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Extraction and Pyrolysis-GC-MS analysis of polyethylene in samples with medium to high lipid content

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Abstract

While it is recognised that humans are constantly exposed to plastics, there are limitations in understanding the extent of this exposure, particularly dietary exposure. This lack of information is partly due to challenges with the analysis of complicated matrices. This study aimed to assess the impact of medium to high lipid content (> 3%) food samples on the accurate quantification of polyethylene (PE), using pyrolysis-gas chromatography mass spectrometry, and develop an alternative sample processing strategy. Analysis of saturated, monounsaturated and polyunsaturated fats was demonstrated to form the same pyrolysis products as PE, producing a significant interference hindering quantification. An extraction protocol was developed that involves enzyme digestion to break the lipids into smaller chain fatty acids, removal of these interferences with pressurised liquid extraction washes, before a final extraction of the PE by pressurised liquid extraction. This new method was validated through the analysis of three medium- to high-fat content foods: cow's milk, eggs and lamb meat, where PE recoveries were acceptable (104% to 127%). Method detection limits were also significantly reduced from 1.9 to 0.05 μg/injection (380 to 10 μg/g) with the new protocol, through the removal of matrix background. PE traces were observed in the three food matrices of 72-240 μg/g, significantly reduced as compared to samples extracted with the old method where concentrations of 12-32 mg/g were calculated, demonstrating the potential for overestimation of dietary exposure. Finally, a simple protocol is reported for future studies to (i) determine if an interference is present and

(ii) sample processing methods to remove identified interferences.

Keywords: Microplastics, polyethylene, lipid interference, food, pyrolysis GC-MS, enzyme digestion

INTRODUCTION

Plastic pollution is arguably one of the largest chemical contaminants to our environment, permeating all environmental compartments[1]. Whilst it is known that humans are exposed to micro (< 5 mm) and nanosized plastics (< 1 μm), the extent of this exposure is relatively unknown, particularly through the diet. Microplastics have been reported in some common foods with a focus primarily on seafood[2,3], but more extensive food survey baskets are needed to fill dietary exposure knowledge gaps. The lack of current information on dietary exposure partly stems from challenges in the analysis of complex food matrices, hindering the ability to produce standardised analysis methods[2].

The vast majority of studies reporting microplastic concentrations in environmental matrices use spectral techniques such as Fourier-Transform Infrared (FTIR) or Raman Spectroscopy for polymer identification and report a particle-count based quantification. For food matrices, these studies have typically used digestion clean-up methods (e.g., HNO_3, H_2O_2, KOH, NaOH, Fenton's reagent, enzymes) followed by identification[2,3]. However, there are limitations with the information these methods can provide, such as only being reliable for a particle size > 20 μm[3] or more realistically, > 150 μm[4], relying on matches to spectral libraries for positive identification and lacking methods to correct for polymer loss during sample processing.

More studies are now using mass spectrometry techniques to report a mass-based quantification of total polymer mass in a sample, such as pyrolysis-gas chromatography mass spectrometry (Pyr-GC-MS). Pyr-GC-MS captures size ranges of both micro and nanoplastics[5], has the benefit of accurate identification with mass spectrometry and the ability to provide internal standard corrected concentrations.

In particular, pressurised liquid extraction (or accelerated solvent extraction, ASE) coupled with Pyr-GC-MS has been demonstrated to be a robust and accurate technique for quantifying polymer concentrations in a wide range of environmental matrices[6-8], and more recently in food matrices with low-fat contents (< 3% fat[9])[4,10]. These previous methods have typically employed a direct extraction with dichloromethane (DCM)[7] or employed a methanol wash of the sample as a clean-up step, followed by a DCM[6] or tetrahydrofuran[8] extraction, to extract and quantify a wide range of polymers.

However, the field of microplastics analysis in environmental matrices is still very much in development, and more complex matrices continue to provide new and unexpected challenges. Recently, Witzig *et al.*[11] reported substantial concentrations of polyethylene (PE) pyrolysis products in extracts of disposable lab gloves. On further investigation, they determined that the leachates contained chemicals with long-chain hydrocarbons such as stearates or fatty acids that were breaking down to the same monitored pyrolysis products as PE, namely 1-pentadecene, 1,14-pentadecadiene and 1-octadecene. They also reported that the interference was not just isolated to analysis with Pyr-GC-MS, but an interference signal was also observed in the analysis with micro-FTIR (μ-FTIR) and micro-Raman (μ-Raman).

