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Fabric phase sorptive extraction for specific migration analysis of oligomers from biopolymers

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ABSTRACT

Oligomers are potential migrants from polymers or biopolymers intended to food packaging and they have to be under control. In order to comply with European regulation 10/2011, their concentration in migration must be below 0.01 μg g^{-1}. In this work, fabric phase sorptive extraction (FPSE) was explored as an effective method for extraction and pre-concentration of oligomers migrated from a blend PLA-polyester material. Both food simulant B (3% acetic acid) and juice, as real food, were used for migration experiments. The parameters of FPSE were optimized and the analysis was done by UHPLC-QTOF and UHPLC-QqQ. A total of 21 oligomers were identified, 9 of them coming from PLA and 12 oligomers from the polyester part. These oligomers were formed by adipic acid (AA), phthalic acid (PA) and/or butanediol (BD), ten were cyclic and 11 were linear molecules. Using the optimized FPSE procedure in 3% acetic acid as food simulant, it was possible to identify 3 new compounds that were not detected by direct injection of the simulant into UHPLC-QTOF. In addition, 2 extra compounds, cyclic $PA\text{-}BD_4\text{-}AA_3$ and cyclic $PA_2\text{-}BD_3\text{-}AA$, were only identified in juice samples after FPSE extraction. Besides, in order to quantify the compounds identified, an isolation procedure for PLA oligomers was carried out. Two oligomers were isolated: cyclic $(LA)_6$ and linear HO-$(LA)_4$-H, both with a purity higher than 90% (LA: lactic acid). The highest concentration value was found for the cyclic oligomer $[AA\text{-}BD]_2$, that showed 22.63 μg g^{-1} in 3% acetic acid and 19.64 μg g^{-1} in juice. The concentration of the total amount of remaining oligomers was below 7.56 μg g^{-1} in 3% acetic acid as well as in juice.

1. Introduction

Packaging can be a source of contaminants in food, since unwanted substances present in the packaging material can be transferred to the product. They can alter the organoleptic characteristics of the food or be toxic to the consumers. For this reason, it is important to carry out migration assays to guarantee the consumer safety and evaluate the risk of these packages [1–3].

In order to perform migration tests, food simulants as well as real food are used. Food simulants are used as substitutes of food to ensure the simplification of chemical analysis. The migration level depends on various factors: the physico-chemical properties of the migrant, the packaging material and the food, temperature, storage time and the ratio between packaging surface and foodstuff weight [4,5].

To ensure the food safety, all materials in contact with food must comply with Commission Regulation Nº 1935/2004/CE [6] and in addition, plastic materials must comply with the Commission Regulation Nº10/2011 and its amendments, that sets the specific migration limits (SMLs) for the allowed substances in food contact plastics. Besides, non-intentionally added substances (NIAS), that are not included in any positive list of this regulation, must not exceed 0.01 μg g^{-1} simulant or food [7], proving that they are not carcinogenic, mutagenic or reprotoxic.

Oligomers are considered as NIAS, since they are collateral sub-products formed during polymer synthesis or sub-products formed by the degradation of the polymer during packaging manufacturing. It has been demonstrated that they can migrate from polymeric materials such as polyethylene terephthalate (PET), polyamide (PA), polyethylene (PE), polyurethane (PU), polypropylene (PP) [8–18], and biopolymer materials as PLA and starch [19,20].

There is not toxicological information about most of the oligomers, and therefore it is not known the adverse harm that they can cause in humans [21–25]. In addition, the analysis of these substances is a very complex task, since in most cases there are not commercial standards for

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their confirmation and quantification. Thus, their chemical structure has to be elucidated based on their fragmentation mass spectra pattern. According to Regulation EU/10/2011 [7], oligomers migration limit should not exceed 0.01 μg g^{-1} of simulant as they are not included in the list of authorized substances, and to achieve these limits it is necessary to use both sophisticated sample preparation techniques and high resolution analytical techniques [26–28].

As it was previously mentioned, both polymers and biopolymers can contain oligomers. Among biopolymers, PLA is the most commonly used and produced at industrial level because it has good mechanical and barrier properties, similar to those of PET. Nevertheless, PLA is usually blended to polyester of petrochemical nature to improve its flexibility or viscosity [29–31]. It has been also found blended to starch and to poly (butylene adipate-*co*-terephthalate) [19,20]. Several oligomers from PLA have been described in the literature [19] and found in migration tests to food simulants but not yet in real food. The analysis of oligomers in food is much more complex, because it is necessary to previously remove those substances that cannot be injected into the UHPLC-MS system (sugars, proteins, fats, vitamins …). An extraction method is then inevitable to facilitate the analysis.

Fabric phase sorptive extraction (FPSE) is a method described by Kabir and Furton in 2014 [32] as a microextraction device with very high sorbent loading in the form of an ultra-thin coating. It is made of a flexible and permeable, natural or synthetic fabric (cotton, cellulose or polyester) used as substrate, whose surface is chemically modified by a sol–gel sorbent coating process, leading to an organic–inorganic sorbent with unique selectivity and affinity towards the target analytes. It results in a highly cross-linked porous material with a good chemical and thermal stability, which leads to a reduction in the extraction time to reach equilibrium. Nowadays, there are a lot of sorbents that can be used in the manufacturing of FPSE membrane so a huge number of substances can be extracted from many matrices and in the process, low solvent volumes are used [33,34].

