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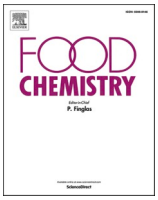

Cooking food in microwavable plastic containers: *in situ* formation of a new chemical substance and increased migration of polypropylene polymers

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ABSTRACT

Microwavable plastic food containers can be a source of toxic substances. Plastic materials such as polypropylene polymers are typically employed as safe materials in food packaging, but recent research demonstrates the migration of plastic substances or their by-products to food simulants, to foodstuff, and, more recently, to the human body through food consumption. However, a thorough evaluation of foodstuff in food contact materials under cooking conditions has not yet been undertaken. Here we show for the first time that plastic migrants present in food contact materials can react with natural food components resulting in a compound that combines a UV-photoinitiator (2-hydroxy-2-methyl-1-phenylpropan-1-one) with maltose from potato starch; this has been identified after cooking potatoes in microwavable plastic food containers. Additionally, polypropylene glycol substances have been found to transfer into food through microwave cooking. Identifying these substances formed *in situ* requires state-of-the-art high-resolution mass spectrometry instrumentation and metabolomics-based strategies.

1. Introduction

Ever since the introduction of the polyvinylidene chloride-based Saran™ wrap by Dow Chemicals in 1953 (Rolling Out Saran Wrap, 1955), the plastic food packaging industry has grown exponentially. Currently, there are a multitude of food contact materials (FCMs), including chemical additives such as plasticisers, antioxidants, photo-initiators, and inks, which can be a source of exogenous, toxic substances (Muncke, 2009). Although risk assessment is performed by the appropriate authorities to ensure the safety of these materials, such evaluation is not always performed under real-use conditions (Muncke et al., 2017). Furthermore, the complete safety assessment of a material becomes even trickier when the plastic material undergoes chemical and/or physical transformation alongside the foodstuff; this is because new, unknown, and unexpected substances can be formed (Muncke et al., 2020). Control, analysis, safety evaluation, and even the very identification of these substances poses a considerable analytical challenge that is often not evaluated during standard risk assessment procedures (Canellas et al., 2021; Muncke et al., 2017; Sapozhnikova, 2021; Seltenrich, 2018). In the European Union, the assessment of products in contact with food is regulated by Commission Regulation (EU) No. 10/2011 (COMMISSION REGULATION (EU) 2020/1245 of 2; COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, 2011).

There are recent reports in the scientific literature on the migration of plastic substances and/or their by-products to food simulants, and even more recently, these have been shown to enter the human body through food consumption (Canellas et al., 2021; Diamantidou et al., 2022; Miralles et al., 2021; Stojanović et al., 2020). The characterisation of FCMs and the evaluation of these materials in contact with simulants using direct analysis in real time mass spectrometry (DART-MS) has been extensively evaluated. One of the advantages of DART-MS analyses is the possibility of performing very fast screening evaluations of the FCM surfaces and foods in contact with them. A recent example of

Abbreviations: R, raw potatoes; BW, potatoes boiled in distilled water; BM, potatoes boiled in the microwave inside a glass beaker; PM, potatoes boiled in the microwave inside a microwavable plastic food container; SR, original packaging before microwave-cooking; SM, original packaging after microwave-cooking; SB, simulant blank.

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applying DART-MS is the work by Lestido-Cardama et al., who employed DART-HRMS to evaluate the migration of chemicals from FCMs to simulants under cooking conditions, and from FCMs to food (Lestido-Cardama et al., 2022). The characterisation of FCMs and their migrants and leachables, such as brominated flame retardants and ink photo-initiators, has also been evaluated using gas chromatography coupled to mass spectrometry (GC–MS) alongside DART-MS (Lago & Ackerman, 2016; Paseiro-Cerrato et al., 2021). The identification of unexpected migrants from FCMs with food simulants using time-of-flight mass spectrometry (ToF-MS) has also been evaluated. To evaluate potential migrants in fruit juice and purées, Gómez-Ramos et al. employed ToF-MS and identified various migrating chemicals, of which cyclic oligomers from polyurethane adhesives constituted most of the newly tentatively identified substances (Gómez Ramos et al., 2019).

Furthermore, there are some scientific publications discussing the transfer of migrants from FCMs to food under cooking conditions, although these have not been extensively evaluated. In the study by Jakob et al., the authors baked various foods on top of non-stick polydimethylsiloxane (PDMS) oven papers, and then analysed the migration of PDMS oligomers onto the cooked foods using DART-MS (Jakob et al., 2016). Nevertheless, to the best of our knowledge, no research has yet been done to substances formed *in situ* in combination with natural food components when foods are cooked inside microwavable plastic food containers (MPFCs).

In our study, we have evaluated the differences in terms of intentionally added substances (IAS) and non-intentionally added substances (NIAS) in potatoes cooked inside MPFCs made of polyethylene terephthalate and polypropylene and also potatoes cooked without MPFCs.

2. Hypotheses

Due to the energetic conditions that foodstuffs and plastic materials are subjected to during microwave cooking, the increased transfer of chemicals from the plastic to the foodstuff when they are in close contact and the potential for *in situ* formation of new, exogenous and potentially toxic substances are expected risks of this cooking practice.

3. Materials and methods

3.1. Reagents

Analytical standards and HPLC-grade ethanol were purchased from Sigma-Aldrich Química S.A. (Madrid, Spain). LC-MS grade water was acquired from Fisher Scientific™ (Fair Lawn, NJ, USA), while LC-MS grade methanol was obtained from Fluka Analytical (Steinheim, Germany). Ammonium formate and formic acid (LC-MS grade) were purchased from Sigma-Aldrich (Steinheim, Germany). Pierce™ FlexMix™ Calibration Solution was provided by Thermo Fisher Scientific™ (Waltham, MA, USA). Anhydrous magnesium sulphate, sodium hydrogenocitrate sesquihydrate, sodium citrate tribasic dihydrate, and sodium chloride were purchased from Sigma-Aldrich.