The effect of fatty acid type lipids (triacylglycerols) on the analysis of PE in food matrices has yet to be investigated. Foods that are classed as medium to high lipid content (> 3% fat[9]) are of particular interest and our recent in-house analyses of these foods (e.g., dairy and meat) have seen unrealistically high

calculated PE concentrations (data not published), suggesting there may be a significant interference. PE is arguably one of the most important polymers to monitor in terms of environmental contamination, as it is the most common plastic currently in use with the highest production rates and has multiple and varied uses[12]. Therefore, providing an accurate method for quantifying PE concentrations in all samples is of high importance.

This study aimed to assess the impact of three different triacylglycerols (saturated, monounsaturated and polyunsaturated fats) on the extraction and analysis of PE using ASE coupled with Pyr-GC-MS and develop an alternative extraction methodology to remove potential interferences and improve confidence in the quantitative mass-based analysis of PE.

EXPERIMENTAL

Chemicals

Analysis grade hexane, isopropanol, methanol, and dichloromethane were purchased from Merck Pty Ltd. (Victoria, Australia), and ethanol was purchased from ChemSupply Australia (Gillman, South Australia). All solvents were purchased in glass bottles to reduce plastic contamination. Ultra-pure water was purified with a MilliQ system (Millipore, Bedford, USA) and additionally filtered through a 47 mm, 0.7 μm pore size Whatman glass fibre filter (ThermoFisher Scientific, Waltham, MA) prior to use.

A PE powder analytical standard (low density, 500 microns) was purchased from Alfa Aesar (ThermoFisher Scientific, Waltham, MA), and a deuterated polystyrene (d_5-PS) analytical standard was purchased from Polymer Source Inc. (Dorval, Canada). Bile salts and sodium carbonate were purchased from Sigma-Aldrich Pty Ltd. (North Ryde, NSW).

Creon® 10,000 (Abbott Laboratories GmbH, Germany, Mylan), containing lipase (10,000 Ph Eur units), amylase (8000 Ph Eur units) and protease (600 Ph Eur units) as active substances from porcine pancreas was purchased over the counter from a local chain pharmacy in Brisbane, Australia.

A monosaturated triglyceride analytical standard (glyceryl trioleate > 97% purity) was purchased from Sigma-Aldrich Pty Ltd. (North Ryde, NSW) and food-grade coconut oil (purchased in a glass jar) and sunflower oil (purchased in a polyethylene terephthalate container) were purchased from a local supermarket chain in Brisbane, Australia. Samples of cow's milk, egg and lamb meat samples were purchased from three local supermarket chains in Brisbane, Australia. The milk was purchased in a polyethylene container, the eggs in a cardboard egg carton and the lamb meat was packaged on a polypropylene tray and covered in a polyethylene film. The three samples of each food type were pooled for one final sample of milk, egg and lamb.

Extraction

The original ASE method for polymer extraction and analysis was previously published by Okoffo *et al.*[7]. Briefly, samples (2 mL or 1 g) were packed into pre-cleaned 10 ml ASE cells with the void volume filled with hydromatrix (ThermoFisher Scientific, Waltham, MA) that had been pre-cleaned with DCM. Samples in the ASE cells were spiked with 40 μg of d_5-PS internal standard and extracted on an ASE 350 system (Dionex, Sunnyvale, CA) with DCM at 180 °C and 1500 psi, a heat and static time of 5 min and two extraction cycles. An 80 μL aliquot of the extracted sample was transferred to a pyrolysis cup (Eco-Cup LF, Frontier Laboratories, Japan), and the cups were covered with aluminium foil and set aside in a laboratory fume cabinet for 30 min to allow the solvent to evaporate prior to analysis with Pyr-GC-MS.

Analysis

Identification and quantification of PE were performed using Pyr-GC-MS operated in double shot mode, as previously published[7]. Briefly, plastic identification and quantification were performed using the double-shot component of a multi-shot micro-furnace pyrolyzer (EGA/PY-3030D) equipped with an auto-shot sampler (AS-1020E) (Frontier Lab Ltd., Fukushima, Japan) coupled to a GC/MS-QP2010-Plus (Shimadzu Corporation, Japan).

The first shot (thermal desorption) was conducted with a starting temperature of 100 °C, ramped to 300 °C at 20 °C min^{-1}, and held at 300 °C for 1 min. The second shot (pyrolysis) was conducted at 650 °C for 0.20 min (12 sec). The pyrolyzer interface and GC injection port temperatures were set at 320 and 300 °C, respectively. The samples were injected with a split of 50:1 on an Ultra Alloy® 5 capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness) (Frontier Lab). The GC oven column temperature program was as follows: starting temperature of 40 °C and held for 2 min, then increased to 320 °C at 20 °C min^{-1}, and held for a further 14 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min with a constant linear velocity. The ion source temperature was kept at 250 °C with an ionisation voltage of 70 eV. Data was collected in full scan mode with a mass range of 40 to 600 m/z monitored.

