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The effect of particle size reduction techniques on extraction and recovery of 16 PFAS in food-contact paper packaging matrices

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Goal

To investigate the effect of two particle size reduction techniques, ball milling and blade cutting-grinding, on the Perfluoroalkyl and Polyfluoroalkyl substances (PFAS) recovery of three types of food contact materials (microwave popcorn bags, molded fiber bowl, and wrappers) using Focused Ultrasound Solid-Liquid Extraction (FUSLE) technique for extraction of 16 targeted fluorinated compounds.

Introduction

Paper and board (P&B) food contact materials (FCMs) have gained special attention lately due to the broad use of commercial additive blends and raw materials of known and unknown nature that have raised health and regulatory concerns.

Natural fibers of bleached or unbleached cellulose are used to make P&B, moreover, it can also be recycled from recovered materials. P&B used as FCMs can be noted as chemically complex matrices, partly due to the naturally occurring substances in P&B, but also due to chemical treatments used to make these materials suitable for food contact (Bengtström et al., 2014). Since P&B are used in a variety of applications, one of the challenges in paper production is to achieve specific technical functionalities. Therefore, the use of chemical additives is widely employed in the manufacturing process to achieve various performance requirements. Among these additives, processing aids and functional additives are some of the main categories. Processing aids are used to improve the efficiency of the paper making processes and are not intended to be transferred into the final product, although traces can be found. Some typical examples are defoamers, biocides, felt cleaners, and deposit control agents.

Commercial fluorochemicals are very useful in P&B production as they impart water, oil, and stain repellency onto these materials and act as dispersion and levelling agents. Polyfluorinated surfactants (PFS) are the main group of chemicals in commercial blends used to improve paper technical performance. Proper quantification of these substances in extracts of P&B are not always possible, as appropriate analytical standards are not commercially available. Although they can be relatively stabilized in paper matrices, they are still of major concern as they can be precursors of poly- and per-fluorinated alkyl substances (PFAS). PFAS can function as monomers or be attached to a polymer backbone in these matrices. PFAS have been used in paper and P&B packaging since the 1950s, mostly as coatings to prevent the paper material from soaking up fats and water, but also in printing inks and as moisture barriers (Trier et al., 2017). Some examples include fast food paper wrappers, microwave popcorn bags, cake forms, sandwich and butter paper, chocolate paper, paper for dry foods and pet foods. PFAS have been linked to a variety of human health issues, including cancers, decreases in fertility, and reduced immune system function, raising concerns for health and toxicology studies as well as regulations (E.P. et al., 2009; Fair et al., 2011; Hines et al., 2009; Macon et al., 2011; Pelch et al., 2019; Rosenmai et al., 2016; Tucker et al., 2015). Long-chain PFAS (8 carbons or higher) have been phased out in the United States and European Union due to these health concerns (United States Environmental Protection Agency, 2006), but they are often replaced in manufacturing processes by short-chain PFAS (Wang et al., 2015). More studies revealed that short-chain PFAS may carry similar health concerns as long-chain PFAS, despite the short-chain compounds reduced bioaccumulation (Scheringer et al., 2014; The Danish Environmental Protection Agency, 2015).

PFAS in general have been found in surface water, groundwater, finished drinking water, rainwater, and air emissions in some areas. Currently there are no maximum contaminant levels established for PFAS in food packaging, US-EPA has established drinking water health advisories for PFOA and PFOS at 70 parts per trillion (States & Protection, 2009). PFAS as non-intentionally added substances (NIAS) can also find their way into P&B matrices. Since PFAS are ubiquitous in the environment, it can be present in processing water in paper mills.

Given the health and migration concerns associated with PFAS in FCM, it is imperative that adequate and good performance analytical methods be developed to quantify different PFAS accurately and efficiently in a variety of matrices.

