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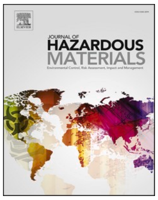

Synthesis and quantification of oligoesters migrating from starch-based food packaging materials

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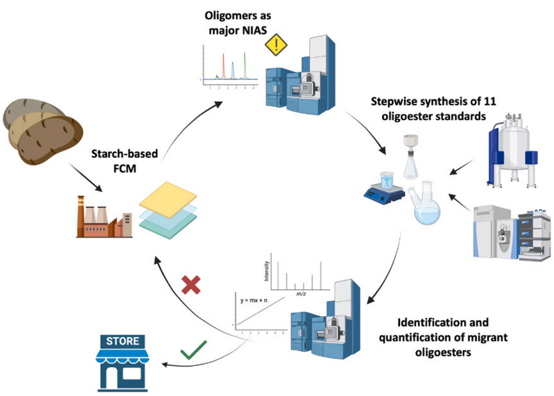
HIGHLIGHTS

- Non-targeted LC-HRMS migrant assessment from FCM starch-based biopolymers.
- Oligoesters appeared as the main NIAS.
- Oligomers from AA, BD, iPA and PG were identified as migrants.
- Stepwise synthesis of eleven cyclic and linear oligomers standards composed of 2 to 8 monomers.
- Quantification raises concern in 2 out of the 3 samples according to the TTC concept.

GRAPHICAL ABSTRACT



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ABSTRACT

The term oligomer refers to structurally diverse compounds coming from incomplete polymerisation or polymer degradation. Their ability to migrate into foodstuffs along with recent studies about their bioavailability and toxicity have risen concerns about the scarcity of standards needed to perform thorough analytical and toxicological studies. In this work, migration extracts of three starch-based biopolymers films for the packaging of fruits and vegetables were analysed according to European legislation 10/2011. Oligoesters analysed by UPLC-MS (QTOF) were the main non-intentionally added substances (NIAS) identified in the food simulants. A stepwise synthesis approach was used to synthesise and isolate eleven cyclic and linear oligoester standards ranging from 2 to 8 monomers based on adipic acid, 1,4-butanediol, isophtalic acid and propylene glycol monomers. These standards were characterised by ^{1}H and ^{13}C NMR as well as high resolution mass spectrometry. An overall high

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Abbreviations: UPLC-MS, ultra-performance liquid chromatography-mass spectrometry; TTC, threshold of toxicological concern; NIAS, Non-intentionally added substances; IAS, Intentionally added substances; PLA, poly(lactic acid); PVA, polyvinyl alcohol; PBAT, poly(butylene adipate-co-terephtalate).

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purity of > 98 % was achieved as detected by UPLC-MS(Orbitrap). The standards were then used to unequivocally identify the oligoesters in the migration assay samples by comparing their UPLC-MS/MS spectra, and to semi-quantify or fully quantify these migrant oligoesters. The oligoester quantification results deemed safe only one out of the three biopolymer films according to their threshold of toxicological concern concept. The work herein described aims to contribute towards the oligomers knowledge gaps, opening the door for comprehensive toxicological risk and absorption, distribution, metabolism, excretion and toxicity (ADMET) studies.

1. Introduction

Regarding plastics, the prefix bio- can refer to polymers coming from renewable sources and/or having a biological origin, to their biodegradability or compostability, or to a combination of both [1]. This feature, together with acceptable physical and mechanical properties and a low carbon footprint, contributes to their increasing market growth rate [2]. Whilst plastic uses comprise consumer goods, electronics, agriculture, etc., the packaging sector represents the major market (48 %) for bioplastic materials [3].

Starch-based biopolymers are one of the most popular (about 18 % of market share) bio-based plastic choices [3]. The widely available starch has to be mixed with plasticizers and other chemical moieties to improve its physical properties. The incorporation of reinforcements, chemical modifications and the blending with other co-polymers is a common practice to minimize the limitations of starch-based materials such as poor mechanical properties or high hydrophilicity [4]. When it comes to food packaging applications, research has focused on developing starch-based blends with poly(lactic acid) (PLA), polyvinyl alcohol (PVA) or poly(butylene adipate-co-terephtalate) (PBAT), among others, to improve both their physical and chemical properties [5]. However, the presence of a large and diverse number of blends represents a challenge for the risk assessment of both intentionally added substances (IAS) and non-intentionally added substances (NIAS) in food contact materials.

IAS as well as NIAS can be transferred from a packaging material into the packaged food, making migration tests a widely applied tool for the proper chemical risk assessment of a plastic food contact material (EU10/2011). IAS are usually well under control with fulfilled risk assessments and defined specific migration limits [6]. However, as diverse substances coming from impurities in the raw materials, incomplete polymerization, or polymer degradation, NIAS are still being discovered, especially in new food packaging materials ([7]; E.L. [8-13]). Oligomers, low molecular weight polymers, constitute one of the primary forms of NIAS [14]. Due to their low molecular weight (generally below 1000 Da), they can migrate from the packaging material matrix into the food and are often overlooked by polymer scientists, who focus their attention on the 10^4 up to 10^6 Da range [14]. As most biopolymers are formed by a polycondensation reaction of various monomers, structurally and chemically diverse oligoesters often represent the dominant form of NIAS [12].

Nonetheless, the lack of isolated oligoester standards results in an analytical challenge for the identification and quantification of NIAS [10,15]. Moreover, it limits the ability to perform toxicological risks assessments that would shed some light into the human and environmental exposure and absorption, distribution, metabolism, excretion and toxicity (ADMET) of these oligoesters. Hence, in recent years, efforts have been made to contribute towards the availability of migrant oligoesters [16-18]. Nevertheless, the need for oligoester reference standards is nothing but increasing [19].

In the present study, migration extracts of three starch-based biopolymer films for packaging fruits and vegetables were analysed by non-targeted LC/HRMS. From this analysis, a variety of eleven linear and cyclic oligoester combinations composed by 1,4-butanediol, propylene glycol, phthalic acid and adipic acid, were identified as possible NIAS. Based on a stepwise synthetic strategy, they were synthesized, and they were used to unequivocally confirm and quantify the NIAS oligoesters migrating from the three starch-based biopolymer samples.

2. Materials and methods

2.1. Reagents

Reagents used for analytical and migration purposes as well as for synthetic purposes are provided in the supplementary information.

2.2. Samples

The food safety of three commercial starch-based films (S1, S2 and S3) for food packaging applications were investigated in this study. Samples were provided by a local manufacturer and their formulation was not disclosed. Their thicknesses were measured using a digimatic micrometer from Mitutoyo (Kanagawa, Japan) as being 26.5 ± 1.1, 25.5 ± 1.8 and 29.8 ± 2.4 µm for films S1, S2 and S3, respectively.

2.3. Migration tests

All the migration experiments were performed in triplicate and according to the European Regulation for food contact materials EU/10/2011 [20]. Migration tests were performed using three different food simulants: ethanol 10 % (*v/v*, simulant A, hydrophilic foods), acetic acid 3 % (*w/v*, simulant B, hydrophilic foods with pH < 4.5) and Tenax® (simulant E, dry foods). For simulants A and B, migration tests were performed by total immersion of 5 × 1 cm cut-offs in 20 mL vials which were filled according to the 6 dm^2 contact surface/kg of simulant rate, established by the Regulation EU/10/2011. For simulant E migration experiments, 4 × 2 cm cut-offs of were placed in direct contact with 0.32 g of Tenax® inside aluminium foil pouches following the 4 $g.dm^{-2}$ ratio established by UNE-EN-14338 [21] and placed inside glass Petri dishes. Migration experiments took place in an oven at 40 °C for 10 days. Migration extracts from simulants A and B were directly injected in the UPLC-MS(QTOF) system. Prior to injection, Tenax® samples from each migration experiment were extracted twice with ethanol following the methodology designed by Vera *et al. Vera et al.,* [22]. The recovered ethanol was then filtered with a PTFE syringe filter (0.45 µm) and concentrated to approximately 0.5 mL under a gentle stream of nitrogen. This concentration step was gravimetrically monitored.