The monitored ions are listed in Supplementary Table 1. Pyrolysis of PE forms triplets of alkanes, α-alkenes, and α,ω-alkenes (dienes) at different hydrocarbon chain lengths. For PE quantification, the alkene was monitored and used for quantification due to increased sensitivity, with the C10, C12 and C14 alkenes monitored. A C21 diene was also monitored[13] as lipids are expected to have a shorter chain length than PE, and are not expected to break down to the longer chain hydrocarbons[11], providing a potentially more selective product. However, the sensitivity decreases with the longer chain length pyrolysis products, and detection limits in this method are ~10 times higher for the C21 pyrolysis product than the other pyrolysis products monitored, Supplementary Table 1.

QA/QC

All laboratory work was conducted in a laboratory fume cabinet and 100% cotton lab coats were worn at all times. Where possible, all laboratory equipment and consumables were glass or stainless steel to minimise or reduce contact of the sample with any plastic material. Laboratory blanks were analysed with every batch of samples (5-8 samples) to assess background contamination from the sample processing methodology. Minimal PE peaks were detected in ASE blanks (< instrument method detection limits to 0.1 μg/injection) and concentrations in the blanks were at least 10-20 times lower than the PE spike experiments or the interfering lipid signal, so they were considered negligible in the PE recovery experiments. Instrument detection limits were calculated as the concentration of a peak in a clean run that had a signal-to-noise (S:N) ratio of 3:1 [Supplementary Table 1]. Method detection limits (MDLs) were calculated as the concentration of a peak with an S:N ratio of 3:1 from the analysis of an extracted sample, and MDLs were calculated from both the original and the new method for comparison [Table 1].

RESULTS AND DISCUSSION

Assessment of lipid interference

Three different triacylglycerols were tested by analysing samples of coconut oil (saturated fat), glyceryl trioleate (monounsaturated fat) and sunflower oil (polyunsaturated fat). Triplicate samples were extracted using the previously developed method by Okoffo *et al.*[7], where 2 mL of each sample was loaded into a 10 mL ASE cell, samples spiked with 40 μg of d_5-PS internal standard, and samples were subsequently extracted with DCM.

Table 1. Calculated method detection limits (MDLs) from the extraction of a PE standard, milk, egg and lamb using the old and new methodologies; PE concentrations (×g/injection and ×g/g) detected in food samples using the new and old methodologies; and PE recovery experiment results from *n* = 3 samples of a PE standard, milk, egg and lamb spiked with 5 ×g/injection (1 mg/g) of PE and extracted with the new method (PE concentrations are represented as mean +/- standard deviation)

	MDLs μg/injection and (μg/g)		PE concentration in sample μg/injection and (μg/g)		PE spiked samples			
	New method	*Old method*	*New method*	*Old method*	Concentration (μg/injection)	Concentration (mg/g)	% Recovery	% RSD
PE only	0.05 (10)	0.05 (10)	*N*/A	*N*/A	5.2 ± 0.4	1.04 ± 0.04	104 %	8 %
Milk (0.5 g)	0.05 (10)	1.1 (220)	0.18 (72)	95 (19,000)	5.6 ± 0.7	1.12 ± 0.14	112 %	12 %
Egg (0.5 g)	0.05 (10)	1.9 (380)	0.60 (240)	62 (12,400)	5.1 ± 0.7	1.02 ± 0.14	102 %	14 %
Lamb (0.5 g)	0.05 (10)	1.4 (280)	0.18 (72)	162 (32,400)	6.0 ± 0.4	1.27 ± 0.10	127 %	6 %

PE: Polyethylene.

For each sample, there was a distinct peak for each PE pyrolysis product monitored, with retention times and ion ratios of quantifier and qualifier ions matching the pyrolysis products generated from a PE standard, Figure 1 and Supplementary Figure 1. This suggests the formation of the same hydrocarbon products from the lipids and PE, not an additional co-eluting matrix interference. The calculated concentration was elevated and highly variable between each pyrolysis product [Supplementary Table 2], with calculated concentrations ranging from 10-2960 μg/injection for coconut oil, 220-355 μg/injection for glyceryl trioleate and 120-1320 μg/injection for sunflower oil. While it is possible the samples may contain trace levels of PE as a contamination from manufacture or processing, the calculated concentrations in these samples are unrealistic. A PE-only sample (2 mg of standard and no matrix) was also extracted and returned a consistent concentration for each pyrolysis product (4.6 to 5.3 μg/injection, Supplementary Table 2). The variable concentrations calculated in the triacylglycerol samples suggest they are breaking down into the same pyrolysis products as PE but at different ratios, likely due to different chain lengths of the parent molecules[11].