This sample preparation technique consists of 2 main steps: extraction and back-extraction. In the first one, the liquid sample is put in contact with the FPSE membrane that traps the analytes in the sorbent. In the second one, the FPSE membrane is immersed in a small volume of organic solvent that extracts the analytes from the membrane. This method has been focused on the determination of drugs, pharmaceuticals, and other compounds mainly in environmental samples as well as in food samples and biological fluids [35–41]. However, FPSE has not been previously applied to the extraction of oligomers.

The aim of this study was to develop a method based on FPSE extraction for the identification and quantification of PLA and polyester oligomers released from packaging materials to real food, such as pineapple juice.

2. Materials and methods

2.1. Reagents and standards

Three cyclic ester oligomers, AA-DEG (C10H16O5) purity 75.8%, AA-DEG-IPA-DEG (C22H28O10) purity 95.0% and IPA-DEG-IPA-DEG (C24H24O10), purity >90%, composed of diethylene glycol (DEG), adipic acid (AA) and isophthalic acid (IPA) were used as standards to optimize the FPSE method. The oligomers were chemically synthesized by a adhesive company and their structures and purity were confirmed by NMR at the University of Zaragoza. Their structures are described in supplementary material 1. Dibutyl sebacate (DBS) and dimethyl sebacate (DMS) standards were from Sigma Aldrich Química (Madrid, Spain). Methanol and acetic acid (LC–MS quality) were purchased to Scharlau Chemie S.A (Sentmenat, Spain). Acetronitrile (LC-MS quality) was supplied by Sigma Aldrich Química and ultrapure water used for all solutions and dilutions was produced by a Wasserlab purification system (GR 216071; Madrid, Spain). Methanol and water for UHPLC analysis (ultra LC-MS quality) were obtained from Baker (Deventer, The Netherlands) and formic acid (CAS: 64-18-6) was from Waters (Milford, MA, USA).

2.2. Samples

2.2.1. PLA biopolymer

A biodegradable polyester, poly (butylene adipate-*co*-terephthalate), with 18% of renewable resources (PLA) was used for this study. The material was certified as a compostable polymer. Film thickness was 0.17 mm. Its mass density was 1.24–1.26 g cm^{-3} and its melt volume rate (190 °C, 5 Kg) was 7–11 mL min^{-1}. Melting points were 110–120 °C to 140–155 °C and permeation rate of water vapor at 38 °C.

2.3. Juice

Pineapple juice from a local market was used as food sample. Its ingredients were: concentrated juice (50%), water, sugar (11.2 g in 100 g of juice), citric acid, pectin, ascorbic acid, proteins and vitamin C.

2.4. FPSE membrane

For the optimization of the FPSE method, ten FPSE membranes coated with different sol-gel based sorbents characterized with different polarities and selectivities were tested: sol-gel Carbowax 20,000 Da (code: CW), sol-gel dimethylsiloxane-ethylene oxide block copolymer 3500–4500 Da with 60%wt of non-siloxane (code: DBE-C25), sol-gel polytetrahydrofuran (code: PTHF), sol-gel dimethylsiloxane-ethylene oxide block copolymer 2500 Da with 50–55% wt of non-siloxane (code: DBE-621), sol-gel polycaprolactone diol (code: PC), sol-gel polyethylene glycol-polyethylene oxide-polyethylene glycol triblock copolymer (PEG-PEO-PEG, code: PGPPG), sol-gel caprolactone-dimethylsiloxane-caprolactone block copolymer (PC-PDMS-PC, code: PCPPC), sol-gel Chitosan (code: Ch), sol-gel dimethylsiloxane (code: PDMS) and sol-gel polyethylene glycol 300 Da (code: PEG).

2.5. Instrumentation and analytical conditions

The separation was carried out in an Acquity™ UPLC system from Waters (Milford, MA, USA) using an Acquity UPLC BEH C18 column of 2.1 mm × 100 mm and 1.7 μm particle size. The column temperature and flow were 40 °C and 0.3 mL min^{-1}, respectively. Injection volume was 10 μL. Two mobile phases were used: water with 0.1% formic acid (phase A) and methanol with 0.1% formic acid (phase B). The gradient used for the analysis by UHPLC-MS-QqQ was: initial composition 30/70 (A/B) and then at 4.5 min it was changed to 70/30 (A/B). At 7 min, initial conditions were selected until 9 min. The gradient used for the analysis by UHPLC-MS-QToF was: initial composition 98/2 (A/B) and then at 8 min it was changed to 100% of solvent B. At 10 min, initial conditions were selected until 14 min. Data analysis was performed using Mass Lynx v.4.1 software (Waters, Milford MA, USA).

For the analysis by UHPLC-MS-QqQ, an electrospray interface (ESI) and a TQ mass spectrometer from Waters (Milford, MA, USA) were coupled to the UHPLC system. The electrospray probe was used in positive mode (ESI+) and acquisition was performed in SIR (single ion recording) mode. The MS parameters used were as follows: capillary voltage was 3.5 kV, source temperature was 120 °C, desolvation gas temperature 450 °C, cone gas flow 60 L h^{-1}, and desolvation gas flow 600 L h^{-1}. The selected ions were 217.1 [MH+] for AA-DEG, 453.18 [MH+] for AA-DEG-IPA-DEG and 473.45 [MH+] for IPA-DEG-IPA-DEG. Cone voltage was optimized from 20 to 70 V for the three compounds. Finally, 30 V cone voltage was selected as the optimum value for the three of them.

