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Determination of Trace Perfluoroalkyl Carboxylic Acids in Edible Crop Matrices: Matrix Effect and Method Development

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Supporting Information

ABSTRACT: A robust method was developed for simultaneous determination of nine trace perfluoroalkyl carboxylic acids (PFCAs) in various edible crop matrices including cereal (grain), root vegetable (carrot), leafy vegetable (lettuce), and melon vegetable (pumpkin) using ultrasonic extraction followed by solid-phase extraction cleanup and high liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). The varieties of extractants and cleanup cartridges, the usage of Supelclean graphitized carbon, and the matrix effect and its potential influencing factors were estimated to gain an optimal extraction procedure. The developed method presented high sensitivity and accuracy with the method detection limits and the recoveries at four fortification levels in various matrices ranging from 0.017 to 0.180 ng/g (dry weight) and from 70% to 114%, respectively. The successful application of the developed method to determine PFCAs in various crops sampled from several farms demonstrated its practicability for regular monitoring of PFCAs in real crops.

KEYWORDS: *perfluoroalkyl carboxylic acids, ultrasonic extraction, solid-phase extraction cleanup, HPLC-MS/MS, matrix effect*

INTRODUCTION

Perfluoroalkyl carboxylic acids (PFCAs) are a class of typical perfluorinated compounds (PFCs) with a fully fluorinated hydrophobic carbon chain linked to a carboxyl group.[1,2] Due to their high inertness and exceptional surface activity, PFCAs have been widely applied in many fields,[1−3] resulting in a great release into environment and ubiquitous occurrence in various environmental media and the human body.[3−5] PFCAs are highly stable and very difficult to biodegrade, because of their high bond energy of carbon–fluorine bonds,[6,7] posing an environmental accumulation and various toxicities to human health,[8] e.g., perfluoro-*n*-nonanoic acid (PFNA) and perfluoro-*n*-decanoic acid (PFDA) were found to be associated with children's impulsivity,[9] and perfluorooctanoic acid (PFOA) with lower birth weight,[10] children's immunotoxicity,[11] and adults' chronic kidney disease and hyperuricemia.[12] PFCAs (≥C6) were even suspected to be carcinogenic.[13] Consequently, PFCAs have received increasing concern worldwide in recent years. European Food Safety Authority (EFSA) announced PFCs as emerging contaminants in the food chain with the tolerable daily intakes (TDI) set as 1500 ng/kg/day for PFOA.[14] Furthermore, EFSA recommended that the member states should supervise the occurrence of PFOA as well as its homologues and precursors in the environment.[15]

Soil PFCAs from sewage sludge application, dry/wet deposition, etc. could be taken up by various edible crops, with root concentration factors (C_{root}/C_{soil}) and translocation factors (C_{shoot}/C_{root}) up to 64 and 1.8, respectively.[16−24] Uptake of PFCAs from soils to edible crops depended on pollution levels, PFCA properties, soil types, crop species, etc. In general, PFCA concentrations in crops increased with pollution levels of soils, and PFCA carryover from roots to shoots increased with their decreasing chain lengths.[17,25,26] In addition, PFOA uptake in crops exhibited a potential active uptake related to anion channels, and the translocation factors of PFCAs in crops showed a positive correlation with the ratios of protein contents in roots to shoots.[20,27]

European Union PERFOOD project reported that crop origin foods including fruits and vegetables contributed mostly to the dietary exposure to perfluorohexanoic acid (PFHxA) and PFOA.[28] However, the risk of dietary exposure to PFCAs via vegetable consumption was low due to low concentrations of most PFCAs detected in vegetables.[28,29] Even so, more work on monitoring PFCAs in actual edible crops is needed, especially for those countries (e.g., China) where massive amounts of PFCs were manufactured or PFC hot-spot regions where thousands of tons of PFC-contaminated biosolids have been applied.[17,20,21,30] Presently, most studies on the uptake of PFCAs from soil to crop were conducted in greenhouses, while data on the behaviors of PFCAs in field soil–crop systems are limited.[17,20] In addition, besides direct intake via crop origin

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Table 1. Matrix-Matched Calibrations and Solvent Calibration of Target PFCAs

