The detection and elucidation of oligomers migrating from biodegradable multilayer teacups using liquid chromatography coupled to ion mobility time-of-flight mass spectrometry and gas chromatography–mass spectrometry

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ABSTRACT

Biodegradable materials are increasingly being used in manufacturing processes due to their environmental benefits. In this work, a study has been performed to assess the migration of compounds from biodegradable multilayer teacups to a tea solution. Liquid chromatography in conjunction with ion-mobility quadrupole time-of-flight mass spectrometry has been used for the elucidation of non-volatile compounds. An orthogonal projection to latent structures–discriminant analysis has been carried out to compare the tea after migration against untreated tea used as blank. Headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry has been optimised to analyse the migration of volatile compounds. Eight migrants were identified in the tea, six of which were non-intentionally added oligomers. The degree of migration for hot tea ranged from 0.05 and 4.68 mg/kg, exceeding the specific migration limit. Nevertheless, the migration to cold tea was an order of magnitude lower (between 0.003 and 0.56 mg/kg).

Keyword: Biodegradable multilayer Disposable teacups Food migration Tea NIAS Oligomers ion mobility MS

1. Introduction

Food packaging protects and preserves the quality of food products. Conventional polymeric materials used for food packaging are non-biodegradable and, in many cases, are not completely recyclable. Increased public awareness of the environmental challenges related to conventional plastic materials has triggered the development of biobased and biodegradable food packaging materials (Nilsen-Nygaard et al., 2021). As with any other food contact material, biodegradable materials are expected to not only protect food and maintain food quality, but they also have to be evaluated for food contact compliance. Therefore, migration studies must be undertaken to evaluate the compliance of the material with legislation. Non-intentionally added substances (NIAS) in food contact materials can pose major issues for food packaging manufacturers. Historically, NIAS have been shown to migrate from packaging to food (Nerin et al., 2014; Vera, Canellas, & Nerin, 2020). NIAS typically include impurities or reaction and degradation products that originate from the manufacturing process (Nerin, Alfaro, Aznar, & Domeno, 2013; Nerin et al., 2013; Nerin et al., 2014). For example, during polymerisation, oligomers may be formed as side-reaction products. The oligomers that have the potential to migrate to food can range in complexity from simple dimers up to decamers in both cyclic and linear formations (Brenz, Linke, & Simat, 2021; Canellas et al., 2021; Lopes, Tsochatzis, Karasek, Hoekstra, & Emons, 2021; Ubeda, Aznar, Rosenmai, Vinggaard, & Nerin, 2020). As such, oligomers could be volatile or non-volatile compounds and therefore different techniques are required to detect and identify them. Volatile oligomers have previously been identified through the combination of several analytical techniques, including gas chromatography–mass spectrometry (GC–MS) and gas chromatography–atmospheric pressure chemical ionisation-quadrupole/time-of-flight mass spectrometry (GC–APCI-Q/ToF) (Liu, Wrona, Su, Vera, Nerin, & Hu, 2021), two-dimensional gas chromatography–mass spectrometry (GC × GC–ToF), atmospheric solid analysis probe (ASAP) and direct inlet probe–atmospheric pressure chemical ionisation mass spectrometry (DIP) (Mahmoud et al., 2020), and Fourier transform infrared spectrometry (FTIR) and size-exclusion chromatography (*SEC*) (Lamers, de Waal, & Meijer, 2020). Non-volatile oligomers have been identified using several of-flight mass spectrometry (LC–Q/ToF) (Hu et al., 2021), liquid chromatography-Orbitrap (LC–Orbitrap) (Luo et al., 2017) and liquid chromatography in conjunction with ion mobility-quadrupole/time-of-flight mass spectrometry (LC–IM-Q/ToF) (Canellas et al., 2021). Several oligomers have been previously reported to migrate from biodegradable food contact materials to food simulants. Most of the studies have been focused on the migration of polylactic acid (PLA) oligomers (Armentano et al., 2015; Aznar, Ubeda, Dreolin, & Nerin, 2019; Burgos, Tolaguera, Fiori, & Jimenez, 2014; Dopico-Garcia et al., 2013; Gavril et al., 2019; Zimmermann, Dombrowski, Volker, & Wagner, 2020). The migration of oligomers from other biodegradable polymers such as poly((R)-3-hydroxybutyrate-*co*-(R)-3-hydroxyhexanoate has also been investigated (Silano et al., 2019). The food contact material used in these studies consisted of several layers of either coextruded polymers or laminates bonded by an adhesive. Very few studies on migration of compounds from biodegradable multilayers have been undertaken (Canellas, Vera, & Nerin, 2015). Tea is the second most consumed beverage in the world. Therefore, several migration studies from tea bags and packaging used in contact with tea have been done in recent years (Alnaimat, Barciela-Alonso, & Bermejo-Barrera, 2020; Cao & Ji, 2013). Nevertheless, the migration study from a biodegradable multilayer to tea has never been performed. In this work, the detection and elucidation of oligomers migrating to tea from a biodegradable multilayer material has been carried out.