3.2. Experimental setup

The experimental setup can be divided into two complementary parts: (*i*) a metabolomics-based approach to identify IAS and NIAS in microwave-cooked potatoes inside MPFCs, and (*ii*) a migration study of the MPFCs using food simulants.

Commercially available ready-to-cook potatoes inside MPFCs were purchased in a local supermarket (Almería, Spain). The plastic bag was made of polyethylene terephthalate (PET) and polypropylene (PP) on the outer and internal layers, respectively. The packaging did not include information about the precise composition of the MPFCs, and this had to be obtained through customer support. Each bag contained approximately 400 g of unpeeled, raw potatoes, which were processed differently as four types of samples: raw (R), boiled-water (BW), boiled-microwave (BM), and plastic-microwave (PM). The PM samples ($n = 9$) were cooked in a microwave at 800 W for 7.5 min within the plastic bags; the BM samples ($n = 3$) were cooked in the microwave under the same conditions, inside a glass beaker instead of the original plastic bag; the BW samples ($n = 3$) were boiled in distilled water in a glass beaker; and the R samples ($n = 3$) consisted of raw, uncooked potatoes. All the potatoes within each sample were milled together, remaining unpeeled and unwashed, and then transferred into glass bottles for storage (a total of 18 bottles: 9 PM, 3 R, 3 BW and 3 BM). All the samples were analysed in triplicate.

The original packaging, before (SR) and after (SM) the microwave-cooking process, was also utilised in a migration experiment using food simulants, with three replicates ($n = 3$) per packaging sample. As per the current European Union regulations, Commission Regulation (EU) No. 10/2011, three identical pieces of the bags were cut and placed in polytetrafluoroethylene (PTFE) tubes containing a 10 % ethanol (EtOH) solution in water (V/V), described as "food simulant A" (COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, 2011). A simulant blank (SB), containing only the food simulant in PTFE tubes, was also included in three replicates. All the PTFE tubes were kept in the dark at 40 °C for 10 days. All the samples were analysed in triplicate.

3.3. Sample preparation

All milled potatoes contained in the glass bottles were extracted in triplicate using the QuEChERS citrate method without a clean-up step (Rajski et al., 2021). Additionally, a reagent blank (RB) was extracted as a quality control measure. In summary, 10 g of sample were weighed in a PTFE tube, 10 mL of acetonitrile (MeCN) were added, and then the tubes were automatically shaken for 4 min. Next, QuEChERS citrate extraction salts (4.0 g magnesium sulphate, 1.0 g sodium chloride, 1.0 g sodium citrate dihydrate and 0.5 g sodium citrate dibasic sesquihydrate) were added to the extraction tubes, vortexed for 10 s and then automatically shaken for 4 min. Once shaken, the tubes were centrifuged for 5 min at 4000 rpm and, finally, the supernatants were transferred into 4 mL glass vials. Prior to injection, all the MeCN extracts were diluted five-fold with water containing 62.5 parts-per-billion (ppb) of an injection standard (dimethoate-D_6). Furthermore, the simulants (including those containing no plastic) were also diluted five-fold, ensuring that the extract concentration was equally diluted, and that the injection vial contained four parts of water and one part of organic solvents (MeCN and EtOH).

3.4. LC-HRMS analyses

The samples were analysed using liquid chromatography coupled to Orbitrap™ high-resolution mass spectrometry (LC-HRMS) with a heated electrospray ionisation source in non-target mode. Every vial was analysed in triplicate using a Thermo Scientific™ Vanquish™ Flex UHPLC System (Thermo Scientific™, Germering, Germany) coupled to a Thermo Scientific™ Orbitrap Exploris™ 240 (Bremen, Germany) equipped with a Thermo Scientific™ OptaMax™ NG (H-ESI II) ion source. The samples were analysed in positive ionisation mode only. The H-ESI source configuration was: spray voltage 3500 V, static gas mode, sheath gas 40 (arbitrary units), aux gas 5 (arbitrary units), sweep gas 1 (arbitrary units), 325 °C for the ion transfer tube temperature and 350 °C for the vaporiser temperature.

The mobile phase used in this work consisted of a water:MeOH (98:2, V/V) mixture containing 5 mM ammonium formate and 0.1 % formic acid (solvent A), and a MeOH:water (98:2, V/V) mixture also containing ammonium formate and formic acid at the same concentrations (solvent B). Chromatographic separation was performed in gradient mode with a total run time of 17 min. Initially, the mobile phase consisted of 100 % solvent A, which was maintained for 1 min. Then, it was gradually modified down to 80 % A at 2 min, 30 % A at 3 min and 0 % A at 14 min. This mobile phase composition was maintained until 17 min, after

which it was again modified to 100 % A to re-equilibrate the column for the next injection. The column oven temperature was set to 30 °C and the mobile phase flow to 0.350 mL/min throughout the analysis. The chromatographic column employed was a Thermo Fisher Scientific™ Accucore™ C8 (100 mm × 2.1 mm × 2.6 μm).