2.4. UPLC-HRMS analysis of migration extracts

Chromatographic separation was carried out on a CORTECS UPLC BEH C18 column (1.6 µm, 2.1 ×100 mm) using an UPLC Acquity system, both from Waters (Milford, MA, USA). Chromatography was performed at 0.3 $mL.min^{-1}$ column flow using water (phase A) and methanol (phase B) both with 0.1 % (*v/v*) formic acid as mobile phases. Column temperature was set at 35 ºC and the injection volume was 10 µL. Chromatography started at A:B gradient of 95:5 (*v/v*), changed to 5:95 (*v/v*) in 6 min and stayed at these conditions for an additional 4 min, going back to the initial 95:5 (*v/v*) gradient conditions to pre-condition the column for 3 min

The UPLC system was connected through an ESI probe to a Xevo G2 QTOF mass spectrometer from Waters. Instrument configuration was as follows: capillary at 2.8 kV, sampling cone at 35 V, extraction cone at 3 V, source temperature at 150 ºC, desolvation temperature at 450 ºC, cone gas flow at 40 L h^{-1} and desolvation flow at 600 L h^{-1}. Acquisition was carried out in sensitivity MS^E mode, allowing the acquisition over a range of collision cell energies (CE) from 15 to 30 V during the same run. Data were recorded using Masslynx® v4.1 software.

The identification of compounds was performed by comparing migration extracts with a migration blank and following a previously described methodology [23] to achieve level 2b of the scale proposed by Schymanski *et al*. Schymanski et al., [24]. Level 5 (lowest) comprises an accurate measurement of the mass-to-charge ratio (*m/z*). Level 4 is achieved with an unambiguous molecular formula. Level 3 is obtained when multiple potential structures are feasible. Level 2 can be claimed by the proposal of a single structure supported by experimental diagnostic evidence such as MS^2 (2b) or matching library spectra (2a). Final confirmation of the structure (level 1) is only reached through comparison with a reference standard. Briefly, using the low energy spectrum, the precise mass of precursor ions was used to determine the lowest mass error and the highest isotopic fit of the elemental composition options proposed by Masslynx. Afterwards, the selected elemental compositions were linked to a chemical structure using different chemical databases (e.g. Chemspider, Scifinder) and freely available software (NIAS-db 1.0, [16]) by paying attention to the chemical criteria and background experience about NIAS and IAS in bio-based polymers. Finally, a candidate molecule was selected using its high-energy mass spectrum. For being selected as a candidate, at least two main fragment ions of the high energy mass spectrum showed a score value below 3 using the MassFragment® tool from Masslynx. The score value was calculated by the software based on fragmentation probabilities.

2.5. Synthesis and characterization of oligoester standards

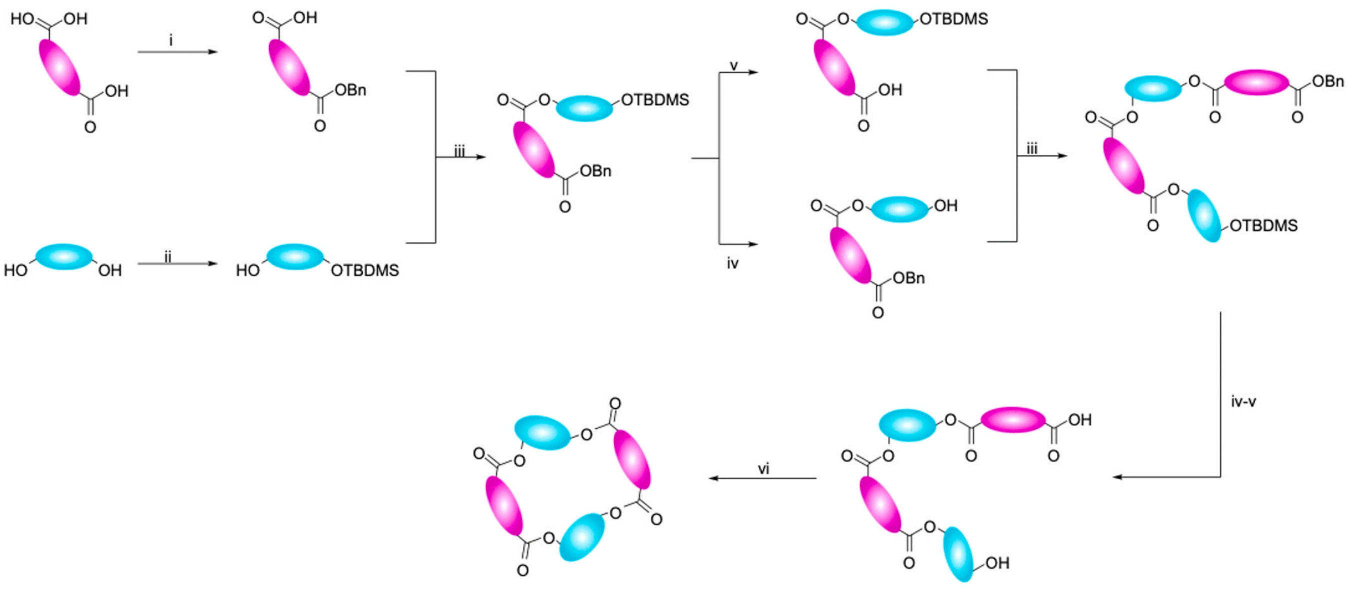
The selection of oligoester candidates to be synthesized was made with regards to obtain as many structurally diverse oligoesters as possible but firstly considering the hypothesised oligoesters in the migration extracts. An optimized stepwise oligoester synthesis strategy previously described [16] was readapted for the preparation of the new identified substances arising from the above-mentioned migration protocols. The synthesis of these oligoester standards involves the use of new diol monomers and new diacid counterparts. Scheme 1 briefly summarises all reactions in the stepwise synthesis sequentially implemented involving successive monosilylation, monobenzylation, debenzylation, desilylation, esterification and macrolactonization. Products were purified after each reaction by flash column chromatography using an automatic Reveleris Büchi apparatus (Flawil, Switzerland) fitted with pre-packed high purity 40 µm silica cartridges (4 to 220 g, Büchi). An in-depth description of the synthesis protocol, monitoring, and characterisation equipment (^{1}H and ^{13}C NMR, ESI-TOF-HRMS) is provided in the supplementary information.