2. Materials and methods

2.1. Reagents and materials

HPLC-grade ethanol was supplied by Scharlau Chemie S.A (Sentmenat, Spain). Benziisothiazolone (98% purity) and tributyl phosphate (98% purity) were purchased from Merck. 1,4,7-Trioxacyclotridecane- 8,13-dione 98% purity, which had been isolated and concentrated from an industrial product by Coim S.p.A (Offanengo, Italy), was used as a standard. Polydimethylsiloxane 100 µm, polyacrylate 85 µm and divinylbenzene/Carboxen/polydimethylsiloxane 50/30 µm solid-phase microextraction (SPME) fibres were used. They were supplied by Supelco (Spain).

A 100% compostable, biodegradable multi-layered material made from a biodegradable polymer, 20 µm biodegradable adhesive 12 g/m2 and paper 120 g/m2 was purchased locally. According to the manufacturer, the composition of the biodegradable polymer was 40% polyester and 60% PLA. The multi-layered material was used to construct teacups. Hacendado tea, a mixture of white tea (35%), black tea (30%) and red tea (30%) was purchased from a local supermarket, and was used as a representative mixture of different teas. One tea bag was submerged in 200 mL of boiling water that was placed in a baker for 5 min to yield a solution used for the migration assays. Migration cells (MigraCell®) with a disk surface of 31.4 cm2 were purchased from FABES Forschungs-GmbH (Munich, Germany).

2.2. Migration studies

The migration studies were set-up by placing 31.4 cm2 of the multilayer material in the migration cells with the face of the material made from the biodegradable polymer in contact with the tea. Assays were prepared to simulate the migration to both hot and cold tea from the compostable cups. In the case of hot tea, 31.2 mL of boiling tea (100 ◦C) were poured over the multilayer material. The volume of 31.2 mL was calculated by taking into account the ratio between the area of the cup in contact with the tea (120.7 cm2) and volume of tea in the cup (120 mL). The solution was retained in the migration cells for 2 h at room temperature (20 ◦C) to mimic the worst contact scenario for the use of the teacups. To simulate the use of the cups with cold tea, the assays were prepared in exactly the same way with the exception of letting the tea cool to 20 ◦C prior to pouring it over the multilayer material. Three replicates of both the hot and cold tea samples were prepared and analysed.

2.3. Ultra-high-pressure-liquid chromatography coupled to ion mobility-quadrupole time of flight analyser (UPLC–IMS-Q/TOF)

Screening analyses were carried out using an Acquity™ UPLC chromatography system coupled to an electrospray interface (ESI) and Vion® ion mobility-quadrupole/time of flight (IMS-Q/TOF) mass spectrometer, from Waters (Manchester, UK). A UPLC BEH C18 column of 1.7 µm particle size (dimensions: 2.1 × 100 mm) was used with a flow rate of 0.3 mL/min and a column temperature of 35 ◦C. The mobile phases were water (phase A) and methanol (phase B), both with 0.1 % formic acid. The gradient used was 95% A to 100% B within 13 min, with 2 min re-equilibration to the initial conditions. The volume of sample injected was 5 µL. The electrospray interface (ESI) was used in positive ionisation, sensitivity mode with a capillary voltage of 3 kV and a sampling cone of 30 V. The temperatures used were 120 ◦C and 500 ◦C for the source block and desolvation gas, respectively, and the desolvation gas flow rate was 800 L/h. The system was calibrated and data were acquired in the range *m*/*z* 50–1000. Leucine-Enkephalin [M + H]+, *m*/*z* 556.2766, was used as the lock-mass compound for real-time mass correction. Data were acquired in data independent analysis (DIA) using high definition MSE mode (HDMSE) in which low collision energy (6 V) and high collision energy (ramp from 20 V to 40 V) data are acquired simultaneously. Argon was used as the collision gas and nitrogen was used as the ion mobility gas. IMS gas flow rate was 25 mL/min, wave velocity 250 m/s, IMS pulse height 45 V. The drift time alignment of high and low energy spectra was performed using a ± 0.28 ms window in the drift cell. Mass and CCS calibration was performed using Major Mix kit (Waters Corp., Milford, MA). The acquisition rate was 10 Hz, and data acquisition and processing were carried out using UNIFI v.1.8 software. For identification a mass tolerance of 5 mDa was used.

2.4. Headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME–GC–MS)

The instrumentation used was a CTC Analytics CombiPal autosampler coupled to an Agilent 6890 N gas chromatograph with a mass spectrometer MS 5975B detector. All equipment was from Agilent Technologies (Madrid, Spain). The capillary column used was an HP-5MS (30 m × 0.25 µm × 250 µm) from Agilent Technologies (Madrid, Spain). The oven program was as follows: 50 ◦C for 2 min, increasing at a rate of 10 ◦C/min up to a maximum of 300 ◦C which was maintained for 2 min. Splitless injections and a helium flowrate of 1 mL/min were used, with the injector temperature set to 250 ◦C. The acquisition was performed using electron ionisation (EI) and SCAN acquisition mode (in the range *m*/*z* 45–350), normal scan speed (0–7 scan rates) was used for the identification of compounds, while SIM mode was used for quantification, and it was set at 4 cycles/s. The solid-phase microextraction conditions were optimized for this study. Three types of SPME fibers were initially tested: 100 µm polydimethylsiloxane fibres, 85 µm polyacrylate fibres and 50/30 µm divinylbenzene/Carboxen/polydimethylsiloxane fibres. For the preliminary study an extraction temperature of 80 ◦C and extraction time of 20 min were selected. Following the selection of the fibre type, the extraction conditions were optimised using the experimental design software MODDE 6.0. Extraction temperatures ranging from 50 to 80 ◦C, extraction time ranging from 10 to 30 min and sodium chloride addition ranging from 0 to 35% in the solution were considered in the experimental design. A volume of 3 mL of 25 ng/g standard solution in tea was placed in the headspace vials.