The HRMS instrument was run using a combination of full-scan mass spectrometry (FS) and data-dependent tandem mass spectrometry ($ddMS^2$) modes. The FS mode employed a resolution of 120,000 full width at half maximum (FWHM) with a scan range of mass-to-charge ratio (m/z) 100–1000. Between every two FS scans, 10 $ddMS^2$ scans were acquired. The $ddMS^2$ analysis trigger algorithm was based on a minimum intensity of 1.0E4 and a dynamic exclusion list. The dynamic exclusion list was created as follows: exclude after 2 times, if it occurs within 5 s, exclusion duration of 15 s, mass tolerance of ± 3 ppm, and exclude isotopes. The $ddMS^2$ scan employed a resolution of 15,000 FWHM with an isolation window of m/z 1 and a stepped collision energy mode with HCD collision energies of 15, 30 and 45 V. The total cycle time was ≤ 700 ms. As described in a previous work (Rajski et al., 2021), Mild Trapping and Advanced Peak Determination were enabled, all spectra acquired in profile mode, and calibration was performed weekly (mass calibration) and monthly (system calibration) using the Thermo Scientific™ Pierce™ FlexMix™ calibration solution, while internal mass calibration was also enabled using RunStart EASY-IC™. During a later step, the instrument was run at a resolution of 240,000 FWHM in the FS mode to confirm the exact mass of the evaluated analytes, and product ion scans were performed at a resolution of 15,000 FWHM, both with stepped (15, 30 and 45 V) and absolute (10, 20, 30, 40, and 50 V) collision energies.

3.5. Data processing and in silico studies

A similar data processing workflow to that reported in previous works was used, shortly described herein (Díaz-Galiano et al., 2022). The compounds originating from the plastic or from the plastic during the microwave cooking process were sought for using Compound Discoverer™ software version 3.2 (Thermo Scientific™) in combination with local and online library searches. All the potato samples replicates were included in the study (R, BW, BM and PM), and the group ratios were calculated for every possible group combination. Solvent and reagent blanks were used to remove interfering signals. The spectra were selected within a t_R window of 0.5 and 16.5 min. A maximum retention time shift of 0.1 min was allowed, and the mass tolerance was set to ±5 ppm. The intensity tolerance was set at 30 %, with a signal-to-noise (S/N) threshold of 5 and a minimum peak intensity of 5.0E5. The adducts to evaluate were $[2M+H]^+$, $[2M+K]^+$, $[2M+Na]^+$, $[2M+NH_4]^+$, $[M+2H]^{2+}$, $[M+H]^+$, $[M+H\text{-}H_2O]^+$, $[M+K]^+$, $[M+Na]^+$ and $[M+NH_4]^+$.

The local databases included in the workflow within the "Search Mass Lists" node were the EFS HRAM Compound Database and the Extractables and Leachables HRAM Compound Database. A "Search mzCloud" node was also added to compare the MS^2 spectra against the mzCloud spectral library. In the "Search ChemSpider" node, the search mode was set to "By Formula or Mass" with a mass tolerance of ±5 ppm, and the following fifteen online databases were included: Alfa Chemistry, CAS Common Chemistry, ChemBank, EPA DSSTox, EPA Toxcast, Excipients Browser, Exposome Explorer, MassBank, Merck Millipore, PurePEG, Sigma-Aldrich, Springer Materials, Submitted chemical data, TCI and Toronto Research Chemicals.

The chemical space of the acetonitrile-based QuEChERS extraction, after the described data processing configuration, provided a total number of 18,860 signals of potential interest. To reduce this number, three filters were applied in Compound Discoverer™: first, the removal of all signals present in the reagent blank, simulant blank and/or the solvent blank, then signals with a $\log_2$-fold change equal to or greater than 2 in the PM samples compared to the rest. With these filters, the number of signals was reduced to 10 (Supplementary Table S1). The centred and scaled principal component analysis (PCA) performed by Compound Discoverer™ showed great differentiation between the PM potatoes and the other three groups (Supplementary Figure S1). The first principal component provided a 59.6 % differentiation between groups, and the second principal component a 26.7 % separation, a cumulative proportion of variance of 86.3 %.

In all cases, the $ddMS^2$ spectra were compared against the *in silico* fragmentation patterns provided by Mass Frontier™ Spectral Interpretation Software 8.0 (Thermo Scientific™). The *in silico* fragments were obtained for the adduct identified by Compound Discoverer™ using General Fragmentation Rules, the HighChem Fragmentation Library, with a maximum number of 10 reaction steps and a reaction limit of 10,000.

Finally, all the data were also manually processed using Xcalibur 4.4 software (Thermo Scientific™) to review the MS and MS^2 data. A minimum of 3 diagnostic ions (an adduct of the precursor and 2 fragment ions) with a minimum mass accuracy of ±5 ppm were employed for tentative identification.

4. Results and discussion

4.1. Photoinitiator derivative in situ formation

Since 2005, when the European Commission's Rapid Alert System for Food and Feed (RASFF) first reported the presence of the photoinitiator 2-isopropylthioxanthone in baby food originating from food contact materials (Gallart-Ayala et al., 2011; Rothenbacher et al., 2007), this class of compounds (found as IAS in FCMs) has been more thoroughly evaluated. One such compound is the photoinitiator 2-hydroxy-2-methyl-1-phenylpropan-1-one (HMPP), also known as Irgacure® 1173 or Darocur® 1173, among other commercial names. HMPP is typically employed in the synthesis and crosslinking of a variety of materials, e.g., various polymers, printing inks, and adhesives for FCMs. Most of the employed mass of this small molecule is retained on the surface of the final products and has been reported as being present on FCMs (Ouali et al., 2018; Sanchis et al., 2019).