For the analysis by UHPLC-MS-QToF, an electrospray interface (ESI) and a Xevo G2 QToF mass spectrometer from Waters (Milford, MA, USA) were coupled to the UHPLC system. The conditions of analysis were: positive mode (ESI+), sensitivity mode, capillary voltage 3 kV, cone

voltage 30 V, source temperature 120 °C, cone gas flow 10 L h^{-1} desolvation gas temperature 350 °C and desolvation gas flow 600 L h^{-1}. The accuracy and reproducibility of all the analyses were guaranteed by the use of a LockSpray™. The mass range considered was from 50 to 1200 *m/z*. The acquisition was carried out in MS^E mode with two functions; acquiring at low collision energy in the collision cell (function 1) to obtain information about the precursor ion, and at high collision energy (function 2) to provide information about the fragment ions. The collision ramp energy was from 15 to 30 V.

2.6. Optimization of FPSE protocol

For the optimization process, solutions at a concentration of 100 μg g^{-1} of each standard (AA-DEG, AA-DEG-IPA-DEG and IPA-DEG-IPA-DEG) were prepared in milliQ water. Different parameters were optimized in order to maximize the extraction efficiency: the fabric phase sorbent, FPSE extraction mode (sequential or simultaneous), initial sample volume and final extract volume and extraction and back-extraction conditions.

2.6.1. Determination of enrichment factor and extraction recovery

Extraction efficiency was evaluated using different values:

- Enrichment factor (EF), calculated according to the following equation:

$$EF = \frac{C_{FPSE}}{Co}$$

where C_{FPSE} is the concentration of the analyte in the final FPSE extract after FPSE membrane procedure and C_0 is the initial concentration of analyte in the sample solution.

- Percentage of extraction recovery (ER%), calculated according to the following equation:

$$ER\% = \frac{C_{FPSE} \cdot V_{FPSE}}{Co \cdot Vo} \cdot 100$$

where C_{FPSE} is the concentration of the analyte in the final FPSE extract, C_0 is the initial concentration of analyte in the sample solution, V_{FPSE} is the final extract volume after FPSE membrane procedure, and Vo is the initial sample volume.

2.7. Final FPSE protocol

After a rigorous optimization, the final FPSE protocol was as follows:

Cleansing of FPSE membrane: the FPSE membrane was placed in a vial with 5 mL of a mixture of methanol/acetonitrile (50:50, v/v) for 1 h in an ultrasound bath Branson 3510 (40 Hz, 80 W) at room temperature. Subsequently, the FPSE membrane was removed from the solvent, rinsed with water and air dried. In the case of using juice, the cleansing was done in two steps of 30 min in order to guarantee the complete cleansing of the FPSE membrane.

Sample extraction step: An aliquot of 18 mL of the sample was placed in a 20 mL vial with a FPSE membrane and a magnetic bar and it was stirred at 700 rpm for 20 min. Afterwards, the FPSE membrane was removed, rinsed with water and air dried.

FPSE back-extraction step: The FPSE membrane was placed in an Eppendorf and 1 mL of the extraction solvent (methanol) was added. The Eppendorf was placed in an ultrasound bath for 10 min at room temperature. Then, the FPSE membrane was removed and the extract was gently evaporated to dryness under a nitrogen current at 40 °C in a Techne sample concentrator (Cole-Parmer Ltd., UK). Afterwards, it was re-dissolved in 500 μL of methanol/water (50/50). The final extracts were analyzed by UHPLC-MS (QqQ) or UHPLC-MS (QToF).

This procedure was carried out by triplicate for each FPSE membrane.

2.8. Migration test

Migration experiments were performed by total immersion of the samples into acetic acid 3% (food simulant B) and into pineapple juice (real food). Food simulant B was selected since it is one of the simulants recommended in Regulation EU/10/2011 [7] for clear drinks with pH lower than 4.5, as is the case of pineapple juice.

For carrying out these assays, cut-offs of films of 5 × 2 cm were introduced into 20 mL vials and immersed in acetic acid 3% (simulant B) or pineapple juice (18 mL). Vials were filled in according to the rate 6 dm^2 kg^{-1} of simulant, established by the Regulation EU/10/2011 [7]. Afterwards, the vials were placed in an oven at 40 °C for 10 days. 40 °C were selected instead of 60 °C because 60 °C is close to the glass transition temperature of PLA and the material would degrade. After this time, PLA was removed and the extracts were analyzed. All migration experiments were performed in triplicate.

2.9. Isolation procedure of PLA oligomers

In order to quantify the compound identified, an isolation procedure of PLA oligomers was carried out. Fifty grams of PLA pellets sample were powdered. They were cryogenically cooled using liquid nitrogen and then grinded using a knife mill under liquid nitrogen. This way, there was an improvement in the extraction efficiency and a better sample homogeneity. Then, oligomers were extracted with 50 mL of methanol at 40 °C overnight. After that, the solution was filtered and evaporated to dryness using a rotary evaporator. The resulting residue was dissolved in 50 mL of 10% methanol. The solution was fractionated using an OASIS HLB 35 cc cartridge (6 g, Waters). For this purpose, the cartridge was previously conditioned with methanol and water. The sample was passed through the cartridge and subsequently fractionated with 50 mL of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% methanol. The eluted fractions were injected into the UHPLC-QTOF in order to check the presence of oligomers in each fraction. Those fractions containing several oligomers were selected, evaporated to dryness and redisolved in 50 mL of 10% methanol. The solution was then added to an OASIS HLB 6 cc cartridge (200 mg, Waters) and each 50 mL of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% methanol was passed through the cartridge and collected separately again. The last fractionation protocol was applied successively until an oligomer was isolated.

When an oligomer was isolated, the eluted fraction was evaporated; the residue was weighted and dissolved in 5 mL of methanol. Confirmation of the purity was achieved by UHPLC-MS-MS.