3. Results and discussion

3.1. Compound identification using UPLC–IM-Q/TOF Vion

UPLC–IM-Q/TOF was selected to detect and identify the compounds present in the tea extracts migrating from the biodegradable multilayer. This technique provides additional peak capacity which results from a combination of retention time, drift time, accurate mass of precursor and product ions simultaneously. With a suitable calibration, a collision cross section (CCS) value can be derived from each drift time measurement. The CCS value is related to the three-dimensional conformation of the chemical structure of a compound and is used as an additional identification point. Due to the complexity of food matrices, it can be difficult to isolate non-intentionally added substances migrating from containing materials. Therefore, a multivariate analysis (MVA) approach was used to compare blank samples (consisting of tea prepared according to Section 2.1.) and the tea migration solution. Markers across the sample set, each uniquely defined by a combination *m*/*z*, retention time and drift time, were determined by UNIFI with the intensity of each marker in each sample used to generate a marker matrix. The marker matrix was automatically transferred to the MVA software, EZInfo, and investigated using orthogonal projection to latent structures-discriminant analysis (OPLS-DA). An OPLS-DA analysis yields two plots; a score plot which shows the separation between groups of samples, and an S-plot, from which the markers responsible for the separation can be determined. The S-plot compares the covariance of each marker, the magnitude of change (*x*-axis), to the correlation of each marker, the consistency of the change (*y*-axis). Fig. 1 shows the S-plot obtained for the tea samples.

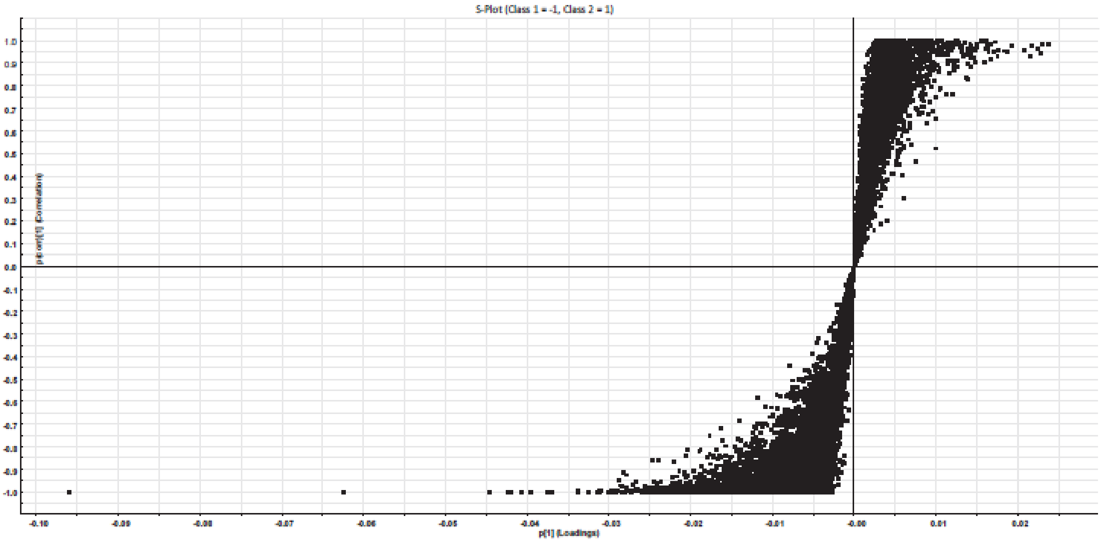

Fig. 1. The S-plot in which markers in the upper right quadrant are elevated in the blank (tea) sample and markers in the lower left quadrant are elevated in the migration sample.

