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RESEARCH PAPER

UPLC–ESI-Q-TOF-MS^E and GC–MS identification and quantification of non-intentionally added substances coming from biodegradable food packaging

Elena Canellas[1,2] · **Paula Vera**[1] · **Cristina Nerín**[1]

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Abstract Biodegradable packagings are made by combination of several materials creating a multilayer with the properties needed. Each material, including the adhesive, could contain substances that could migrate to the food. In this work, gas chromatography coupled with mass spectrometry and ultra-high-pressure liquid chromatography coupled with quadrupole time-of-flight mass spectrometry were used to identify the biodegradable adhesive compounds. Five of the 13 compounds identified were nonintentionally added substances; they were neoformed compounds created by the reaction of added compounds in the adhesive. Moreover, the migration of the compounds through four different biodegradable materials—paper, polylactic acid, ecovio®, and polyvinyl alcohol—was studied for the first time. Three of the 13 compounds identified in the adhesive migrated from the adhesive to Tenax®, which was used as a solid food simulant. One of them, 2,4,7,9-tetramethyl-5-decyne-4,7-diol, was an intentionally added substance, and the other two were 1,6-dioxacyclododecane-7,12-dione and 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone, which were nonintentionally added substances identified in this work. Higher migration values (ranging from 0.81 to 2.07 mg/kg) were observed for migration through ecovio® than through the multilayer made by combination of ecovio® and polyvinyl alcohol (0.07–0.39 mg/kg) owing to the barrier effect provided by polyvinyl alcohol. The migration values for migration through paper and polylactic acid were below the limits of detection.

Keywords Ultra-high-pressure liquid chromatography coupled with quadrupole time-of-flight mass spectrometry · Biodegradable packaging · Nonintentionally added substances · Migration

Introduction

The food packaging industry is becoming increasingly interested in the use of bioplastics for packaging owing to growing environmental awareness. According to European Bioplastics, bioplastics can be defined as plastics based on renewable resources (biobased) or as plastics which are biodegradable and/or compostable [1]. Biopolymers are polymers produced by living organisms; they are polymeric biomolecules but are not necessarily biodegradable. It is the type of chemical bond which defines the biodegradability. There are several synthetic polymers that are biodegradable and compostable [2, 3].

Most food packages are formed by combination of several materials creating a multilayer. This is due to the packaging requirements, such as surface characteristics, thickness/body of the package, gas or aroma barrier, chemical resistance, sealability, and formability or shrink properties, which are not found in a single material. To have the desired mechanical or physical properties, it is necessary to combine the materials that provide specific characteristics. Depending on the materials used in the multilayer, they are created by coextrusion or lamination using adhesives [4]. Biodegradable plastics are also combined in order to obtain multilayers. Biodegradable adhesives have recently been designed in order to produce totally compostable multilayers [5–14]. In this work, the

✉ Cristina Nerín
cnerin@unizar.es

[1] GUIA Group, Department of Analytical Chemistry, University of Saragossa, I3A, María de Luna 3, 50018 Saragossa, Spain

[2] Samtack Adhesivos Industriales, C/ Cerámica, n°3, Pol. Ind. Magarola Sud, 08292 Esparreguera, Barcelona, Spain

multilayers studied are built by the combination of several compostable materials bonded by a compostable adhesive.

Each material, including the adhesive that joins the different layers, is made of many components, such as plasticizers, antioxidants, thermal stabilizers, tackifiers, thickeners, fillers, surfactants, emulsifiers, waxes, slip agents, light stabilizers, biocides, solvents, monomeric and oligomeric carriers, or plasticizers that provide the properties needed [4, 15]. Moreover, not only the previously mentioned intentionally added substances could appear in the material, nonintentionally added substances (NIAS) can also be present. These NIAS could appear as impurities from the raw materials used, from decomposition of the initial components, or because of chemical interactions between them (neoformed compounds) or even with the food in contact with them [16–19].

When multilayers come in contact with foods, all these chemicals, including NIAS, can migrate to the food and contaminate it. Consequently, the identification of the migrant compounds from food packaging material is an essential step. Migration from plastics, paper, and adhesives has been broadly studied [20–23]. Nevertheless, no attention has been paid to the migration from compostable multilayers. This work deals with the migration of compounds coming from a new biodegradable adhesive used to build several compostable multilayers. The different behavior of these migrant compounds on the different compostable materials used to build the multilayers is also studied and discussed.

Experimental

Materials

The adhesive selected for the study was a water-based biodegradable adhesive. This adhesive is compostable according to EN 13432 [24]. It is normally used for the production of multilayer films based on a broad variety of compostable films and papers. The adhesive was supplied by Samtack (Barcelona, Spain).

