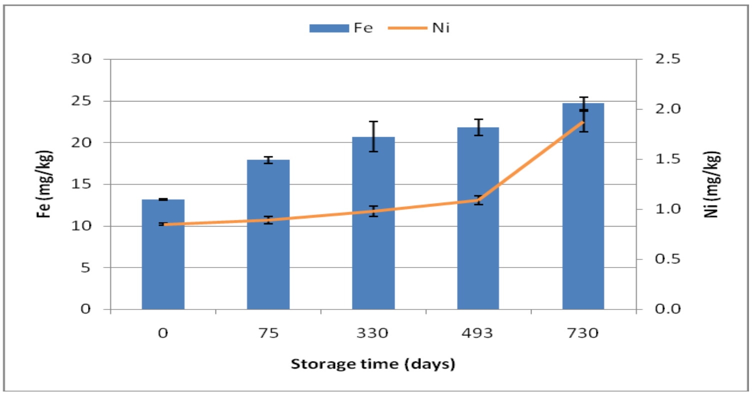


Figure 6.4: Evolution of Fe and Ni content in canned sterilized hummus as a function of storage time

Knowing that sesame paste is prepared through crushing sesame seeds with rocks, this action could have contributed to the release Pb, Cd, Ni and Zn.

Table 6.4: Average concentrations of metal elements in canned legumes, vegetable and ready to eat houmous calculated between 0 days after sterilization until expiry, i.e. 730 days.

	Average concentration* ± S.D (mg/kg)							
	Sn	**Fe**	**Pb**	**Cd**	**Cr**	**Ni**	**Cu**	**Zn**
Houmous	ND	19.66 ± 3.90	0.06 ± 0.001	0.05 ± 0.004	0.25 ± 0.01	1.14 ± 0.38	3.63 ± 0.21	12.94 ± 0.36
Chickpeas	ND	18.31 ± 5.65	0.02 ± 0.001	0.04 ± 0.001	0.24 ± 0.01	0.62 ± 0.06	5.51 ± 0.76	3.46 ± 1.27
Okra	ND	19.84 ± 9.13	0.05 ± 0.003	0.005 ± 0.001	0.19 ± 0.03	0.12 ± 0.10	0.57 ± 0.06	2.90 ± 0.43
Fava beans	ND	11.26 ± 1.60	0.03 ± 0.002	0.02 ± 0.004	0.14 ± 0.01	0.35 ± 0.03	1.76 ± 0.20	6.79 ± 0.53
Red beans	ND	19.84 ± 3.94	0.03 ± 0.002	0.02 ± 0.002	0.15 ± 0.01	0.33 ± 0.04	2.24 ± 0.20	5.38 0.97

5. Difference between packaging and brand

Throughout previous chapters, it is noticed that there is important differences in the levels of bisphenol compounds and metal elements between types of packaging (i.e. coated cans, uncoated cans and glass jars) and brands.

5.1. Contents of targeted contaminants between food packaging

5.1.1. Bisphenol compounds between types of coated cans and glass jars

In chapter 2, two types of cans from same brand were designated for the study of BPA migration. The first type of cans (labeled L, 423 mL capacity) is used for packing vegetables (fava beans, okra) and fruits; these cans are coated with epoxy-phenolic resin and titanium oxide pigmentation. The second type of cans (labeled S, 222 mL capacity), are used to pack processed meats; their coating is based on epoxy-phenolic resin pigmented with carbon black and covered with a wax layer to facilitate meat sliding from the can.

At first, upon comparing the level of BPA between the two types of cans in µg/L of water simulant, the student t-test (p-value <0.0001) clearly indicated a significantly higher BPA mean concentration in (S) cans (122.9 µg/L) in comparison to (L) cans (92.3 µg/L). Yet, taking into account the food surface contact area, there is no more evidence of difference since BPA migration is around 10.5 µg/dm^2 for both types of cans. Hence, BPA migration was probably neither affected by the modifications in epoxy-phenolic coating nor by the geometry of the cans.

Another comparison also could be deduced from chapter 5, on the levels of BPA and BADGE.2H_2O in okra stored in coated cans or glass jars. Interestingly, although the caps of okra jars are coated with polymeric coating (unknown), the levels of BPA and BADGE.2H_2O remained below their detection limits in these samples, even after 330 days of storage. In the mean time, BPA in canned okra was in the range of 12.3-32.7 µg/kg, and BADGE.2H_2O in the range of 122.1-147.2 µg/kg (see chapter 5-Table 5.2). As expected, food stored in glass jars should be preferred over canned foods in order to reduce the dietary intake of bisphenol compounds.

5.1.2. Release of metal traces from coated and uncoated food cans

In general, metal cans are coated with an organic layer to protect the integrity of food cans from effects of the food (e.g. highly acidic foods and some food ingredients promote corrosion of metal leading to potential release of metal traces). Yet, tin cans without internal coating are used for light colored, acidic juices and fruits (e.g. pineapple, pears, peaches), where, under these conditions, tin act as an antioxidant preventing darkening and flavor changes [24].

In this project, both types of cans were considered: migration of metals from uncoated tinplate cans into fruits (detailed in chapter 4), and migration from coated cans into legumes and okra (see chapter 5). A synthesis of our results is presented in **Table 6.5**. Higher average concentrations of Fe, Pb, Cd, Cu and Zn were observed in canned legumes and okra than in canned fruits, whereas Cr level was lower in legumes and okra than fruits, and Ni content was similar between these food categories. Indeed, the difference in the content in the metal elements arise from the food itself and the properties of the tinplate can [25]. Comparison for the same food between metal cans and glass jars will give some indication about metal release, so as the trends of metal contents over storage duration.