An *in situ*-formed (or neoformed) maltose derivative of HMPP, present only in potatoes cooked in the microwave inside the MPFCs, was identified as the probable structure (level 2b) (Schymanski et al., 2014) of an ion with m/z 506.2232, corresponding to compound 6 in Supplementary Table S1. This m/z was identified as the ammonium adduct, $[M+NH_4]^+$, of a $C_{22}H_{32}O_{12}$ chemical formula, which was further confirmed by the identification of the proton ($[M+H]^+$) and sodium ($[M+Na]^+$) adducts of the expected m/z (489.1967 and 511.1786, respectively). The probable structure identification is also further supported by the identification of 34 fragment ions in the tandem mass spectrometry spectra (Fig. 1 and Supplementary Figure S2) matching the proposed structure, 21 being of significant size ($m/z \geq 100$). Four of these fragments were key to the assignation: (*i*) an m/z 325.1125 ion, corresponding to a dehydroxylated maltose moiety –with the ammonium adduct of this fragment also being found at m/z 342.1395–; (*ii*) an m/z 165.0910 ion, corresponding to the proton adduct of the photoinitiator moiety; (*iii*) an m/z 309.1333, corresponding to the HMPP backbone plus a dehydrated glucose moiety; and (*iv*) an m/z 185.0808 ion, a fragment whose structure assignation corresponds to the bonded section of the maltose and photoinitiator moieties. The proposed *in silico* structures for the m/z 309.1333 and 185.0808 ions suggest that the bond between maltose and HMPP takes place through the 6′ maltose carbon. These and the rest of the assigned fragments can be found in Supplementary Table S2, supporting the assigned structure. Several fragments pertaining to the maltose moiety could be identified thanks to previously developed strategies for identifying glucose derivatives in non-target analyses (Díaz-Galiano et al., 2022).

Most interestingly, this HMPP-maltose derivative could not be detected in any of the food simulant experiments (SR, SB or SM), nor in any other potato sample (R, BW or BM). It has been previously reported

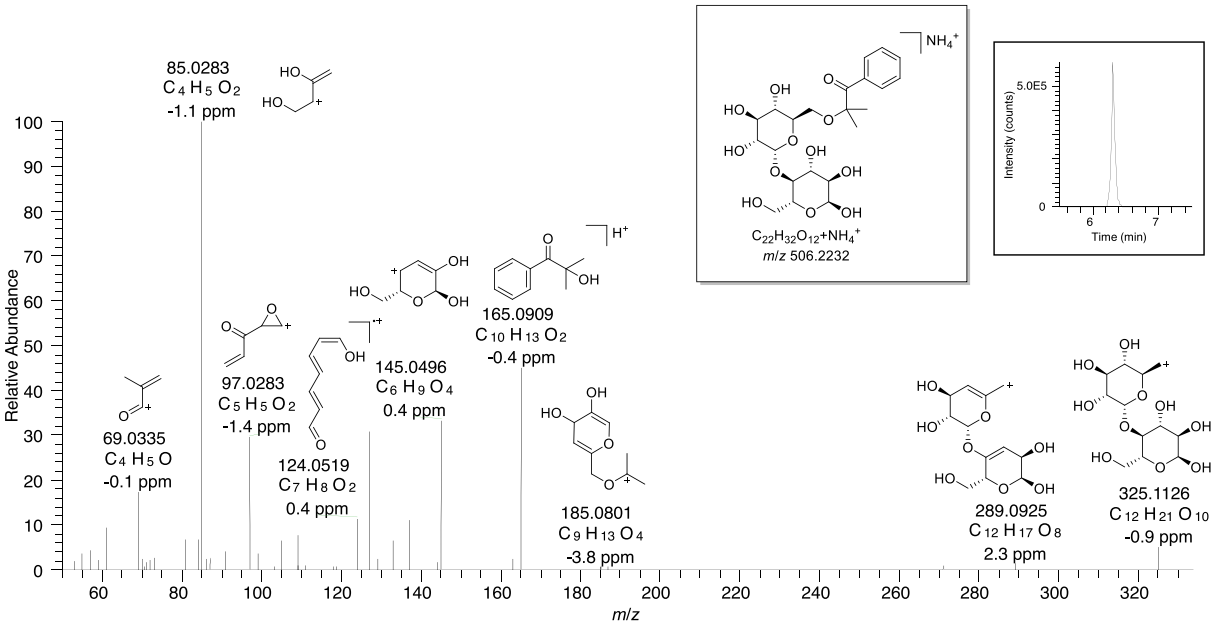

Fig. 1. Tandem mass spectrometry spectrum of the HMPP-maltose derivative in a microwave cooked potato within MPFCs with some of the fragments labelled between *m/z* 50 and 330.

that free maltose is released in starch-containing foods when cooked in a microwave and when these foods are soaked in water (Negi et al., 2001). While the free maltose content must increase for all processed potatoes (i.e., except for raw potatoes), the tentatively identified HMPP-maltose derivative is only found when potatoes are cooked inside the MPFC. The leaching and transfer of chemicals from FCMs to food is thoroughly described in the literature and has also been demonstrated in this work, as shown in Section 4.2. This further supports the claim that HMPP-maltose derivative synthesis is taking place on the potato during microwave cooking as free maltose is released from starch and the UV-photoinitiator migrates from the MPFC to the potato during said process (Fig. 2); it also demonstrates that leaching and risk assessment of FCMs with food simulants does not suffice.