The following compostable multilayer complexes made with the biodegradable adhesive were provided by Samtack for the migration study:

- Multilayer 1: polylactic acid (PLA) film of thickness 20 μm–adhesive 4 g/m^2–offset paper with a grammage of 90 g/m^2
- Multilayer 2: ecovio® EXP 0.5 SL® film of thickness 40 μm–adhesive 4 g/m^2–ecovio® EXP 0.5 SL® film of thickness 40 μm
- Multilayer 3: ecovio® EXP 0.5 SL® film of thickness 40 μm–adhesive 4 g/m^2–polyvinyl alcohol) (PVA) film of thickness 40 μm–adhesive 4 g/m^2–ecovio® EXP 0.5 SL® film of thickness 40 μm

The following reagents were purchased from Sigma–Aldrich Química (Madrid, Spain): 1,4-butandiole, 2,4,7,9-tetramethyl-5-decyne-4,7-diol, butylated hydroxytoluene, bis(2-ethylhexylmaleate), adipic acid, 2-methyl-2*H*-isothiazol-3-one, 1,2-benzisothiazol-3(2*H*)-one, and 1,4,7-trioxacyclotridecane-8,13-dione.

High-performance liquid chromatography–mass spectrometry (MS) quality methanol and ultrapurified water were supplied by J.T.Baker (Deventer, The Netherlands). Tenax® TA 80/100 mesh was supplied by Supelco (Bellefonte, PA, USA).

Sample preparation and migration tests

To identify the compounds in the adhesive, solutions of the adhesive were prepared. One gram of adhesive was dissolved in 100 g of methanol. The solution was filtered. Then 1 μL of this solution was analyzed by gas chromatography(GC)–single-quadrupole MS (Q-MS), and 5 μL was analyzed by ultra-high-pressure liquid chromatography (UPLC)–quadrupole time-of flight (Q-TOF) MS.

The migration study was done with poly(2,6-diphenyl-*p*-phenylene oxide) (Tenax®) following the procedure optimized in previous work [25–27]. The cutouts of each multilayer, 5 cm×5 cm, were placed in Petri dishes and covered with 1 g of Tenax®, forming a uniform layer (4 g Tenax® per square decimeter of laminate in accordance with UNE-EN 14338 [28]). This system was kept in an oven at 40 °C for 10 days. Then, it was extracted twice with 3.4 mL of methanol. The total solution containing the two extracts was concentrated under a stream of N_2 to 200 μL and analyzed by GC–Q-MS and UPLC–Q-TOF-MS. Three replicates of each laminate and substrate were studied.

Instrumentation

Gas chromatography–single quadrupole mass spectrometry

The equipment consisted of a CTC Analytics CombiPal autosampler coupled to an Agilent 6890 N gas chromatograph with an MS 5975B mass spectrometer detector. All instruments were from Agilent Technologies (Palo Alto, CA, USA).

The capillary column used was an HP-5MS column (30 m×0,25 μm×250 μm) from Agilent Technologies (Madrid, Spain). The oven program was as follows: 40 °C for 2 min, at a rate of 10 °C/min to 300 °C, maintained for 2 min. The injection type was splitless, the injection volume was 1 μL, and the helium flow rate was 1 mL/min. The acquisition was done in electron impact ionization mode. The mass detector was set in scan mode (in the range *m/z* 45–350).

NIST 08 mass spectral search program version 2.0 was used for the identification of the compounds.

Ultra-high-pressure liquid chromatography–quadrupole time-of-flight mass spectrometry

Chromatography was done with an Acquity™ system with use of an Acquity UPLC BEH C_{18} column of 17-μm particle size (2.1 mm×100 mm), both from Waters (Milford, MA, USA). The column flow rate was 0.3 mL/min and the column temperature was 35 °C. The gradient used was 5–95 % methanol (0–10 min), and the volume of sample injected was 5 μL.

The detector consisted of an atmospheric pressure ionization source with an electrospray ionization (ESI) interface coupled to a mass spectrometer consisting of a hexapole, a quadrupole, a collision cell, and a time-of-flight analyzer (Xevo G2) from Waters (Milford, MA, USA).

The electrospray probe was set in both positive mode (ESI+) and negative mode (ESI-). The corona voltage was 2.5 kV for ESI+ and 0.5 kV for ESI-. The sampling cone voltage was optimized between 20 and 50 V, and 30 V was selected for the screening because more peaks were detected. Nitrogen was used as the desolvation gas: the flow rate was 500 L/h at 400 °C. The cone gas flow rate was 20 L/h.

MS^E mode was selected for the acquisition; a collision energy ramp from 5 to 30 V was used. The mass range considered was 10–1200 Da. Data were collected in centroid mode, and the sensitivity analyzer mode was selected. The accuracy and reproducibility of all the analyses were guaranteed by use of a LockSpray. Leucine-enkephalin was used as the lock mass at a concentration of 2 ng/mL in water–acetonitrile with 0.1 % formic acid and a flow rate of 5 μL/min. MassLynx version 4.1 (Waters, Milford, MA, USA) was used to analyze the samples.