2.6. Purity assessment of oligoester standards

To perform a purity assessment of the synthesized oligoester compounds, each standard was first solubilised with DCM and further diluted gravimetrically with ACN to obtain a 10 µg.g^1 solution. Each solution was characterised on a UHPLC UltiMate 3000 coupled to an Orbitrap Q Exactive instrument fitted with a heated electrospray ionisation source (UHPLC-ESI-MS-Orbitrap), both from Thermo Scientific (Waltham, MA, USA). Chromatographic separation was achieved at 40 ºC on a C_{18} Hypersil Gold column (1.9 µm, 2.1 × 100 mm) from Thermo Fisher Scientific (San José, CA, USA). The flow rate was set at 0.4 mL. min^{-1} and the mobile phase was composed of 10 mM ammonium acetate in both water (A) and acetonitrile (B). Separation began with a A:B gradient of 95:5 (*v/v*) (1 min) and ramped to a gradient of A:B 100:0 (*v/v*) over 16 min, to be maintained over 9 min before going back to the initial conditions (2 min). The ionization parameters were as follows: sheath gas flow, 50 arbitrary units (AU); auxiliary gas flow, 10 AU; capillary temperature, 350 °C; heater temperature, 300 °C; spray voltage, 3.5 kV; S-lens radio frequency, 70 AU. Data were acquired in full scan by using the positive/negative switching mode over the *m/z* range 100 – 1064 at a nominal resolving power of 70,000. Automatic gain control (AGC Target) was set at high dynamic range (1×10^5) and maximum injection time (IT) at 250 ms. Purity percentage was determined by measuring the peak area of the $[M + H]^+$, $[M + Na]^+$, $[M + K]^+$, $[M + NH_4]^+$, $[M – H]^-$ and $[M + HAc – H]^-$ present adducts of the oligoester and the impurities.

2.7. Identification and quantification of oligoesters in migration extracts

In the present study, synthesized oligoester standards were used to achieve Schymanski's level 1 identification, putting together the information obtained from Section 2.4. and the relative retention time,



Scheme 1. Reaction sequence involved in the stepwise synthesis of oligoester standards. Method A: (i) BnBr, NaHCO3, Dioxane/DMF; Method B: (ii) TBDMSCl, Et3N, DMAP, DCM; Method C: (iii) EDC.HCl, DMAP, DCM; Method D: (iv) HF.Pyr, THF; Method E: (v) H2, Pd(OH)2, iPrOH; Method F: (vi) 2,4,6-Trichlorobenzoyl chloride, Et3N, DMAP, 10–3 M in THF.

chromatographic peak shape and MS^2 fragmentation pattern. To this end, migration extracts, migration blanks and a 10 μg g^{-1} solution containing the synthesized oligoester were analysed by UPLC-MS (QTOF). Chromatographic parameters and instrument configuration were kept the same as in Section 2.3. However, data acquisition was performed using the MS^2 function by selecting the most abundant adduct (as observed in full scan mode) as parent ion to then apply a CE potential ramp (20 to 50 V) to favour both low and high mass fragments. To avoid co-eluting interferences, only one parent ion was fragmented at each time window.

Quantification was conducted using the external calibration method. To account for impurities between oligoester standards, different sets of 14 points calibration curves were gravimetrically prepared at the following concentrations: 10,000, 5000, 2500, 1000, 750, 500, 300, 150, 100, 50, 20, 10, 5 and 1 ng.g^{-1}. The analysis method was the same as that employed for the analysis of migration samples in Section 2.3. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as the smallest concentration of analyte that provided a signal to noise ratio three times and ten times the blank signal, respectively.

3. Results and discussion

3.1. NIAS migration in starch-based biopolymer films

Detected compounds in food simulants A, B and E after contact with the three starch-based biopolymer film samples are shown in Table 1. No IAS were detected in any of the samples through the non-targeted analysis. However, 22 oligoester combinations were identified as suggested by MassFragment, which accounted for 90 % of the cumulative area of signals after blank subtraction ($n = 23$, 175×10^3 AU). Migrant oligoesters added up to 97 %, 96 % and 85 % in simulant A ($n = 11$, 75×10^3 AU), B ($n = 18$, 54×10^3 AU) and E ($n = 10$, 46×10^3 AU), respectively.

In terms of monomeric units, even cyclic combinations were dominated by 4-unit combinations ($n = 4$, 91×10^3 AU), followed by 6-units ($n = 2$, 13×10^3 AU), 8-units ($n = 2$, 9×10^3 AU) and 2-units ($n = 2$, 8×10^3 AU) combinations. The only odd cyclic combinations hypothesised were composed of lactic acid monomers alone and were a 7-units (11×10^3 AU) and a 5-units (3×10^3 AU) combination. Linear oligoesters were less abundant, ranging from 2 to 7 units oligomers, the most intense being the 5-units ($n = 2$, 13×10^3 AU), followed by 2-units ($n = 2$, 10×10^3 AU) and 4-units ($n = 2$, 8×10^3 AU). Only one combination for each of the 3-units (2×10^3 AU), 6-units (0.1×10^3 AU) and 7-units (0.2×10^3 AU) could be found. It could be highlighted that cyclic oligoesters are favoured compared to linear oligoesters, the latter being diols or hydroxy acids. No diacid linear combinations were found, maybe due to their higher reactivity [25]. Oligoester combinations involved 5 diols: butanediol (BD), ethylene glycol (EG), propylene glycol (PG), diethylene glycol (DEG) and trimethylolpropane (TMP); and 3 diacids: adipic acid (AA), sebacic acid (SA) and phthalic acid (PA, undefined isomer). Lactic acid (LA) did not combine with any other monomer as it was not found in combination with any diol or diacid monomers. BD and PG were the most abundant diols, being present in 42 % and 21 % of the oligomers, respectively. Likewise, AA was the most common diacid, being 64 % of the oligoester combinations, followed by phthalic acid (8 %) and sebacic acid (4 %).

Linear and cyclic combinations of AA and BD were common across samples 1 and 3, which indicated a type of blend using poly(1,4-butylene adipate) (PBA), a biopolymer commonly blended with other polyesters to increase their biodegradability and mechanical properties [26]. Various oligoester forms of AA and BD have been reported in other biodegradable food contact materials, with the most common being the

Table 1

Compounds hypothesised in the migration of three biopolymers samples (S1, S2 & S3) in food simulants A (ethanol 10 % *v/v*), B (acetic acid 3 % *w/v*) and E (Tenax®). Molecular formula (MF); linear (lin) and cyclic (c) proposed candidates; remarks, main fragments, and their scores (S) obtained by MassFragment. ID level 1 * was achieved by using the herein developed oligoesters standards due to the unavailability of reference standards. LA: lactic acid, BD: 1,4-butanediol, AA: adipic acid, EG: ethylene glycol, PG: propylene glycol, DEG: diethylene glycol, SA: sebacic acid, PA: phthalic acid, TMP: trimethylolpropane, Rt: retention time, nd: not detected. The symbol "-" was used when no information could be obtained.