Finally, two oligomers of PLA were isolated: cyclic $(LA)_6$ and linear HO-$(LA)_4$-H with a purity higher than 90%.

3. Results and discussion

3.1. Optimization of FPSE protocol

The protocol was designed according to previous works performed in the laboratory [39,40]: 10 mL of the sample volume, 20 min of sample extraction time, 1 mL of methanol as back-extraction solvent and 10 min as back-extraction time. AA-DEG, AA-DEG-IPA-DEG and IPA-DEG-IPA-DEG were used as standards in the optimization method because they are available in our laboratory.

3.1.1. Selection of FPSE membrane

Ten different FPSE membranes coated with polymers of different polarities were tested. Their compositions are described in section 2.4. In order to evaluate the extraction efficiency of the different FPSE absorbents, the enrichment factor (EF) was calculated. The results are shown in Fig. 1. According to these results, the best extractions were provided by CW, PTHF and PDMS with EF values between 3 and 12. The

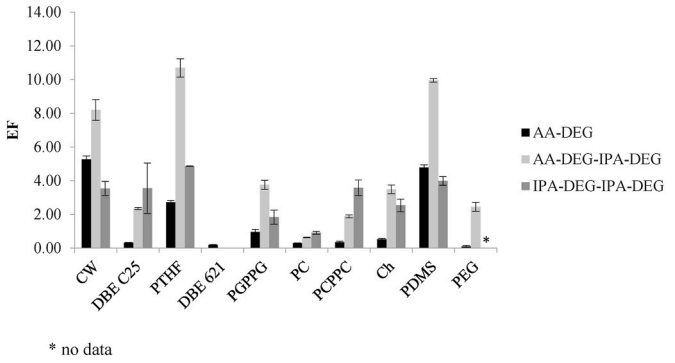


Fig. 1. Enrichment factor values (EF) of three oligomers (AA-DEG, AA-DEG-IPA-DEG and IPA-DEG-IPA-DEG) after FPSE extraction with ten different membranes.

remaining FPSE membranes were removed from the following experiments because their EF values were not high enough.

3.1.2. Combination of FPSE membranes

In order to select the best FPSE membranes, either alone or in combination, an initial study measuring the decrease percentage of oligomers in the standard solution was carried out. The effect of working in sequential or simultaneous modes with the selected FPSE membranes was also studied. The objective of combination was to improve the extraction capacity and therefore to obtain higher recoveries. In order to make the evaluation, the relative decrease of the analytes area in the sample solution (decrease %) was calculated as follows:

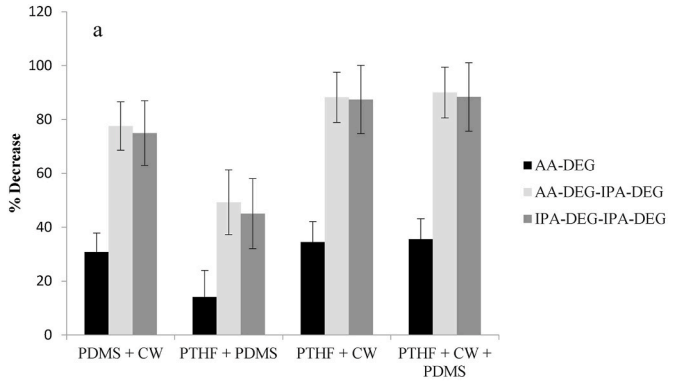
$$Decrease\% = \frac{Ao - A_i}{Ao} \; x \; 100$$

where A_o is the area of the analyte in the initial sample solution before the extraction procedure and Ai is the area of analyte in the same sample solution after the extraction procedure. This value was calculated to facilitate the optimization procedure since its calculation requires fewer steps than the global procedure and allows a quicker optimization. For measuring A_0 and A_i, a small aliquot of the aqueous sample was taken before the procedure and analyzed; and another aliquot of the same aqueous solution was also taken after the extraction procedure. The higher the % decrease, the higher the FPSE extraction capacity.

The combinations tested were: PDMS + CW, PTHF + PDMS, PTHF + CW, and PTHF + CW + PDMS. In this experiment, the use of the different FPSE membranes was sequential. Firstly, the first FPSE membrane was introduced in 10 mL of the sample with a magnetic bar and it was stirred at 700 rpm for 20 min. After that, this membrane was removed and the second FPSE membrane was introduced in the same sample and stirred using the same conditions. Finally, the two FPSE membranes were back-extracted together with 1 mL of the extraction solvent during 10 min at room temperature. Fig. 2a shows the percentage decrease of the analytes added to the sample solution after being submitted to the FPSE extraction for each type of FPSE membranes combination. The best results were obtained for the combination of PTHF + CW and PTHF + CW + PDMS. Since very similar results were obtained for both combinations, PTHF + CW was selected because the use of three FPSE membranes involved a longer analysis time.

In order to check if the analysis time could be shortened, a simultaneous extraction with both FPSE membranes (PTHF_CW), instead of a sequential extraction (PTHF + CW), was studied. The results are shown in Fig. 2b. Both methods showed a similar percentage decrease for both compounds: around 90% decrease for AA-DEG-IPA-DEG and 35% decrease for AA-DEG. Thus, the simultaneous extraction was selected (PTHF_CW) because the extraction time was 20 min shorter.