Finally, in screening or non-target analyses, evaluating whether a given analyte is amenable –and hence feasible– for the sample processing or extraction procedure employed is important as an additional identification criterion. In a previous work, the octan-1-ol:water partition coefficient (log P) and the water solubility in mg/L (log S) of 244 QuEChERS-amenable pesticides were evaluated to estimate which compounds are amenable for this type of sample extraction. The experimentally determined log P range was [-2.0, 9.0] and the log S range was [-12.0, 2.0] (Rajski et al., 2021). To evaluate whether the HMPP-maltose *in situ* formed NIAS would be theoretically amenable to the QuEChERS extraction used in this work, EPI Suite™ software was

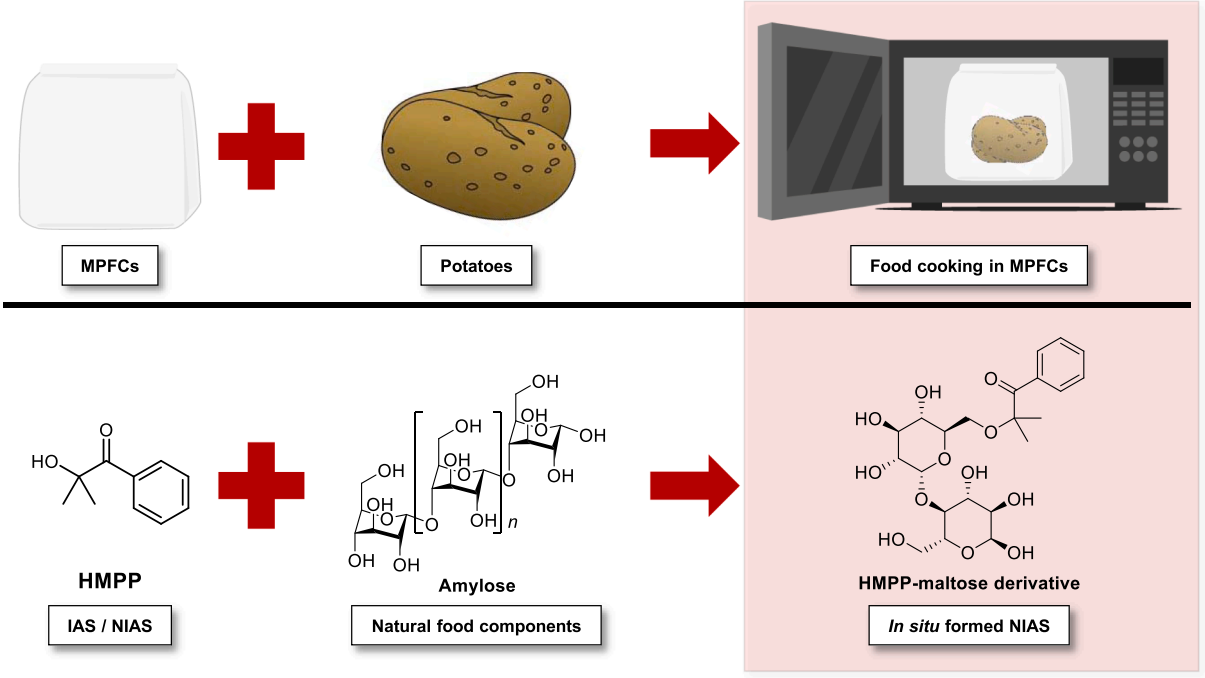

Fig. 2. Proposed mechanism for the *in situ* formed HMPP-maltose derivative.

employed to calculate its log P and log S. The obtained values, −2.0 and 4.6 for log P and log S, respectively, indicate that the HMPP-maltose compound is indeed reasonably amenable to the sample preparation procedure herein described.

4.2. Migration of polypropylene glycols

Two polypropylene glycol (PPG) compounds, heptapropylene glycol (PPG n7) and octapropylene glycol (PPG n8), could be identified among the signals of interest (compounds 4 and 5 in Supplementary Table S1). Identification was possible thanks to the use of the mzCloud library node in the study setup. Although *m/z* 442.3377 and 500.3798 were initially assigned as proton adducts of *m/z* 441.3305 and 449.3726, respectively, manual data revision allowed a different compound assignation based on MS^2 data: compound 4 was tentatively identified as the ammonium adduct of PPG n7 (424.3036 Da, $C_{21}H_{44}O_8$), while compound 5 was tentatively identified as the ammonium adduct of PPG n8 (482.3455 Da, $C_{24}H_{50}O_9$).

The identification of these two compounds was supported in several ways. First, even though it was at much lower intensity, the proton, sodium and potassium adducts (*m/z* 425.3111, 447.2933 and 463.2668 for PPG n7; *m/z* 483.3527, 505.3349 and 521.3087 for PPG n8) were identified in the full scan MS spectra with retention times that matched the ammonium adducts. Second, the $ddMS^2$ spectra matched the MS^2 spectra found in the mzCloud library in terms of mass fragments ($\leq \pm 5$ ppm) and their relative abundances – 4 fragments in the case of PPG n7 and 7 fragments in the case of PPG n8. Finally, all the fragments found in the $ddMS^2$ were also found in the *in silico* fragmentation patterns obtained using Mass Frontier™, and the spectra matched those found in the literature (Thurman et al., 2017). The identity of these compounds was later confirmed with the use of an analytical standard, as shown in the PPG n8 example in Fig. 3.