Rt	*m/z* $[MNa]^+$	MF	S1	S2	S3	Candidate	ID level	Remarks/Fragments (scores)
4.37	213.0738	$C_8H_{14}O_5$	E	E	E	lin[2LA+C_2H_5]	2a	PLA oligomer. 161.0450 (S0.5) 158.0256 (S1)
4.4	241.1047	$C_{10}H_{18}O_5$	A	nd	A	lin[BD+AA] (86923 −79 −7)	1 *	Polyester oligomer. 202.1232 (S0.5) 147.0637 (S.05)
4.9	367.1349	$C_{16}H_{24}O_8$	nd	A,B,E	nd	c[2EG+ 2AA]	2a	Polyester oligomer. 346.1604 (S0.5) 174.0846 (S0.5)
4.96	313.1623	$C_{14}H_{26}O_6$	A	nd	A	lin[2BD+AA] (20985 −13 −1)	1 *	Polyester oligomer. 155.0724 (S1.5) 111.0431 (S1)
5.00	281.1478	-	E	nd	E	-	-	259.1628, 143.0996
5.08	413.1784	$C_{18}H_{30}O_9$	nd	A	nd	lin[2PG+ 2AA]	1 *	Polyester oligomer. 331.1837 (S1) 245.1081 (S1)
5.14	301.2847	$C_{17}H_{37}N_2O_2$	nd	nd	A,E	-	-	301.2847, 149.0060
5.54	395.1679	$C_{18}H_{28}O_8$	nd	A,B,E	nd	c[2PG+ 2AA]	1 *	Polyester oligomer. 203.0996 (S2) 115.0793 (S2)
5.60	357.1162	$C_{14}H_{22}O_9$	nd	E	nd	lin[4LA+C_2H_5]	2a	PLA oligomer. 315.0782 (S3) 119.0626 (S3)
5.67	383.0966	$C_{15}H_{20}O_{10}$	E	nd	E	c[5LA]	2a	PLA oligomer. 158.0196 (S2) 89.0230 (S2)
5.71	369.1543	$C_{16}H_{26}O_8$	A	nd	A	-	-	329.1606, 201.1153
5.85	441.2094	$C_{20}H_{34}O_9$	A	nd	A	lin[2BD+ 2AA]	1 *	Polyester oligomer. 401.2176 (S0.5) 291.1791 (S0.5)
5.87	455.1153	$C_{18}H_{24}O_{12}$	A,B,E	nd	E	c[6LA]	2a	PLA oligomer. 307.1044 (S2) 273.0987 (S2)
5.90	295.1526	$C_{14}H_{24}O_5$	nd	nd	E	c[DEG+SA]	2a	Polyester oligomer. 227.1252 (S2) 203.1207 (S2)
5.92	513.2682	$C_{24}H_{42}O_{10}$	nd	nd	A	lin[3BD+ 2AA]	1 *	Polyester oligomer. 458.2516 (S1) 329.1677 (S1)
6.01	567.2431	$C_{28}H_{38}O_{12}$	nd	A,B	nd	lin[2BD+ 2AA+PA]	2a	Polyester oligomer. 228.1633 (S1) 129.0535 (S0.5)
6.15	527.1379	$C_{21}H_{28}O_{14}$	A,B	A,B	A	c[7LA]	2a	PLA oligomer. 158.0272 (S2) 175.1000 (S3)
6.23	423.1999	$C_{20}H_{32}O_8$	A,B,E	nd	A,B,E	c[2BD+ 2AA] (CAS 78837 −87 −3)	1 *	Polyester oligomer. 311.1474 (S2) 213.1034 (S3)
6.27	739.3167	$C_{34}H_{52}O_{16}$	nd	A,B	nd	c[2EG+ 2PG+ 4AA]	2a	Polyester oligomer. 373.1862 (S0.5) 315.1352 (S0.5)
6.30	641.3145	$C_{30}H_{50}O_{13}$	nd	nd	A	lin[3BD+ 3AA]	1 *	Polyester oligomer. 547.2840 (S0.5) 431.2293 (S1)
6.31	595.2734	$C_{28}H_{44}O_{12}$	nd	A,B	nd	c[2BD+ 3AA+EG]	2a	Polyester oligomer. 402.2205 (S2) 301.1345 (S2)
6.49	767.3488	$C_{36}H_{56}O_{16}$	nd	A,B	nd	c[4PG+ 4AA]	1 *	Polyester oligomer. 333.1578 (S2) 261.1282 (S2)
6.58	279.1573	$C_{14}H_{24}O_4$	nd	nd	E	c[AA+TMP]	2a	Polyester oligomer. 257.1755 (S0.5) 130.1387 (S0.5)
6.63	443.1689	$C_{22}H_{28}O_8$	A,B	nd	A,B,E	c[2BD+AA+PA]	1 *	Polyester oligomer. 307.1150 (S2) 221.0857 (S2)
6.67	623.3052	$C_{30}H_{48}O_{12}$	A,B	nd	A,B	c[3BD+ 3AA]	1 *	Polyester oligomer. 457.2471 (S3) 429.2100 (S3)
7.03	545.1516	$C_{21}H_{30}O_{15}$	A	nd	nd	lin[7LA]	2a	PLA oligomer. 319.1362 (S1.5)
7.74	256.2641	$C_{16}H_{34}NO$	nd	nd	B	-	-	125.9877, 158.0055
7.86	282.2764	$C_{16}H_{37}NO$	nd	nd	B	-	-	125.9877, 247.2442
8.36	284.2945	$C_{18}H_{38}NO$	nd	nd	B	-	-	125.9865, 158.0121
8.45	319.1955	-	nd	nd	B	-	-	125.9862, 365.2050
10.6	536.1666	-	E	nd	nd	-	-	125.9881, 369.2711

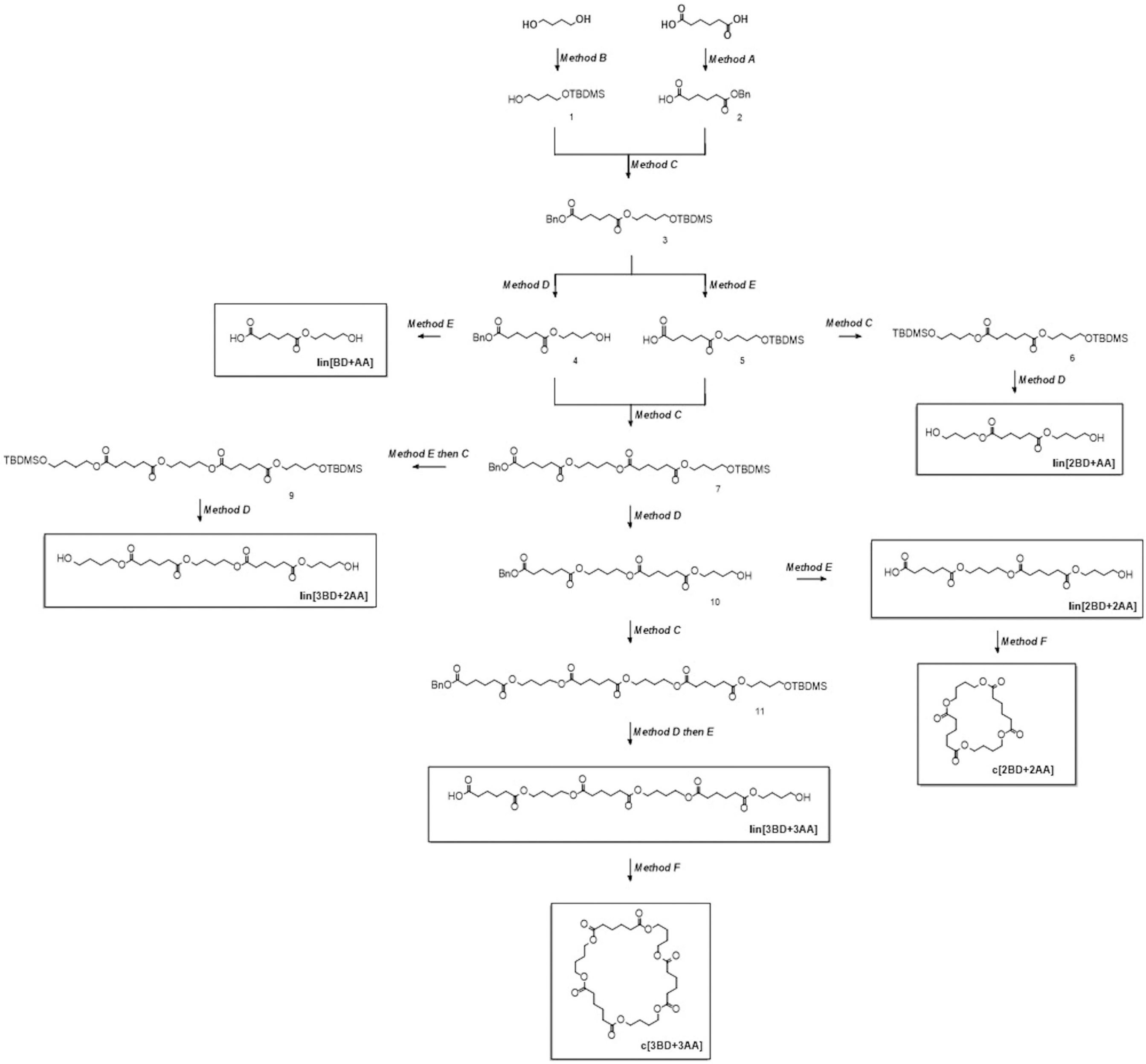
cyclic form of the tetramer c[2BD+ 2AA] ([7,27,16,26]; E.L. [8]). As blends of PLA and PBA with PBAT increase the barrier properties of the resulting material ([28]; H. [29]), we hypothesized that oligomer combinations containing BD, AA, and PA indicate an attempt to improve the barrier properties of the resulting starch-based material.