Results and discussion

Identification

Nontargeted analysis of the sample was done to identify all the components that are included in the adhesive before their migration was studied.

First, the volatile compounds in the adhesive were identified by GC–Q-MS. NIST 08 returned a "hit list" of matching chemical compounds from the library. Then, standards of the compounds were analyzed for confirmatory purposes. Figure 1 shows the chromatogram obtained by GC–Q-MS. Six peaks were observed in the chromatogram, and they were identified as follows. Five of the six compounds were normal constituents of an adhesive formulation: 1,4-butandiole (retention time 7.7 min), which is a common monomer used in adhesives [29], 2,4,7,9-tetramethyl-5-decyne-4,7-diol (retention time 14.1 min), which is the nonethoxylated derivative of 2,2′-((2,4,7,9-tetramethyldec-5-yne-4,7-diyl)bis(oxy))diethanol (retention time 16.7 min), butylated hydroxytoluene (retention time 15.4 min), which is a common antioxidant [30], 2,2′-((2,4,7,9-tetramethyldec-5-yne-4,7-diyl)bis(oxy))diethanol (retention time 16.7 min), which is a surfactant commonly used in water-based adhesives [31], and bis(2-ethylhexylmaleate) (retention time 22.0 min), which is a common plasticizer [30]. The compound with the dominant peak in the chromatogram was 1,6-dioxacyclododecane-7,12-dione (retention time 16.3 min). This compound was not found as a normal constituent of an adhesive formulation. This compound is not commercially available, but Watanabe et al. [32] reported the identification of this substance as a degradation product in resins, so it could be an NIAS. However, Watanabe et al. did not investigate the exact reaction that led to this compound.

The identification of nonvolatile compounds was done by UPLC–Q-TOF-MS. This technique provides molecular fragmentation combined with mass accuracy in order to elucidate the molecular structure that could lead to the identification of the compounds. The conditions used for the acquisition were as explained before. The mass range acquired was limited to up to 1200 Da because it was considered unlikely that compounds more massive than 1000 Da would migrate from the adhesive to the food. The chromatogram was acquired in MS^E mode. MS^E mode is a method of data acquisition that involves the fast alternation between two energy conditions, thus providing the accurate mass of the precursor ion, in addition to fragment ions, for further confirmatory purposes. As a result, two functions are obtained in a single run: function 1, which is the low-energy function that provides the precursor ion spectra that can lead to the molecular formula of the compound; function 2, the high-energy function, which is the result of the application of a collision voltage ramp in the collision cell. Mass fragments that can lead to elucidation of a structure are obtained with this function.

Figure 2 shows the chromatogram obtained by UPLC–Q-TOF-MS in ESI+ mode. The spectra obtained with function 1 contained the accurate mass for the molecular ion. Two criteria were used to establish its elemental formula: (1) the i-Fit, which is the probability that the isotope pattern of a particular elemental composition in the list of results matches the peaks in the measured spectrum, and (2) the mass tolerance, which was set at 3 mDa. Once the molecular formulas of each accurate mass were known, it was necessary to use a database of chemical compounds and to know the typical composition of an adhesive in order to elucidate the compounds that could be present in the sample. ChemSpider [33] and SciFinder [34] were used to obtain a list of candidates for the identification. A bibliographic search was done to see if these compounds are commonly used to manufacture adhesives. Then, with use of function 2, the fragmentation spectra were obtained. The