In order to confirm these results in the overall process, ER% was calculated in the final FPSE extract (Fig. 3). The final extraction was 2



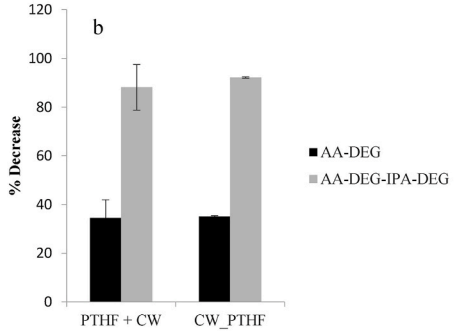


Fig. 2. Percentage decrease of several oligomers (AA-DEG, AA-DEG-IPA-DEG and IPA-DEG-IPA-DEG) in the sample solution after FPSE extraction with (a) membranes combinations used sequentially: PDMS + CW, PTHF + PDMS, PTHF + CW and PTHF + CW + PDMS, and (b) membranes combination used sequentially (PTHF + CW) and simultaneously (PTHF_CW).

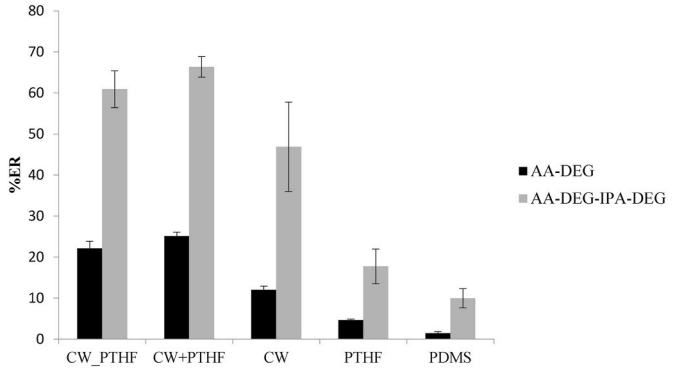


Fig. 3. Extraction recovery (%ER) for AA-DEG and AA-DEG-IPA-DEG using different membranes combination after FPSE extraction.

fold diluted with water to improve the chromatography. The ER% results confirmed that using a combination of membranes was better than using the individual ones, reaching values over 60% for AA-DEG-IPA-DEG and over 20% for AA-DEG.

3.1.3. Selection of sample volume and final extraction volume

Once the FPSE membranes were selected, three different experiments were carried out in order to improve the overall enrichment factor (EF). In these experiments, the sample volume and the extraction volume were optimized:

Experiment 1. (Exp 1): The initial protocol conditions were used. Initial sample volume was 10 mL and final extraction volume was 1 mL.

Experiment 2. (Exp 2): Initial sample volume was 10 mL and final extraction volume was of 500 μL (obtained by the concentration of 1 mL final extract under a nitrogen current and under gravimetric control).

Experiment 3. (Exp 3): Initial sample volume of 18 mL and final extraction volume of 500 μL (obtained by the concentration of 1 mL final extract to dryness under a nitrogen current and redilution with 500 μL of methanol/water).

Fig. 4 shows the results obtained from these three experiments. Experiment 3 results showed the highest EF values, reaching 1.4 for AA-DEG and 3.3 for AA-DEG-IPA-DEG.

3.1.4. Optimization of extraction and back-extraction conditions

Finally, different back-extraction conditions were optimized. All the assays were carried out in triplicate:

- One or double back-extraction. It was carried out with 1 mL of methanol (one back-extraction) and twice 1 mL of methanol (double back-extraction) in order to guarantee the maximum extraction of analytes. In both cases, final extraction volume was gently evaporated to dryness under nitrogen current and redissolved in 500 μL of methanol/water. Finally, one extraction was selected because EF values for AA-DEG and AA-DEG-IPA-DEG in the second extraction were only slightly higher (Supplementary material 2).

- Back-extraction solvent volume. The back-extraction was performed with 1 and 1.5 mL solvent volume. It was considered that since 2 FPSE membranes were used in the process, 1 mL-volume could not be enough to back-extract the analytes and a higher extraction volume could be necessary. The ER% was compared for both extraction solvent volumes and the results did not show any significant differences (p-value > 0.05).

- Sample extraction time. Due to the increase of the initial sample volume, a series of increasing extraction times was examined: 20, 30, 40 and 60 min. For the study, an aliquot of 500 μL was taken and analyzed in each studied time. A t-student test was performed in order to test if there were significant differences between the analyte areas at the 4 extraction times. The results did not show significant differences (p-value $= 0.80 > 0.05$) for any of the 3 oligomers. Therefore, the extraction equilibrium was reached at 20 min and no longer extraction times were necessary.

3.1.5. Efficiency of FPSE extraction in real samples

The next step was to check the efficiency of the FPSE extraction in a real sample such as pineapple juice. For this purpose, extraction recovery and enrichment factor studies were performed using 4 standards with similar structure to the oligomers found in migration from the biopolymer in previous studies: 2 cyclic oligomers (AA-DEG and AA-DEG-IPA-DEG) and 2 linear esters (DMS and DBS). The two linear esters were selected for the study since no oligomer standards with a linear structure were available.

