Identification of bisphenol A diglycidyl ether derivatives after *in vitro* digestion process by LC-MS/MS

L. Pazos-Soto[1,2], P. Vázquez-Loureiro[1,2], S. Mariño-Cortegoso[1,2], A. Lestido-Cardama[1,2], J. Bustos[3], P. Paseiro-Losada[1], A. Rodríguez-Bernaldo de Quirós[1,2], R. Sendón[1,2], L. Barbosa-Pereira[1,2]

[1] FoodChemPack Research Group, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, Spain
[2] Instituto de Materiales (iMATUS), University of Santiago de Compostela, 15782 Santiago de Compostela, Spain
[3] National Food Centre, Spanish Agency for Food Safety and Nutrition, 28220, Majadahonda, Spain

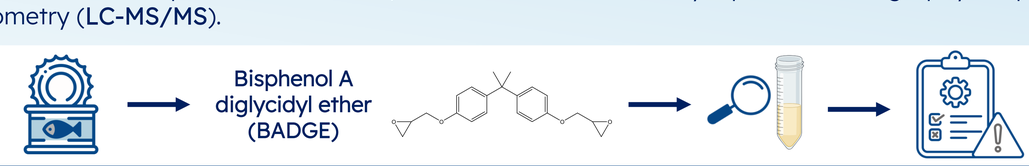
1. INTRODUCTION

Epoxy-based resins, obtained by polymerization between bisphenol A (BPA) and epichlorohydrin, are well-known compounds used for **coating** applications in food metal cans [1]. This chemical group has arisen as a public concern because of the **endocrine disruptor** character of their monomer, BPA, along with the possible health effects that their major derivatives may pose on consumers [2].

For a correct estimation of the exposure, the **bioaccessibility** must be considered, this means, the fraction of the contaminant available to be absorbed. With that purpose, the INFOGEST protocol [3] was applied.

Objectives

- → Perform an accelerated **stability test** to simulate the storage of tuna cans.
- → Study the **gastrointestinal fate** of bisphenol A diglycidyl ether (BADGE), one of the main contaminants related to the cited resins.
- → **Identify** the transformation products of BADGE, with water and chloride by liquid chromatography coupled to tandem mass spectrometry (**LC-MS/MS**).

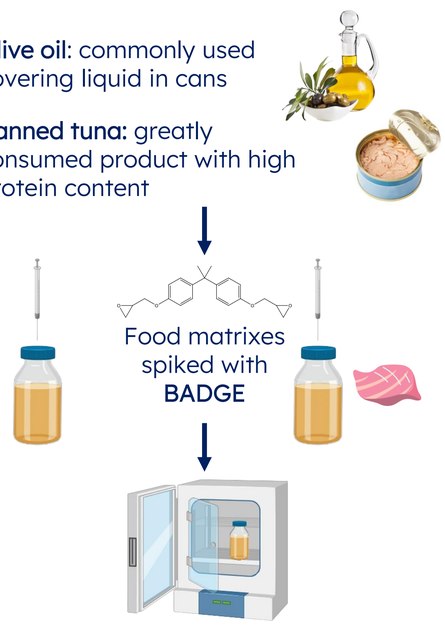


2. MATERIAL AND METHODS

2.1. Stability test

Olive oil: commonly used covering liquid in cans

Canned tuna: greatly consumed product with high protein content

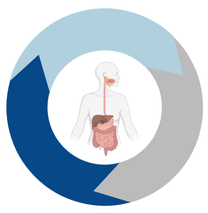


60° for 10 days, according to **(EU) No 10/2011** on plastic materials and articles intended to come into contact with food [4].

2.2. *In vitro* digestion

INFOGEST protocol

Gastrointestinal digestion through three phases testing the influence of gastric pH



SSF: simulated salivary fluid
SGF: simulated gastric fluid
SIF: simulated intestinal fluid

1. Oral phase
5 g sample, amylase
SSF, 2 min pH 7

2. Gastric phase
Pepsin, HCl
SGF, 2 hr pH 1,2,3 and 4

3. Intestinal phase
Bile, pancreatin, NaOH
SIF, 2 hr pH 7

2.3. Identification with LC-MS/MS

Table 1. Experimental conditions of HPLC-FLD and LC-MS/MS

Equipment	LC-MS TSQ Quantum Access MAX and Agilent Technologies 1100 series HPLC
Column	Phenosphere 80Å ODS (150 mm x 4.6 mm, 3µm)
Mobile phase	ACN:H2O
Flow rate	1mL/min
Injection volume	10µL
Fluorescent detection	Excitation: 225 nm Emission: 305 nm
MS data acquisition	Selected reaction monitoring (SRM) and Full Scan
Source	Positive and negative atmospheric pressure chemical ionization (APCI)
Spray voltage	2500 V
Vaporizer T°	340°C
Capillary T°	350°C

Phase mobile gradient		
Time (min)	ACN (%)	H_2O (%)
0	40	60
3	40	60
19	75	25
24	100	0
26	100	0

Figure 1. LC-MS/MS Thermo Scientific

Figure 2. Triple quadrupole and data acquisition diagram

3. RESULTS AND DISCUSSION

Table 2. Parameters of identification of the standard mix using LC-MS/MS

Compound	CAS	APCI mode	Parent ion	Product ions	Collision energy (V)
BADGE·2H2O	5581-32-8	-	421.4	226.8	30
				300.6	17
BPA	80-05-7	-	227.0	134.5	29
				213.1	22
BADGE·HCl·H2O	227947-06-0	-	439.4	82.5	14
				393.5	15
BADGE·H2O	76002-91-0	+	400.0	107.1	41
				135.1	25
BADGE·2HCl	4809-35-2	-	457.0	82.4	15
				393.2	21
BADGE·HCl	13836-48-1	+	418.0	106.9	40
				135.1	28
BADGE	1675-54-3	+	382.3	135.1	25
				191.1	19
CycloDiBADGE	20583-87-3	+	569.0	135.1	27
				107.1	43