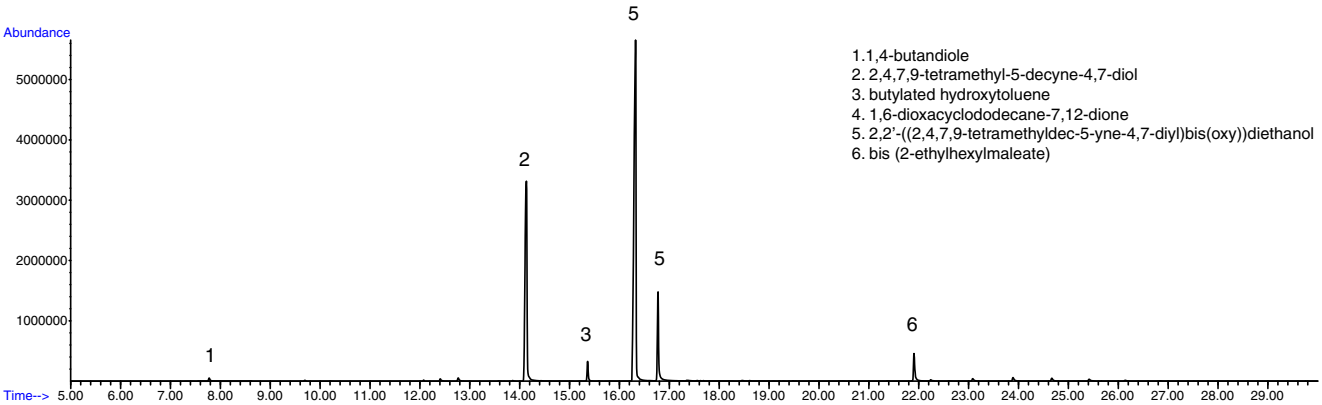

Fig. 1 Chromatogram of the biodegradable adhesive obtained by gas chromatography–mass spectrometry (MS)

accurate masses of the fragments were considered in order to find out if they could be generated from the candidates obtained in the databases and then confirm their identification.

With use of these procedures, we identified three nonvolatile compounds. Adipic acid was identified with ESI- mode. It is a common monomer used in adhesives [29]. On the other hand, with ESI+ mode, 2-methyl-2*H*-isothiazol-3-one (retention time 1.7 min) and 1,2-benzisothiazol-3(2*H*)-one (retention time 3.8 min) were identified. These compounds are two common biocides used in water-based adhesives [30], However, the compounds with the greatest areas in the chromatogram (retention times 5.7, 6.3, 6.6, and 6.8 min) remained unknown as the fragments of the candidates proposed by the databases did not match the fragments obtained experimentally by Q-TOF-MS.

We hypothesized that both the volatile compound 1,6-dioxacyclododecane-7.12-dione and the four unknown compounds could be neoformed compounds coming from the reaction between some volatile and nonvolatile compounds identified.

Figure 3 shows the reaction of the two compounds identified in the sample, butane-1,4-diol and adipic acid, proposed for the formation of 1,6-dioxacyclododecane-7,12-dione. In our first attempt we assumed that these two compounds could react to form bigger lactones with the same structure (Fig. 3). Taking this hypothesis into account, we calculated the molecular masses of these neoformed compounds. They matched the *m*/*z* of the unknown compounds minus the sodium mass since these compounds formed sodium adducts by ESI (Fig. 2; *m*/*z* 423.2022, 623.3059, 823.4108, and 1023.5125). Then, the accurate masses of the fragments of these compounds were considered in order to find out if they could be generated from these candidates obtained by the reaction between butane-1,4-diol and adipic acid.

Figure 4 shows the high-energy spectrum (function 2) of 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone (retention time 5.7 min). As can be seen, all the fragments match the fragments of the proposed candidate. The same was found with the other three compounds shown in Table 2: 1,6,13,18,25,30-hexaoxacyclohexatriacontane-7,12,19,24,31,36-hexone (retention time 6.3 min); 1,6,13,18,25,30,37,42-octaoxacyclooctatetracontane-7,12,19,24,31,36,43,48-octaone (retention time 6.6 min), and 1,6,13,18,25,30,37,42,49,54-decaoxacyclohexacontane-7,12,19,24,31,36,43,48,55,60-decaone (retention time 6.8 min). There are no standards commercially available for these compounds for the

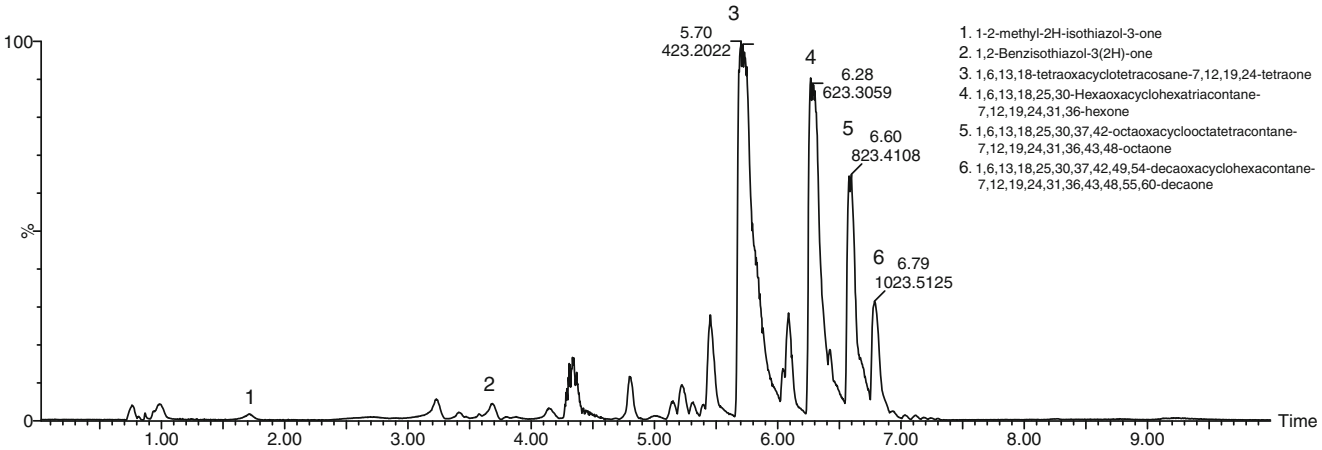

Fig. 2 Chromatogram of the biodegradable adhesive obtained by ultra-high-pressure–quadrupole time-of flight MS (UPLC–Q-TOF-MS)