This interference was noted for all pyrolysis products monitored, including the C21 diene, although the diene was close to detection limits with a high background observed in these samples. It has previously been reported that dienes may be negligible in samples with interferences from stearates/fatty acids, as dienes only originate from the thermal decomposition of long-chain parent compounds such as PE[11]. The C21 diene in these samples was present, suggesting that interferences are highly variable, originating from a range of parent chemicals, and quantifying PE using alternative pyrolysis products will not be a solution that works for all matrices.

It was also observed that there was an interference in at least one confirmation ion for the d_5-PS internal standard (m/z 82), Figure 1, resulting in ion ratios that were 70% to 250% higher than the average ion ratios for the calibration standards. This interference was not observed in the extract of a sample with low lipid content, e.g., basmati rice[10], Figure 1. There were no observable matrix effects on the d_5-PS quantification ion (m/z 109) and the calculated PE concentrations were not affected, but it is suggested this ion ratio be monitored to provide an initial indicator of potential lipid interference on sample analysis.

The interferences observed in these samples significantly impacted the ability to accurately quantify PE in these high lipid samples. It also indicates that the first shot of the Pyr-GC-MS analysis method (thermal desorption conducted at 300 °C), which acts as a pre-clean of the sample by removing more volatile matrix components, does not remove these lipids. Interferences were not observed for pyrolysis products of the other six common polymers validated in the previous method (polystyrene, polypropylene, polyethylene