The upper right quadrant of the S-plot contains markers with intensities elevated, or only present, in the blank (tea) samples, while the lower left quadrant shows markers with intensities elevated, or only present, in the migration samples. The further a marker is from the origin on the *x*-axis, the greater its contribution to the variance between the groups and the further a marker is from the origin on the *y*-axis, the higher the confidence of the analytical result. Markers only present, or at elevated intensities, in the migration samples were selected from the lower left quadrant of the S-plot (Fig. 1) and submitted to the structural elucidation process. The first step in the identification of a marker is to determine an elemental composition from the m/z of the molecular ion. The elements considered for this process were carbon, oxygen, hydrogen, nitrogen, chlorine, bromine, fluorine, sulfur, phosphorus, silicon and sodium. Sodium was included because the compounds have a high tendency to form sodium adducts with the mobile phase. Two criteria were used to establish the reliability of an elemental composition for an unknown: (1) the i-FIT, which is a measure of the goodness-of-fit of the theoretical isotope pattern of a particular elemental composition to the measured isotope pattern in the low energy data; (2) the mass tolerance, which was set at 3 mDa. Once a molecular formula for an accurate mass had been determined, a database search was performed to find compounds with a formula matching the elemental composition. The database searching is carried out automatically by the Discovery tool integrated in UNIFI software. The tool proposes candidate compounds for a given elemental composition by connecting to Chemspider database (Williams, 2007). For each proposal, the structure of the compound is retrieved from Chemspider and chemically intelligent algorithms are used to predict likely substructures. The mass of each substructure is then compared to the masses of the ions in the high energy spectrum associated with the marker and matches are assigned as fragment ions. Competing compounds with the same elemental composition can subsequently be compared using the number of fragments assigned in the high-energy data, the percentage of the high-energy spectral intensity accounted for by the assignments and the number of references for the compound in the Chemspider database.

A total of seven markers were selected for investigation from the S-plot; however, only benzisothiazolone and tributyl phosphate were successfully identified by applying the abovementioned approach. The identification of these compounds was confirmed by acquiring data for corresponding standards under the same experimental conditions. Benzisothiazolone is a common biocide used in adhesives and plastics (Canellas, Vera, Domeno, Alfaro, & Nerin, 2012; Rosero-Moreano, Canellas, & Nerin, 2014) that can cause allergic reactions (Damstra, Vanvloten, & Vanginkel, 1992). Tributyl phosphate is deemed to be a global environmental contaminant, that has been used as a plasticizer or flame retardant in plastics and paper (Cortejade, Kiss, Cren, Vulliet, & Bulete, 2016; Liu, Lin, Dong, & Li, 2019). The five markers not identified using the automated process described were suspected to be oligomers not present in the Chemspider database (Williams, 2007). A bibliographic search was undertaken to determine which NIAS, with elemental formulae matching those proposed by the automated procedure, might be present in a multilayer material containing polymer, adhesive and paper materials. Structures for candidate compounds were drawn in Chemdraw and the mol files generated were imported manually into UNIFI. Substructures were generated using the Fragment Match tool in UNIFI and their proposed *m*/*z* values were automatically compared to the *m*/*z* values of ions in the a confident assignment could be made. The biodegradable polymer used in multilayer material has a composition of 40% polyester and 60% PLA. The polyester used for the blend is synthesized from 1,4-butanediol, adipic acid and terephthalic acid, and is a poly(butylene adipate co-butylene terephthalate) (PBAT) (Siegenthaler, Kunkel, Skupin, & Yamamoto, 2012). Combination of acids, several diols and glycols were also considered as candidates, since it has been demonstrated that oligomers can be formed as reaction products and such oligomers have been found in many adhesives and polyesters (Tsochatzis, Gika, & Theodoridis, 2020; Ubeda et al., 2017; Vera et al., 2020; Zhang, Kenion, Bankmann, Mezouari, & Hartman, 2018) used for multilayers. The compounds considered as potential candidates for the markers were ethylene glycol, 1,4 butanediol (1,4- BD), 2-methyl-1,3-propanediol, diethylene glycol (DG), 1,6 hexanediol (1,6-HD), neopentyl glycol (NPG), propylene glycol, dipropylene glycol, tripropylene glycol, adipic acid (AA), azelaic acid, sebacic acid, suberic acid, phthalic acid, isophthalic acid and terephthalic acid. One advantage of using ion mobility in HDMSE is that precursors and fragment ions are aligned in both the retention time and drift time dimensions. This enables high-energy ions from coeluting compounds with different drifts time to be filtered from the high energy spectra, thus aiding spectral interpretation. Fig. 2 shows the low and high-energy spectra of the candidate observed at 6.46 min (*m*/*z* 429.2482) with and without drift-time alignment.