Table 6.5: Comparison of average metal contents between foods in uncoated cans, coated cans and glass jars

		Fe	**Sn**	**Pb**	**Cd**	**Cr**	**Ni**	**Cu**	**Zn**
Uncoated fruit cans (pH: 3.5-4)	Min (mg/kg)	3.14	28.20	ND	ND	0.19	0.28	0.25	1.15
	Max (mg/kg)	8.54	138	ND	0.02	0.45	1.24	0.82	3.19
	Average (mg/kg)*	5.91	73.86	-	0.01	0.29	0.58	0.45	2.13
	Range of %increase [a]	**0-46.2**	**54-208**	-	**31-306**	**0-62**	**26-104**	**33-228**	**0-103**
Coated legumes cans (pH: 5.1-5.9)	Min (mg/kg)	5.59	ND	0.02	0.01	0.05	0.11	1.62	2.98
	Max (mg/kg)	23.37	ND	0.26	0.04	0.22	1.11	6.23	7.67
	Average (mg/kg)*	12.70	-	0.10	0.02	0.12	0.47	3.29	5.14
	Range of %increase [b]	**21-108**	-	**0-205**	**0-80**	**0-57**	**0-129**	**0-41**	**0-69**
Coated okra cans (pH: 3.9)	Min (mg/kg)	9.88	ND	0.05	ND	0.13	0.06	0.48	2.21
	Max (mg/kg)	33.16	ND	0.06	ND	0.21	0.30	0.65	3.30
	Average (mg/kg)*	19.84	-	0.05	-	0.19	0.12	0.57	2.90
	Range of %increase [c]	**235**	-	NA	-	**62**	**406**	**20**	**47**
Okra glass jars (pH: 3.8)	Min (mg/kg)	5.47	ND	0.04	ND	0.15	0.09	0.52	2.79
	Max (mg/kg)	6.86	ND	0.04	ND	0.16	0.10	0.52	3.34
	Average (mg/kg)*	6.16	-	0.04	-	0.15	0.10	0.52	3.06
	Range of %increase [d]	**25**	-	NA	-	NA	NA	NA	**20**

** Average concentration of all data obtained as a function of time, including brands when present*
[a] *Range increase considering all brands between purchase date till expiry (2 years)*
[b] *Range increase considering all brands between day 0 till expiry (2 years)*
[c] *No range is presented since only one band was analyzed day 0 till expiry (2 years)*
[d] *No range is presented since only one band was analyzed between 90 and 330 days*
NA: no significant increase was noticed

The predominant effect of coating presence was on Sn, where its release was completely avoided in coated cans (Sn levels approximately dropped from above 100 mg/kg in uncoated cans to non-detected levels (<25 mg/kg) in all coated cans). Although Sn release is favored in acidic medium, it was not even observed in coated cans of acidic okra (pH about 3.9). Our results are thus in agreement with previous study reporting that in lacquered cans Sn release is lowered by 99.5% [26].

Interestingly, higher release of Fe and Pb was observed in coated cans, suggesting that the application of coating is not sufficient to avoid metal release in this case. On the other hand, higher increases of Cd, Cu and Zn were recorded in uncoated cans, with some discrepancy among the brands. In the case of Cr and Ni, results were similar between coated and uncoated food cans.

Finally, the release of trace metals was the lowest in okra glass jars, where among the targeted metals, only Fe and Zn increased by 20-25% as a function of time, in agreement with previous study [27].

5.2. Contents of targeted contaminants between brands of canned food

As mentioned previously, the migration trends of bisphenol compounds and metal elements depend on the properties of packaging materials [28], [29] that significantly varies between manufacturer and another.

5.2.1. Difference in the levels of bisphenol compounds between brands

As clearly noticed from the results obtained in chapter 3, there are important differences between cans from different brands (C1 to C4). Results from one-way ANOVA performed on BPA and BADGE.2H_2O levels in food simulants, along with quantiles, are shown in **Figure 6.5**. Cans C2 possess the least contents of BADGE.2H_2O (average = 349 ± 45 µg/kg) and BPA (average = 47 ± 5.4 µg/kg) followed by C4 cans. Highest concentrations of BPA (average = 197 ± 85 µg/dm^2) were detected in C1 cans and highest concentrations of BADGE.2H_2O (average = 94.4 ± 16.4 µg/dm^2) in C4.

Therefore the quality of the cans concerning the migration of bisphenol compounds in simulants is in the following decreasing order: for BPA: C2 > C4 > C3 > C1, for BADGE.2H_2O: C2 > C4 > C1 > C3. Cans C1 and C2 are locally produced, but C4 cans are imported cans.

As described previously, cans C1, C2 and C4 in chapter 3 correspond to same cans used by the local food brands A, B and D mentioned in chapter 5.

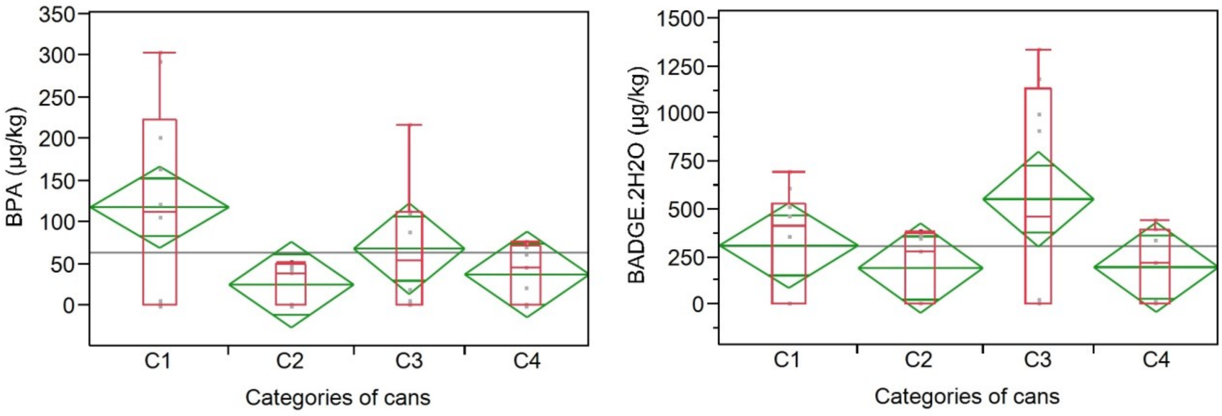


Figure 6.5: One-way ANOVA analysis and quantiles plot of BPA and BADGE.$2H_2O$ levels in food simulants by type of cans. NB: the legend for ANOVA and quantiles plots is the same as in Fig. 6.2.

Based on the average concentrations of BPA and BADGE.$2H_2O$ found in different food brands, **Table 6.6** (canned fava beans and chickpeas in chapter 5 – see **Table 5.2**), we can notice that the order of cans quality has changed and became: for BPA: B (C2) > A (C1) = D (C4), for BADGE.$2H_2O$: D (C4) > A (C1) > B (C2). This change may be related to the differences in food composition and ingredients that are not present with simulants. Yet, C2 cans from brand B still show the least content of BPA. In practice, canned food production companies should work with can manufacturers who provide the best coatings with least or absolutely no contamination occurring from the can to the food.