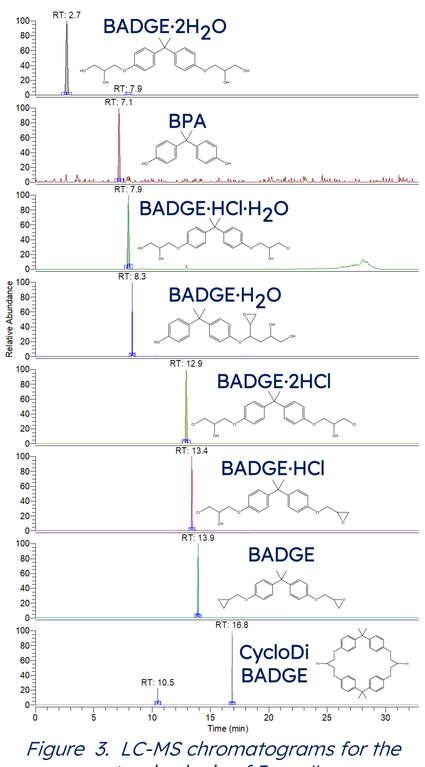


Figure 3. LC-MS chromatograms for the standard mix of 5 mg/L

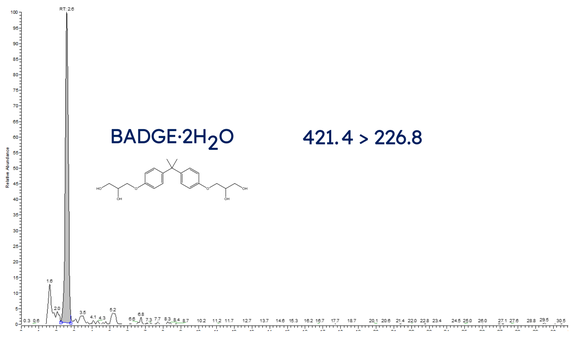


Figure 4. LC-MS/MS chromatogram using negative APCI ionization mode of digested olive oil spiked with BADGE

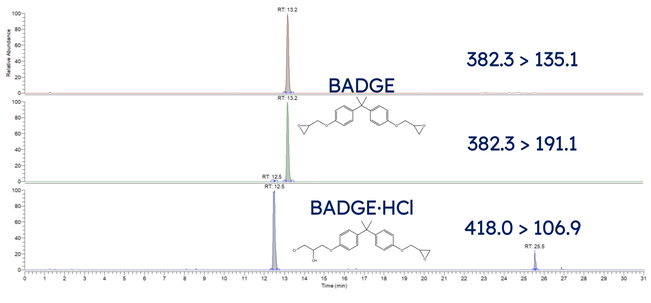


Figure 5. LC-MS/MS chromatogram using positive APCI ionization of digested olive oil spiked with BADGE

BADGE·2H2O was identified after analyzing the bioaccessible fraction of the sample of olive oil spiked with BADGE using negative APCI as ionization mode.

BADGE and **BADGE·HCl** were found in the bioaccessible fraction of the sample of olive oil spiked with BADGE using positive APCI as ionization mode.

4. CONCLUSIONS

- ❑ Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) allowed the identification of BADGE and its derivatives in the samples obtained after the *in vitro* digestion process.
- ❑ The preliminary results showed that the studied chemical, BADGE, reacts with water and chloride during gastrointestinal digestion to form **BADGE·2H2O** and **BADGE·HCl**, respectively.
- ❑ These compounds have been identified in the bioaccessible fraction of the spiked olive oil, one of the most common covering liquids used in canned food.
- ❑ BADGE derivatives present in the standard mix were not found in tuna samples. The possible biotransformation of BADGE in presence of this food matrix will require more investigation.
- ❑ Further research should be undertaken to study the effect of different food matrices on the transformation of BADGE, together with the tentative identification of other substances yet unknown.

5. REFERENCES

[1] Poças, M. F., & Hogg, T. (2007). Trends in Food Science & Technology, 18, 219-230.
[2] Lestido-Cardama A, Sendón R, Bustos J, Nieto MT, Paseiro-Losada P, Rodríguez-Bernaldo de Quirós A. Compr. Rev. Food. Sci. Food. Saf., 2022, 21(4), 3558–611.
[3] Minekus M, et al. (2014). Food & function, 5, 1113-1124.
[4] Commission Regulation (EU) No 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food. Off. J. Eur. Union 2011, 12, 1-89.

6. ACKNOWNLEDGEMENTS

BACFood4Expo project (PID2020-114569RJ-I00) funded by Spanish Ministry of Science and Innovation (10.13039/501100011033) under the competitive State R&D Program Oriented to the Challenges of the Society, "R&D Projects 2020" - Modalities "Research Challenges and Knowledge Generation".

In vitro oral bioAccessibility of Chemicals from food contact materials to estimate Human Exposure through the Diet (ACHED) (CNS2022-135887) funded by Spanish Ministry of Science (10.13039/50110001103) and European Union NextGenerationEU/PRTR.

L. Pazos-Soto is grateful for her grant "Programa de axudas á etapa predoutoral" da Xunta de Galicia.