Method sensitivity for detection of PFAS have been improved dramatically in the last few decades by the use of advanced analytical technologies such as triple quadrupole tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS) and liquid chromatography quadrupole high resolution accurate mass spectrometry (LC-HRAM). However, efforts to improve extraction and clean-up of solid-matrix samples for subsequent analysis on those technologies are still needed in order to minimize uncertainties and assure instrumental reproducibility and accuracy in workflows. Several extraction methods have been reported for the extraction of PFAS in different matrices (Nakayama et al., 2019). Examples of methods used on solid matrices include: solid-liquid extraction (SLE), pressurized liquid extraction (PLE), ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), and focused ultrasound solid-liquid extraction (FUSLE) (Martínez-Moral & Tena, 2013; Monge Brenes et al., 2019; Zabaleta et al., 2014). Among all these methods, FUSLE has been validated and shown to be a low-cost, fast, simple and safe extraction technique with PFAS recoveries on food matrices and popcorn bags of nearly 100% (Moreta & Tena, 2013, 2014). Although it is known that a reduction in particle size could always lead to increased extraction efficiency, none of these studies address this variable, which seem to pose a challenge whenever sampling solid matrices.

This application note describes a direct analysis workflow for the determination of 16 targeted PFAS in three types of food contact matrices (microwave popcorn bags, molded fiber bowl, and food wrappers), evaluating their recovery through extractions employing two particle size reduction techniques.

Experimental

Sample preparation

Sample cutting was performed on each packaging material using a trimmer (Swingline™ ClassicCut™ Lite Guillotine) with a stainless-steel blade to obtain a sample material consisting of rectangles of 25–50 mm^2. Sections of popcorn (no susceptor was used as part of the sample) and sandwich bags that contained adhesives were removed prior to cutting. Compressed house air was used to remove excess

sample from cutting surfaces, and all cutting, and sample preparation surfaces were cleaned with methanol between matrix types to avoid cross-contamination.

Cutting grinding—each pre-cut packaging material was pulverized into uniform particle powder using an IKA™ A11 Analytical Mill with a stainless-steel fiber cutting blade attached (30 seconds for popcorn bags; 1 minute for sandwich bags; 1 minute for the paper bowls). The mill and its components were cleaned completely with methanol between samples of different materials to avoid cross-contamination.

Ball milling—each pre-cut packaging material was pulverized into uniform particle powder using a Retsch™ MM 400 ball mill with a 25 mm diameter stainless-steel ball in each 50 cm^3 stainless steel jars and stainless-steel balls, with each type requiring two 1-minute intervals to be ground into a fine powder at 30 Hz. Alconox® detergent solution was used to clean the ball/jar followed by methanol rinsing between samples of different materials to avoid cross-contamination.

Spiked samples were prepared by adding a methanolic PFAS native standard solution to each powdered packaging material (20 ppb) dispersed in ethyl acetate (Monge Brenes et al., 2019). The suspended and spiked samples were mixed thoroughly, then evaporated to dryness using a water bath set at 45 °C, and ground again to ensure homogeneity. Spiked and non-spiked samples were stored in polyethylene bags wrapped in aluminum foil and refrigerated (Frigidaire™, FFTR1814TWO) at 4 °C for subsequent analyses.

Extraction

Extractions of the different PFAS-spiked samples were carried out by following a validated method (Moreta & Tena, 2014) with few modifications. A focused ultrasonic liquid extraction (FUSLE) procedure using a Misonix™ S-4000 Ultrasonic Sonicator with a power of 600 W and an operating frequency of 20 kHz, equipped with a 3 mm titanium tip, was utilized to extract the PFAS from the samples. Each different sample material spiked with the native PFAS standard cocktail had undergone three extractions. A known amount of ground paper (~1.000 ± 0.001 g of homogenized sample) was placed into a 50 mL (34 × 100 mm) glass centrifuge tube and 24 mL of HPLC grade ethanol was added to each sample. Before each extraction, 100 µL of 300 ng mL^{-1} of mass labeled PFAS standard solution was added to each tube. The weight of sample used in each extraction was accurately recorded and used to normalize the concentration of PFAS obtained per gram of paper. The sonicator probe was inserted in the mixture to a depth of 2 cm from the bottom of the test tube. Each individual tube was then secured in an ice bath and subsequently sonicated. Samples were exposed to 30% amplitude at 50% pulsed cycle for 10 s. Extracts were filtered through a 60 mL Pyrex® Buchner funnel with fritted disc and porosity 10–15 µm using a vacuum pump at 550 in Hg vacuum. The probe, glassware, and extracted samples were washed twice with 2.5 mL of ethanol each rinse. The total amount of filtered extract with rinses was transferred to a 40 mL Pyrex scintillation vial without cap and immediately evaporated to dryness under a nitrogen stream using a nitrogen evaporator (N-EVAP™ 111) equipped with water bath set at 45 °C. The dry residue was reconstituted with 1 mL of LC-MS grade methanol and filtered into a 300 µL polypropylene LC vial using a disposable polypropylene medical sterile syringe equipped with a 13 mm diameter, 0.22-µm nylon filter.