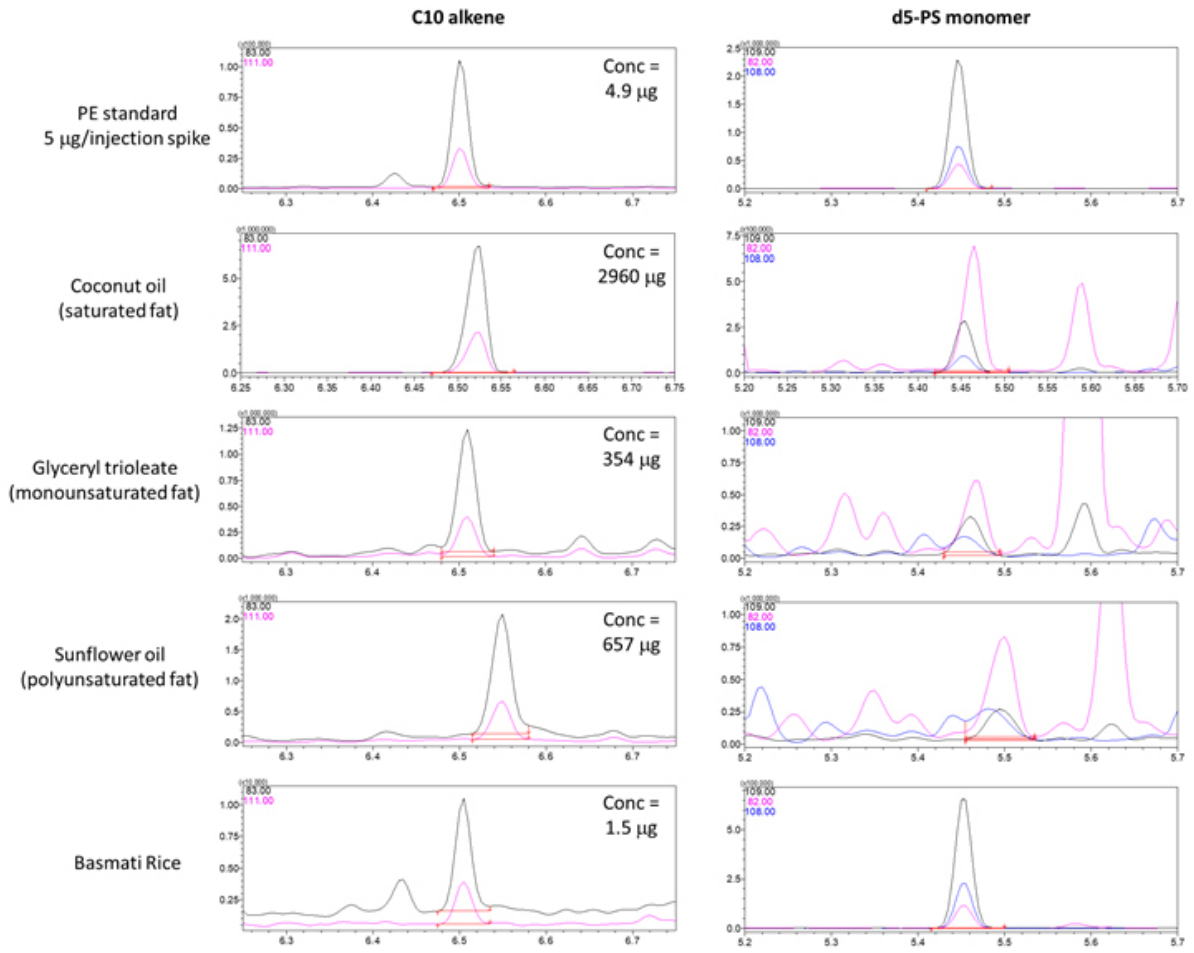

Figure 1. Pyrograms of the C10 alkene pyrolysis product of PE and d_5-styrene monomer pyrolysis product of d_5-PS for extracts of (i) PE standard (ii) coconut oil (iii) glyceryl trioleate (iv) sunflower oil and (v) basmati rice using the old ASE method. The calculated concentration (Conc) as µg/injection from the C10 alkene pyrolysis product is listed on the left graphs. PE: Polyethylene.

terephthalate, polyvinyl chloride, poly(methyl methacrylate) and polycarbonate). Therefore, the previous method is still valid for these polymers in medium to high lipid matrices, but an alternative clean-up or analysis method is required for the analysis of PE in matrices with substantial lipid content.

Temperature

The temperature of the thermal desorption (first) shot of the Pyr-GC-MS analysis method was investigated to determine if the triacylglycerols could be removed in this shot, leaving the PE in the sample cup for analysis in the second pyrolysis shot. The decomposition temperature of PE was assessed first by analysing PE standards with maximum thermal desorption temperatures of between 350 °C and 450 °C. The PE started to decompose at 400 °C, with significant peaks observed in the thermal desorption shot at 420 °C, Supplementary Figure 2. Extracts of the three triacylglycerol samples were then analysed with thermal desorption methods reaching maximum temperatures of 380 °C and 400 °C, i.e., just prior to PE decomposition. Coconut oil was removed in both thermal desorption methods with no PE pyrolysis product interferences in the subsequent pyrolysis run. However, interferences were observed in both analyses of the glyceryl trioleate and sunflower oil. Similar signals were observed for sunflower oil in the pyrolysis run at both temperatures, but a smaller peak was observed in the 400 °C run for glyceryl trioleate,

indicating a higher but incomplete removal at the higher temperature Supplementary Figure 3.

This is in line with the previous study by Brebu *et al.*[14], who demonstrated that the maximum degradation rate of grape seed biomass occurs when the degradation of PE starts at 400 °C, and the maximum PE degradation at 475 °C occurs before the grape seed degradation ends; therefore, the decomposition cannot be separated. They also demonstrated that there were interactions between components of grape seed and PE, with radicals formed in the initial stages of PE degradation that interfered with the degradation of the grape seed, retarding the degradation of PE. Therefore, the focus of this study was shifted to removing the lipid interference in the extraction protocol prior to Pyr-GC-MS analysis.

ASE sample wash

An ASE washing step was trialled, where the triacylglycerols were ASE extracted with a range of different solvents, then extracted again with DCM following the original protocol. The new ASE wash method consisted of extraction at 60 °C and 1500 psi, with heat and static time of 5 min and one extraction cycle.

This method was trialled with, (i) hexane; (ii) isopropanol; (iii) hexane:isopropanol (3:2, v/v); (iv) methanol; and (v) ethanol. These solvents were chosen as they covered a range of polarities and have had success in previous studies that ASE extracted and quantified lipids from various complicated matrices[15-18]. Aliquots of both the wash and the DCM extraction were then analysed by Pyr-GC-MS.

The only solvent trial to effectively remove any of the lipids before the DCM extraction was the hexane:isopropanol (Hex:IPA) mix, which successfully removed the interference from both coconut oil and glyceryl trioleate. However, this method did not remove the interference signal in the sunflower oil with a similarly high signal in both the Hex:IPA wash and subsequent DCM extract, Supplementary Figure 4.

It has also been noted in previous studies that different solvent combinations are required to remove different lipids with[17,18], reporting that Hex:IPA (3:2 v/v) effectively extracted egg yolk related lipids, but a combination of chloroform:methanol (2:1, v/v) was required to remove lipids in muscle tissues. Methods involving halogenated solvents (chloroform, DCM) were avoided in this study for use as a wash step to avoid extraction of the PE as well under temperature and pressure in the ASE system. As a wash removal step was not successful for the polyunsaturated fat, methods were then investigated to digest the triacylglycerols into smaller molecule fatty acids that may be removed more successfully.