When comparing the 3 starch-based materials, two lactic acid oligomers, lin[2LA+C_2H_5] and c[7LA], were the only combinations found across all three samples, suggesting a common PLA component. Ethoxylation (+C_2H_5) of LA oligomers occurs during the migration process in contact with ethanol, meaning no ethoxylated oligoesters would have migrated into the foodstuff [7]. Sample 2 showed forms of PG and EG with adipic acid, pointing in this case to the presence of poly(propylene glycol adipate) (PPA) and poly(ethylene glycol adipate) (PEA) as plasticisers [30,31]. Only one combination of SA and DEG, commonly used as raw materials in polyester manufacturing ([32]; N. [33]), was found forming a cyclic dimer in sample 3. TMP is a substance used in the production of hyperbranched polyesters, a type of polymer with good properties as coating agents [34]. Interestingly, another cyclic dimer containing AA and TMP, could also be hypothesised in sample 3.

3.2. Synthesis of oligoester standards

3.2.1. Selection of combinations

Overall, the lack of NIAS reference compounds represents a major obstacle not only for their use as analytical standard to unequivocally identifying and quantifying them in samples, but also for the need to perform mechanistic and toxicological studies [14]. When it comes to oligoesters, only a few syntheses have been attempted. Regarding oligoesters containing AA as diacid and BD as diol, only c[2BD+ 2AA] was available to purchase (LGC standards, Middlesex, UK). However, to the best of our knowledge, no attempt has been performed to synthesize a series of AA and BD oligoesters, so the stepwise synthesis (Scheme 2) was seen as an opportunity to obtain not only c[2BD+ 2AA], but the rest of linear and cyclic oligoesters ranging from 2 to 6 units. As some oligoesters containing EG combined with terephthalic acid [17] and LA oligomers [35] have already been synthesized, the efforts were focused



Scheme 2. Stepwise synthesis of linear and cyclic oligoesters derived from butanediol (BD) and adipic acid (AA).

in attempting the synthesis of oligomers containing PG as diol (Scheme 4). Most of the described oligomeric syntheses involve the use of PA (both isophthalic, iPA, and terephthalic, tPA) as diacid [16,36,17,18]. However, as none involved the production of c[2BD+iPA+AA], it was decided to attempt it specially because oligoesters containing an aromatic moiety have a higher toxicity potential [37]. In this case, iPA was chosen as PA isomer due to its preferable use to improve barrier properties in biopolymer production [38].

3.2.2. Synthesis

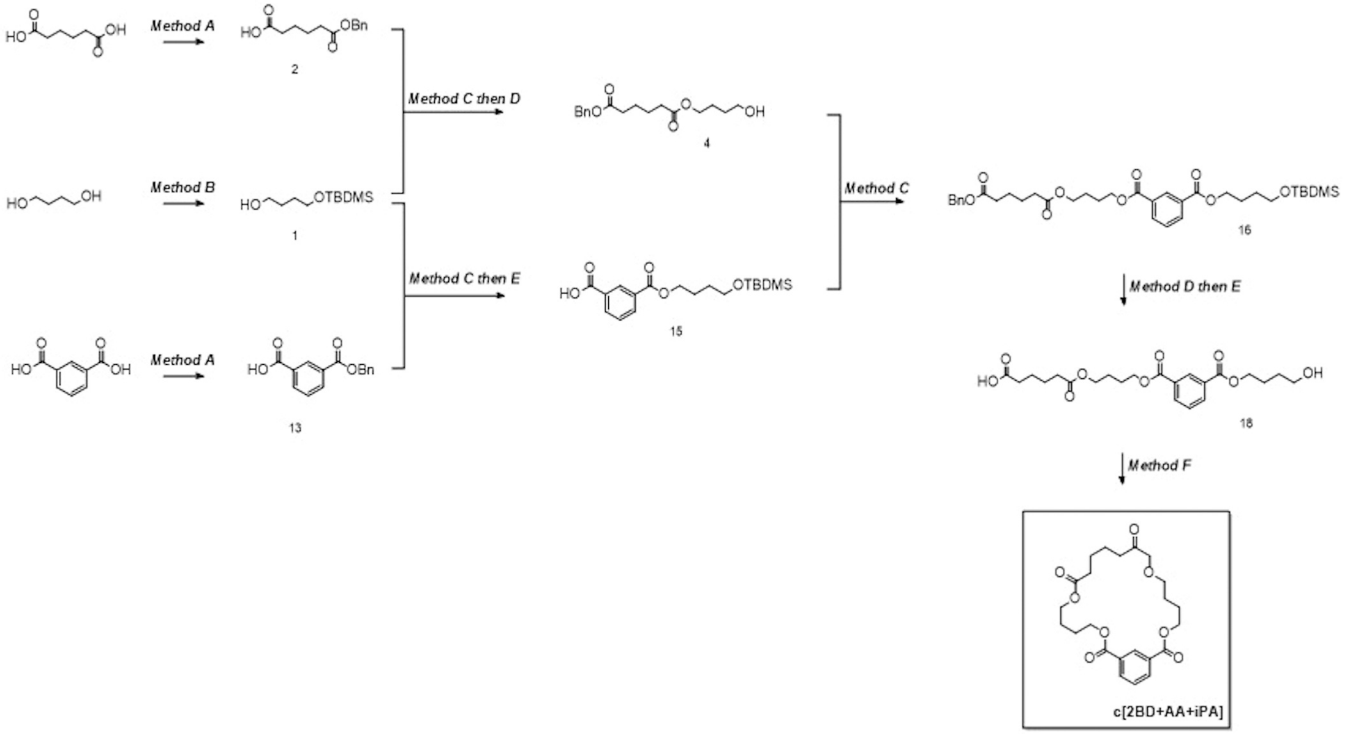
Similarly to the procedure described by [16], a stepwise synthetic approach was applied in this work to prepare new linear or cyclic oligomers based on new diacid monomer such as adipic acid, or new diols units such as butane-1,4-diol or propylene glycol. This robust and well-adaptable multistep synthesis allowed us to yield the expected oligomers with an excellent control of the size and with clean purity for further semi-quantitative analysis.