System configuration

A Thermo Scientific™ Vanquish™ Flex Binary UHPLC system coupled to a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer was employed for the separation and quantification of the PFAS. The setup for the UHPLC and gradient profile used to elute and separate the PFAS is shown in Table 1.

Table 1. LC parameters for chromatographic separation of the PFAS

Analytical column	Thermo Scientific™ Hypersil GOLD™ aQ column, 100 × 2.1 mm, 1.9 µm (P/N 25302-102130)	
PFAS Upgrade Kit for Vanquish Flex	PN 80100-62144	
Trap column	Hypersil GOLD column, 50 × 3 mm, 1.9 µm (P/N 25002-053030)	
Column temperature	30 °C	
Autosampler temperature	7 °C	
Flow rate	300 µL/min	
Solvent A	LC-MS grade water acidified with formic acid to 0.1% (v/v) with ammonium formate to obtain 10 mM	
Solvent B	LC-MS grade methanol	
Injection volume	1.0 µL	
Gradient	Time (min)	% Solvent B
	0	60
	4	100
	6	100
	7	20
	8	100
	9	20
	10	100
	11	20
	12	60
	13.5	60

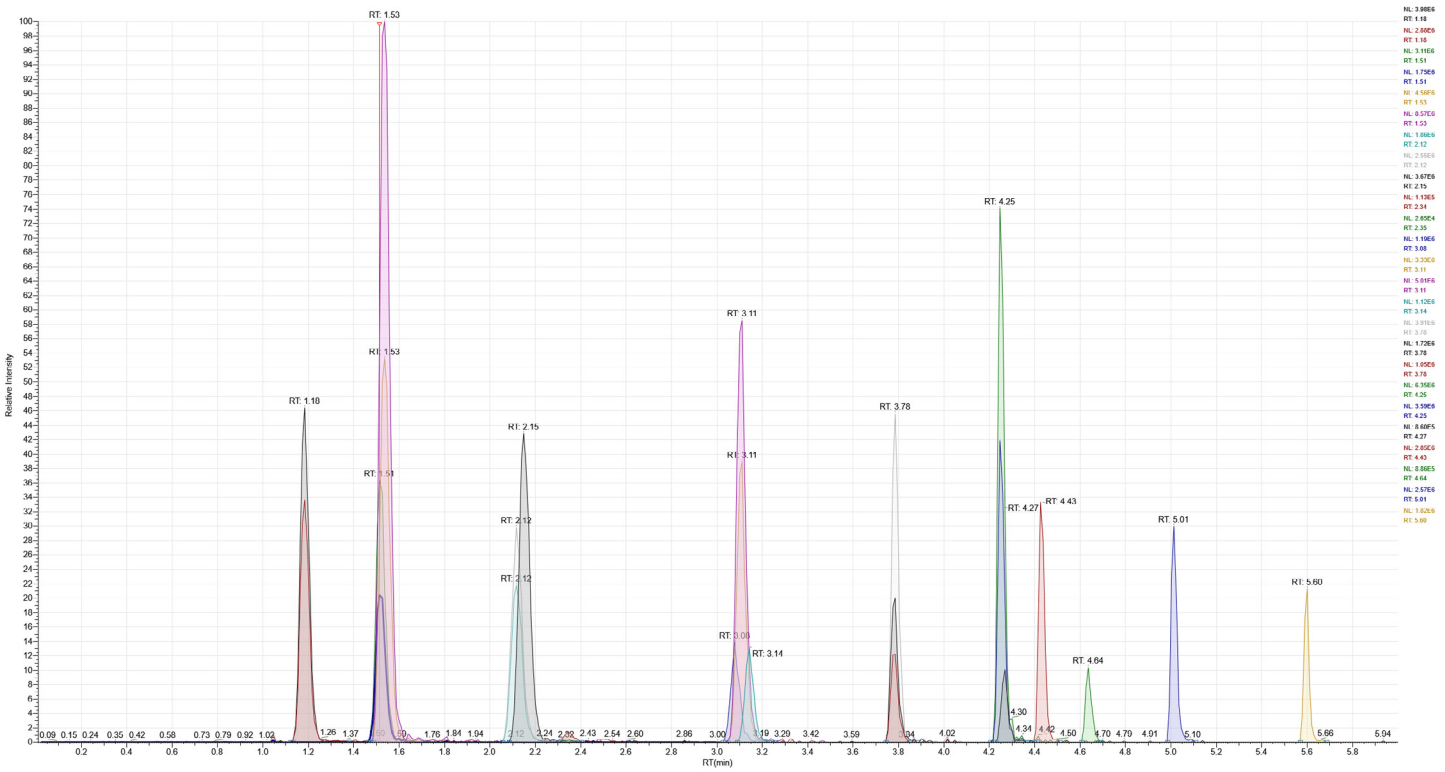

Figure 1. Full Scan Extracted Chromatogram separation for PFAS analytes using the ball mill grinding technique.

The chromatographic separations took place in less than 6 minutes, with retention times between 1.2 and 5.6 minutes. The remaining 7.5 minutes was used to remove contaminates (or possible carry over) and conditions the column prior to the next sample injection.

The ion source was equipped with a heated electrospray (HESI) probe, and the Orbitrap Exploris 120 was tuned and calibrated using a Thermo Scientific™ Pierce™ FlexMix™ Calibration Solution for negative ions on the days of the analyses. Instrument method was created using a Full Scan with Data Independent Acquisition (FS-DIA). Quantitation was done on the precursor mass