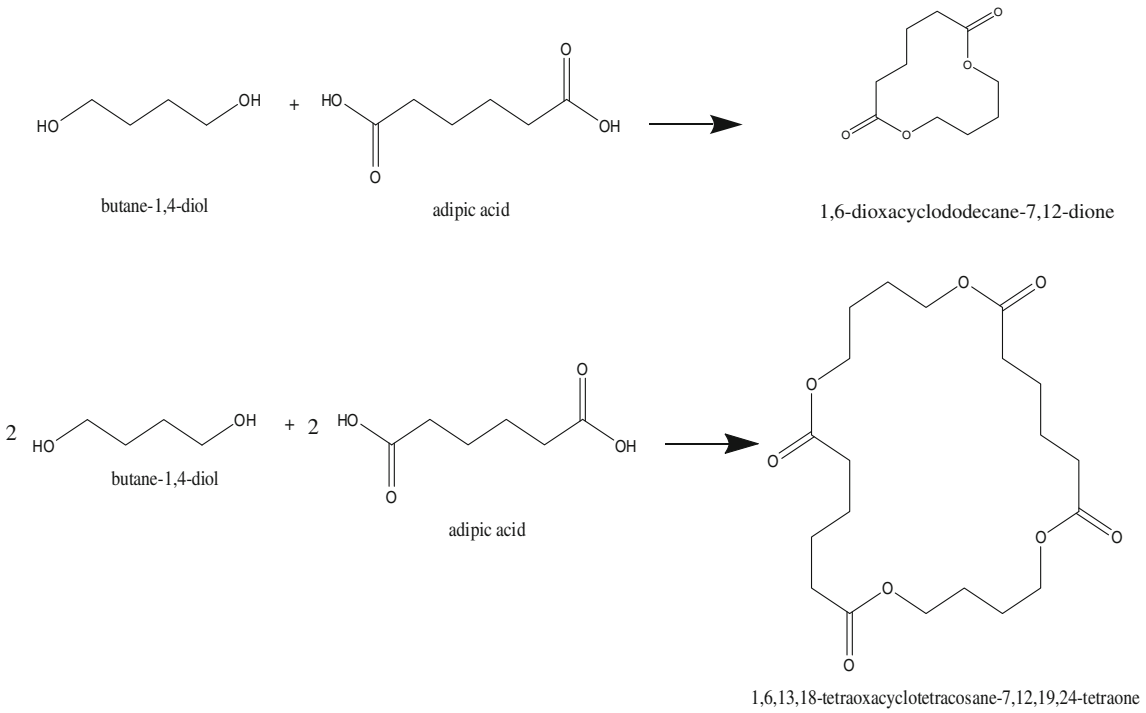

Fig. 3 Reaction of adipic acid with butane-1,4-diol to form several nonintentionally added substances identified by UPLC–Q-TOF-MS