Our efforts were first dedicated to the preparation of oligoesters derived from butanediol (BD) and adipic acid (AA) (Scheme 2). In comparison to the original synthesis described by [16], a slight modification was implemented for the monobenzylation reaction to furnish the monobenzylated ester **1** in view of facilitating the treatment of the reaction while also improving the yield [39]. Likewise, the rest of the synthesis worked very effectively for the size elongation of the linear oligomer in a well-controlled fashion. Along with this sequential synthesis, the linear oligomers lin[BD+AA], lin[2BD+AA], lin[3BD+ 2AA], lin[2BD+ 2AA], lin[3BD+ 3AA] were successfully obtained as highly valuable intermediates which are also part of the identified NIAS detected as migrants from the biopolymer films. Our results showed that the final and tedious macrolactonization was well amenable for different size of cyclic oligomers to obtain either the 4-units c[2BD+ 2AA] or the 6-units c[3BD+ 3AA], with yields of 26 % and 18 %, respectively.

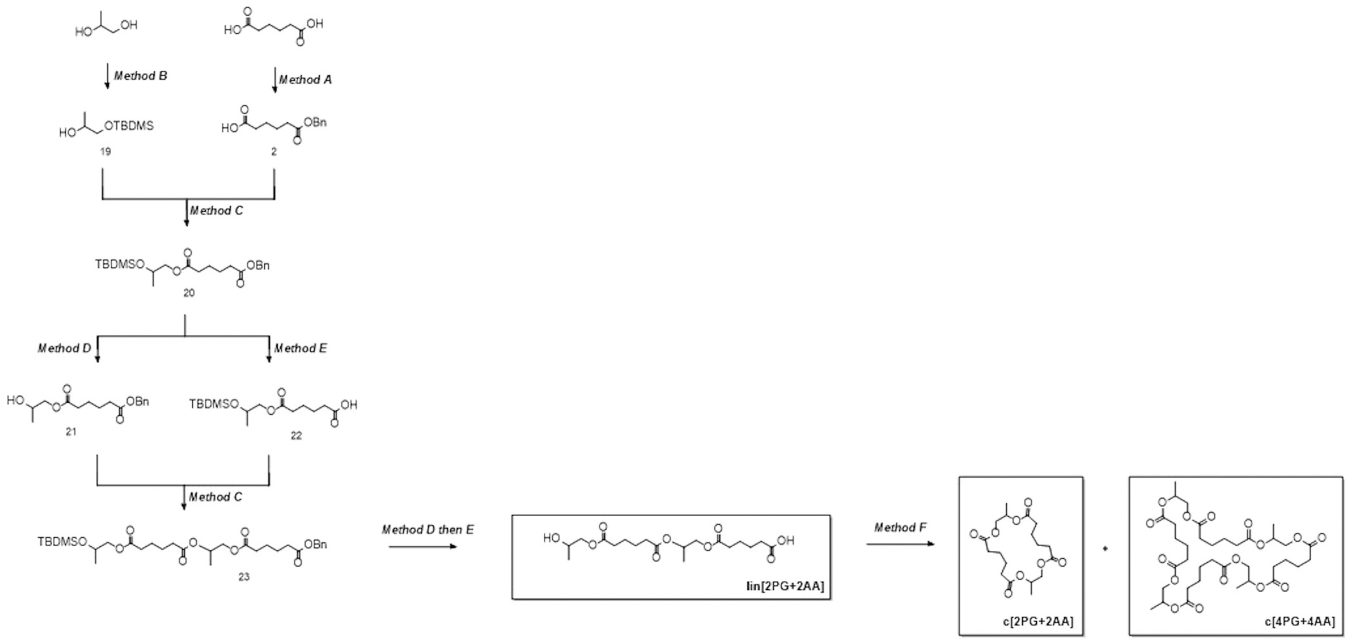
As represented by the following cyclic oligoester c[2BD+iPA+AA] (Scheme 3), the stepwise synthesis allowed the preparation of this expected cyclic 4-units-compound with a global yield of 38 % over 11 synthetic steps. This result showed the great efficacy of the synthesis and that this synthetic strategy was well-adaptable to produce hybrid oligomers based on several diacid units (i.e. AA and iPA). Finally, our results showed that this stepwise synthesis was highly suitable for the preparation of non-symmetrical oligomers bearing a diol unit represented with two differential reactivity of the hydroxyl groups such as a primary and a secondary alcohol (Scheme 4). This type of dual reactivity of the diol partner was applied for the preparation of propylene glycol-based oligomers which are consistent with other NIAS arising from biofilms. Once again, the stepwise synthesis was very appropriate for the synthesis of linear and cyclic oligoesters derived from propylene glycol (PG) and adipic acid (AA). The linear oligomer lin[2PG+ 2AA] was cleanly obtained with a global yield of 52 % over 9 steps. This compound was used as precursor of the macrolactonization for the most critical step as intramolecular reactions can be favoured over intermolecular ones. While good macrolactonization yields were generally observed (up to 80 %), the macrolactonization of lin[2PG+ 2AA] afforded the expected cyclic tetramer c[2PG+ 2AA] with a lower yield (73 %), because of the concomitant formation of the octamer c[4PG+ 4AA] (15 %) along with the dodecamer c[6PG+ 6AA] (5 %). The complex mixture components were separated by flash chromatography and only c[2PG+ 2AA] and c[4PG+ 4AA] reached adequate quantities for a complete characterisation and further use as reference standards.

3.2.3. Characterisation

Characterisation data of the 11 synthesized oligoesters and their reaction intermediates (^{1}H and ^{13}C NMR, ESI-TOF-HRMS and melting point if applicable) can be found in the supplementary information. Table 2 showcases the chemical purities (*w/w*) of the final products. No impurities were identified for c[2BD+ 2AA], and most of the compounds showed a purity above 98 % *w/w*. The three hydroxy acids from AA and BD; lin[BD+AA], lin[2BD+ 2AA] and lin[3BD+ 3AA] were the products with the highest impurity percentages (29.7 to 98.8 %). Most of the impurities involved side esterification reactions that resulted in larger chain oligoesters. This might be due to the fact that these impurities were formed during co-evaporation of the products under heat and vacuum, as continued removal of water can pull the equilibrium of the



Scheme 3. Stepwise synthesis of cyclic tetramer oligoester c[2BD+iPA+AA].



Scheme 4. Stepwise synthesis of linear and cyclic oligoesters derived from propylene glycol (PG) and adipic acid (AA).

Table 2
LC-ESI-MS(Orbitrap) purity percentages of the synthesised oligoesters (*w/w*) and their impurities.

Compound	Purity	Impurities
lin[BD+AA]	29.7 %	lin[2BD+ 2AA] 54.3 %, lin[3BD+ 3AA] 11.4 %, lin[BD+AA+C_2H_5] 3.0 %, lin[2BD+ 2AA+C_2H_5] 1.7 %
lin[2BD+AA]	99.3 %	lin[3BD+ 2AA] 0.7 %
lin[2BD+ 2AA]	81.6 %	lin[4BD+ 4AA] 13.6 %, lin[3BD+ 3AA] 2.3 %, lin[2BD+ 2AA+C_3H_8] 1.0 %, c[2BD+ 2AA] 0.8 %, lin[2BD+AA] 0.6 %, lin[BD+AA] 0.1 %
c[2BD+ 2AA]	100 %	-
lin[3BD+ 2AA]	97.3 %	$C_{18}H_{24}NO$ 2.3 %, $C_{12}H_{28}NO_3$ 0.4 %, lin[2BD+AA] 0.1 %
lin[3BD+ 3AA]	98.8 %	$C_{34}H_{60}NO_{14}$ 1.0 %, lin[3BD+ 2AA] 0.1 %, lin[2BD+ 2AA] 0.1 %
c[3BD+ 3AA]	98.6 %	$C_{16}H_{36}NO_2$ 1.1 %, 650.3743 *m/z* [M+NH_4] 0.3 %
c[2BD+AA+iPA]	98.7 %	c[2BD+ 2AA] 1.3 %
lin[2PG+ 2AA]	98.7 %	lin[3PG+ 3AA] 0.9 %, c[2PG+ 2AA] 0.4 %
c[2PG+ 2AA]	98.0 %	$C_{48}H_{20}O_2$ 1.0 %, $C_{49}H_{22}O_2$ 1.0 %
c[4PG+ 4AA]	98.2 %	$C_{47}H_{56}NO_{23}$ 1.3 %, $C_{49}H_{57}O_6$ 0.4 %, c[2PG+ 2AA] 0.1 %

esterification reaction [40].