Table 6.6: Comparison of mean concentration of BPA and BADGE.$2H_2O$ between food brands

	Mean concentration* ± S.D (µg/kg)		
Brands	**A**	**B**	**D**
BPA	41.4 ± 8.2	22.1± 3.5	44.2 ± 1.4
BADGE.$2H_2O$	147 ± 26.6	164 ± 66.9	88.6 ± 4.6

** Mean concentration includes all results obtained on canned fava beans and chickpeas in chapter 5*

5.2.2. Reduction of bisphenol compounds in can coating

After contacting manufacturers of brands A and B in 2017, they informed us that they are importing BPA-free or BPA-reduced content lacquers from Germany and Turkey, respectively. Unfortunately, they couldn't provide us with the chemical formula of these lacquers. According to the analysis of sterilized water, none of the targeted bisphenol compounds were detected in brand B-BPA free lacquer. On the opposite, BPA and BADGE.$2H_2O$ were found (confirmed using MS detection) in brand A-reduced BPA coating with quite similar levels as for cans coated with BPA containing lacquer (see **Table 6.7**).

Additionally, we rinsed few cans from brand B with hot water to test if this practice could remove some residues of bisphenol compounds; unfortunately no reduction in the levels of BPA or BADGE.2H_2O could be observed.

Table 6.7: Mean concentrations of BPA and BADGE.2H_2O in cans with different lacquers (sterilized cans filled with water) and rinsed cans (with hot water)

	Mean concentration ± S.D (µg/kg)	
Type of cans	**BPA**	**BADGE.2H_2O**
Brand A+ BPA	80 ± 5	360 ± 33
Brand A + reduced BPA	69 ± 8	301 ± 39
Brand B + BPA	42 ± 10	271 ± 60
Brand B - BPA	ND	ND
Brand B + BPA + rising	39 ± 5	233 ± 31

+ with
- without
n=3 replicates of cans

5.2.3. Difference in the levels of trace metals between brands

Similar to bisphenol compounds, the materials used in tinplate cans between brands along with the source of raw foods, play a major role on the content and release of metal trace elements in canned foods [20]. According to the results presented in chapters 4 and 5, it is difficult to judge which of the brands had the best container quality. Among the studied brands of legumes and vegetable (see chapter 5), brand A seems to be the best choice for canning non-acidic food, since it showed the least release of metals (Pb migration was insignificant in brand A), while brand B showed the highest release of Pb and Ni.

The overall comparison of the evolution of key trace metals in uncoated fruit cocktail cans (see chapter 4) showed a significant difference in the migration tendencies between brands. Surprisingly, as indicated in **Table 6.8**, migration of Sn, Cd and Cu was the highest in brand B, although the cans of this brand were partially coated on their side seam (which was not beneficial on reducing the release of these elements).

Table 6.8: Comparison in the average contents and percentage increases of metal elements between brands

Food	Brand		Sn	Fe	Cd	Pb	Cr	Ni	Cu	Zn
Fava beans [a]	A	Average [d]	ND	11.26 ± 1.79	0.02 ± 0.004	0.03 ± 0.002	0.14 ± 0.01	0.35 ± 0.03	1.76 ± 0.22	6.79 ± 0.59
		%increase [e]	-	21	80	NA	18	NA	32	14
	B	Average [d]	ND	10.20 ± 4.12	0.02 ± 0.004	0.14 ± 0.06	0.07 ± 0.01	0.19 ± 0.07	2.60 ± 0.11	4.42 ± 0.02
		%increase [e]	-	31	44	86	14	69	NA	NA
	C	Average [d]	ND	8.80 ± 1.93	0.02 ± 0.01	0.18 ± 0.04	0.08 ± 0.002	0.46 ± 0.06	2.67 ± 0.11	4.37 ± 0.05
		%increase [e]	-	37	56	39	NA	22	NA	NA
Chickpeas [b]	A	Average [d]	ND	18.32 ± 5.65	0.04 ± 0.001	0.02 ± 0.001	0.24 ± 0.01	0.62 ± 0.06	5.51 ± 0.76	3.46 ± 1.28
		%increase [e]	-	91	NA	NA	NA	23	41	69
	D	Average [d]	ND	10.64 ± 2.21	0.04 ± 0.001	0.02 ± 0.001	0.24 ± 0.01	0.94 ± 0.24	4.98 ± 0.07	7.02 ± 0.95
		%increase [e]	-	34	48	21	NA	44	NA	21
Fruits [c]	A	Average [d]	94.24 ± 23.36	4.14 ± 0.69	0.003 ± 0.001	ND	0.33 ± 0.07	0.72 ± 0.19	0.32 ± 0.05	3.10 ± 0.41
		%increase [e]	53	46	72	-	64	102	32	22
	B	Average [d]	61.30 ± 34.51	8.48 ± 0.09	0.02 ± 0.01	ND	0.22 ± 0.03	0.35 ± 0.08	0.54 ± 0.40	2.69 ± 0.71
		%increase [e]	132	NA	176	-	20	38	228	46
	C	Average [d]	100.6 ± 52.8	6.47 ± 0.55	0.01 ± 0.001	ND	0.44 ± 0.01	1.09 ± 0.21	0.50 ± 0.21	2.05 ± 0.21
		%increase [e]	118	NA	31	-	NA	32	86	103

[a] *Analyzed between day 0 after sterilization and expiry (2 years)*
[b] *Analyzed between day 0 after sterilization and expiry (2 years)*
[c] *Analyzed between purchase date (at 7 to 9 months) and expiry (2 years)*
[d] *Average concentration obtained as a function of time*
[e] *%increase as a function of time*
NA: no significant increase was noticed
N.B. Brands of fruits and different from brands of legumes

6. Effect of storage temperature

It was reported that storage temperature can have a direct influence on both rate and extent of migration of chemicals from food contact materials. For instance, increased temperatures lead to higher migration rates and rapid establishment of equilibrium [27].

In our study we considered storage temperature as a main factor due to its high variability between seasons and places in Lebanon. As already mentioned in previous chapters, three conditions were tested: refrigerating temperature at 5 ± 1°C, room temperature at 22 ± 4°C, and high storage temperature at 40 ± 4°C.

6.1. Influence of storage temperature on the migration of bisphenol compounds

As for storage time, storage temperature was shown to have insignificant effect on the migration of bisphenol compounds (see chapters 1 and 2), whatever the food simulant or brand. This observation was also noticed elsewhere [5], [30]. However, in case of real food, an interaction effect was noticed between pH (i.e. acidic) and storage temperature (see chapter 5). As an illustration minor effect of storage temperature for fava beans (aqueous food) was noted, whereas BPA and BADGE.2H_2O levels were significantly affected by high storage temperature (40°C) in canned okra (acidic food). Consequently, after storage of canned okra for 730 days at 40°C, the content of BPA increased by about 40% and that of BADGE.2H_2O by about 280%. The effect of high storage temperature was also reported by Errico et al. [31] on the migration of BPA in canned tomato paste (acidic as well). In contrast, the effect of refrigerating temperature (5°C) was minor on both types of foods.