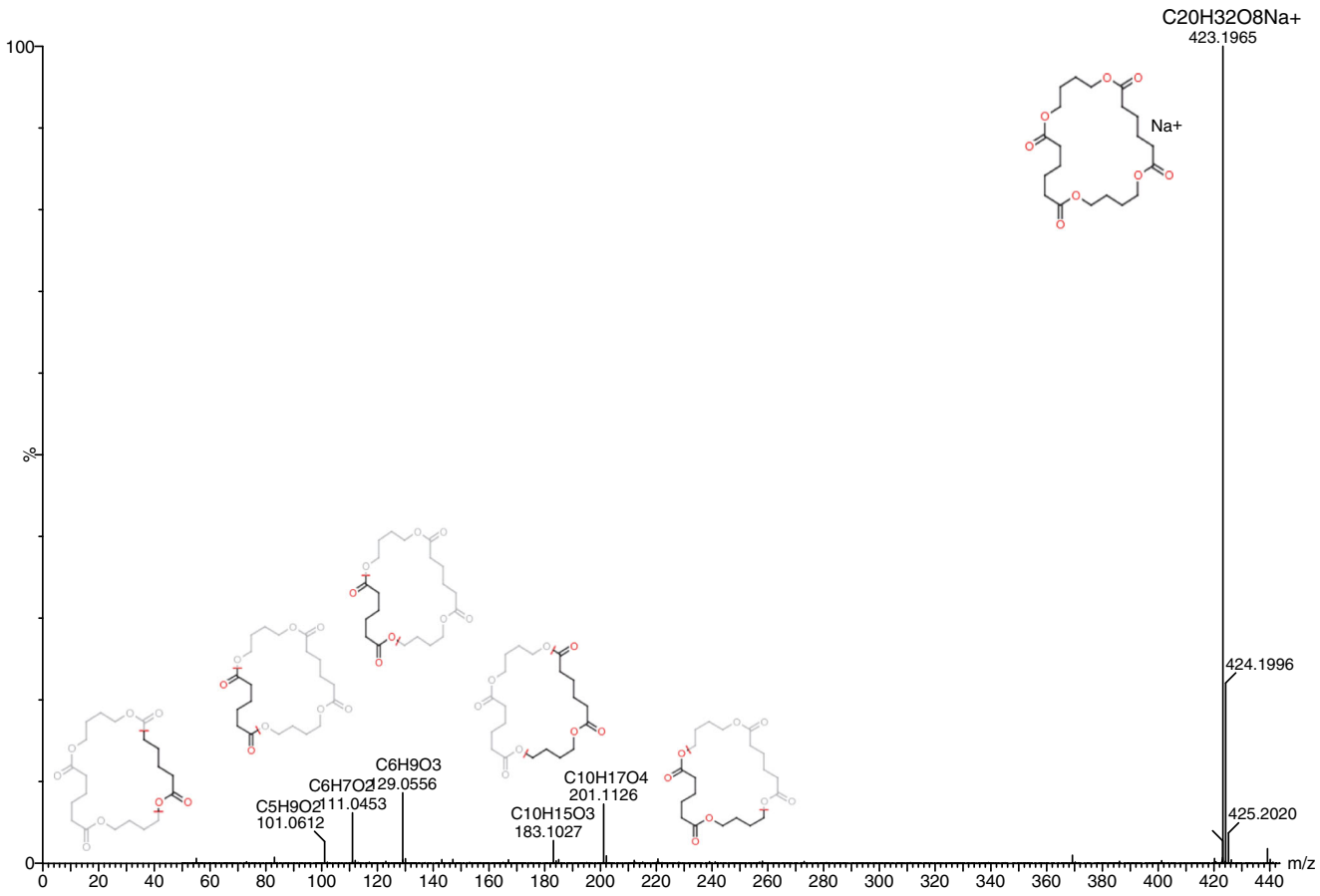

Fig. 4 High-energy spectrum of 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone obtained by UPLC–Q-TOF-MS

confirmation step. Nevertheless, the identification of 1,6-dioxacyclododecane-7,12-dione, butane-1,4-diol, and adipic acid and the match with accurate masses of the experimentally obtained fragments by UPLC–Q-TOF-MS with these lactones led us to conclude that the identification was reliable.

Migration study

Once the composition of the compostable adhesive was known, the migration of these compounds was studied when the adhesive is part of a multilayer. This adhesive is a new compostable adhesive, so it may be used to form multilayer complexes with compostable materials in order to form compostable packaging.

The multilayer complexes studied were based on several compostable films found on the market: PLA, ecovio® [which consists of the biodegradable ecoflex® (fossil basis) and PLA], compostable PVA, and paper. The samples studied are described in "Materials."

None of these multilayer complexes are barriers to liquids. They are intended to be used for dry food. For that reason, we decided to do the migration assays with Tenax® following the indications described in both Regulation (EU) No 10/2011 and UNE-EN 14338.

Tables 1 and 2 show the limits of detection, the migration values for migration to Tenax®, and the estimated daily intake (EDI) for both sides of multilayer 1, from multilayer 2, and from multilayer 3. The EDI was calculated according the equation established by the US Food and Drug Administration [35]

$$\begin{aligned}\text{EDI (mg/person} \times \text{day)} = {} & \text{migration(mg/kg)} \\ & \times 3\,\text{kg(food intake per person and day)} \\ & \times C_{\text{F}},\end{aligned}$$

where C_{F} is the fraction of the daily diet expected to be in contact with a specific packaging material (for adhesives this is 0.14).

Migration of all the compounds previously identified was below the limits of detection in multilayer 1. However, three compounds migrated from multilayers 2 and 3: 2,4,7,9-tetramethyl-5-decyne-4,7-diol, 1,6-dioxacyclododecane-7,12-dione, and 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone (the migration values were 0.21, 2.07, and 0.81 mg/kg, respectively, for multilayer 2 and 0.04, 0.39, and 0.07 mg/kg, respectively for multilayer 3). Two of these compounds were neoformed substances, NIAS, coming from the adhesive. This highlights the importance of this study since without the previous identification, the pattern recognition of these NIAS would not have been possible.