analyte	matrix	linear range (ng/mL)	R^2	ME[a]	IDL[b] (ng/mL)	MDL[c] (ng/g, dw[d])	MDL (ng/g, fw[e])
PFHxA	methanol	0.1−50	0.999		0.010		
	lettuce	0.1−50	0.999	0.37		0.083	0.005
	pumpkin	0.1−50	0.999	0.38		0.053	0.005
	carrot	0.1−50	0.999	0.34		0.081	0.008
	grain	0.1−50	0.999	0.42		0.096	0.089
PFHpA	methanol	0.1−50	0.999		0.010		
	lettuce	0.1−50	0.998	0.39		0.021	0.002
	pumpkin	0.1−50	0.999	0.48		0.034	0.003
	carrot	0.1−50	0.997	0.47		0.072	0.007
	grain	0.1−50	0.999	0.42		0.017	0.016
PFOA	methanol	0.1−50	0.999		0.017		
	lettuce	0.2−50	0.995	1.00		0.114	0.005
	pumpkin	0.1−50	0.993	1.07		0.027	0.002
	carrot	0.1−50	0.998	1.13		0.044	0.005
	grain	0.1−50	0.999	1.25		0.071	0.067
PFNA	methanol	0.1−50	0.999		0.010		
	lettuce	0.1−50	0.999	0.71		0.067	0.004
	pumpkin	0.1−50	0.999	1.16		0.083	0.008
	carrot	0.1−50	0.999	0.82		0.069	0.006
	grain	0.1−50	0.999	1.07		0.025	0.023
PFDA	methanol	0.1−50	0.999		0.012		
	lettuce	0.2−50	0.999	0.84		0.180	0.009
	pumpkin	0.1−50	0.999	0.89		0.066	0.006
	carrot	0.2−50	0.999	0.97		0.124	0.013
	grain	0.1−50	0.998	0.85		0.057	0.053
PFUnA	methanol	0.1−50	0.999		0.010		
	lettuce	0.1−50	0.996	0.94		0.110	0.005
	pumpkin	0.1−50	0.997	1.01		0.091	0.010
	carrot	0.2−50	0.999	0.76		0.155	0.016
	grain	0.2−50	0.998	0.56		0.104	0.096
PFDoA	methanol	0.1−50	0.999		0.010		
	lettuce	0.1−50	0.999	0.88		0.081	0.003
	pumpkin	0.1−50	0.998	0.65		0.051	0.005
	carrot	0.1−50	0.999	0.98		0.076	0.007
	grain	0.2−50	0.999	0.85		0.105	0.098
PFTrDA	methanol	0.1−50	0.999		0.010		
	lettuce	0.1−50	0.999	0.66		0.028	0.002
	pumpkin	0.1−50	0.999	0.22		0.045	0.004
	carrot	0.1−50	0.999	0.71		0.037	0.004
	grain	0.1−50	0.998	0.47		0.023	0.021
PFTeDA	methanol	0.1−50	0.999		0.010		
	lettuce	0.1−50	0.999	0.40		0.031	0.002
	pumpkin	0.1−50	0.999	0.35		0.050	0.005
	carrot	0.1−50	0.996	0.60		0.036	0.003
	grain	0.1−50	0.998	0.57		0.029	0.027

[a]ME = $\text{slope}_{\text{matrix}}/\text{slope}_{\text{methanol}}$. [b]Instrument detection limit (IDL). [c]Method detection limit (MDL). [d]Dry weight. [e]Fresh weight.

food consumption, indirect intake of PFCAs in crops via animal origin food consumption was of concern.[21] Once PFCAs were absorbed in human bodies, they would diminish slowly, and the estimated arithmetic mean half-lives of PFCAs (C7−C11) ranged from 1.5 to 9.2 years.[3]

Determination of PFCAs in crops is challenging due to complex matrix constituents (e.g., sugar, chlorophyll, protein, etc.) and trace levels of PFCAs in crops.[28,29,31] Most previous analytical methods on PFCAs in crops were developed based on limited crop species, and the matrix effect, especially its influencing factors (e.g., matrix constituents and PFCAs chain length) that could severely interfere with the reliability and stability of an analysis method, was rarely discussed.[2,32−35] Recently, superior detection equipment, e.g., HPLC combined with a quadrupole time-of-flight QToF Premier HRMS instrument (HPLC-QTOF-HRMS) and ultraperformance liquid chromatography−tandem mass spectrometry (UPLC-MS/MS), was successfully applied to detect PFCAs in crops at pg/g level (fresh weight).[32,34] However, these superior instruments are much too expensive to be commonly used in the ordinary laboratory for regular monitoring. Therefore, an improved robust analysis method using a common instrument (HPLC-MS/MS) needs to be developed for the determination of PFCAs in various types of crops.

Ultrasonic extraction and solid-phase extraction could provide a simple and reliable analysis procedure, and the

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lyophilized crop samples could concentrate analytes as well as decrease the consumption of both sample and solvent.[2,32,36,37] Therefore, the purpose of the present work was to develop a robust method for simultaneous determination of nine PFCAs (C6−C14) in various edible crop matrices (lyophilized samples) including cereal, root vegetable, leafy vegetable, and melon vegetable using ultrasonic extraction followed by solid-phase extraction cleanup (SPE) and HPLC-MS/MS. Optimization of the method included adjustment of HPLC-MS/MS parameters and sample pretreatment followed by the method validation. Furthermore, the matrix effect and its potential influencing factors in the developed method were also discussed comprehensively. Consequently, the developed method was successfully applied to determine PFCAs in various types of real edible crops with satisfactory results.

MATERIALS AND METHODS

Materials. Nine PFCAs (C6−C14) including perfluoro-*n*-hexanoic acid (PFHxA), perfluoro-*n*-heptanoic acid (PFHpA), perfluoro-*n*-octanoic acid (PFOA), perfluoro-*n*-nonanoic acid (PFNA), perfluoro-*n*-decanoic acid (PFDA), perfluoro-*n*-undecanoic acid (PFUnA), perfluoro-*n*-dodecanoic acid (PFDoA), perfluoro-*n*-tridecanonic acid (PFTrDA), and perfluoro-*n*-tetradecanoic acid (PFTeDA) were selected as the target analytes in the present study, because the detectable amounts of PFCAs (≥C6) were usually much higher than those of PFCAs (<C6), e.g., only ≤6% of PFCAs (<C6) contribution to the total perfluorinated acids in sediments and soils in Shanghai, China.[13] The nine PFCA standards and six isotopically labeled standards (ISs), i.e., $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnA, and $^{13}C_2$-PFDoA, were purchased from Wellington Laboratory (Ontario, Canada). The purities of the standard PFCAs are ≥98%. HPLC-grade acetonitrile (ACN), methyl *tert*-butyl ether (MTBE), tetrahydrofuran (THF), methanol, and ammonium hydroxide (NH_4OH) were purchased from Sigma-Aldrich (Steinheim, Germany). The solid-phase extraction (SPE) instrument containing 24-port vacuum manifolds was bought from Sigma-Aldrich (St. Louis, MO). The Oasis WAX cartridges (6 mL, 150 mg), Oasis HLB (6 mL, 150 mg), and Florisil cartridges (6 mL, 1000 mg) were bought from Waters Corporation (Milford, MA). The Supelclean graphitized carbon (ENVI-Carb) was purchased from Supelco (Bellefonte, PA, USA). Acrodisc LC13 GHP Pall filter (0.2 μm) was obtained from Pall Corp (Port Washington, NY, USA). All other reagents, i.e., tetrabutylammonium hydrogen sulfate (TBA), sodium hydrogen (NaOH), and sodium hydrogen carbonate ($NaHCO_3$) bought from Guangzhou Chemical Reagent Co., Ltd. (Guangzhou, China), were of analytical grade. Ultrapure water obtained from a Unique-R20 instrument (Research Scientific Instruments Corporation, Xiamen, China) was applied throughout the entire experiment.