It is noteworthy that the study of storage temperature effect was carried only for two months (60 days) in food simulants, whereas it was monitored up to 2 years (730 days) for canned fava beans and okra. The effect of storage temperature for canned okra was noticed at day 493 that is far beyond the storage duration of food simulants. Therefore, the significant interaction effect between acidic medium and 40°C in the case of food simulants may be related to the limited storage duration considered in our experiments.

6.2. Influence of storage temperature on the migration of trace metals

The effect of storage temperature on the release of trace metals was carried on different types of foods and cans, including: fruits in uncoated and partially coated cans, okra and fava beans in coated cans, and hummus in coated cans. The results discussed in previous chapters showed that, in case there is an influence of storage temperature, 40°C had a predominant effect on accelerating the release of metals from food cans, whereas 5°C had a minor effect on slowing down metal release.

Storage at 40°C caused severe changes in the appearance of these products especially at long storage time as illustrated in **Figure 6.6**. Fruit cans were blown after few months (2 to 5 months) due to important corrosion caused by high temperature and aggressive acidic nature of canned fruits. In fact, under high storage temperature, the tin layer is more likely to be released leaving unprotected steel to corrode very rapidly, with vigorous evolution of hydrogen [32]. This behavior was also observed by Parkar et al. [20] upon storing uncoated mango cans at 38°C for 6 months. In contrast, can damage was not noticed with canned okra, knowing that both fruits and okra are considered acidic food (pH between 3.5 and 4). Therefore, the presence of coating inside the okra cans avoided severe corrosion and allowed the metallic can to withstand the high storage temperature for a long time (up two years). Only slight corrosion appeared on the external walls of okra cans stored at 40°C for 730 days, with noticeable darkening in the color of okra. Interestingly, after 730 days at 40°C, a little part of side seam coating was sloughed off into the canned hummus (this was noticed on two out of three cans). Perhaps, at thigh storage temperature and after long duration the fatty nature of hummus tends to dissolve part of the organic coating. Dark color of canned hummus was also observed upon storage for 730 days at 40°C. On the other hand, no changes appeared on fava beans cans stored at 40°C, not even at long storage times, probably thanks to the less aggressive nature of fava beans (pH of 5.9).

On the scale of metal analysis, the results revealed a huge increase Fe and Sn (up to 118 times) in canned fruits at a time prior to their blowing. In this case, the content of Fe and Sn reached unacceptable and worrying values (104-127 mg/kg for Fe and 345-538 mg/kg for Sn) as shown in chapter 4.

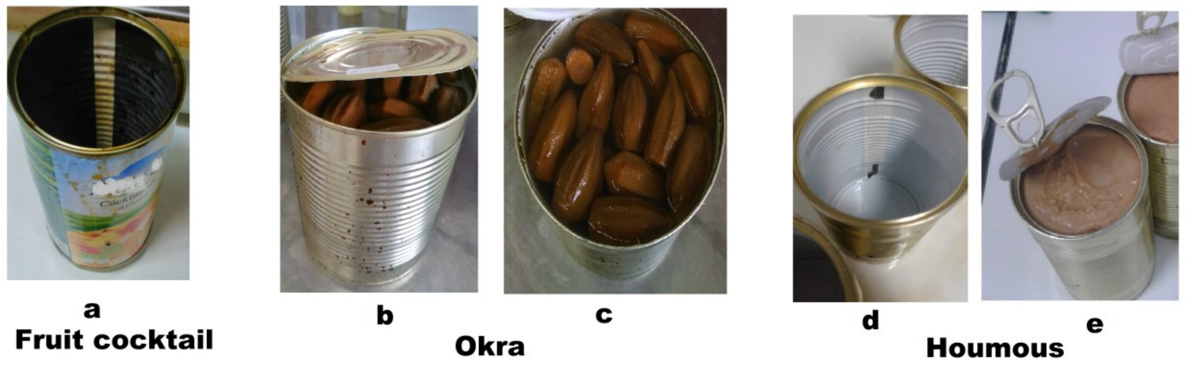


Figure 6.6: Aspect of food and cans after storage at 40°C for long time (fruit cocktail after 5 months, while okra and hummus after 2 years). NB: the natural colors of okra and hummus are green and light beige, respectively.

Significant effect of high temperature was also noted for Fe in canned fava beans, okra and hummus, but this effect was much lower than that observed in canned fruits. Among legumes and vegetables, Fe in okra was most affected by storage temperature due to its low pH. Yet, the presence of coating helped in minimizing the migration of Fe, such that it was 1.1 to 1.4 times higher at 40°C reaching up to 67.8 mg/kg after 730 days (see chapter 5-Table 5.2).

Table 6.9: Content of metal elements in hummus cans stored under the three designated temperatures for 730 days.

Temperature (°C)	**Concentration ± S.D (mg/kg)**							
	Fe	**Sn**	**Cd**	**Pb**	**Cr**	**Ni**	**Cu**	**Zn**
5	23.20 ± 0.73	ND	0.05 ± 0.002	0.06 ± 0.002	0.26 ± 0.04	1.96 ± 0.13	3.47 ± 0.13	13.47 ± 0.07
22	24.70 ± 0.73	ND	0.05 ± 0.003	0.05 ± 0.001	0.27 ± 0.03	1.88 ± 0.10	3.64 ± 0.14	13.33 ± 0.07
40	55.88 ± 4.11	ND	0.05 ± 0.005	0.05 ± 0.003	0.26 ± 0.02	1.84 ± 0.12	4.22 ± 0.23	12.99 ± 1.08

Considerable release of Fe was also noticed in canned hummus under 40°C of storage as shown in Table 6.9. Interestingly, Fe was the only metal affected by high storage temperature in hummus. On the other hand, the effect of 40°C of storage was minor on Fe migration in fava beans (see chapter 5-Table 5.2) due to its nonaggressive pH (5.9), absence of citric acid and good appearance of the can and its coating. Interestingly, Sn levels in coated food cans remained below the detection limit even at high storage temperature, either in low pH-okra or in hummus cans with a sloughed part of coating.

In short, the effect of storage temperature, specifically 40°C, on targeted metals was highly dependent on packaging and food content as summarized in Figure 6.7.