Multilayer 1 was built from paper and PLA, and these two materials seem to act as a barrier for these compounds.

Table 1 Compounds identified by gas chromatography–mass spectrometry (MS), limits of detection (*LOD*), migration values for migration to Tenax® from both sides of multilayer 1 [polylactic acid (*PLA*)–adhesive 4 g/m^2–paper], from multilayer 2 (ecovio®–adhesive 4 g/m^2–ecovio®), and from multilayer 3 [ecovio®–adhesive 4 g/m^2–poly(vinyl alcohol) (PVA)–adhesive 4 g/m^2–ecovio®], and estimated daily intake (*EDI*)

RT (min)	Compound	LOD (mg/kg)	Multilayer 1 (PLA side)		Multilayer 1 (paper side)		Multilayer 2		Multilayer 3	
			Migration value (mg/kg)	(EDI (mg/kg/day)	Migration value (mg/kg)	EDI (mg/kg/day)	Migration value (mg/kg)	EDI (mg/kg/day)	Migration value (mg/kg)	EDI (mg/kg/day)
7.7	1,4-Butandiole	0.1	<LOD		<LOD		<LOD		<LOD	
14.1	2,4,7,9-tetramethyl-5-decyne-4,7-diol	0.03	<LOD		<LOD		0.21	0.08	0.04	0.02
15.4	Butylated hydroxytoluene	0.3	<LOD		<LOD		<LOD		<LOD	
16.3	1,6-Dioxacyclododecane-7,12-dione[a]	0.6[1]	<LOD		<LOD		2.07	0.86	0.39	0.16
16.7	2,2'-((2,4,7,9-Tetramethyldec-5-yne-4,7-diyl)bis(oxy))diethanol[b]	0.03	<LOD		<LOD		<LOD		<LOD	
22.0	Bis(2-ethylhexyl)maleate)	3	<LOD		<LOD		<LOD		<LOD	

RT retention time

[a] Compound quantified with 1,4,7-trioxacyclotridecane-8,13-dione

[b] Compound quantified with 2,4,7,9-tetramethyl-5-decyne-4,7-diol

Table 2 Retention times (*RT*) of the compounds identified by ultra-high-pressure–quadrupole time-of flight MS, number of the compound in the chromatogram, measured mass, type of ion found, ΔmDa (measured mass - calculated mass from the formula), formula, compound name, LOD, migration values for migration to Tenax® coming from both sides of multilayer 1 (PLA–adhesive 4 g/m^2–paper), from multilayer 2 (ecovio®–adhesive 4 g/m^2–ecovio®), and from multilayer 3 (ecovio®–adhesive 4 g/m^2–PVA–adhesive 4 g/m^2–ecovio®), and EDI

RT(RT (mi(min)No. and ESI mode		Measured mass and ion found	ΔmDa	Formula	Compound	LOD (mg/kg)	Multilayer 1 (PLA side)		Multilayer 1 (paper side)		Multilayer 2		Multilayer 3	
							Migration value (mg/kg)	EDI (mg/kg/day)	Migration value (mg/kg)	EDI (mg/kg/day)	Migration value (mg/kg)	EDI (mg/kg/day)	Migration value (mg/kg)	EDI (mg/kg/day)
1.9, ESI-		145.0505 [-H]	0.4	$C_6H_{10}O_4$	Adipic acid	0.05	<LOD		<LOD		<LOD		<LOD	
1.7, ESI +	1	116.0178 [+H]	0.8	C_4H_5NOS	2-Methyl-2*H*-isothiazol-3-one	0.002	<LOD		<LOD		<LOD		<LOD	
3.8, ESI+	2	152.0180 [+H]	1.0	C_7H_5NOS	1,2-Benzisothiazol-3(2*H*)-one	0.004	<LOD		<LOD		<LOD		<LOD	
5.7, ESI+	3	423.2022 [+Na]	2.7	$C_{20}H_{32}O_8$	1,6,13,18-Tetraoxacyclotetracosane-7,12,19,24-Tetraone	0.005[a]	<LOD		<LOD		0.81	0.34	0.07	0.03
6.3, ESI+	4	623.3059 [+Na]	1.6	$C_{30}H_{48}O_{12}$	1,6,13,18,25,30-Hexaoxacyclohexatriacontane-7,12,19,24,31,36-hexone	0.005[a]	<LOD		<LOD		<LOD		<LOD	
6.6, ESI+	5	823.4108 [+Na]	1.6	$C_{40}H_{64}O_{16}$	1,6,13,18,25,30,37,42-Octaoxacyclooctatetracontane-7,12,19,24,31,36,43,48-octaone	0.005[a]	<LOD		<LOD		<LOD		<LOD	
6.8, ESI+	6	1023.5125 [+Na]	1.6	$C_{50}H_{80}O_{20}$	1,6,13,18,25,30,37,42,49,54-Decaoxacyclohexacontane-7,12,19,24,31,36,43,48,55,60-decaone	0.005[a]	<LOD		<LOD		<LOD		<LOD	