Mixture stock solution (1000 μg/L) of the nine target PFCAs (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, and PFTeDA) was prepared in methanol and then stored in a refrigerator at 4 °C for use within one month. Working standard solutions of the nine target analytes at 0.1, 0.2, 0.5, 1, 2.5, 5, 10, 25, 50 ng/mL for calibration were obtained by freshly diluting the stock solution with methanol. Considering the effect of matrix constituents on the detector responses of the analytes, various edible crop species including cereal (grain), root vegetable (carrot), leafy vegetable (lettuce), and melon vegetable (pumpkin) were selected to estimate the matrix effect on the analysis of PFCAs in crops. Therefore, matrix-matched standard solutions (0.1, 0.2, 0.5, 1, 2.5, 5, 10, 25, 50 ng/mL) were prepared by diluting the standard solution with the crop sample extracts. All the crop samples for developing the analysis method of PFCAs were purchased from organic farms in Guangzhou in October 2015, and they were free of the target PFCAs based on the instrument detection limits (IDLs) (Table 1). The physicochemical properties of the crop samples including chlorophylls, total dissolved sugar, fiber, and protein were measured according to the methods described in the Supporting Information.

Sample Pretreatment Procedure. *Spiked Sample Preparation.* Fresh vegetables and cereals were chopped after being washed by tap water and ultrapure water successively. Approximately 2 kg of each crop sample was homogenized using a crusher (Jiu Yang Co., Ltd., China). An aliquot of about 500 g of the homogenized sample was lyophilized in a vacuum freeze drier (Jiangsu Hengfeng equipment manufacture Co., Ltd., China), and then ground to powders (0.45 mm) in a mill. These powder samples were stored in brown glass bottles in a refrigerator at 4 °C for use. The spiked samples at four concentration levels (0.5, 10, 25, and 50 ng/g) were prepared by spiking mixture standard working solutions (1 mL) of 0.25, 5, 12.5, and 25 ng/mL to the lyophilized powder samples (0.5 g) in centrifugal tubes, respectively. After being shaken for 2 h for homogenization, the spiked samples were left for 10 h at room temperature in a fume hood to volatilize the solvent (methanol).

Extraction. A half-gram aliquot of each lyophilized sample powder was weighed and then added into a 50 mL polypropylene centrifugal tube. Fifty microliters of internal standards containing the six ISs (100 ng/mL for each one) were spiked to the sample in each centrifugal tube with equilibrium for half an hour. Three solvents [i.e., MTBE, ACN/water (90:10, v/v), THF/water (75:25, v/v)] were used to obtain a satisfactory extractant. For MTBE extraction, the sample was mixed in an MS3 digital vortex (IKA group, German) for 2 min and held for 8 h after adding 0.2 mL of 0.5 M NaOH. Subsequently, 2 mL of ion-pairing agent (i.e., TBA, 0.25 M) and 4 mL of buffer solution (Na_2CO_3/$NaHCO_3$, pH = 10) were added to each sample and mixed for 2 min using an MS3 digital vortex. Afterward, 5 mL of MTBE was added, mixed (1 min), sonicated (10 min), and then centrifuged at 8000 rpm (10 min). The supernatant (i.e., MTBE layer) was transferred to a polypropylene tube (15 mL). For ACN/water (90:10, v/v) or THF/water (75:25, v/v) extraction, 5 mL of extractant was added into each centrifugal tube and the tube was mixed in an MS3 digital vortex (IKA group, German) for 2 min, and then sonicated for 10 min in ultrasound equipment (Kunshan ultrasonic instrument, Co., Ltd., China). After centrifugation at 8000 rpm for 10 min, the supernatant was collected and transferred to a 15 mL polypropylene tube. The extraction procedures using the above three solvents were repeated twice, respectively. All the supernatants of each sample obtained by the three extracts were collected, combined, and then concentrated to a volume of approximately 1 mL using a gentle stream of nitrogen gas at 40 °C, respectively.

Solid-Phase Extraction Cleanup (SPE). The obtained extract (1 mL) of each sample was diluted with 9 mL of ultrapure water. Three cartridges (i.e., WAX, florisil, and HLB) with the same cleanup process were used to gain a satisfactory cleanup cartridge. The diluted extract was introduced into a cleanup cartridge (6 mL, 150 mg) loaded with ENVI-Carb (10 mg) and preconditioned with 5 mL of methanol and 5 mL of ultrapure water in turn before cleanup. The eluent was discarded. Afterward, the target compounds were eluted with 4 mL of methanol and 4 mL of 0.1% of ammonia methanol (ammonia/methanol, v/v) in turn. The eluent was collected and concentrated to dryness under a gentle stream of nitrogen gas, and then was redissolved in 1 mL of methanol, mixed by a vortex for 30 s, and filtered through a 0.22 μm Acrodisc LC13 GHP Pall filter for HPLC-MS/MS analysis.