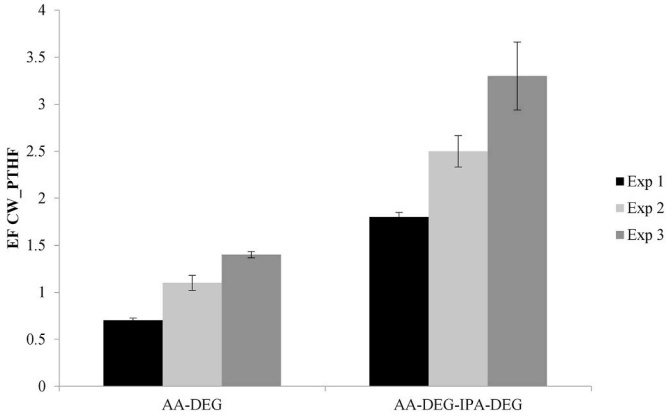


Fig. 4. Enrichment factor values (EF) for AA-DEG and AA-DEG-IPA-DEG using the membrane combination CW_PTHF after FPSE extraction according to Experiments 1, 2 and 3 (section 3.1.3).

The procedure was as follows: Aliquots of 18 mL of juice or simulant B were spiked with the 4 standards mixture, so that the final concentration was 2 mg kg^{-1} of each standard. After that, the FPSE procedure was applied and the EF and ER% of each compound was calculated using the corresponding calibration curves (Table 2).

In both simulant B and juice, EF and ER% values of linear esters (DMS and DBS) were higher than for the cyclic oligomers (AA-DEG and AA-DEG-IPA-DEG). ER% values of DMS were 45% and 62% in simulant B and in juice, respectively. The results observed in Table 2 show that EF and ER% values in acetic acid and juice were similar. This indicates that, although the composition of juice with sugar and proteins is complex, similar extraction recoveries of oligomers are expected for both matrices.

3.2. Oligomers identified in migration from the PLA/polyester sample

In this study, the FPSE extracts obtained from migration in 3% acetic acid and pineapple juice were analyzed by UHPLC-QToF. In the case of 3% acetic acid migration samples, they were also analyzed by direct injection without FPSE method. The oligomers were identified according to their retention time (rt) and exact mass and based on previous oligomer identification studies [19]. The oligomers identified are shown in Table 1.

A total of 21 oligomers (10 cyclic and 11 linear) were identified, 9 of them came from PLA and 12 from polyesters. Compounds with an asterisk "*"were identified after the FPSE procedure and compounds with double asterisk "**" were only identified in juice. PLA oligomers are made of repeated monomer units of [LA] ($C_3H_4O_2$) and show different structures: linear oligomers with the structure HO-$[LA]_n$-H (n = 4–8) and cyclic oligomers with the structure $[LA]_n$ (n = 6–9). The polyester oligomers showed also cyclic and linear configurations and were composed by adipic acid (AA), phthalic acid (PA) and/or butanediol (BD). In total, 6 linear and 6 cyclic compounds were identified. The oligomers structures included 2 or 3 types of monomers, AA and BD or AA, BD and PA.

3.3. Efficiency of FPSE membrane in the extraction of PLA and polyester oligomers

Migration samples in 3% acetic acid were used to evaluate the efficiency of the FPSE protocol method applied to the extraction and concentration of oligomers coming from a PLA/polyester packaging sample. For this purpose, EF values were calculated (EF = area after FPSE/area before FPSE) and the results are shown in Fig. 5. Fig. 6 shows the chromatograms obtained for the migration samples with simulant B before and after FPSE extraction.

All the oligomers detected in the analysis of the 3% acetic acid solution by direct injection increased their area when the FPSE method was applied. Ten out of 16, with EF values close or higher than 10. Some oligomers like $[LA]_6$, linear HO-$[LA]_8$-H, $[LA]_7$ and cyclic $[LA]_8$ even reached values close to 20. Cyclic $[LA]_9$ oligomer reached a value over 30. In addition, it was possible to identify 3 new oligomers (linear PA_2-BD_3-AA, cyclic $[AA\text{-}BD]_3$ and cyclic PA-BD_3-AA_2). They are marked with an asterisk in Table 1. These EF values were much better than those obtained for the oligomer standards during optimization (Fig. 4), which was very positive. The results demonstrated the efficacy of the FPSE method for the concentration of migrants coming from packaging materials. Since similar recovery values are expected for acetic acid 3% and juice, as it was proved in section 3.1.5, this method will be appropriate for the evaluation of migration of oligomers from this kind of biopolymers.

Table 1
Identification and quantification of oligomers in migration assays of a PLA/polyester sample. Retention time (rt), measured mass (mass), molecular formula (MF) and enrichment factor (EF).