in Full Scan, while in DIA a specified mass range of precursors is fragmented in the IRM. Once the MS^2 ions are acquired, the fragments (product ions) are then used to confirm the selected PFAS (Table 2) using Thermo Scientific™ TraceFinder™ software. The optimized MS parameters are shown on Table 3.

Each paper matrix extract was sampled three times. From each of these samples, repeated measurements from the same vial were run through the LC two consecutive times. UHP grade nitrogen was used as nebulizer, drying and collision gas. Instrumental operations and data acquisition were performed with the TraceFinder software.

Analytical QA/QC

A continuing calibration verification standard was injected every 12 injections immediately after an instrument blank, which was used to monitor potential carryover between injections.

Laboratory reagent blanks were used to monitor whether method analytes or other interferences were present in the environment, reagents, or apparatus.

Native (target) and surrogate PFAS standard mixtures in methanol were purchased from Wellington Laboratories. Calibration standards at 1, 5, 10, 25, 50, 75, and 100 $ng.mL^{-1}$ of native PFAS were prepared with a mix of stable isotope mass-labeled standards to correct for the matrix effects, and the extraction recovery. Calibration curves were run at the beginning of every sample batch. Blanks were run before and after calibration curves and in between samples of different matrices. All materials demonstrated to be free from interferences by analyzing method blanks. All glassware, including syringes and filters, were thoroughly rinsed with LC-MS grade methanol prior to sample preparation.

Table 2. List of targeted PFAS included in this method.