Chromatographic and Mass Spectrometric Condition. Analysis of the target PFCAs was performed on a HPLC-MS/MS composed of a 1260 liquid chromatograph (Agilent, USA) interfaced with an API 4000 Q-Trap spectrometer (Applied Biosystems, Foster City, CA, USA). Sample was injected with 5 μL of volume. HPLC separation of the target PFCAs was performed on a solid-core particle C_{18} column (4.6 × 100 mm, i.d., 2.7 μm, Waters, USA). The mobile phase included (a) water and (b) acetonitrile, both containing 5 mM ammonium acetate. In the gradient program, the C_{18} column was eluted with a linear gradient of (b) at a flow rate of 500 μL/min. The linear gradient of (b) was set as follows: start at 3% (held 0.5 min), increase to 95% at 6 min (held 3.1 min), and then back to 3% at 9.5 min (held 3 min), in a total run time of 12.5 min. All the target PFCAs were eluted within 8.0 min. The electrospray ionization source in scheduled negative multiple-reaction-monitoring (MRM) mode was

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Table 2. Optimal HPLC-MS/MS Parameters for Target PFCAs and Internal Isotopically Labeled Standards

analyte	retention (min)	precusor ion	product ion	IS used	DP[a]	CE[b]
PFHxA	5.59	312.9	269.0[c]/168.9	$^{13}C_2$-PFHxA	−40	−13
PFHpA	5.94	362.8	318.8[c]/168.9	$^{13}C_2$-PFHxA	−49	−15
PFOA	6.26	412.8	369.0[c]/168.8	$^{13}C_4$-PFOA	−45	−15
PFNA	6.55	462.9	418.8[c]/218.7	$^{13}C_5$-PFNA	−38	−16
PFDA	6.85	513.0	469.0[c]/269.0	$^{13}C_2$-PFDA	−50	−17
PFUnA	7.06	563.0	519.1[c]/269.1	$^{13}C_2$-PFUnA	−45	−17
PFDoDA	7.30	613.1	569.0[c]/268.8	$^{13}C_2$-PFDoA	−50	−19
PFTrDA	7.53	663.0	619.0[c]/269.0	$^{13}C_2$-PFDoA	−55	−20
PFTeDA	7.75	713.0	669.1[c]/319.0	$^{13}C_2$-PFDoA	−55	−23
$^{13}C_2$-PFHxA	5.60	315.0	269.0/168.9		−40	−13
$^{13}C_4$-PFOA	6.27	416.8	371.8/171.8		−40	−16
$^{13}C_5$-PFNA	6.54	468.0	423.0		−38	−16
$^{13}C_2$-PFDA	6.85	514.9	470.0/269.0		−45	−15
$^{13}C_2$-PFUnA	7.05	565.0	520.0		−50	−17
$^{13}C_2$-PFDoA	7.29	615.0	570.0		−50	−19

[a]DP, decluster potential. [b]CE, collision energy voltage. [c]Quantitative ion.

applied for the identification and quantitation of the target PFCA variants. The typical parameters of the electrospray ionization source were as follows: ion source spray voltage (IS), −4500 V; air curtain gas pressure (CUR), 200 psi (nitrogen); collision gas (CAD), high; entrance potential (EP), −10 V; collision cell exit potential (CXP), −15 V; atomization gas pressure (GAS1), 45 psi (nitrogen); auxiliary gas pressure (GAS2), 50 psi (nitrogen); atomization temperature (TEM), 550 °C. The optimized HPLC-MS/MS parameters for target PFCAs and used ISs are listed in Table 2.

Validation Study. The developed method was validated with linearity, instrument detection limits (IDLs), method detection limits (MDLs), specificity, accuracy, and precision using different edible crop matrices including cereal (grain), root vegetable (carrot), leafy vegetable (lettuce), and melon vegetable (pumpkin). Concentrations of the target PFCAs in the edible crop matrices were determined by the internal standard isotope method, with $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA,$^{13}C_2$-PFDA, $^{13}C_2$-PFUnA, and $^{13}C_2$-PFDoA as internal standard compounds (Table 2). The linearity was estimated for each target PFCA by determining its standard containing the internal standards (5 ng/mL) in solvent (methanol) and each edible crop matrix, respectively, in triplicate at nine concentration levels (0.1, 0.2, 0.5, 1, 2.5, 5, 10, 25, 50 ng/mL). The calculated parameters including the slope and determination coefficient (R^2) of linear regression equations are shown in Table 1. Matrix effect (ME) was quantified by computing the slope ratio between each matrix-matched calibration curve (n = 3) and the methanol curve (n = 3).[35,37] The IDLs were set as the concentration which produces a signal-to-noise (S/N) ratio of three in the solvent (methanol).[29] To fully exhibit the efficiency of the pretreatment procedure and the variability of the instrument noise, both the procedural blanks of the four edible crop samples and the samples spiked with a trace PFCA concentration (0.05 or 0.2 ng/g) in sextuplicate were determined. Because no detectable signals for the target analytes were recorded in all the procedural blanks, the MDLs were set as the five times standard deviation of sextuplicate analysis of spike levels at the trace concentration, according to the previous studies.[32,34] The specificity of the developed method was evaluated in accordance with the absence of interfering peaks related to the characteristic m/z at the retention time of the target PFCAs in the procedural blanks and the edible crop matrix blanks.[37,38] Accuracy and precision of the developed method were evaluated using recovery tests conducted by the four crop matrices spiked with target PFCAs at four concentrations, i.e., 0.5, 10, 25, and 50 ng/g. The accuracy was determined by calculating the percentages of actual levels to theoretical levels in the spiked tests.[34] The precision was expressed as the percentage relative standard deviation (RSD) for 5 replicates.[34] In order to avoid sample contamination, all experimental utensils used were rinsed by ultrapure water and HPLC-grade methanol in turn.[31] In the sample analysis, a sample matrix spike and a procedural blank were included in the analytical procedures for every batch of six samples.[31]