Enzyme digestion

An enzyme digestion method was investigated to break the polyunsaturated fats into long-chain fatty acid units that could then be removed in either an ASE wash extraction or the first (thermal desorption) shot of the Pyr-GC-MS analysis. As a cost-effective method, the pancreatic enzyme supplement CREON® 10,000 was trailed. While containing a high concentration of lipase to target the digestion of lipids, this enzyme mixture also contains amylase and protease to digest starch and proteins, adding a further clean-up step to food samples. Pancreatic enzyme mixtures have been used for digestion of complicated matrices in previous microplastics analyses[19], and CREON® 40,000 (a more concentrated prescription version) has been demonstrated to effectively digest bivalve tissue for microplastics analysis[20]. Enzyme digestion was chosen over other digestion methods such as KOH, NaOH, H_2O_2 as it is a potentially softer digestion (not damaging the microplastics) and specifically targets the source of the interference in these samples - lipids.

The enzymes were prepared by emptying the contents of capsules (~250 mg per capsule of mini microspheres containing active enzymes) and grinding in a ceramic mortar and pestle to a fine powder. A

subsample of the ground material was analysed for potential interferences, and the PE pyrolysis products were all below detection limits. A 5% enzyme solution was prepared in MilliQ water, immediately adjusting the pH to 7.5 with 1M sodium carbonate solution to retain the enzymes at optimum conditions. A 5% solution was chosen as this concentration was observed to be approaching the solubility limits of the CREON powder in MilliQ water, but this solubility was not investigated further.

Samples consisting of 2 mL of sunflower oil were then mixed with 1 mL of a 5% bile salt solution in MilliQ water to form an emulsion with the oil. The sample pH was adjusted to ~10 with a 1M sodium carbonate solution, and samples were heated to 37 °C for 5 minutes in a Thermoline Orbital Incubator shaker (Thermoline Scientific, Wetherill Park, NSW). Once samples were at temperature, 2 mL of the 5% enzyme solution was added and the samples vortexed to mix thoroughly, then incubated at 37 °C with gentle rotation (100 rpm) for 24 hours. After 0.5 hours, the solution turned a milky/opaque consistency with no further visual changes for the remainder of the incubation period. The pH of the sample also dropped to between 8 to 9, suggesting the formation of fatty acids after 0.5 hours of incubation.

Following incubation, the samples were loaded into 10 mL ASE cells with the void volume filled with hydromatrix, pre-cleaned with DCM. The samples were then extracted with i) methanol (60 °C, 1 cycle) to remove water and the more polar matrix components and ii) Hex:IPA (3:2 v/v, 60 °C, 1 cycle) to remove remaining non-polar interferences. The samples in the ASE cells were then spiked with 40 µg of d_5-PS internal standard and extracted with the previously validated DCM method (180 °C, 2 cycles). Aliquots of the methanol, Hex:IPA and DCM extracts were subsequently analysed. A large interference was observed in both the methanol and Hex:IPA washes, suggesting both wash steps were necessary for full removal of the interference. The DCM extract, meanwhile, had a clean baseline with all interferences removed.

The incubation time was further optimised by repeating the above digestion and extraction protocol with sunflower oil samples incubated for 0.5, 2 and 24 h. In all cases, full removal of the interference was observed in the DCM extract, indicating that only 0.5 h is needed for complete digestion, and a 1-hour digestion time was chosen for the following experiments. The final optimised method is listed as Text S1 in the Supplementary Information, and depicted as a schematic in Figure 2.

Validation of the final method with complex food matrices

The method was further validated by testing lipid interference removal efficiency and PE recovery from three medium-high lipid content food matrices: cow's milk (10% total fat), egg (6% fat) and lamb tissue (23% fat). The samples were freeze-dried and then ground following previously published protocols[7], and the lamb was processed as bought with the fat layer still attached to the tissue.

To analyse a mass of 0.5 g of freeze-dried sample, the new extraction method was scaled up. Firstly, 2 mL of MilliQ water and 1 mL of 5% bile salts solution were added to 0.5 g of the dried sample to reconstitute and form an emulsion. The samples were homogenised with a Polytron® PT 1200 E homogeniser unit (Kinematica, Switzerland), and the pH was adjusted to 10 with 1M sodium carbonate solution. After samples were heated to 37 °C, 3 mL of the 5 % enzyme solution was added. The samples were vortexed thoroughly, followed by incubation with gentle shaking at 37 °C for 1 h. Samples were then extracted following the new ASE protocol. For comparison, further duplicate 0.5 g subsamples of the freeze-dried food matrices were extracted with the original ASE method. Subsamples were weighed into ASE cells containing pre-cleaned hydromatrix and the samples in the ASE cells spiked with 40 µg of d_5-PS internal standard prior to extraction with the original ASE method (DCM only).