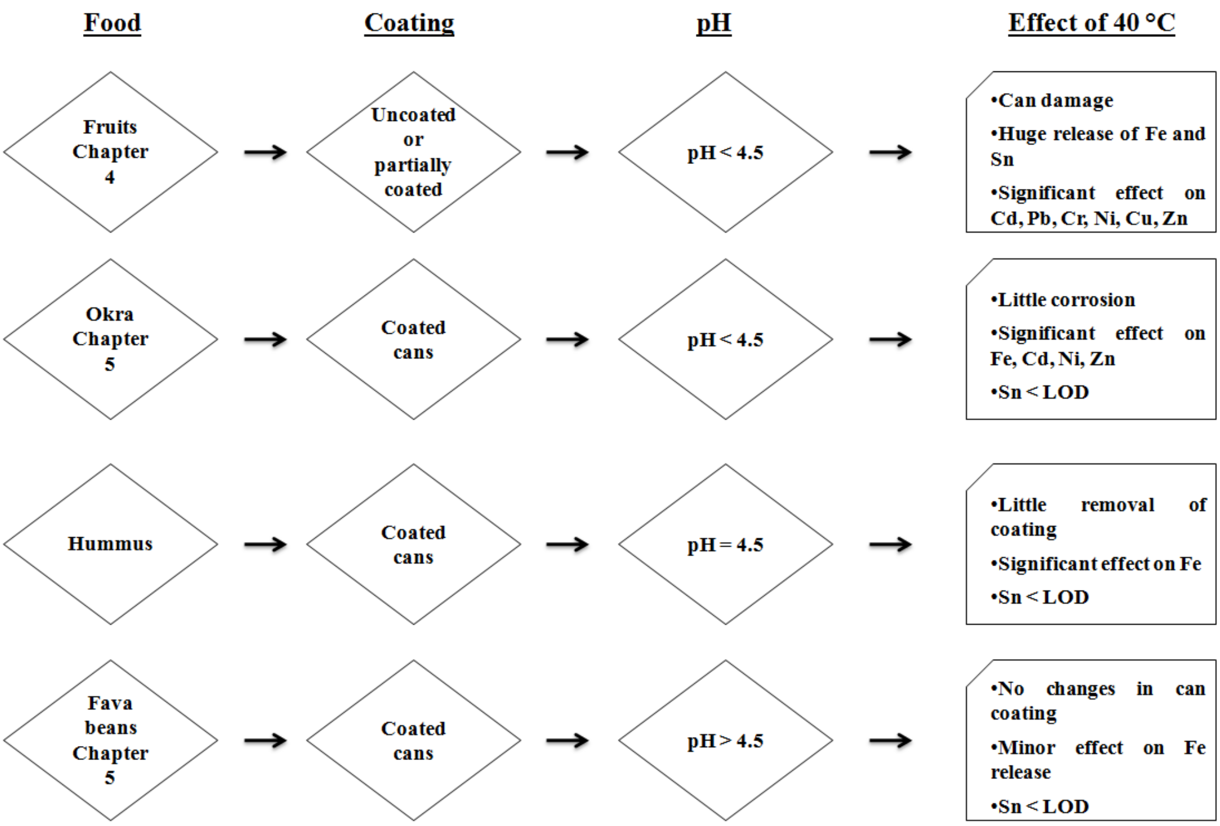


Figure 6.7: Summary of main effects observed under high storage temperature (40°C) depending on food content and type of tinplate cans

7. Effect of can denting

Obviously, the concern about dented cans is that the food they contain may not be safe to eat, such that microorganisms like bacteria and molds are ready to dive in if given the chance. According to the USDA [33], if a can containing food has a small dent, but is otherwise in good shape, the food should be safe to eat. However, the effect of mild can denting on the migration of chemicals from cans coating and metal substrate is doubtful. To address this questioning, the effect of can denting on the migration of trace metals and bisphenol compounds was investigated (see chapters 4 and 5). The study was carried upon denting uncoated fruit cans as well and coated cans of fava beans and okra.

As detailed in chapter 5, the effect of can denting on the migration of BADGE.$2H_2O$ was highlighted in case of acidic food (i.e. canned okra), where can denting can cause deterioration in the lacquer and ruin its integrity leading to higher interaction between food and coating. In this case BADGE is susceptible to rapid hydrolysis in the presence of acidic medium. On the other hand, BPA was not influenced by can denting neither in canned fava beans nor in okra, which is agreement with the results obtained by Goodson et al. [5].

Considering metal release, our results (shown in chapters 4 and 5) indicate an interaction effect between denting and presence of coating, pH and food ingredients. Indeed, upon can denting, the tin layer is scratched, causing a higher attack of acidic medium at this area, thus leading to corrosion and significant release of Sn and underlying metals [16]. This behavior was observed in uncoated fruit cans (see chapter 4). However, interestingly, can denting of coated food cans didn't lead to any increase in Sn content, even in acidic okra.

Another interesting finding underlines the high increase in Fe content in coated okra cans upon denting (its concentration is approximately doubled), while this parameter was less influencing, still significant, on Fe release in uncoated fruit cans (increased by about 1.2 times at the early period of denting). In fact, in case of lacquered tinplate cans, any defect in the protective lacquer (such as scratches, tearing and discontinuity) would result in a concentrated attack of the base steel [16], therefore the effect of can denting was expected to be higher in case of coated cans. In this case the release of Sn could have occurred, yet due to the comparably high quantification limit of Sn (25.4 mg/kg) we couldn't detect this change. In case of non acidic aqueous food like fava beans, Fe was not influenced by can denting.

Other metal elements, such as Cd, Cu and Zn, were also significantly affected by can denting, mainly in acidic foods. Zn levels slightly decreased in dented cans with fava beans, and tend to decrease in canned fava beans from brand A at long storage time (see Chapter 5-Table 5.3). This is probably due the presence of sulfites as mentioned on the product label of brand A fava beans since sodium metasulfite (E223) is used as antioxidant: the released free Zn can probably react with sulfur dioxide resulting from the sulfites to produce zinc sulfate that forms white spots on the coating (which already has white appearance) [16].

8. Other parameters

People sometimes keep leftovers in opened cans in the refrigerator for few days, or heat their foods directly in the cans during meal preparation. So, these actions have been both considered since they are suspected to affect the migration of bisphenol compounds and metal elements.