Data Analysis. PFCA concentration data derived from HPLC-MS/MS analysis were obtained using AB Sciex Analyst 1.6 software (Applied Bioscience). Calculation of mean, RSD (%), Pearson correlation, and regression equation were performed on SPSS 21.0 (International Business Machines Co, USA). Tables and figures exhibited in the present paper were finished by Microsoft Excel 2013 (Microsoft Co., Redmond, WA, USA).

RESULTS AND DISCUSSION

HPLC-MS/MS Optimization. The reverse-phase HPLC instrument containing a C_{18} stationary phase and acetonitrile in the mobile phase was usually used to separate PFCA variants.[30,39] Three kinds of C_{18} columns, i.e., Phenomenex C_{18} column (4.6 × 100 mm, i.d., 2.7 μm,), Waters Solid-core particle C_{18} column (4.6 × 100 mm, i.d., 2.7 μm), and Waters Atlantis T3 C_{18} column (2.1 × 150 mm, i.d., 3 μm), were evaluated for separation of the PFCA variants. The solid-core particle C_{18} column gave the best sensitivity, resolution, and reproducibility of the PFCA variants owing to using the solid-core particle technology (Figure S1), so it was selected as the stationary phase. As for mobile phase, a linear gradient of acetonitrile in a certain concentration of ammonium acetate solution was capable of separating PFCA variants better than pure acetonitrile, because ammonium acetate increased both the protonation of carboxyl in the analytes and the interactions of alkane groups between the PFCA variants and the C_{18} column.[39] The ammonium acetate solutions at different concentrations, i.e., 1, 5, 10, and 20 mM, were compared to obtain the optimized sensitivity. It was observed that the solution with 5 mM ammonium acetate exhibited the optimal sensitivity (Figure S1). Under the optimal HPLC conditions, the retention time of the analytes ranged from 5.59 to 7.75 min (Table 2, Figure S1).

The single standard solutions of each PFCA variant were infused into the electrospray ionization source to produce an ion transition under the scheduled negative MRM mode. The deprotonated molecules ($[M - H]^-$, i.e., precursor ions) with m/z in the range of 312.9−713.0 were formed by the PFCA (C6−C14) in the full-scan mass spectrum.[31,40] Subsequently, the first fragment ions ($[M - COOH]^-$) with m/z ranging from 269.0 to 669.1 and the second fragment ions with m/z

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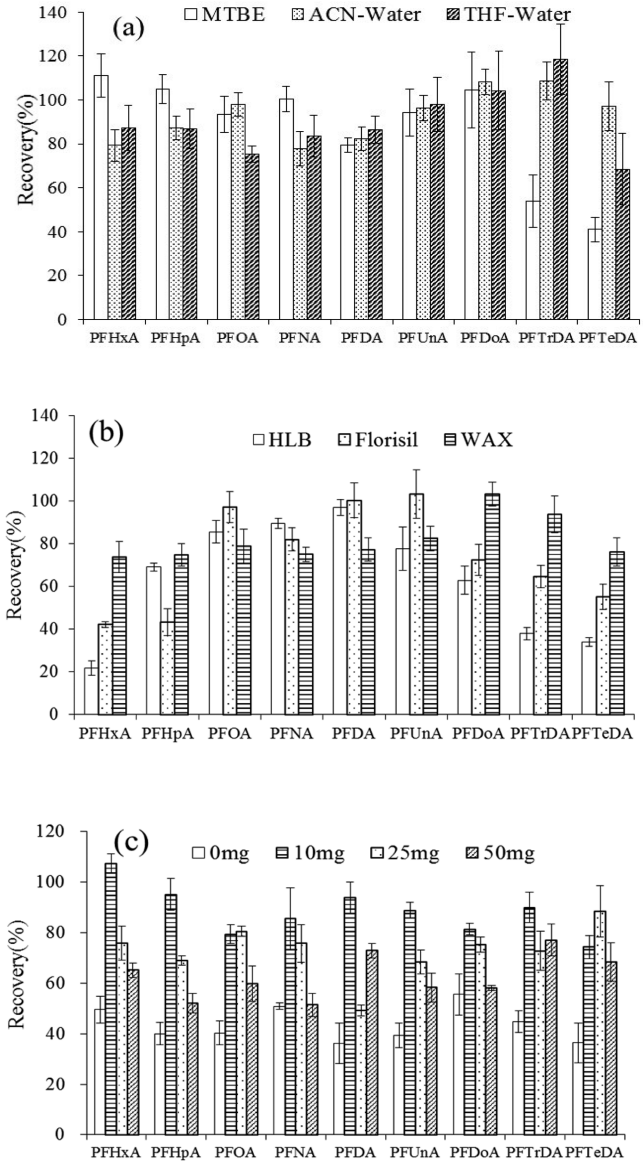

Figure 1. Effects of extractant (a), cleanup cartridge (b), and ENVI-Carb usage (c) on the recoveries of PFCAs in lettuce sample spiked at 10 ng/g. MTBE, ACN−water, and THF−water indicate methyl *tert*-butyl ether, acetonitrile/water (90:10, v/v), and tetrahydrofuran/water (75:25, v/v), respectively.

ranging from 168.9 to 319.0 were formed from the precursor ions.[31,40,41] The precursor ions, fragment ions, and optimal MS/MS parameters for the each PFCA variant are presented in Table 2.