The calculated exact mass of compound 1 in the Supplementary Table S1 (134.0943 Da) also matched that of a propylene glycol derivative, the exact mass of dipropylene glycol (PPG n2), as did the assigned formula $C_6H_{14}O_3$. After identifying these three polypropylene glycol substances with software-based tools, the raw data were studied to evaluate the presence of other polypropylene glycol derivatives within the *m/z* 100–1000 range, from tripropylene glycol (PPG n3) to hexadecapropylene glycol (PPG n16). The information for these compounds on the FS level is summarised in Table 1, and their $ddMS^2$-level data in Supplementary Table S3. The $ddMS^2$ experiments combine collision

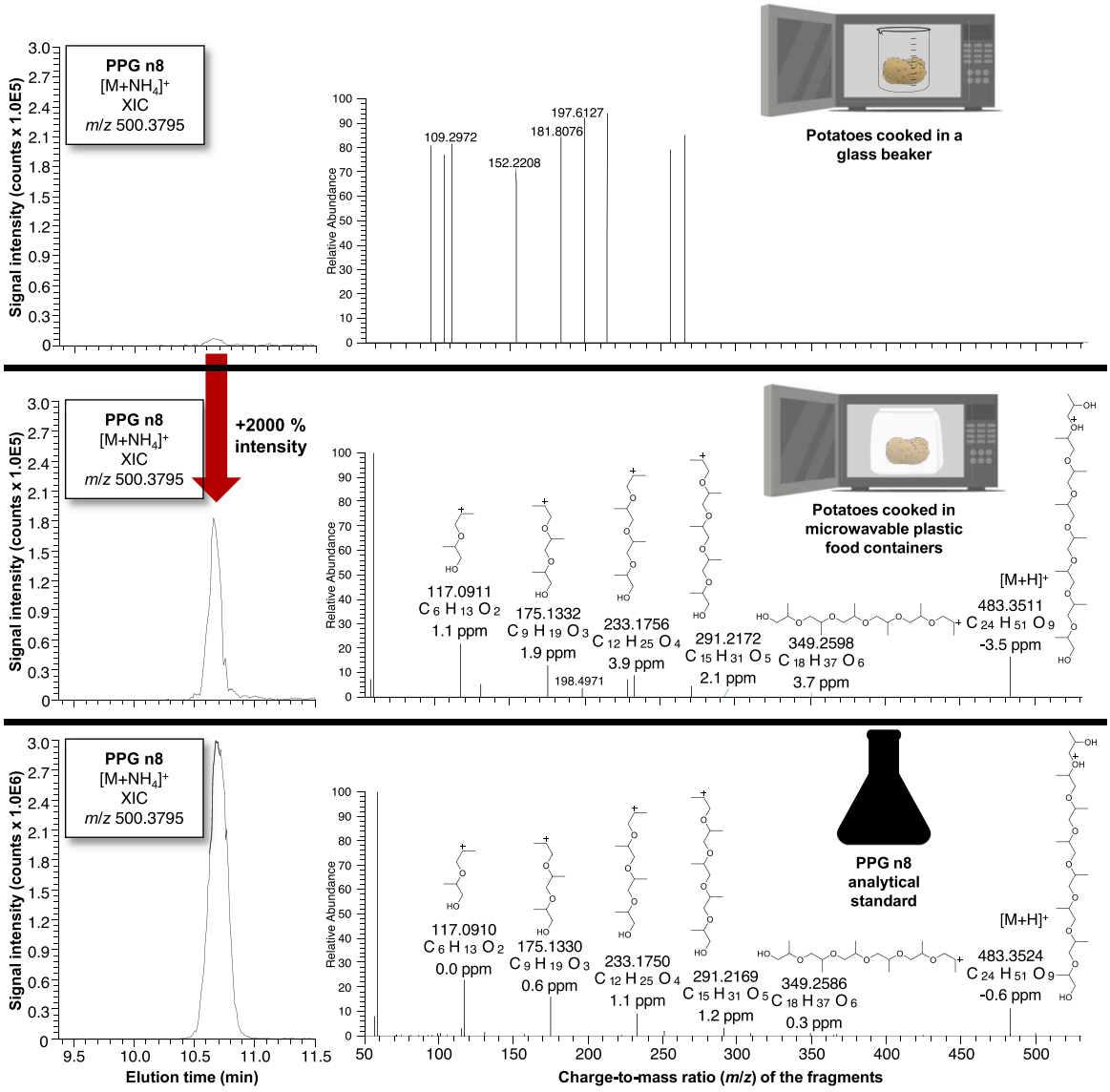

Fig. 3. Comparison of PPG n8 presence by extracted ion chromatograms (XIC) and mass fragments in microwave-cooked potatoes (*top*), in-plastic microwave-cooked potatoes (*centre*) and in a PPG n8 analytical standard (*bottom*).

Table 1
Summary of polypropylene glycol compounds evaluated in this work.