In chapter 4, the effect of leaving opened cans in the fridge was tested on the migration of metal elements in uncoated fruit cocktail cans. After few days, the inner tin layer was sloughed off (see Fig. 6.8), due to the increased content of oxygen and subsequent oxidation of tin layer in the absence of protective coating [20]. Consequently, Fe levels increased by 35 times (up to108 mg/kg) and those of Sn increased by 3 times (up to 223 mg/kg), while the levels of other key metals were not affected. In fact, Petropoulos et al. [34] showed that the release of Sn, Fe, Cd and Pb starts after six hours from opening. Therefore, keeping opened cans in the refrigerator should be avoided, especially with acidic food packed in uncoated cans.

Figure 6.8: Effect of keeping opened cans of fruit cocktail in the refrigerator

Heating foods directly in cans was investigated in chapter 5 on both bisphenol compounds and metal elements. For this purpose canned fava beans were heated in their cans for few minutes. Overall, no significant effect of this practice was noticed on the considered contaminants levels. Similar conclusions were drawn for BPA in a previous study [5], while to our best knowledge the effect of heating food directly in the cans has never been investigated for trace metals before. So, our results do confirm that, under the tested conditions, this practice is safe concerning the migration of potentially toxic metals and bisphenol compounds.

9. Worrying concentrations

As described in previous paragraphs, BPA increased after sterilization to a range of 47-197 µg/kg in food simulants and of 12.3-104.3 µg/kg in real foodstuffs. These results comply with the recommended SML of BPA (600 µg/kg) stated by the regulation EU 10/2011 [9]. However, BPA range obtained in food simulants exceeded the revised SML of 50 µg/kg for food or food simulant [35]. On contrary, BADGE.2H$_2$O, either in food (101-210.8 µg/kg) or its simulants (349-1106 µg/kg), was still lower than the SML of the sum of BADGE and its hydrolysis products (BADGE·H$_2$O and BADGE·2H$_2$O) set at 9000 µg/kg for food or food simulant according to the directive EC 1895/2005 [36]. No alerting concentrations of bisphenol compounds were associated with high storage temperature (i.e. 40°C) or long storage time (up to expiry date).

In contrast, under many conditions worrying levels of trace metal were recorded that were most related to worst case scenarios. For instance, Sn and Fe were highly affected by the interaction effect of absence of coating, low pH (<4.5), high storage temperature (40°C) in canned fruits (see chapter 4), where the important can corrosion led to drastic increase of Fe and Sn levels in these samples (up to 118 times as indicated), exceeding both their SRL value (40 and 100 mg/kg for Fe and Sn respectively) [37] and their maximum permitted levels in canned food (15 mg/kg of Fe [38] and 250 mg/kg of Sn [39]). Similarly, Pb increased at 40°C in brand B fruit cocktail (see chapter 4) up to 57.05 µg/kg, exceeding its SRL value of 10 µg/kg [37], but remaining within its maximum permitted level in canned food (100 µg/kg) [40]. Under the same conditions, i.e. 40°C and pH <4.5 (canned okra), but in the presence of protective coating, Sn and Pb remained below their detection limit while Fe exceeded its recommended levels after 2 years of storage (see chapter 5).

Threatening concentrations of Fe and Sn were also observed upon leaving opened fruit cans in the refrigerator as shown in the previous paragraph. In this case Fe (reached 108 mg/kg) and Sn (reached 223 mg/kg) levels crossed the above recommended limits.

Upon storage at room temperature or lower, most metals remained within their permissible limits. Yet, few unacceptable levels of metals were still obtained depending on the brand of food cans; for example, in brand B fruit cocktail (see chapter 4), Cd increased by 15.36 µg/kg between 7 and 24 months to exceed its SRL value of 5 µg/kg [37]. In contrast, over same storage duration, Ni release exceeded its SRL value of 0.14 mg/kg in most fruit cans except in fruit cocktail brand B and pears halves. Likewise, Pb released values over 493 days exceed its recommended SRL (i.e. 10 µg/kg) in fava beans (brands B and C) and chickpeas (brand D), as described in chapter 5. Its concentrations were still remaining below the maximum level allowed (i.e. 1000 µg/kg) in canned vegetables according to Codex [40] and Libnor [41].

10. Valuable advices for industries and consumers

Under appropriate storage conditions the levels of bisphenol compounds and metal elements in canned manufactured in Lebanon respected the recommended limits, which suggests that the Lebanese production of tinplate cans is controlled. However, to further reduce the dietary exposure to potentially toxic bisphenol compounds and metal elements, the above findings of the present study provide valuable advices and precautions for industries and consumers, such that:

- Any canned food should be purchased fresh and under moderate temperature, especially acidic food with pH < 4.5

- It is better to pack and buy foods in glass jars, if available

- The purity of metal substrates as well as the polymerization and formulation of polymeric coating should be controlled, since our results confirm major differences in the packaging quality between brands.

- Canned foods production companies should be aware of the findings obtained and work with can manufacturers who are developing better coatings for absolutely no contamination occurring from the can to food,

- Upon applying good-quality coating, coated cans should always be preferred over uncoated cans as it protect against corrosion and limits the migration of Sn.

- The storage conditions across the supply chain need to be taken into consideration for better quality canned foods. For instance, cans with defects, like dented cans, should be discarded since high release of Fe is expected.

- In case of uncoated cans, it is important to avoid storing leftovers in their cans in the fridge as it will cause unacceptable oxidation of tin layer.

Some authors, **[18], [20]**, mentioned that it is advisable to ensure adequate heat treatment for minimizing migration of bisphenol compounds and metal elements. Yet our results showed that migration of metal elements was not affected by the duration time and temperature of heat treatment (pasteurization vs. sterilization). On the other hand pasteurization might slow down the migration of residues of bisphenol compounds, but the migration is soon completed during the first few months of storage. Thus, at the end, lowering the time and temperature of heat treatment might not have appreciable effect on lowering levels of contaminants in consumed canned foods.

11. Conclusion

The original aspect of this chapter was to combine the results obtained through the different chapters of the presented thesis in order to find answers to the main argued issues. Based on the data collected through experimental work, this project was able to interpret clearly the effect of heat treatment, storage time, food type, food packaging and brand, storage temperature, can denting, opening cans in fridge and heating foods in their cans on the migration of bisphenol compounds and metal elements. Finally, from the interesting outputs of this study, valuable advices and instructions were provided to industries and consumers to reduce the dietary exposure to potentially toxic contaminants released from tinplate cans.

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Conclusions and Perspectives

According to EU 1935/2004 recommendations, food contact materials must not release constituents in amounts that can endanger human health or bring about unacceptable changes in the composition of the food or deterioration of its organoleptic characteristics. In this research project we have assessed the migration of metal elements and bisphenol compounds from coated and uncoated cans into different categories of foods (fruits and vegetable) and their simulants. We have worked on tinplate cans manufactured in Lebanon, and highly consumed and exported Middle Eastern foods also produced in this country. We mainly focused on investigating the impact of parameters related to heat treatment, storage conditions, type of food/food simulant, can brand and inappropriate handling of canned foods.