Native Analyte	Native Acronym (CAS)	Formula (native)	Surrogate Analyte
Perfluorobutanoic acid	PFBA (375-22-4)	$C_4F_7O_2$	M3PFBA
Perfluoropentanoic acid	PFPeA (2706-90-3)	$C_5HF_9O_2$	M3PFPeA
Perfluorobutanesulfonic acid	PFBS (375-73-5)	$C_4HF_9O_3S$	M3PFBS
Perfluorohexanoic acid	PFHxA (2706-90-3)	$C_6HF_{11}O_2$	MPFHxA
Perfluoropentanesulfonic acid	PFPeS (2706-91-4)	$C_5HF_{11}O_3S$	MPFHxS
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3- heptafluoropropoxy) propanoic acid (GenX)	HPFO-DA (62037-80- 3)	$C_6HF_{11}O_3$	M3HFPO-DA
Perfluoroheptanoic acid	PFHpA (375-85-9)	$C_7HF_{13}O_2$	MPFHxA
Perfluorohexanesulfonic acid	PFHxS (355-46-4)	$C_6HF_{13}O_3S$	MPFHxS
Sodium dodecafluoro-3H-4, 8- dioxanonanoate	NaDONA (958445- 44-8)	$C_7H_5F_{12}NO_4$	M8PFOA
Perfluoroheptanesulfonic acid	PFHpS (375-92-8)	$C_7HF_{15}O_3S$	MPFHxS
Perfluorooctanoic Acid	PFOA (335-67-1)	$C_8HF_{15}O_2$	M8PFOA
Perfluorooctanesulfonic acid	PFOS (1763-23-1)	$C_8HF_{17}O_3S$	M8PFOS
Perfluorononanoic acid	PFNA (375-95-1)	$C_9HF_{17}O_2$	M8PFOA
Potassium 9-chlorohexadecafluoro-3- oxanonane-1-sulfonate	9Cl-PF3ONS (73606-19- 6)	$C_8ClF_{16}KO_4S$	MPFHxS
Perfluorodecanoic acid	PFDA (335-76-2)	$C_{10}HF_{19}O_2$	M8PFOA
11-chloroeicosafluoro-3-oxaundecane-1- sulfonic acid	11Cl-PF3OUdS (763051- 92-9)	$C_{10}HClF_{20}O_4S$	MPFHxS

Table 3. Mass spectrometry conditions.

Source Parameter	Value
Ionization	H-ESI Negative mode
Spray voltage	2.5 kV
Ion transfer tube temperature	256 °C
Sheath gas	48
Aux gas	11
Vaporizer temperature	413 °C
Sweep gas	1
RF lens	70
Acquisition Type	**Full MS**
Resolution	60,000
AGC target	Standard (1e6)
Maximum injection time	Auto
Mass range	65–650 *m/z*
Acquisition Type	**DIA**
Resolution	30,000
AGC target	Standard (1e6)
Maximum injection time mode	Auto
Loop Count	1
Isolation window	200 *m/z*
Fragmentation mode	HCD
HCD collision energy (%) / stepped NCE	10, 30
Number of Scan Events	5

Data analysis

Identification and quantitation of PFAS targets

TraceFinder software was used to process the targeted screening quantitative data. Identification of the PFAS compounds was done by matching the retention time of the native standards, and confirmation identity was accomplished using accurate-mass measurements with spectral library matching. Confirmation of native PFAS compounds and their respective surrogate analytes are shown in Table 4. The concentration of each PFAS was determined using the response ratio of the PFAS quantitation (abundance of the precursor using MS^1 filtering mode) from the inclusion list (Table 4) to that of the relevant labeled surrogate standard.

Statistical analysis

Statistical analyses were performed using JMP Pro™ software (v. 14). Student t-test was used to compare whether the means of the two sets of data were statistically significantly different from each other.

Table 4. Inclusion list used for targeted PFAS analysis in data processing.