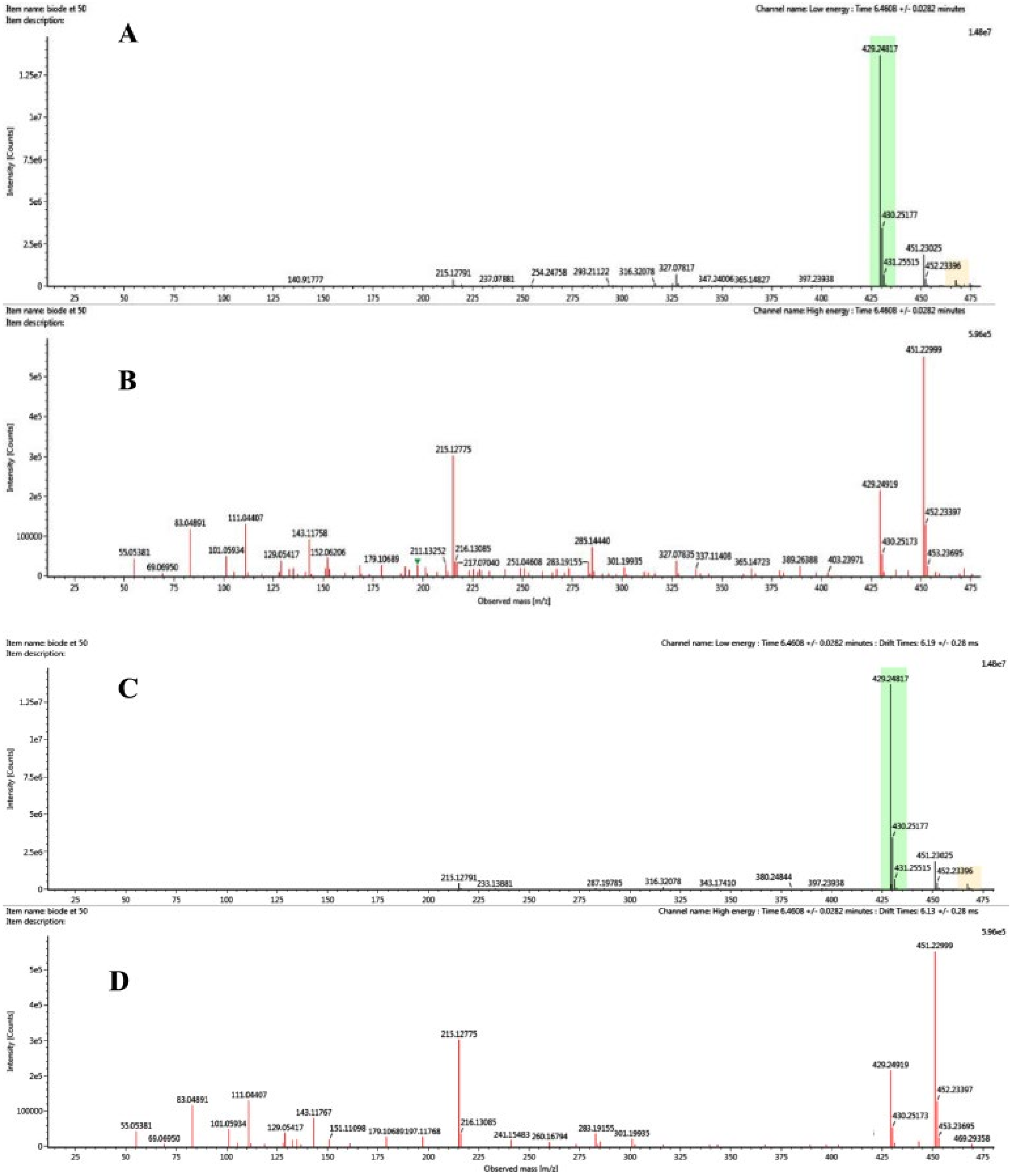

Fig. 2. Low (A) and high energy spectra (B) of the cyclic oligomer NPG-AA-NPG-AA in the absence of drift-time alignment (top) and low (C) and high energy spectra (D) of the cyclic oligomer NPG-AA-NPG-AA with drift-time alignment (bottom).

It can be appreciated that the drift time-aligned fragment spectrum presents a much lower number of ions, which are not related to the precursor ion, thus allowing a more efficient distinction between true fragment ions and background ions. Having well-defined assignment together with precursor ion and fragment ion alignment provided additional confidence to the identification of unknowns. Fig. 3 shows the assignment of the cyclic oligomer NPG-AA-NPG-AA to the low and high energy spectra of the marker with a protonated *m*/*z* of 429. 2472.

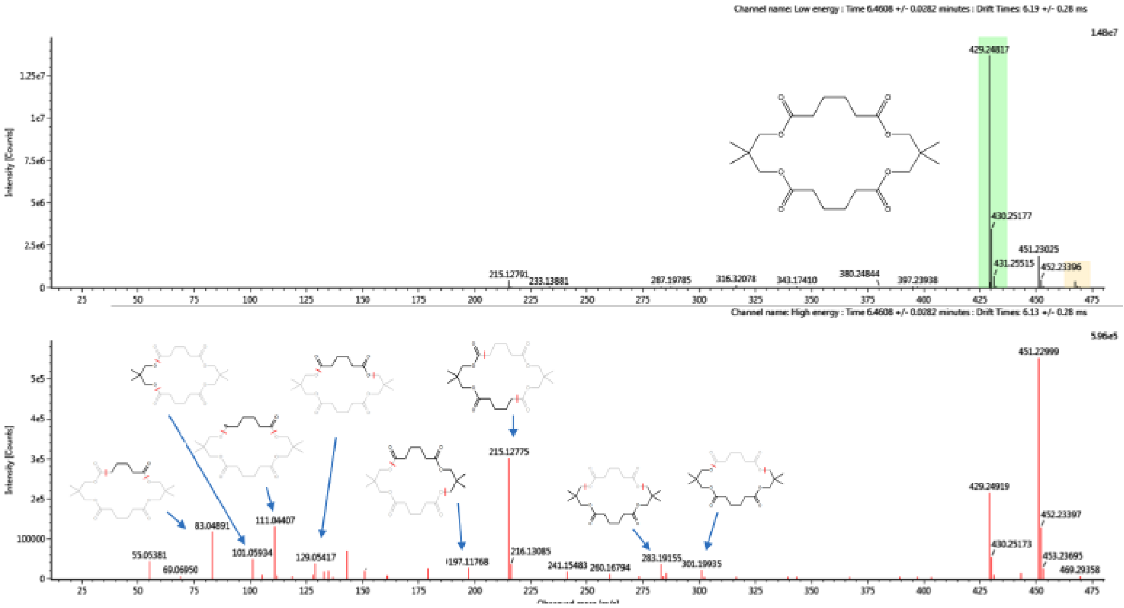

Fig. 3. Low (above) and high energy (below) spectra of the cyclic oligomer NPG-AA-NPG-AA with drift-time alignment was applied.