Sample Extraction Optimization. *Extractant Optimization.* Recovery experiments for each single factor, namely, varieties of extractants and cleanup cartridges, and usage of ENVI-Carb were performed using PFCAs-spiked leaf vegetable matrix (lettuce) at 10 ng/g in order to achieve an optimized extraction procedure. Correspondingly, the optimized procedure was assessed in the other three edible crop matrices.[31,38] First, three commonly used solvents [MTBE, ACN/water (90:10, v/v), THF/water (75:25, v/v)] for PFCA extraction were separately evaluated in the extraction procedure to obtain a satisfactory extractant.[32−34] Extraction was conducted three times for each extractant. As shown in Figure 1a, good recoveries (79%−111%) and RSDs (3.3%−17.1%) of PFHxA, PFHpA, PFOA, PFNA, PFDA, PFDoA, and PFUnA were obtained when MTBE was used as the extractant, but low recoveries (41%−54%) of PFTrDA and PFTeDA were not satisfactory. Meanwhile, good recoveries (68%−118%) for all the target PFCAs except relatively high RSDs (16%−18%) of PFDoA, PFTrDA, and PFTeDA were gained when using THF/water (75:25, v/v) as extractant. On the other hand, both recoveries (78%−108%) and RSDs (5.2%−11%) for all the PFCAs were satisfactory when ACN/water (90:10, v/v) was applied. Higher extraction efficiencies of ACN/water than those of MTBE and THF/water might be attributed to strong affinity of PFCAs to the former.[22] It is noted that PFCAs contain acidic group (carboxyl) and tend to distribute in polar solvent, but their polarities decrease with increasing carbon chain lengths,[32,42] resulting in relatively lower recoveries of the PFCAs with long (≥C10) carbochains (57%−68%) when extracting thrice using ACN/water (90:10, v/v) (Table S1). Therefore, when ACN/water (90:10, v/v) was used as the extractant in the present study, pure acetonitrile with relatively lower polarity was also applied to ensure the sufficient extraction of the PFCAs with long carbochains (C10−C14). Consequently, the optimized extraction procedure was set as follows: ACN/water (90:10, v/v) was used for first extraction, followed by pure acetonitrile for the second extraction and ACN/water (90:10, v/v) for the third extraction.

Cleanup Cartridge Optimization. SPE is an efficient procedure for removing interfering matrix constituents of the samples in the analysis of PFCAs.[43] Three frequently used cleanup cartridge for PFCAs analysis, i.e., WAX cartridge, HLB cartridge, and Florisil cartridge, were separately investigated to gain the optimized cleanup cartridge.[2,43] When HLB cartridges or Florisil cartridges were used, good recoveries (63%−97%) with RSDs (5.3%−11%) for PFOA, PFNA, PFDA, PFUnA, and PFD_OA but bad recoveries (22%−55%) with RSDs (1.4%−6.3%) for the other four PFCAs were observed in Figure 1b. On the other hand, WAX cartridges presented satisfactory recoveries (73%−103%) with RSDs (5.3%−8.7%) for all the target PFCAs. Due to its mixed retention mechanism including reverse phase and anion exchange, WAX cartridge displayed higher selectivity and recovery for the acidic analytes than HLB and Florisil cartridge.[2,43] So, desirable recoveries for the PFCAs were achieved owing to their acidic group (carboxyl) when using WAX cartridge, which was chosen as the cleanup cartridge in this study.

ENVI-Carb Usage Optimization. ENVI-Carb can retain aromatic compounds via π-electron interaction, but in vain for PFCAs owing to their strong electronegative C−F bonds.[32,42] Therefore, SPE followed by ENVI-Carb cleanup can further remove interfering matrix constituents and improve the recoveries of PFCAs in environmental samples such as soil, sludge, and vegetable.[13,34] The effects of ENVI-Carb usages (i.e., 0, 10, 25, and 50 mg) on the PFCA recoveries were tested in the present study. As shown in Figure 1c, significantly better PFCA recoveries (52%−112%) were observed when using 10−50 mg of ENVI-Carb compared with control (40%−56%), indicating that efficiency of combined WAX cartridge and ENVI-Carb was more satisfactory than the individual effect of WAX cartridge. However, the PFCA recoveries generally decreased with increasing ENVI-Carb usage owing to the sorption of PFCAs to the excessive ENVI-Carb (25 and 50 mg). Thus, 10 mg of ENVI-Carb was selected for cleanup. It was noted that the SPE and ENVI-Carb cleanup for PFCAs were usually set as two separate procedures in the previous