Nº	rt	mass [MNa^+]	MF	EF	Oligomer	Concentration 3% acetic acid (μg g^{-1})	Concentration juice (μg g^{-1})
1	3.92	241.1043	$C_{10}H_{18}O_5$	1.11	Linear AA-BD	1.61 ± 0.06	<LOD
2	4.29	329.0842	$C_{12}H_{18}O_9$	3.33	Linear HO-$[LA]_4$-H	<LOQ	<LOD
3	4.64	313.1620	$C_{14}H_{26}O_6$	2.55	Linear AA-BD_2	1.94 ± 0.18	0.14 ± 0.05
4	4.85	401.1053	$C_{15}H_{22}O_{11}$	9.60	Linear HO-$[LA]_5$-H	0.08 ± 0.00	<LOQ
5	5.25	473.1271	$C_{18}H_{26}O_{13}$	11.76	Linear HO-$[LA]_6$-H	0.11 ± 0.02	<LOQ
6	5.56	545.1499	$C_{21}H_{30}O_{15}$	12.91	Linear HO-$[LA]_7$-H	0.13 ± 0.01	0.03 ± 0.00
7	5.76	455.1159	$C_{18}H_{24}O_{12}$	17.31	Cyclic $[LA]_6$	0.68 ± 0.08	0.09 ± 0.05
8	5.78	617.1703	$C_{24}H_{34}O_{17}$	20.0	Linear HO-$[LA]_8$-H	0.04 ± 0.00	<LOQ
9	5.79	513.2671	$C_{24}H_{42}O_{10}$	4.79	Linear AA_2-BD_3	0.81 ± 0.04	0.57 ± 0.08
10	6.00	527.1373	$C_{21}H_{28}O_{14}$	18.78	Cyclic $[LA]_7$	1.05 ± 0.15	0.18 ± 0.11
11	6.02	461.1782	$C_{22}H_{30}O_9$	4.48	Linear PA-BD_2-AA	0.56 ± 0.02	0.22 ± 0.03
12	6.13	423.2400	$C_{20}H_{32}O_8$	8.39	Cyclic $[AA\text{-}BD]_2$ CAS 78837-87-3	22.63 ± 2.15	19.64 ± 1.79
13	6.19	599.1591	$C_{24}H_{32}O_{16}$	18.5	Cyclic $[LA]_8$	0.57 ± 0.12	<LOQ
14	6.24	533.2356	$C_{24}H_{42}O_{10}$	2.53	Linear PA-BD_3-AA	0.30 ± 0.02	0.22 ± 0.03
15	6.41	671.1793	$C_{27}H_{36}O_18$	31	Cyclic $[LA]_9$	0.12 ± 0.03	<LOQ
16*	6.51	681.3568	$C_{38}H_{50}N_4O_6$		Linear PA_2-BD_3-AA	0.4 ± 0.02	0.22 ± 0.02
17	6.64	443.1676	$C_{22}H_{28}O_8$	9.03	Cyclic PA-BD_2-AA	7.56 ± 0.64	3.39 ± 0.58
18*	6.77	601.3229	$C_{30}H_{48}O_{12}$		Cyclic $[AA\text{-}BD]_3$ CAS 1135871-65-6	4.52 ± 0.96	3.25 ± 0.82
19*	7.11	643.2741	$C_{32}H_{44}O_{12}$		Cyclic PA-BD_3-AA_2	1.51 ± 0.25	1.57 ± 0.33
20**	7.36	843.3785	$C_{42}H_{60}O_{16}$		Cyclic PA-BD_4-AA_3	<LOD	<LOQ
21**	7.48	663.2416	$C_{36}H_{36}O_{12}$		Cyclic PA_2-BD_3-AA	<LOD	<LOQ

PA: phthalic acid. AA: adipic acid. BD: butanediol. [LA]: $C_3H_4O_2$.
*compounds only identified in acetic acid 3% after FPSE method ** compounds only identified in juice after FPSE method.

Table 2
Enrichment factors (EF) and extraction recovery percentage (ER %) of 4 standards in simulant B and juice after FPSE.

	Compound	EF	ER %
Simulant B	AA-DEG	0.4 ± 0.13	1.0 ± 1.0
	AA-DEG-IPA-DEG	2.7 ± 0.15	7.5 ± 1.1
	DMS	16.0 ± 0.84	45 ± 6.4
	DBS	13.1 ± 0.70	36 ± 5.4
Juice	AA-DEG	0.83 ± 0.082	2.3 ± 0.64
	AA-DEG-IPA-DEG	3.9 ± 0.13	11 ± 1.0
	DMS	22 ± 1.6	62 ± 12
	DBS	8.1 ± 0.39	23 ± 3.0

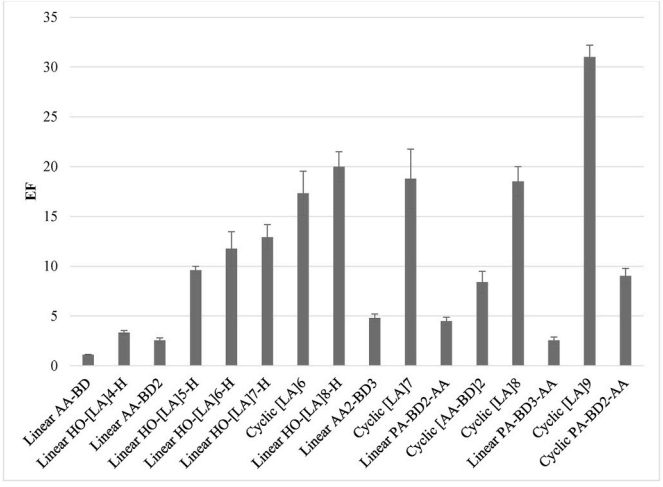


Fig. 5. Enrichment factor values (EF) of oligomers present in migration from PLA-polyester sample to simulant B.

3.4. Semi-quantification of migration of PLA and polyester oligomers

Next step was the semi-quantification of the oligomers present in migration using the isolated oligomers as standards for the calibration curves. Cyclic oligomers were semi-quantified with $(LA)_6$ and linear oligomers with HO-$(LA)_4$-H. The instrumental limits of quantification (LOQ) were 3.6 μg g^{-1} for $(LA)_6$ and 0.45 μg g^{-1} for HO-$(LA)_4$-H and the instrumental limits of detection (LOD) were 1.2 μg g^{-1} for $(LA)_6$ and 0.15 μg g^{-1} for HO-$(LA)_4$-H.

LOD and LOQ of method were calculated taking into account the concentration factor of the method ($C_F = m_0/m_f$ being m_f the final grams of extract (500 mg) and m_o the initial grams of the extraction dissolution (18 g). LOD and LOQ of method of $(LA)_6$ were 0.033 and 0.1 μg g^{-1} respectively and LOD and LOQ of HO-$(LA)_4$-H were 0.004 and 0.012 μg g^{-1}.