The sodiated adduct of the oligomer can also be observed in the spectrum (*m*/*z* 451.2302). Sodium adducts do not fragment readily, as shown by the presence of the 451.23 ion in the high energy spectrum, however they can be used as diagnostic features for the confirmation of an identification. The fragmentation of the protonated adduct (*m*/*z* 429.2472) is clearly displayed in high-energy spectrum and eight accurate *m*/*z* values were assigned to fragments of the cyclic oligomer NPG-AA-NPG-AA. Table 1 shows the five cyclic oligomers that were identified following this procedure. Two of the cyclic oligomers identified were formed by the combination of AA and 1,4-BD (cyclic oligomer 1,4-BD-AA-1,4-BD-AA and cyclic oligomer 1,4-BD-AA-1,4-BD-AA-1,4-BD-AA), one oligomer identified was formed by the combination of AA and NPG (cyclic oligomer NPG-AA-NPG-AA), one oligomer was formed by the combination of AA and 1,6-HD (cyclic oligomer 1,6-HD-AA-1,6-HD-AA) and the last was formed by the combination between AA, 1,6-HD and NPG (cyclic oligomer NPG-AA-1,6-HD-AA). The limits of detection for all of the assigned oligomers are shown in Table 1 and were found to be below 0.01 mg/kg. Since there is no commercial standard of any of these oligomers, the assigned oligomers were semi-quantified using the cyclic oligomer, 1,4,7-trioxacyclotridecane-8,13-dione (cyclic oligomer AA-DG) as a reference standard. The limits of detection were calculated with a calibration-based method, using the standard deviation of the response (sy) of the curve and the slope of the calibration curve (b) at levels approximating the LOD according to the formula: LOD = 3.3*(sy/b). For the semi-quantification, an external calibration was used with a determination coefficient of 0.998 and a linear range from 0.016 mg/kg (LOQ) to 5 mg/kg using the cyclic oligomer 1,4,7-trioxacyclotridecane-8,13-dione (cyclic oligomer AA-DG) as a reference standard. Stock solutions were prepared by dissolving the solid standard in methanol. Serial dilutions were prepared to cover the concentration range 0.016–5.0 mg/kg.

3.2. Identification by HS-SPME–GC–MS HS-SPME–GC–MS was used to identify volatile compounds present in the tea migration extracts from the biodegradable multilayer. Headspace solid-phase microextraction was selected to minimise interferences from the tea matrix. A screening was initially applied to get as many as possible volatile migrants. For this purpose, a preliminary SPME method was created to compare three SPME fibre coatings so that the fibre coating most appropriate for this analysis could be selected. For this method, the extraction temperature and the extraction time were set to 80 ◦C and 20 min, respectively. The first fibre tested was a 85 µm polyacrylate fibre that is used for the analysis of polar, semi-volatile compounds (MW 80–300) (Merck, 2021). No migration products from the biodegradable multilayer were detected in the extract when using this fibre. The second fibre tested was a 100 µm polydimethylsiloxane fibre which is commonly used to analyse volatile compounds (MW 60–275). One migration product was detected in the extract with this fibre. The third fibre tested was a 50/30 µm divinylbenzene/Carboxen/ polydimethylsiloxane fibre which is used for trace compound analysis (MW 40–275). One migration product was also detected with this fibre in the tea extract but with a greater response compared to that observed using the 100 µm polydimethylsiloxane fibre. Therefore, the 50/30 µm divinylbenzene/Carboxen/polydimethylsiloxane fibre was selected for the subsequent analysis. Only one compound was found to have migrated from the biodegradable multilayer to the tea. Compound identification was performed by comparing the measured spectrum to the NIST 2014 (Version 2.2) library with a forward-fit acceptance criterion of at least 900. Fig. 4 shows a peak at 20.3 min that corresponds to the cyclic oligomer formed by AA and 1,4-BD (AA-1,4-BD), a volatile NIAS from the same family as that previously identified using UPLC–IM-Q/TOF.

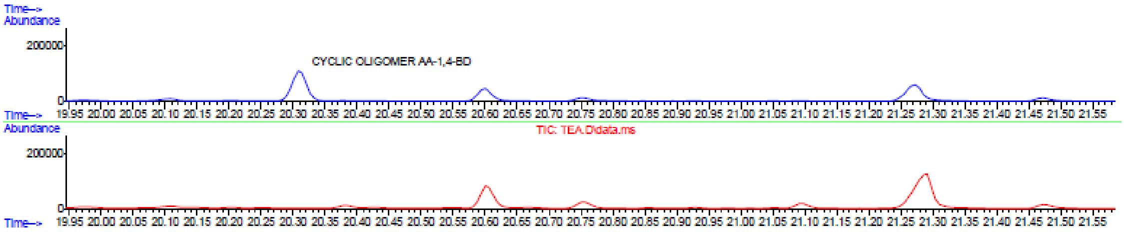

Fig. 4. Chromatogram obtained using HS-SPME-GC–MS of the tea solution after migration (above) and blank tea (below).