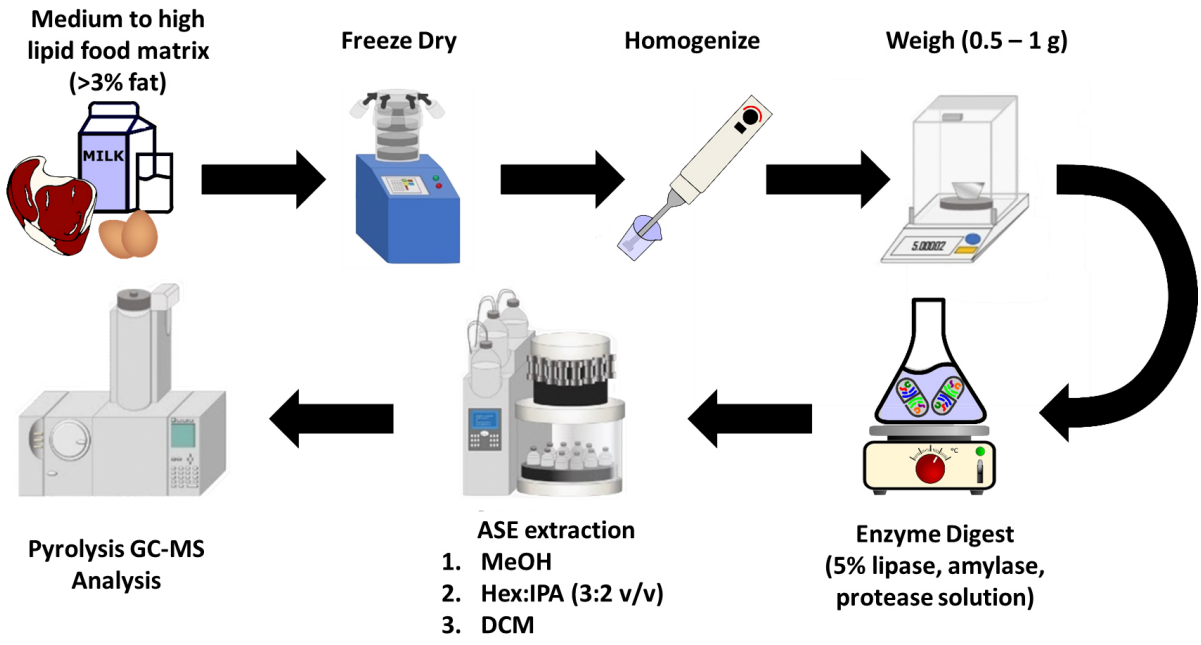

Figure 2. Schematic of final sample extraction and analysis method for quantification of PE in medium to high lipid matrices. PE: Polyethylene.

In all samples, the PE pyrolysis product interferences were removed using the new method, with trace levels of PE present at 0.18, 0.6 and 0.18 µg/injection [Figure 3], equating to 72, 240 and 72 µg/g dry weight in the milk, egg and lamb respectively [Table 1]. Large interferences were evident in the samples extracted with the original method, with concentrations calculated from the C10 alkene of 95, 62 and 162 µg/injection, equating to 19,000, 12,400 and 32,400 µg/g dry weight of PE in the milk, egg and lamb respectively, and demonstrating the significant overestimation in exposure if these interferences are not considered. Again, highly variable concentrations were calculated from the different pyrolysis products with 95-157 µg/injection, 62-134 µg/injection and 162-333 µg/injection calculated for milk, egg and lamb, respectively, from the C10 to C14 alkenes.

The background level in the acquired pyrograms was also noticeably lower in the samples extracted with the new method, and this can be seen in the almost 40 times difference in calculated MDLs from the two methods [Table 1]. While these are only three common food types, the applicability of the method to provide reliable quantitative data with lipid interferences removed from food matrices is clearly demonstrated.