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Table 3. Recoveries (n = 5) and RSD (%) of Target PFCAs in Various Crop Matrices

		lettuce		pumpkin		carrot		grain	
analyte	spiked levels ng/g	recovery	RSD	recovery	RSD	recovery	RSD	recovery	RSD
PFHxA	0.5	105	9.9	107	2.9	83	6.1	107	5.2
	10	79	7.2	92	9.6	103	11	79	7.0
	25	78	3.2	103	1.3	110	6.8	94	11
	50	72	1.9	96	6.4	107	11	96	4.3
PFHpA	0.5	105	5.6	103	12	91	7.3	102	4.2
	10	92	5.3	107	11	108	12	91	8.9
	25	82	59	106	3.3	112	12	89	6.2
	50	77	5.4	105	5.6	104	2.8	98	4.8
PFOA	0.5	82	1.9	93	2.0	81	11	103	12
	10	103	8.0	99	11	101	12	88	4.6
	25	111	8.3	110	1.1	109	13	98	4.1
	50	93	5.9	106	4.1	103	5.3	95	9.8
PFNA	0.5	86	2.5	92	5.4	99	3.0	90	13
	10	72	7.9	101	11	109	7.8	102	2.3
	25	90	6.7	92	4.3	110	4.5	101	5.5
	50	88	7.0	82	5.8	94	6.4	95	7.6
PFDA	0.5	103	5.8	103	12	99	3.0	87	12
	10	79	5.3	89	9.0	104	10	89	3.6
	25	114	9.4	94	9.9	108	6.1	87	8.4
	50	104	1.7	103	9.3	98	7.5	90	6.4
PFUnA	0.5	102	4.9	107	9.3	101	10	105	5.6
	10	101	5.8	99	8.1	106	7.5	111	2.6
	25	96	6.6	110	9.8	112	6.1	91	4.2
	50	81	1.1	111	12	114	12	86	5.0
PFDoDA	0.5	91	5.8	89	8.7	89	8.7	101	10
	10	113	9.2	86	11	109	5.0	77	6.1
	25	76	2.8	98	12	81	5.6	77	12
	50	77	8.0	105	10	113	6.4	71	12
PFTrDA	0.5	94	2.0	88	6.8	75	9.2	77	2.7
	10	114	8.7	78	3.2	113	5.6	70	5.6
	25	72	5.5	75	6.7	107	5.2	86	5.2
	50	75	12	80	9.2	107	9.6	76	6.3
PFTeDA	0.5	104	6.2	87	5.8	84	10	71	1.1
	10	95	9.9	76	4.7	88	11	73	4.3
	25	71	5.2	73	10	108	2.0	75	6.8
	50	71	2.5	74	4.7	101	11	73	9.5

Table 4. Pearson's Correlation Coefficients between Matrix Effect (ME = $\text{slope}_{\text{matrix}}/\text{slope}_{\text{methanol}}$) and Crop Matrix Constituents (n = 12)

matrix effect	chlorophyll a	chlorophyll b	carotenoids	total dissolved sugar	fiber	protein
ME_{PFHxA}[a]	−0.149	−0.130	−0.538	−0.938**	0.606*	−0.213
ME_{PFHpA}	−0.776**[b]	−0.783**	−0.032	0.609*	0.293	−0.803**
ME_{PFOA}	−0.728**	−0.721**	−0.993**	−0.487	0.934**	−0.665*
ME_{PFNA}	−0.741**	−0.731**	−0.388	−0.367	−0.698*	−0.835**
ME_{PFDA}	−0.509	−0.526	−0.067	0.814**	0.041	−0.416
ME_{PFUnA}	0.419	0.413	0.954**	0.559	−0.744**	0.315
ME_{PFDoA}	0.225	0.216	−0.247	0.105	−0.131	0.386
ME_{PFTrDA}	0.442	0.432	−0.013	0.132	−0.360	0.586*
ME_{PFTeDA}	−0.422	−0.425	−0.799**	−0.115	0.532	−0.271

[a]Matrix effect. [b]"*" and "**" indicate $P < 0.05$ and $P < 0.01$, respectively.

studies,[24,33,44] which might increase the analytical procedures and thus lead to more losses of the analytes in the pretreatment processes including transfer centrifugation etc. So, in the present study, the ENVI-Carb was directly loaded in the WAX cartridge, and the cleanup combined with ENVI-Carb and SPE achieved satisfactory recoveries (72%−112%, RSD ≤ 10%), comparable with the results using mixed-mode SPE (C8 mixed with quaternary amine or Florisil mixed with ENVI-carb, recoveries 50%−95%, RSD ≤ 22%) or by inline application of WAX cartridge combined with ENVI-carb (recoveries 61%−116%, RSD ≤ 13%),[32−34] and better than the results using the two separate cleanup processes (recoveries 48%−158%, RSD ≤ 30%).[24,44]

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Table 5. Concentrations (ng/g, dry weight/fresh weight) of the Target PFCAs in Real Edible Crops