Following the identification, the HS-SPME–GC–MS conditions were optimiszed in order to improve the sensitivity of the analysis. The software application MODDE 6.0 was used to model the extraction conditions for the standard oligomer AA-DG, which was used for the semi-quantification since there is no commercial standard of the identified oligomer. The design was one central composite on face with a total of 17 runs. The detected compound showed an excellent fit because the fraction of the variation of the response explained by the model (R2) and the fraction of the variation of the response that can be predicted (Q2) were close to 1. Using the response surface plots for the compound, shown in Fig. 5,

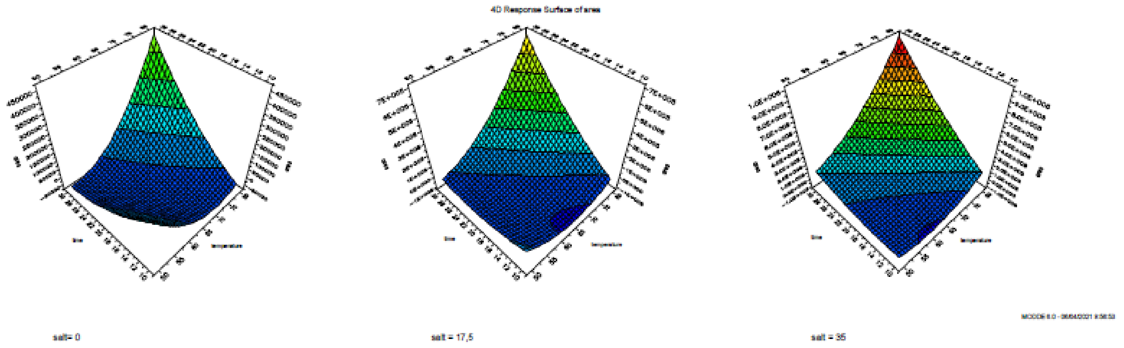

Fig. 5. Response surface plots (temperature vs. extraction time) obtained with MODDE 6.0 for the cyclic oligomer AA-DG for analyses three different percentages of salt (0, 17.5 and 35%).

it was concluded that the optimum conditions for HS-SPME were an extraction temperature of 80 ◦C, an extraction time of 30 min and an extraction solution containing 35% sodium chloride. Using these conditions, it was established the limit of detection of the compound AA-1,4-BD in tea was 0.09 mg/kg. *3.3. Migration results* Specific migration limits (SML) do not exist for the NIAS identified here. However, Regulation https://doi.org/10/2011/EC (Commision, 2011) states that any potential health risk in the final material or article, arising from reaction and degradation products, should be assessed in accordance with internationally recognised scientific principles of risk assessment. For such purposes the concept of a threshold of toxicological concern (TTC) has been devised which sets the maximum level of exposure for each Cramer class, as described by Kroes et al. (2004). The European Food Safety Authority (EFSA) confirms that the TTC is a pragmatic screening and prioritisation tool for use in food safety assessment (EFSA, 2019). The Cramer rules classify the compounds, based on their chemical structure, into one of three classes (I, II and III) reflecting a presumption of low, moderate or serious toxicity, respectively (Cramer, Ford, & Hall, 1978). With the exception of tributyl phosphate, the NIAS identified here were classified as Cramer class I, which sets the maximum estimated daily intake (EDI) at 1.8 mg/person/ day. Tributyl phosphate belongs to Cramer class III for which the EDI is set at 0.09 mg/person/day. Using the assumption that each day in Europe the average adult consumes 1 kg of food that has been in contact with 1 dm3 of packaging material, a worst-case estimate for the EDI can be calculated (Europe, 2013). Based on these assumptions, the calculated SML using the TTC approach was determined to be 0.09 mg/kg for tributyl phosphate and 1.8 mg/kg for the remaining identified NIAS. Table 1 shows the SML and the migration results to both hot and cold tea for all the compounds identified by UPLC–IM-Q/TOF.

Table 1 Compounds identified by UPLC–IM-Q-TOF in the migration solution, together with retention time, accurate mas of detected adduct, collision cross section value (CCS), limit of detection (LOD), specific migration limit (SML), and migration results for hot (100 ◦C left to cool to 20 ◦C over 2 h) and cold (20 ◦C over 2 h) tea.