3.3. Unequivocal identification and quantification

In order to verify the chemical structure of the oligomer candidates proposed during the identification of migration extracts, MS^2 spectra of precursor ions were compared with the spectra of a 10 mg.kg^{-1} oligoesters solution. All synthesised oligoesters were identified with the retention time criteria and a minimum of 4 matching MS fragments. The application of the collision energy ramp proved to be a good tool to generate low and high mass fragments (see supplementary information). To the best of our knowledge, this is the first time that lin[BD+AA], lin [2BD+AA], lin[2BD+ 2AA] c[2BD+ 2AA], lin[3BD+ 2AA], lin [3BD+ 3AA], c[3BD+ 3AA], c[2BD+AA+iPA], lin[2PG+ 2AA], c [2PG+ 2AA] and c[4PG+ 4AA] have been unequivocally identified as migrant oligoesters from biopolymer samples. As isolated compounds characterized by NMR and UPLC-MS from a stepwise controlled synthesis, and the impossibility of achieving a full level 1 ID on the Schymanski scale due to the unavailability of reference standards, the in-house oligoesters standards were used to propose a 1 * ID level (Table 1).

For quantification purposes, linearity and sensitivity were first determined on standard calibration curves. Gravimetrically prepared solutions of the oligoester standards were analysed by UPLC-MS(QTOF) and the area of the most abundant adduct for each compound ([M + H]$^+$ or [M + Na]$^+$) was obtained with a 10 ppm *m/z* tolerance. Linearity parameters for each analyte are presented in Table 3. Relative error or bias in the proposed linear range was within the acceptable order of ± 20 %. Limits of detection (LOD) ranged from 0.03 to 2.54 µg.kg^{-1}, which is in range of what other authors found for similar compounds [41]. Given that the slope represents the sensitivity of a method towards an analyte, we found differences between slopes of up to 30 times of c [2BD+AA+iPA] and lin[3BD+ 3AA]. Although the variation is less pronounced when comparing more chemically alike oligoesters, it is still relevant and will affect the final concentration values and, consequently, the safety compliance of the material. For example, between cyclic oligomers differences in response ranged from 1.1 to 6 times and for linear oligomers from 1.1 to 14 times. Even though differences in the ionization efficiency and transmission between analytes are known to be dependent on several factors (e.g. polarity, size, mobile phase composition), the understanding of the ESI process is still limited [42,43]. Moreover, the lack of oligomer standards makes semi-quantification the only alternative strategy to be applied for reporting purposes [12].

Migration values of migrant oligoesters (µg.kg^{-1} of food simulant) are summarised in Table 4. For oligoesters for which a standard was not synthesised, a quantifying standard from Table 3 was chosen according to their structure-type (linear or cyclic), size and free functional groups.

Comparing migration results from the 3 food simulants, simulant E showed the lowest migration values. In addition, due to Tenax different migration profile compared to simulants A and B, simulant E values were

Table 3
Linearity data of the oligoester standards analysed by UPLC-MS(Q-TOF). Linear range, LOD and LOQ values are expressed in µg kg^{-1}. QS: quantifying standard.

QS no.	Compound	Quant *m/z*	Adduct	Slope	Intercept	R^2	Linear range	LOD	LOQ
Q1	lin[BD+AA]	241.1046	$[M + Na]^+$	10.18	21.87	0.999	6.2 - 1000	1.87	6.22
Q2	lin[2BD+AA]	313.1622	$[M + Na]^+$	18.02	206.84	0.998	1.5 - 1000	0.44	1.47
Q3	lin[2BD+ 2AA]	441.2095	$[M + Na]^+$	12.80	107.87	0.999	1 - 1000	0.31	1.04
Q4	c[2BD+ 2AA]	401.2170	$[M + H]^+$	8.73	35.35	0.999	1.7 - 1000	0.52	1.74
Q5	lin[3BD+ 2AA]	513.2670	$[M + Na]^+$	24.39	62.91	0.999	0.4 - 150	0.13	0.44
Q6	lin[3BD+ 3AA]	619.3324	$[M + H]^+$	1.66	−29.39	0.998	8.5 - 1000	2.54	8.46
Q7	c[3BD+ 3AA]	601.3219	$[M + H]^+$	7.86	−56.86	0.999	1.1 - 1000	0.33	1.11
Q8	c[2BD+AA+iPA]	443.1676	$[M + Na]^+$	48.52	477.25	0.999	0.1 - 500	0.03	0.09
Q9	lin[2PG+ 2AA]	413.1782	$[M + Na]^+$	14.19	29.18	0.998	3.5 - 1000	1.04	3.46
Q10	c[2PG+ 2AA]	373.1857	$[M + H]^+$	13.29	126.19	0.998	0.7 - 1000	0.19	0.65
Q11	c[4PG+ 4AA]	745.3641	$[M + H]^+$	8.166	−145.625	0.998	50 –1000	0.39	1.28

Table 4
Mean concentration values of oligomers (µg kg^{-1}) with standard deviation (n = 3) in the three biopolymers (S1, S2 & S3) migration extracts in simulant A, B and E. In brackets in the first column: assigned quantification standard when different (see Table 3 for code). Concentration values in bold represent those surpassing their TTC value. The symbol "-" indicates the absence of the oligoester in the given food simulant.