Prior to migration studies, appropriate analytical methods were first developed, optimized and validated to assess the reliability of our data. The method based on SPE extraction followed by LC-Fluorescence detection and MS confirmation was efficient for the determination of bisphenol compounds in food simulants (RSDs <10% and recoveries in the range 81-120%). Likewise, good precision (RSDs <15%) and recoveries (82-120%) were obtained for bisphenols from complex food matrices using simple solvent extraction followed by SPE purification prior to LC analysis. In the case of trace metals, their determination in canned food based on microwave-assisted digestion followed by elemental analysis with AAS achieved similar performances (RSDs <10% and recoveries 94-111%).

Among the targeted bisphenol compounds, only BPA and BADGE.2H_2O were confirmed by MS detection in all sterilized canned foods and their simulants. Heat treatment (especially sterilization, as compared to pasteurization), was the dominant influencing parameter on their migration (their levels drastically increased from non-detected up to ten and hundreds of µg/kg). Controlling parameters of heat treatment (i.e. time and temperature) is crucial to manage the quantity of migrants, and ensure conformity with regulation. For that purpose, empirical predictive models were developed and validated based on response surface methodology to predict the influence of can processing and storage conditions on the migration of bisphenol compounds into food simulants. Nevertheless, concentrations of BPA (47-197 µg/kg) and BADGE.2H_2O (355-520 µg/kg) in sterilized food simulants were overestimated in comparison with real food (namely sterilized legumes and vegetables: 22.1-44.2 µg/kg of BPA and 88.6-170 µg/kg BADGE.2H_2O).

Several other interesting results were drawn from our work. In particular, the behavior of metal elements toward heat treatment and storage conditions was quite different from that of bisphenol compounds. Hence, heat treatment had moderate effect on trace metals release in food (maximum of 17-34% increase in few samples). On the other hand storage conditions were found critical with regards to trace metals: long storage under high temperature (i.e. 40°C) favors their release. However, remarkable differences in the migration trends were observed between coated and uncoated food cans, since Sn release was prevented in presence of coating, where higher migration of Fe and Pb was observed. Fe release was also found to be favoured in acidic food (such as okra with $pH < 4$). On the opposite, metal migration did not seem to be influenced by high fat content of canned hummus. Another finding of our work is the major effect of the size of canned food pieces, since our results show that large halves of fruits lead reduction in metal content due to limited can-food contact surface, as compared to small pieces of fruits.

Principal component analysis was a valuable tool to help understanding the effect of the different parameters tested on the contaminants release. Clear discrimination between can brands was noticed, that could arise from the variability of can coatings (e.g. formula, porosity, thickness, etc.) and purity of the metallic steel and tin layers.

As expected, storing food in glass jars is a convenient way to limit consumer exposure to migrants from packaging. Indeed, our results give evidence of that assumption for both bisphenols (undetected levels in glass jar okra) and metal elements (Fe and Zn increase were limited to 20-25%). Discarding dented cans should also be recommended, both for uncoated and coated cans, since our results show an overall higher release of both trace metals (Fe, Cd, Cu, Zn) and bisphenols (BADGE.2H_2O) in that case. Surprisingly, the common practice of heating the food (here fava beans) directly in the can before food consumption had no effect on contaminant levels. On the opposite, leaving opened uncoated fruit cans in the refrigerator had a dramatic effect on Fe and Sn levels in the food, due to the tin layer that was sloughed off with subsequent serious corrosion.

Finally, our work also provides informative data about the regulatory compliance of both tinplate cans and canned vegetable food produced in Lebanon. The average migration of BPA and BADGE.2H_2O between production and expiry dates (2 years) in considered canned foods did comply with the recommended SML limits stated by the regulation EU n°10/2011 (i.e. 600 µg/kg of BPA and 9,000 µg/kg of BADGE plus its hydrolysis products), but considering the new amended regulation EU n°2018/213 some samples exceeded the SML of BPA (50 µg/kg). For trace metals, even under appropriate storage conditions, some levels exceeded the authorized limits, especially for Pb and Cd that are of great health concern. So, food companies should work with can manufacturers who deliver high-quality coatings and high purity tinplated steel sheets, to minimize food contamination and ensure regulation compliance. Our results also provide canned fruit and vegetable contamination data that may be useful for Lebanese authorities to support their food exposure assessment to undesirable and dangerous chemicals in the Lebanese population diet.

While providing interesting and new data about food packaging migrants and the main parameters influencing their release, with novelty linked to the simultaneous analysis of both organic and inorganic contaminants, our work still faces some limits.

Firstly, additional work should consider the coating characteristics (formula, thickness and porosity) and the initial concentration of metal elements in the tinplated steel layer, where these two factors are crucial for tracking the migration rates. Besides, our empirical models for bisphenol compounds release from tinplate cans should be tested on more cans, collected from the national and international markets, in order to generalize their application aiming at predicting the effect of heat treatment, storage conditions and packaging and food content properties on the migration of bisphenol compounds. However, the simplicity and easy to apply approach of these models should still be respected.

It would be also interesting to assess the effect of incubation at 50°C for 15 days after production, which is usually done by canned food industries to ensure the aseptic property of the product before distribution. This practice may influence migration since our results confirmed that, at elevated temperature, food contamination from packaging increases.

Then, this project was limited to a number of canned food samples and conditions. Therefore it would be of great interest to complete this work on other canned food types present on the Lebanese market. This work could also be expanded to ready-to-eat canned meals (such as baba ghanouge (eggplant mixed with tahina) and stuffed vine leaves) that are famous Lebanese products exported worldwide. Indeed, food rich in fat (such as hummus and baba ghanouge) are expected to solubilize higher levels of bisphenol compounds than non-fatty food. However, for that purpose, analytical development will be required to validate a quantitative method for bisphenol compounds determination in fatty food.

Finally, since several peaks were observed in our chromatograms, we are convinced of the presence of other migrants in our samples. So, future work focusing on other non-intentionally added substances (NIAS) (here only BADGE and BFDGE derivatives were considered) is mandatory to our point of view to get more knowledge on that emerging field. Here, the development of non-targeted analytical methods will be highly valuable.