Compound	CAS No.	Chemical formula	Monoisotopic mass (Da)	Main adduct	Adduct experimental *m/z*	Mass error (ppm)	Other detected adducts $[M+A]^+$	t_R(min)	$ddMS^2$ spectrum
PPG n2	106–62-7	$C_6H_{14}O_3$	134.0943	$[M+H]^+$	135.1017	0.7	NH_4, Na, K	3.89	No
PPG n3	45096–22-8	$C_9H_{20}O_4$	192.1362	$[M+H]^+$	193.1433	−0.5	NH_4, Na, K	5.20	No
PPG n4	24800–25-7	$C_{12}H_{26}O_5$	250.1780	$[M+H]^+$	251.1853	0.0	NH_4, Na, K	6.71	Yes
PPG n5	21482–12-2	$C_{15}H_{32}O_6$	308.2199	$[M+H]^+$	309.2273	0.4	NH_4, Na, K	7.94	Yes
PPG n6	74388–92-4	$C_{18}H_{38}O_7$	366.2618	$[M+H]^+$	367.2692	0.5	NH_4, Na, K	9.35	Yes
PPG n7	14362–16-4	$C_{21}H_{44}O_8$	424.3036	$[M+NH_4]^+$	442.3376	0.5	H, Na, K	9.94	Yes
PPG n8	45308–36-9	$C_{24}H_{50}O_9$	482.3455	$[M+NH_4]^+$	500.3795	0.4	H, Na, K	10.69	Yes
PPG n9	2172326–56-4	$C_{27}H_{56}O_{10}$	540.3873	$[M+NH_4]^+$	558.4211	−0.2	H, Na, K	11.21	Yes
PPG n10	2413933–22-7	$C_{30}H_{62}O_{11}$	598.4292	$[M+NH_4]^+$	616.4630	0.0	H, Na, K	11.68	Yes
PPG n11	*No number*	$C_{33}H_{68}O_{12}$	656.4711	$[M+NH_4]^+$	674.5052	0.4	H, Na, K	12.09	Yes
PPG n12	*No number*	$C_{36}H_{74}O_{13}$	714.5129	$[M+NH_4]^+$	732.5471	0.4	H, Na, K	12.43	Yes
PPG n13	*No number*	$C_{39}H_{80}O_{14}$	772.5548	$[M+NH_4]^+$	790.5890	0.5	H, Na	12.74	No
PPG n14	*No number*	$C_{42}H_{86}O_{15}$	830.5967	$[M+NH_4]^+$	848.6307	−0.5	H, Na	13.02	No
PPG n15	*No number*	$C_{45}H_{92}O_{16}$	888.6385	$[M+NH_4]^+$	906.6725	0.1	H, Na	13.27	No
PPG n16	*No number*	$C_{48}H_{98}O_{17}$	946.6804	$[M+NH_4]^+$	964.7141	−0.3	H, Na	13.47	No

energies of 15, 30 and 45 V, and given the low intensity of most fragments at higher collision energies (Supplementary Figure S3), this results in some of the common fragments being absent from some of the PPG spectra.

All the polypropylene glycols from PPG n4 to PPG n11 could be identified in the PM and SM samples. Additionally, PPG n2, PPG n3, PPG n13, PPG n14, PPG n15 and PPG n16 could also be tentatively identified. However, no $ddMS^2$ spectra were obtained in any of the analysed samples. In the case of PPG n2 and PPG n3, the low intensity of their detected adducts is the reason why no $ddMS^2$ scan was triggered. In the case of PPG n13 to PPG n16, the low sensitivity of the peaks was not an issue, but rather the very intense baseline at this time point in the chromatogram (Supplementary Figure S4). As a side effect of many ions being coeluted at the high organic solvent concentration in the mobile phase the minimum intensity required to trigger a $ddMS^2$ scan for a given ion is significantly higher than earlier in the chromatogram. An argument can be made that for non-target analyses using data-independent acquisition with dynamic exclusion list strategies, gradient optimisation may be a key parameter in the number of MS^2 spectra obtained - with very steep gradients, a higher degree of ion coelution results in less time to perform $ddMS^2$ scans for less intense ions, resulting in missed identification opportunities.

Regarding the distribution of PPGs in the different samples, their relative abundance is shown in Fig. 4. This figure shows the abundance of PPGs in the various potato samples on the left side, and the abundance of PPGs in the simulant migration experiment on the right. Polypropylene glycols were barely detected above the instrumental limit of detection in potatoes cooked in the microwave in glassware, and no PPG was detected in either the raw potatoes or the water-boiled potatoes. This is in stark contrast with their detection in potatoes microwaved inside MPFCs, for which PPGs from lengths n2 to n11 were detected. The fundamental conclusion from this figure is that cooking the potatoes inside MPFCs in the microwave causes a very significant migration of PPGs onto the potatoes. The most intense signals for PPGs were found for chain lengths n7 to n10. This conclusion is further supported by the relative intensities of PPGs n7 to n11 in the non-microwaved MPFCs (migration experiment SR) compared to the microwaved MPFCs (migration experiment SM). The lower intensities of these PPGs in the SM samples compared to the SR samples are indicative of their transfer into the MPFCs-cooked potatoes. Abundances for PPGs n12 to n16 presented little to no variation, indicating that the microwaving process had no effect on their migration to food.

4.3. Cooking mode affecting chemical intake

The *in situ* formation of new NIAS has previously been reported,

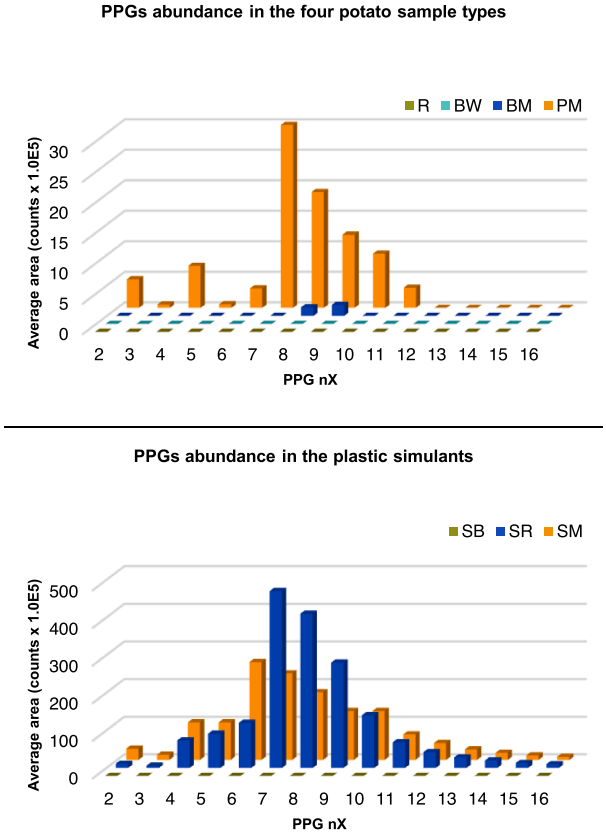

Fig. 4. Average areas of polypropylene glycol substances from PPG n2 to PPG n16 in potato samples and in plastic food simulants.

although as reactions between other IAS or NIAS exclusively, and not with natural food components (Canellas et al., 2019). Furthermore, research has also been carried out into the increased and/or exclusive migration of IAS and NIAS when food contact materials are subjected to microwave heating (Sapozhnikova et al., 2021), but only in the case of simulants. A plethora of chemicals have been identified in these and other works looking at IAS and NIAS, including plasticisers, heat stabilisers and lubricants, among others (García Ibarra et al., 2018; Sapozhnikova, 2021).