The PE method recovery was also tested from all three matrices. Triplicate 0.5 g samples of the freeze-dried matrices were spiked with 2 mg of PE standard, and all samples were extracted with the new optimised method. A PE-only sample was also prepared by spiking 2 mL of MilliQ water with 2 mg of PE (equating to 5 µg/injection) and extracting with the samples. Recoveries of the extracted PE standard were acceptable at 5.2 ± 0.4 µg/injection (104%), and there were no observable losses of PE in the ASE wash steps. Concentrations of PE recovered from the three matrices were also acceptable with 5.6 ± 0.7 µg/injection for milk, 5.1 ± 0.7 µg/injection for egg and 6.0 ± 0.4 µg/injection for lamb (112%, 102% and 127% recovery respectively, Table 1). The average recoveries of the lamb tissue were elevated as compared to the other matrices (127%), and while a substantial interference was not observed in the non-spiked lamb samples

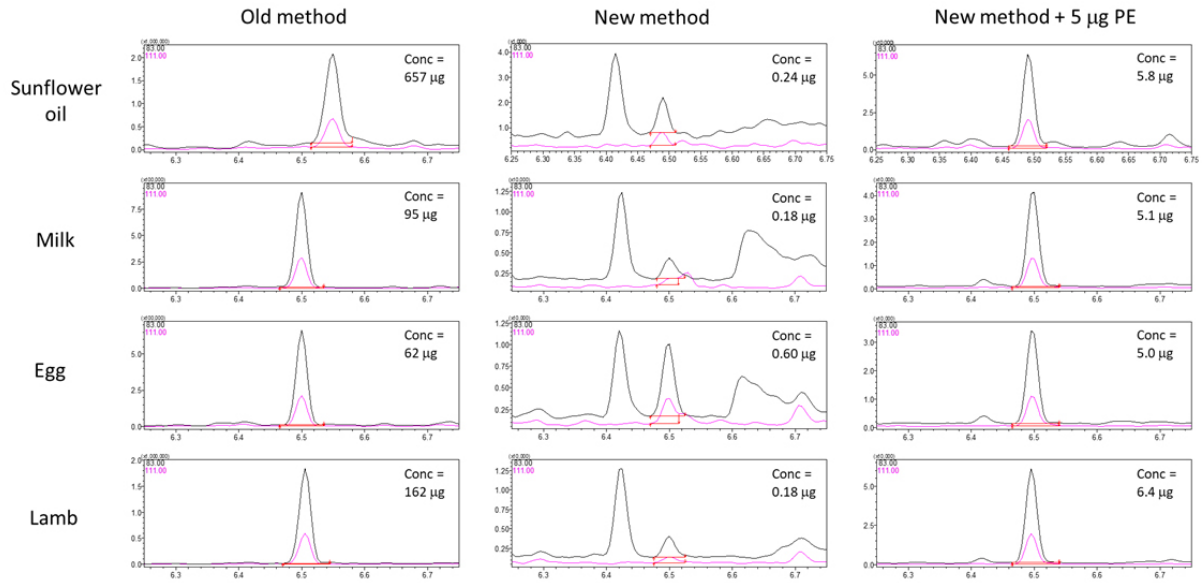

Figure 3. Pyrograms of C10 pyrolysis product from analysis of sunflower oil, milk, egg and lamb extracted with i) the old ASE method, ii) the new method and iii) spiked with PE and extracted with the new method. PE: Polyethylene; ASE: accelerated solvent extraction.

extracted with the new method, this may indicate that food samples with > 20% fat are reaching the limits for reproducible enzyme digestion with this method.

It is recognised that this study only includes one sample of each matrix, and further analyses of these and other food matrices are required to understand dietary exposure to plastics. It is also noted that this methodology is aimed at removing fatty acid type lipids, and there are other molecules containing long-chain hydrocarbons that can also break down to the same PE pyrolysis products during analysis, such as sodium dodecyl sulfate[11], which should be considered when analysing different matrices.

CONCLUSIONS

This study presents for the first time an effective and optimised method for extraction and analysis of PE from medium to high lipid content matrices. The potentially substantial interference that molecules such as fatty acid type lipids can have on quantification of PE is demonstrated, and for matrices of medium to high lipid content, additional clean-up steps are required to remove these interferences.

Based on the results from this study, a simple protocol was developed for future sample analyses that will determine if an interference is present and the subsequent procedure to remove the interferences if needed.

(1) *Extraction of sample with original validated method.* The previous method utilising ASE extraction coupled with Pyr-GC-MS method is effective for a wide range of polymers, including PE in matrices with a low lipid content (< 3% lipid, [9]). This method should be used for initial extraction and quantification of polymers.

(2) *Assessing potential interferences.* A number of pyrolysis products of PE should be monitored to look for highly variable calculated concentrations. The m/z 82 ion should also be monitored for d_5-PS as high ion ratios provide an additional indication of potential interferences.

(3) *PE specific extraction*. If a potential interference is identified, a second subsample should be extracted with the new digestion and ASE extraction protocol and analysed for PE, providing confidence in the accuracy of the reported results.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study and performed sample extractions and data analysis and interpretation: Rauert C, Pan Y, Okoffo ED

Made substantial contributions to conception and design of the study and review/edit of the manuscript: Thomas KV, O'Brien JW

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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