Analyte	Retention Time (min)	Precursor Ion (*m/z*)	Product Ion (*m/z*)
PFBA	1.18	212.9792	168.98937
M3PFBA	1.18	215.98926	171.9997
PFPeA	1.52	262.97601	218.9862
M3PFPeA	1.52	265.98636	222.9900
PFBS	1.54	298.94299	79.9574 98.9558 298.9430
M3PFBS	1.54	301.95306	79.9574 98.9558
PFHxA	2.12	312.97281	268.9830
MPFHxA	2.12	314.97952	268.9835 269.9867
PFPeS	2.14	348.9398	79.9574 98.9558 118.9925
HPFO-DA	2.34	328.96773	168.9895 284.9783
M3HFPO-DA	2.34	331.97779	286.9849
PFHpA	3.07	362.96962	318.9798
PFHxS	3.09	398.9366	79.9574 98.9558 118.9925
MPFHxS	3.09	402.945	84.9907 169.9891 250.9761 376.9688 398.9358
NaDONA	3.13	376.96887	84.9907 250.9761
PFHpS	3.77	448.93341	79.9574 98.9558 168.9894
PFOA	3.77	412.96643	168.9894 368.9766
M8PFOA	3.77	420.99326	118.9926 171.9995 375.9997
PFOS	4.23	498.93022	79.9574 98.9558
M8PFOS	4.23	506.95706	79.9573 98.9557 418.9731 498.9295
PFNA	4.25	462.96323	168.9894 268.983 418.9734
9Cl-PF3ONS	4.41	530.89558	82.9609 98.9557 198.9492 350.9442
PFDA	4.62	512.9606	168.9894 268.9835 318.97979 468.9701
11Cl-PF3OUdS	4.99	630.8902	82.9609 198.9493 450.9386
d5-N-EtFOSAA	5.59	531.0093	168.9896 218.9864 268.9835 330.0905

Results and discussion

Recovery of the 16 PFAS compounds spiked into the three different food contact matrices using two different particle-size reduction techniques is summarized in Table 5. The recovery values were higher or lower for each analyte within each food contact material between the two particle-size reduction techniques used in this study.

The great majority of compounds analyzed in these methods were within the recovery range of 70–150%, except for three compounds (HPFO-DA, PFHpA, PFHxA) that resulted in lower recoveries depending on the type of matrix that were analyzed. The lower recovery observed might be related to co-eluting matrix components that may have caused signal suppression since minimal sample clean-up was used in these methods.

It can be observed that very low coefficient of variation values were achieved through independent extractions. The values for % recoveries of the compounds PFBA and L-PFBS analyzed in the microwave popcorn bag are not available since copious amounts of these analytes were already present in this matrix. Those abundances were out of the concentration range of the analyte's responses used in the standard calibration curves.

An evaluation of the total mass of all 16 PFAS recovered (spiked + already present) per mass of the food contact matrix using both particle-size reduction techniques can be found in Table 6. The use of the ball analytical mill and blade analytical mill for particle-reduction of samples from the molded fiber bowl and brown sandwich bags show no statistical differences between the two techniques for total recovery of PFAS and also from microwave popcorn bag samples. The amounts of PFBA and L-PFBS analyzed in the microwave popcorn bag were not taken into account for this evaluation for the reason explained earlier in this section.

Table 5. PFAS recoveries (%) and (%CV) of three different food contact matrices using two particle-size reduction techniques for extraction.

	Molded Fiber Bowl			
Analyte	Ball Analytical Mill (%Recovery)	%CV>10%	Blade Analytical Mill (%Recovery)	%CV>10%
PFBA	84.60	1.04	75.78	13.59
PFPeA	88.04	0.25	84.72	4.56
L-PFBS	85.82	3.32	85.76	1.32
PFHxA	117.81	3.34	129.02	6.22
L-PFPeS	82.56	0.83	81.93	2.02
HPFO-DA	73.08	0.65	49.32	2.72
PFHpA	94.09	3.30	99.53	2.85
L-PFHxS	90.55	2.00	89.33	2.10
NaDONA	89.71	4.22	87.78	5.14
L-PFHpS	96.75	1.73	97.38	0.42
PFOA	88.80	4.95	93.25	4.23
PFOS	91.09	2.31	90.38	2.87
PFNA	92.80	5.30	91.72	6.16
9Cl-PF3ONS	98.81	0.06	96.55	3.56
PFDA	88.85	3.69	93.41	6.07
11Cl-PF3OUdS	111.30	0.35	111.33	1.66

Table 5. Continued.