In contrast, our work has evaluated for the first time (at least to the best of our knowledge) the migration or *in situ* formation of NIAS to food

during the microwave cooking process when using MPFCs, and also for the first time, we have identified *in situ* NIAS linked to natural food components.

The bioavailability and fate of the HMPP-maltose derivative when ingested are not known, nor are those of other potential *in situ*-formed NIAS. The tentatively identified compound, for which there is no available data on risk or toxicity, is a Cramer class III structure, suggesting potentially high toxicity (Roberts et al., 2015). This classification is in line with the data provided for HMPP by the European Chemicals Agency (ECHA) through the REACH regulation, which states the harmful nature of the photoinitiator (Registration Dossier - ECHA, n.d.; Substance Information - ECHA, n.d.). The short, mid and long-term effects on human health of this *in situ*-formed maltose derivative are unknown. However, the linking of exogenous molecules to glucose moieties has been shown in the past to enhance their accumulation in various organs such as the liver or the spleen (Yoshiyuki et al., 1992). Furthermore, once the HMPP-maltose derivative enters the body, it may undergo partial or full hydrolysis, resulting in maltose and/or HMPP, amongst other unknown metabolization products.

The results presented here continue to raise the question of how safe foods cooked inside MPFCs (and foods packed within FCMs in general) are for human consumption. The migration of pre-existing known and unknown substances, in addition to *in situ*-formed compounds from materials deemed safe, indicates that stricter controls on these materials are urgently needed. The lack of analytical standards for some IAS, NIAS, and (of course) *in situ*-formed NIAS, whether comprised of two or more NIAS or combined with natural food components, makes the control of these type of food commodities and FCMs a challenge, both now and in the future. The current legislation involves using food simulants with different solvents that emulate the real commodities, because the migration assessment of FCMs under real conditions is extremely complicated (Guerreiro et al., 2018). Thus, FCMs may have been deemed safe as a result of experiments that did not expose these materials to common-use situations, with risk assessment legislation lacking in this regard in most Western countries (COMMISSION REGULATION (EU) 2020/1245 of 2; COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, 2011; Muncke et al., 2017; Yang et al., 2011). The HMPP-maltose derivative described in this work would not have been detected if only food simulants had been employed in the experimental workflow and if advanced mass spectrometry techniques and omics-based strategies had not been used. Moreover, this is certainly not an isolated case. Concerns about the lack of evidence regarding the effective evaluation of FCMs are readily available in the recent literature, particularly regarding the challenge that the evaluation of unknown NIAS (such as the HMPP-maltose derivative described here) pose for the food safety field (Muncke et al., 2017; Pieke, Granby et al., 2018; Pieke, Smedsgaard et al., 2018; Seltenrich, 2018). In this regard, Muncke et al. concluded that "[…] *comprehensive qualitative and quantitative chemical analyses of plastic* [materials in contact with food] *are currently impossible*.", a sentiment shared in other scientific works (Pieke, Granby et al., 2018; Pieke, Smedsgaard et al., 2018).

5. Conclusions

Fundamentally, this work highlights the importance of everyday habits in terms of human chemical exposure, particularly through ingestion. Small changes in how meals are prepared can have a significant effect on long-term chemical exposure, as evidenced by the presence of the *in situ*-formed HMPP-maltose derivative and the increased transfer of PPGs from the food packaging onto MPFC-microwaved potatoes. Even if short-term and acute toxicities are deemed to be low for some of these compounds (Fiume et al., 2012, 2016; Fowles et al., 2013), their long-term effects at subchronic or sublethal concentrations, in combination with other chemicals, remain unknown. In fact, this type of combined evaluation may remain forever unattainable under most circumstances, as the possible combinations of known IAS and NIAS far exceed the research and risk assessment capabilities. Furthermore, alongside IAS and NIAS, *in situ*-formed NIAS generated during the cooking process are extremely difficult to evaluate *a priori* due to their nature (Canellas et al., 2021), thus making it very challenging to predict their presence in food (and hence the application of corrective measures). The synergistic effects present amongst IAS, NIAS, and *in situ*-formed NIAS are, for the same reasons, even more difficult to determine (Pieke, Smedsgaard, & Granby, 2018). The most appropriate strategy, therefore, is to consider which daily practices influence increased chemical consumption, and then modify them to prevent any currently unforeseeable consequences. The knowledge on chemical additives and FCMs continues to grow as new research is undertaken. PPGs, as previously discussed, are considered to be safe additives (Fiume et al., 2012, 2016). Nonetheless, recently it has been shown that they are not biologically inert and are capable of crossing human cell membranes (Shi et al., 2022). Given the recent findings in the scientific literature, food packaging should alert the public to the potential risks that these practices pose.

The use of advanced instrumentation and techniques, such as high-resolution mass spectrometry and non-target analyses, along with different approaches including suspected, library search and omics-based strategies, will be of utmost importance over the coming years to better understand the chemical composition of food that is packaged and cooked inside these materials, and the new unknown and unexpected *in situ*-formed substances.

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. Supplementary data

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