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Biologie Biology
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Title : Study of the parameters influencing the migration of organic and inorganic contaminants from metallic packaging into food consumed in Lebanon

Keywords : Canned food, migration, contaminants, bisphenol compounds, trace metal elements, sterilization, storage conditions, experimental design

Abstract :

Tinplate cans represent an important source of food contaminants due to their multi-materials structure. Endocrine disrupting chemicals can migrate from the organic coating, whereas metal trace elements may be released from metal substrate. This issue has be extensively studied worldwide, yet, data relative to canned food from the Lebanese market are scarce, although Lebanese cuisine is appreciated globally with annual exportation of canned food. So, this work investigates the effect of several parameters on the migration of bisphenol compounds (bisphenol A and F, bisphenol A and F diglycidyl ethers and their derivatives) and metal trace elements (Fe, Sn, Pb, Cd, Cr, Ni, Zn, Cu) from tinplate cans into canned foods consumed in Lebanon. Sterilization, storage conditions, food product properties and inappropriate handling of canned food have been studied. Different analytical methods were validated and applied. Liquid chromatography with fluorescence detection (and mass spectrometry confirmation) was useful for studying the migration of bisphenol compounds in foods and their simulants. Metal elements were analyzed using atomic absorption spectrometry after microwave-assisted digestion of canned foods.

Among target bisphenol compounds, only BPA and BADGE.2H2O were detected in the studied foods/food simulants. Empirical models were built and validated, based on response surface methodology, to predict the influence of sterilization and storage on their migration into food simulants. In addition, principal component analysis was used to examine the correlation between the content of targeted contaminants and the studied influencing parameters. Their levels drastically increased upon sterilization (121°C), where the majority of bisphenol residues have migrated. Consequently, the migration of bisphenol compounds was not influenced by storage time (until shelf life) and temperature; the only significant effect was noticed in pasteurized canned okra (100°C). Most of BPA (12.3-197 µg/kg) and BADGE.2H2O (101-1106 µg/kg) levels comply with the European recommended limits; yet, BPA in some cases exceeded its recently revised limit (50 µg/kg). All target metals were found, with increased levels upon storage. Storage temperature had an important impact on the release of metals. In particular, 40°C (storage in hot areas) should be avoided, especially for uncoated cans holding acidic food (e.g. fruits) since serious corrosion occurred within 2 to 5 months, releasing large amounts of Fe and Sn. Storing food cans under refrigerating temperature can successfully limit the migration of metal elements during early storage time. Thus it is advisable to purchase fresh and refrigerated canned foods. Another recommendation is to discard dented cans, since high release of metal elements is observed. Among inappropriate practices, keeping opened uncoated fruit cans in the fridge can cause threatening release of Sn and Fe.

Overall, migration of bisphenol compounds and metal elements was dependent on food properties (ex: pH), and the variability of packaging materials used between brands. Under appropriate storage conditions, the levels of target contaminants respected the recommended limits suggesting that the Lebanese production of tinplate cans is acceptable.

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Titre : Étude des paramètres influençant la migration de contaminants organiques et inorganiques de l'emballage métallique vers les denrées alimentaires consommées au Liban

Mots-clés : Aliments en conserve, migration, contaminants, composés de bisphénol, éléments traces métalliques, stérilisation, conditions de stockage, plan d'expériences

Résumé :

Les boîtes de conserve métalliques représentent une source importante de contaminants alimentaires en raison de leur structure multi-matériaux. Des substances chimiques peuvent migrer du revêtement organique, et des éléments traces métalliques peuvent être libérés du substrat métallique. Ce problème a fait l'objet de nombreuses études, mais les données relatives aux aliments en conserve sur le marché libanais sont rares, bien que la cuisine libanaise soit appréciée mondialement avec l'exportation annuelle d'aliments en conserve. Ainsi, ce travail étudie l'effet de plusieurs paramètres sur la migration de composés phénoliques (bisphénol A et F, diglycidyl éther de bisphénol A et F et leurs dérivés) et éléments traces (Fe, Sn, Pb, Cd, Cr, Ni, Zn, Cu) des boîtes en fer blanc aux aliments en conserve consommés au Liban. La stérilisation, les conditions de stockage, les propriétés des produits alimentaires et la manipulation inappropriée des aliments en conserve ont été étudiées. Différentes méthodes analytiques ont été validées et appliquées. La chromatographie liquide avec détection par fluorescence (et confirmation par spectrométrie de masse) était utile pour étudier la migration des composés de bisphénol dans les aliments et leurs simulants. Les éléments métalliques ont été analysés dans les aliments en conserve par spectrométrie d'absorption atomique après digestion assistée par micro-ondes.

Parmi les bisphénols cibles, seuls le BPA et le BADGE.2H2O ont été détectés dans les aliments ou les simulants d'aliments. Des modèles empiriques ont été construits et validés, basés sur la méthodologie de surface de réponse, pour prédire l'influence de la stérilisation et le stockage sur leur vers les simulants d'aliments. En outre, l'analyse en composantes principales a été utilisée pour examiner la corrélation entre les contaminants cibles et les paramètres étudiés. Les teneurs en BPA et BADGE.2H2O ont considérablement augmenté lors de la stérilisation (121°C), traitement durant lequel la majorité des résidus de bisphénols ont migré. Par conséquent, la migration des bisphénols n'a pas été influencée par la durée et la température de stockage (jusqu'à la date d'expiration) ; le seul effet significatif a été observé pour les Okra (gombo) en conserve pasteurisés (à 100°C). La plupart des teneurs obtenues pour le BPA et le BADGE.2H2O sont conformes aux limites européennes recommandées, mais dans certains cas le BPA dépasse la limite récemment révisée (50 µg/kg). Tous les métaux ont vu leurs teneurs augmenter avec le temps. La température de stockage a eu un impact important. Une température de 40°C devrait être évitée, surtout pour des boîtes non revêtues et contenant des aliments acides (fruits). En effet, une corrosion importante a été obtenue entre 2 et 5 mois, libérant de grandes quantités de Fe et Sn. Il est conseillé d'acheter les boîtes avec une date de production récente et de les stocker dans un endroit frais. Une autre recommandation est d'éviter les boîtes endommagées, puisqu'une forte libération d'éléments métalliques a été observée. Enfin, la conservation des boîtes de conserve non revêtues et ouvertes dans le réfrigérateur a entraîné une libération préoccupante de Sn et Fe.

Globalement, la migration dépend des propriétés des aliments (ex : pH), et de la variabilité des matériaux d'emballage selon les marques. Dans des conditions de stockage appropriées les teneurs de contaminants cibles respectent les limites recommandées, ce qui suggère que la production libanaise de boîtes de fer blanc est acceptable.