Analyte	Brown Sandwich Bag			
	Ball Analytical Mill (%Recovery)	%CV>10%	Blade Analytical Mill (%Recovery)	%CV>10%
PFBA	81.75	3.84	69.63	5.79
PFPeA	85.82	3.13	75.94	1.78
L-PFBS	81.91	4.36	79.42	0.14
PFHxA	110.62	27.14	47.60	4.54
L-PFPeS	76.10	3.05	69.94	2.56
HPFO-DA	88.10	1.57	70.50	3.06
PFHpA	106.67	0.49	101.74	4.27
L-PFHxS	89.97	2.22	86.32	2.80
NaDONA	82.61	0.11	80.44	4.35
L-PFHpS	94.52	0.51	87.72	4.03
PFOA	97.18	3.13	90.01	5.67
PFOS	93.22	0.58	87.93	4.52
PFNA	119.64	0.30	117.18	3.83
9Cl-PF3ONS	95.18	0.20	89.36	5.84
PFDA	130.49	1.24	120.92	3.85
11Cl-PF3OUdS	99.14	3.22	93.57	6.46
Analyte	Microwave Popcorn Bag			
	Ball Analytical Mill (%Recovery)	%CV>10%	Blade Analytical Mill (%Recovery)	%CV>10%
PFBA	NA	NA	NA	NA
PFPeA	156.52	33.99	78.10	5.30
L-PFBS	88.11	2.41	87.07	0.74
PFHxA	NA	NA	NA	NA
L-PFPeS	90.36	3.65	93.89	1.20
HPFO-DA	87.03	10.98	80.61	0.60
PFHpA	123.48	11.56	130.21	4.28
L-PFHxS	94.14	2.39	92.63	1.77
NaDONA	75.70	1.52	70.99	7.78
L-PFHpS	109.42	5.75	112.72	1.44
PFOA	89.74	8.88	81.02	4.56
PFOS	91.60	0.27	92.17	1.14
PFNA	116.91	4.72	108.22	6.26
9Cl-PF3ONS	114.97	5.36	117.61	2.07
PFDA	159.79	1.50	151.41	10.76
11Cl-PF3OUdS	115.13	2.22	116.21	0.88

Table 6. Total PFAS recovered (ng/g) and (%CV) in three different food contact matrices using two particle-size reduction techniques for extraction.

Molded Fiber Bowl				Brown Sandwich Bag				Microwave Popcorn Bag			
Ball Analytical Mill (ng/g)	%CV	Blade Analytical Mill (ng/g)	%CV	Ball Analytical Mill (ng/g)	%CV	Blade Analytical Mill (ng/g)	%CV	Ball Analytical Mill (ng/g)	%CV	Blade Analytical Mill (ng/g)	%CV
325.7	1.56	316.6	0.11	322.9	3.52	305.3	1.92	314.7	1.32	313.1	2.06

Conclusion

Particle size reduction techniques for extraction and spike recovery of 16 Perfluoroalkyl and Polyfluoroalky substances (PFAS) including seven perfluorocarboxylic acids (PFCAs), five perfluoroalkylsulfonates (PFSAs), two chloroperfluoroether sulfonates (Cl-PFESAs), one polyfluoroether carboxylate (PFECAs), and one hexafluoropropylene oxide dimer acid (GenX) on sampling of different cellulosic-based food contact materials were developed in the present study.

No statistical differences were found between the two milling techniques for recovery of the total amount of PFAS spiked in the microwave popcorn bag, molded fiber bowl, and sandwich wrapper matrices. The ultra-high performance liquid chromatography and electrospray ionization (UHPLC/ESI) quadrupole Orbitrap Exploris 120 high-resolution mass spectrometry was reliably able to detect and quantify the analytes in relatively heavy matrices that used minimal clean up during extraction with the surrogate dilution method to correct for matrix effects. The applicability of this methodology in PFAS analysis can be improved in the future by developing strategies for more comprehensive sample clean up depending on the type of matrix used. Among those, solid phase chromatography can be suited for extraction of analytes of different polarities and matrix-interactions. As laboratories are often focused on method detection of analytes, sample preparation procedure studies are of great value to obtain reliable data and to streamline the workflow and reduce turnover time.

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