Concentrations were calculated taking into account the EF of each compound. For those compounds that did not have EF, the value of the compound with the most similar structure was used. For compounds numbered in Table 1 as 19, 20 and 21, EF value of compound 17 was used; for compound 18, it was used the EF value of compound 12; and for compound 16, the EF value of compound 14 was used.

The concentration of oligomers (μg g^{-1}) in 3% acetic acid and juice is shown in Table 1. Sixteen out of 21 compounds were identified in acetic acid 3% before FPSE method and 19 out of 21 compounds were identified in acetic acid 3% after FPSE method. This is a positive result because this optimized FPSE procedure is able to detect 3 new compounds (marked with an asterisk in Table 1) that were initially below their detection limit. On the other hand, 19 out of 21 compounds were identified in juice: 17 compounds were common with acetic acid 3% and 2 compounds were only identified in juice (marked with double asterisk in Table 1). They corresponded to cyclic PA-BD_4-AA_3 and cyclic PA_2-BD_3-AA.

The highest concentration value was found for cyclic $[AA\text{-}BD]_2$ with 22.63 μg g^{-1} in 3% acetic acid and 19.64 μg g^{-1} in juice. The rest of oligomers were below 7.56 μg g^{-1}. The total content of cyclic and linear oligomers was calculated. The results showed that the total concentration was higher for cyclic oligomers than for the linear ones, both in acetic acid (38.64 μg g^{-1} and 5.98 μg g^{-1}) and pineapple juice (28.12 μg

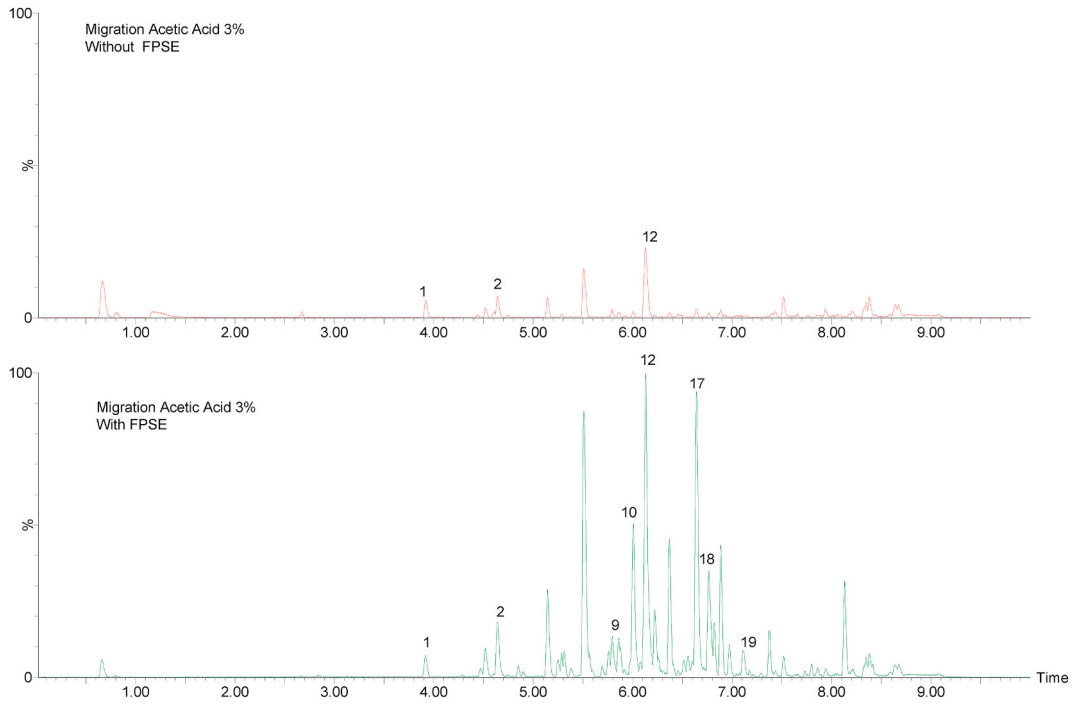


Fig. 6. Chromatograms of the extracts of migration with simulant B (acetic acid 3%) before and after FPSE method.

g^{-1} and 1.4 μg g^{-1}).

4. Conclusions

FPSE was an effective method for pre-concentration and extraction of oligomers in migration from blend PLA-polyester material in food simulants because it allowed detecting oligomers not detected in the direct injection of the sample. FPSE with UHPLC-MS-QTOF was used for the analysis of oligomers in pineapple juice and it was demonstrated that it is an effective method, without requiring additional sample treatment, even though the juice contained sugars and proteins as potential interfering ingredients. Only 2 new compounds (cyclic PA-BD_4-AA_3 and cyclic PA_2-BD_3-AA) were identified in juice respect to those found in acetic acid 3%, what demonstrates the feasibility of simulant B for this migration study. Concerning the oligomers, not only PLA oligomers migrated but also those from polyester, whose migration values were higher than PLA oligomers and could have a critical role in food safety. Due to the increasing concern of oligomers in migration from food contact materials, it is important to have a fast and effective method that evaluates consumer safety. FPSE method has demonstrated to be a good technique for these types of compounds.

Credit author statement

Sara Ubeda: Conceptualization, Methodology, Investigation, Writing - Original Draft. Margarita Aznar: Conceptualization, Methodology, Writing, Supervision Review & Editing. Cristina Nerín: Resources, Writing - Review & Editing, Supervision, Project administration Funding acquisition. Abuzar Kabir: Resources, Writing - Review & Editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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