ESI electrospray ionization

[a] Compounds quantified with 1,4,7-trioxacyclotridecane-8,13-dione

Nevertheless, in the case of multilayers 2 and 3, the compounds were able to pass through the materials they were made of. This is the first time that migration in these kinds of multilayers has been studied. Ecovio® is a material that consists of ecoflex® (fossil basis) and PLA. Ecoflex® provides better mechanical properties to PLA, but this work evidences that it provides a worse barrier to some compounds than PLA itself.

In addition, the effect on the migration through the multilayer provided by the biodegradable PVA was studied. The migration through multilayer 2, which was built with ecovio® and adhesive, was compared with the migration through multilayer 3, which also contained PVA, with the objective to minimize the water and oxygen permeability through the multilayer [4]. Higher migration values were observed in multilayer 2 than in multilayer 3. Therefore, the presence of PVA decreases the migration. Then it can be concluded that PVA not only decreases the permeability of water and oxygen but also the migration of these compounds coming from the adhesive.

Adhesives are not yet covered by specific European legislation, but as any food contact material they have to comply with the frame of Regulation (EC) No 1935/2004. Then, for adhesives to be used in plastic materials and articles, adequate information should be provided to the manufacturer of the final plastic article that would enable that manufacturer to comply with both Regulation (EC) No 1935/2004 and Regulation (EU) No 10/2011. This means that the migration of the compounds should be below the limit established in the latter regulation. Table 3 shows the specific migration limit for the substances found that are included in the positive list in Regulation (EU) No 10/2011. To evaluate if the rest of the compounds found could endanger human health, a bibliographic search for their lowest observed adverse effect level was done. However, the lowest observed adverse effect level or the toxicity of these neoformed compounds has not been studied yet. Then, the evaluation of the safety of these materials poses a problem because toxicity of the compounds is difficult to predict. Therefore, only a theoretical evaluation could be done. A theoretical evaluation based on the Cramer rules and the Threshold of Toxicological Concern was done. This classification assigns the toxic effect of a substance according to its molecular structure [36]. The neoformed compounds found here were classified in the lowest toxicity class (Table 3). The EDI of these migrant compounds (Tables 1 and 2) was below the threshold established by the International Life Sciences Institute for this class of toxicity [36]. Then we can concluded that, taking into account this theoretical toxicity data, the adhesive used in these multilayers will be safe for food contact applications.

Table 3 Specific migration limits (*SML*), lowest observed adverse effect level (*LOAEL*), and human exposure threshold (*HET*) of the compounds studied

Compound	SML (mg/kg)	LOAEL (mg/kg/day)	HET (mg/kg/day)
1,4-Butandiole	5 [Regulation (EU) No 10/2011]		
2,4,7,9-Tetramethyl-5-decyne-4,7-diol		200	
Butylated hydroxytoluene	3 [Regulation (EU) No 10/2011]		
1.6-Dioxacyclododecane-7,12-dione			1.8 (class I)
2,2'-((2,4,7,9-Tetramethyldec-5-yne-4,7-diyl)bis(oxy))diethanol		200	
Bis(2-ethylhexyl)maleate)		30	
Adipic acid	No limit [Regulation (EU) No 10/2011]		
2-Methyl-2*H*-isothiazol-3-one	0.5 [Regulation (EU) No 10/2011]		
1,2-Benzisothiazol-3(2*H*)-one	0.5 (EFSA)		
1,6,13,18-Tetraoxacyclotetracosane-7,12,19,24-tetraone			1.8 (class I)
1,6,13,18,25,30-Hexaoxacyclohexatriacontane-7,12,19,24,31,36-hexone			1.8 (class I)
1,6,13,18,25,30,37,42-Octaoxacyclooctatetracontane-7,12,19,24,31,36,43,48-octaone			1.8 (class I)
1,6,13,18,25,30,37,42,49,54-Decaoxacyclohexacontane-7,12,19,24,31,36,43,48,55,60-decaone			1.8 (class I)

EFSA European Food Safety Authority

Conclusions

This work has established that the identification of the compounds present in a sample intended to be in contact with food is an essential step, since the compounds can react between themselves, producing neoformed substances that could be present in the final sample even at higher concentrations than the intentionally added substances.

This work has studied for the first time the migration of compounds coming from a compostable adhesive through different industrial biodegradable materials. It has been proved that the NIAS that have been identified in the adhesive studied here are able to diffuse through some biodegradable materials, migrating to the food simulant used here for the migration assays.

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