Compound name	Retention time (min)	*m/z* detected	Elemental composition	CCS (Å^2)	LOD \(mg/kg)	SML/derived SML for Cramer class (mg/kg)	Migration to hot tea (mg/kg)	Migration to cold tea (mg/kg)
Benzisothiazolone	3.40	152.0165	C_7H_5NOS [H]$^+$	128.9	0.003	0.5	0.05 ± 0.005	<LOD
Cyclic oligomer 1,4-BD-AA-1,4-BD-AA (1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone)	5.84	423.2001	$C_{20}H_{32}O_8$ [Na]$^+$	188.4	0.005*	1.8 (Class I)	3.21 ± 0.28	0.23 ± 0.01
Cyclic oligomer NPG-AA-1,6-HD-AA (3,3-dimethyl-1,5,12,19-tetraoxacyclopentacosane-6,11,20,25-tetraone)	6.38	443.2650	$C_{23}H_{38}O_8$ [H]$^+$	201.6	0.005*	1.8 (Class I)	2.56 ± 0.18	0.18 ± 0.01
Cyclic oligomer NPG-AA-NPG-AA (3,3-dimethyl-1,5,12,19-tetraoxacyclopentacosane-6,11,20,25-tetraone)	6.46	429.2482	$C_{22}H_{36}O_8$ [H]$^+$	198.6	0.005*	1.8 (Class I)	3.47 ± 0.24	0.35 ± 0.02
Cyclic oligomer 1,4-BD-AA-1,4-BD-AA-1,4-BD-AA (1,6,13,18,25,30-hexaoxacyclohexatriacontane-7,12,19,24,31,36-hexaone)	6.52	623.3043	$C_{30}H_{48}O_{12}$ [Na]$^+$	234.5	0.005*	1.8 (Class I)	3.15 ± 0.12	0.28 ± 0.02
Cyclic oligomer 1,6-HD-AA-1,6-HD-AA (1,8,15,22-tetraoxacyclooctacosane-2,7,16,21-tetraone)	6.79	457.2796	$C_{24}H_{40}O_8$ [H]$^+$	206.4	0.005*	1.8 (Class I)	4.68 ± 0.42	0.56 ± 0.04
Tributyl phosphate	6.79	289.1540	$C_{12}H_{27}O_4P$ [Na]$^+$	184.1	0.008	0.09 (Class III)	0.07 ± 0.01	<LOD

NPG: neopentyl glycol, AA: adipic acid, 1,6-HD: 1,6-hexanediol 1,4-BD: 1,4-butanediol.

* Compounds quantified with 1,4,7-trioxacyclotridecane-8,13-dione (cyclic oligomer AA-DG).

Table 1 shows the migration results of the compounds identified and their SML established by the European Regulation (Commision, 2011) or their calculated SML. The migration of benzisothiazolone and tributyl phosphate was below their SML. Nevertheless, the migration of cyclic oligomers 1,4-BD-AA-1,4-BD-AA, NPG-AA-1,6-HD-AA, NPG-AA-NPG-AA, 1,4-BD-AA-1,4-BD-AA-1,4-BD-AA and 1,6-HD-AA-1,6-HD-AA to hot tea, ranged from 2.56 to 4.60 mg/kg exceeding the SML established for Cramer class I compounds of 1.8 mg/kg. The migration of these compounds to cold tea was more than an order of magnitude lower than that for hot tea with values ranging from 0.18 to 0.56 mg/kg, all below SML. As such, this work demonstrates that the biodegradable multilayer material is more suitable for cold tea, and highlights the importance of the detection and identification of NIAS. The cyclic oligomer AA-1,4-BD detected using HS-SPME–GC–MS had a migration concentration of 1.3 ± 0.2 mg/kg for hot tea, which is below the SML of 1.8 mg/kg. For cold tea, the migration for this oligomer was below the limit of detection (0.09 mg/kg). It should also be considered that estimations of specific migration limits using the TTC approach assume the average population diet to include foods and beverages contained in packaging materials made (or partially made) of the biodegradable multilayers studied in this work. However, due to the dissimilarities between different geographical regions, a comprehensive exposure study should be performed to confidently assign a specific migration value to these compounds. It was stated earlier that the biodegradable polymer used for the multilayer analysed here, is a blend of PLA and polyester. The glass transition temperature (Tg) of PLA is 60 ◦C and previous work demonstrated that migration of compounds is higher when the temperature is over the Tg (Canellas, Aznar, Nerin, & Mercea, 2010). This could be the reason why a greater degree of migration was observed for hot tea. Additionally, high temperatures kinetically promote the migration of compounds from two phases as, according to the Stokes-Einstein equation and its derivations, the diffusion coefficient is directly proportional to the absolute temperature (Schiller, 1991).

4. Conclusions

LC coupled to IMS-Q/TOF has been successfully used to identify the non-volatile compounds migrating from biodegradable multilayer cups used for hot beverages such as tea. An orthogonal projection to latent structures-discriminant analysis has been demonstrated to be a powerful approach to compare tea samples before and after migration. An HS-SPME–GC–MS method has also been developed. High sensitivity was obtained for the migrants detected and identified, and the migration results obtained for hot tea demonstrated that some of the compounds identified exceeded the migration limits established by European Regulations. This work highlights the significance of the detection and identification of NIAS in food migration extracts, which should be performed on new and novel biodegradable as well as conventional materials.

CRediT authorship contribution statement Elena Canellas: Conceptualization, Investigation, Supervision. **Paula Vera:** Investigation, Supervision. **Cristina Nerin:** Supervision, Funding acquisition, Methodology. **Nicola Dreolin:** Investigation, Supervision. **Jeff Goshawk:** Investigation, Supervision.

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