Compound	Cramer Class	Acetic Acid 3 % (v/v)			Ethanol 10 % (v/v)			Tenax®		
		S1	S2	S3	S1	S2	S3	S1	S2	S3
lin[2LA+C_2H_5] (Q1)	I	-	-	-	-	-	-	18.7 ± 2.2	28.7 ± 10.1	32.4 ± 13.8
lin[BD+AA]	I	86.9 ± 9.4	-	156.5 ± 4.9	-	-	-	-	-	-
c[2EG+ 2AA] (Q10)	I	-	801.6 ± 9.1	-	-	1064.9 ± 5.9	-	-	34.0 ± 8.2	-
lin[2BD+AA]	I	23.6 ± 2.3	-	51.8 ± 2.7	-	-	-	-	-	-
lin[2PG+ 2AA]	I	-	301.1 ± 3.6	-	-	-	-	-	-	-
c[2PG+ 2AA]	I	-	692.2 ± 10.0	-	-	1282.8 ± 5.7	-	-	15.1 ± 4.1	-
lin[4LA+C_2H_5] (Q9)	I	-	-	-	-	-	-	-	3.8 ± 1.7	-
c[5LA] (Q4)	I	-	-	-	-	-	-	19.6 ± 1.1	-	10.2 ± 2.3
lin[2BD+ 2AA]	I	124.2 ± 9.9	-	201.1 ± 6.9	-	-	-	-	-	-
c[6LA] (Q7)	I	84.6 ± 6.7	-	-	60.5 ± 3.5	-	-	15.8 ± 0.7	-	11.7 ± 2.8
c[DEG+SA] (Q4)	I	-	-	-	-	-	-	-	-	12.4 ± 3.2
lin[3BD+ 2AA]	I	-	-	165.8 ± 3.3	-	-	-	-	-	-
lin[2BD+ 2AA+PA] (Q6)	I	-	385.5 ± 9.7	-	-	840.3 ± 20.5	-	-	-	-
c[7LA] (Q7)	I	147.1 ± 12.9	501.8 ± 6.9	59.4 ± 4.2	133.7 ± 9.3	764.1 ± 39.7	-	-	-	-
c[2BD+ 2AA]	I	374.2 ± 17.5	-	472.1 ± 13.4	814.6 ± 46.8	-	971.3 ± 41.6	46.1 ± 3.9	-	90.4 ± 16.3
c[2EG+ 2PG+ 4AA] (Q11)	III	-	**251.5 ± 7.0**	-	-	**688.4 ± 10.5**	-	-	-	-
lin[3BD+ 3AA]	III	-	-	**143.5 ± 8.7**	-	-	-	-	-	-
c[2BD+ 3AA+EG] (Q7)	III	-	**205.1 ± 2.5**	-	-	**684.9 ± 17.8**	-	-	-	-
c[4PG+ 4AA]	I	-	65.2 ± 6.2	-	-	273.8 ± 4.8	-	-	-	-
c[AA+TMP] (Q10)	I	-	-	-	-	-	-	-	-	12.1 ± 2.1
c[2BD+AA+PA]	III	2.5 ± 0.8	-	11.6 ± 0.8	11.4 ± 0.7	-	19.5 ± 1.2	-	-	0.5 ± 0.1
c[3BD+ 3AA]	I	50.8 ± 2.1	-	99.6 ± 1.6	168.8 ± 8.3	-	255.6 ± 6.9	-	-	-
lin[7LA] (Q6)	I	31.4 ± 1.6	-	-	-	-	-	-	-	-
Total linear oligoesters		266.1 ± 20.2	683.6 ± 8.6	724.7 ± 21.0	-	840.3 ± 20.5	-	19.1 ± 2.4	32.5 ± 9.0	32.44 ± 13.8
Total cyclic oligoesters		659.2 ± 39.2	2517.2 ± 20.8	543.0 ± 16.2	1140.4 ± 103.0	4758.8 ± 30.6	1246.5 ± 45.0	82.0 ± 4.9	49.1 ± 12.2	137.3 ± 26.6
Total oligoesters		925.2 ± 59.3	3200.9 ± 25.5	1267.7 ± 35.1	1140.4 ± 103.0	5599.1 ± 40.5	1246.5 ± 45.0	101.1 ± 6.9	81.54 ± 10.1	169.7 ± 14.5

similar across all three samples. Despite being considerably higher than in Tenax, migration values in simulant B were lower than in simulant A, probably due to a lower solubility and the acid effect on the stability of oligoesters [41].

Considering samples, overall oligoester migration was higher in S2 than in S1 and S3, which were more similar. For example, c[2PG+ 2AA] was the dominant contributor to the migration from S2, while c[2BD+ 2AA] was the dominant for both S1 and S3. Concentrations of corresponding linear oligoesters were lower than their cyclic counterparts but, in both cases, still exceeded the value established by EU/10/2011 for non-listed substances (10 µg.kg^{-1}) except for c[2BD+AA+iPA] (S1, simulant B & S3, simulant E) and lin[4LA+C_2H_5] (S2, Simulant E). As recently stressed by the scientific community, there is hardly any toxicological data available on oligoesters [16,44] which hinders a correct risk assessment. Recent studies showed androgen receptor (AR) activity of cyclic oligomers from food packaging adhesives [41] and the potential of PLA oligomers to bioaggregate in the liver, intestine, and brain [45]. However, for the oligoesters found in this study, we could not find any data regarding any mutagen, carcinogen, organophosphate, or carbamate activity. In these situations, a *read-cross* approach, such as the threshold of toxicological concern (TTC), could be used [46]. TTC assigns a theoretical toxicity class to each compound depending on its chemical structure [47]. For this purpose, the software Toxtree v3.1.0 (Ideaconsult Ltd., Sofia, Bulgaria) was used. All compounds are divided into three classes according to their theoretical toxicities – low (I), intermediate (II), and high (III) – and are subject to a maximum daily intake above which further in vitro testing is required: 1.8, 0.54 and 0.09 mg per person per day, respectively. Assuming a food consumption of 1 kg/person/day and no other exposure routes, these intakes can be transformed to maximum recommended migration values. Most of the migrant compounds were listed as Class I, having a maximum recommended migration of 1800 $ng.g^{-1}$, which was not exceeded for any of the oligomers. However, four oligoesters (c [2EG+2PG+4AA], lin[3BD+3AA], c[2BD+3AA+EG] and c [2BD+AA+PA]) were classified as class III, having a recommended migration limit of 90 $ng.g^{-1}$. From these four class III oligoesters, only c [2BD+AA+PA] did not exceed the limit, making S1 the only biopolymer sample to comply with the legislation under these premisses.

Although TTC values tend to be conservative, underestimation can occur for some compounds [48,49], evidencing the need of a thorough toxicological assessment of oligoester migrants regardless of their Cramer class.

4. Conclusions

In this work, the food safety of three starch-based biopolymer films has been assessed using three food simulants: ethanol 10 %, acetic acid 3 % and Tenax. A series of non-volatile migrant oligoesters arising mainly from adipic acid, 1,4-butanediol, propylene glycol, ethylene glycol and phthalic acid were detected in the migration extracts by UPLC-MS(QTOF). For identification and quantification purposes, 11 linear and cyclic oligoester combinations (lin[BD+AA], lin[2BD+AA], lin[2BD+2AA] c[2BD+2AA], lin[3BD+2AA], lin[3BD+3AA], c [3BD+3AA], c[2BD+AA+iPA], lin[2PG+2AA], c[2PG+2AA] and c [4PG+4AA]) were successfully synthesized by a multi-step approach and characterized by ^{1}H and ^{13}C NMR and LC-MS(Orbitrap). For the first time, these synthesised oligoester standards allowed to unequivocally identify and quantify the migrant oligoesters detected in biopolymer films. This allowed a more precise and concrete analysis of their theoretical toxicity, with only one of the three samples compliant according to the TTC approach. The protocol herein described aims to contribute towards the availability of oligoester standards, a prerequisite for the highly demanded toxicological studies of these packaging oligomers that will be able to shed some light into a grey area in the food packaging sector.

Environmental implication

The use of bio-based polymers as food contact materials is rapidly increasing. To guarantee consumer safety, these novel materials require thorough risk assessments to prevent any potential hazards from compounds migrating into food. In this study, the safety of starch-based polymers was assessed by identifying and quantifying for the first time eleven cyclic and linear oligoesters, major migrants commonly found in these materials. For this purpose, a stepwise synthesis approach was developed, contributing towards the lack of commercial standards and opening the doors for further toxicological studies that will allow the development of safe bio-based polymers.

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CRediT authorship contribution statement

David Rupérez: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Matthieu Rivière:** Writing – review & editing, Formal analysis, Conceptualization. **Filomena Silva:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jacques Lebreton:** Writing – review & editing, Supervision, Funding acquisition. **Margarita Aznar:** Writing – review & editing, Supervision. **Cristina Nerin:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Arnaud Tessier:** Writing – review & editing, Supervision, Resources, Conceptualization. **Ronan Cariou:** Writing – review & editing, Supervision, Resources, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.135202.

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