analyte	lettuce[a]	mustard leaf[b]	pakchoi[c]	celery[d]	pumpkin[e]	cucumber[f]	eggplant[g]	bitter dish[h]	*Lactuca sativa*[i]	loofah[j]
PFHxA	0.32 ± 2.1/ 0.02 ± 0.11	ND[l]	0.46 ± 0.21/ 0.03 ± 0.01	ND	ND	ND	0.14 ± 0.02/ 0.015 ± 0.002	0.17 ± 0.12/ 0.03 ± 0.02	0.42 ± 0.27/ 0.11 ± 0.07	ND
PFHpA	0.77 ± 1.2/ 0.04 ± 0.06	ND	0.99 ± 1.7/ 0.06 ± 0.10	0.23 ± 0.39/ 0.01 ± 0.02	ND	ND	1.4 ± 1.3/ 0.15 ± 0.14	0.90 ± 0.12/ 0.08 ± 0.01	ND	ND
PFOA	5.3 ± 8.4/ 0.26 ± 0.42	0.82 ± 0.59/ 0.04 ± 0.03	8.7 ± 14.2/ 1.1 ± 2.4	14 ± 18/ 0.61 ± 0.82	2.1 ± 1.6/ 0.20 ± 0.15	0.22 ± 0.16/ 0.01 ± 0.01	0.12 ± 0.02/ 0.008 ± 0.001	3.0 ± 1.5/ 0.51 ± 0.25	0.38 ± 0.20/ 0.10 ± 0.05	0.56 ± 0.29/ 0.04 ± 0.02
PFNA	ND	ND	ND	ND	ND	ND	ND	0.10 ± 0.007/ 0.01 ± 0.001	0.10 ± 0.03/ 0.03 ± 0.01	ND
PFDA	ND	ND	0.11 ± 0.18/ 0.01 ± 0.01	ND	ND	ND	ND	0.33 ± 0.19/ 0.06 ± 0.03	0.30 ± 0.18/ 0.08 ± 0.05	ND
PFUnA	0.38 ± 0.18/ 0.21 ± 0.17	0.22 ± 0.05/ 0.01 ± 0.002	0.35 ± 0.21/ 0.02 ± 0.01	1.1 ± 0.16/ 0.20 ± 0.05	ND	ND	ND	0.23 ± 0.16/ 0.04 ± 0.03	0.23 ± 0.05/ 0.06 ± 0.01	ND
PFDoA	0.21 ± 0.17/ 0.05 ± 0.04	0.13 ± 0.13/ 0.01 ± 0.01	0.18 ± 0.05/ 0.01 ± 0.003	0.13 ± 0.16/ 0.01 ± 0.01	0.19 ± 0.01/ 0.02 ± 0.001	ND	0.14 ± 0.004/ 0.02 ± 0.001	0.15 ± 0.05/ 0.03 ± 0.01	0.19 ± 0.11/ 0.05 ± 0.03	ND
PFTrDA	ND	ND	ND	ND	0.11 ± 0.01/ 0.01 ± 0.001	ND	0.21 ± 0.002/ 0.02 ± 0.001	ND	0.10 ± 0.02/ 0.03 ± 0.01	ND
PFTeDA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
∑PFCAs	7.1 ± 2.7/ 0.36 ± 0.14	1.3 ± 1.4/ 0.06 ± 0.07	11 ± 5.4/ 0.65 ± 0.33	15 ± 8.2/ 0.67 ± 0.36	2.4 ± 1.6/ 0.02 ± 0.15	0.22 ± 0.16/ 0.01 ± 0.01	1.9 ± 1.3/ 0.22 ± 0.15	4.9 ± 1.3/ 0.98 ± 0.26	1.8 ± 0.3/ 0.47 ± 0.08	0.56 ± 0.29/ 0.04 ± 0.02

[a] $n = 7$. [b] $n = 9$. [c] $n = 3$. [d] $n = 3$. [e] $n = 9$. [f] $n = 9$. [g] $n = 10$. [h] $n = 12$. [i] $n = 20$. [j] $n = 9$. [l] Not detected.

Once the optimized pretreatment procedure for the PFCAs in lettuce spiked at 10 ng/g was obtained, it was tentatively applied to determination of the analytes in the other three edible crop matrices, i.e., carrot, pumpkin, and grain spiked at 10 ng/g. Satisfactory recoveries in the range of 70% to 113% with low RSDs (<12%) for all nine PFCAs were observed in the three matrices (Table 3), showing high feasibility of the optimized pretreatment procedure to determine the analytes in various edible crop matrices including cereal, root vegetable, leafy vegetable, and melon vegetable.

Method Validation. *Linearity, IDLs, MDLs, and Specificity.* The peak area ratios (the analyte peak area/relative IS peak area) obtained from the MS/MS mode were used to gain the linearity. The linearity was evaluated by determining solvent calibration curve and various matrix-matched calibration curves (lettuce, carrot, pumpkin, and grain) at nine concentrations, i.e., 0.1, 0.2, 0.5, 1, 2.5, 5, 10, 25, 50 ng/mL for each analyte. High correlation coefficients (R^2 > 0.993) were found in all cases (Table 1), showing desirable linearities for each analyte in all the edible crop matrices within the range of 0.1−50 ng/mL except for PFOA, PFDA, and PFUnA in lettuce, PFDA and PFUnA in carrot, and PFDoA in grain (linear range 0.2−50 ng/mL). The IDLs for the target PFCAs varied from 0.010 to 0.017 ng/mL in solvent (methanol) based on the peak-to-peak $S/N = 3$ (Table 1). The matrix dependent MDLs (dry weight, dw) of the target PFCA variants ranged from 0.017 to 0.180 ng/g in various crop matrices, up to 50 times lower than previous report (0.24−8.2 ng/g, dw),[2,44] showing the high sensitivity of the developed method. Considering water content in edible crop matrix, the matrix dependent MDLs (fresh weight, fw) were determined using an equation as follows:

$$\text{MDLs (fw)} = \text{MDLs (dw)} \times (100 - W)/100 \quad (1)$$

where W indicates water content (%), which is described in Table S2. According to Table 1, the matrix dependent MDLs (fw) for the target PFCAs in vegetables and grain ranged from 0.002 to 0.016 ng/g and from 0.016 to 0.098 ng/g, respectively. The MDLs (fw) in vegetables using the common equipment HPLC-MS/MS (Q-Trap) in the present study were generally better than the results using the same equipment (0.006−0.016 ng/g),[2,24] equaling or nearing the sensitivity of the superior analytical equipment, e.g., HPLC-MS/MS (Q-ToF-HRMS, 0.002−0.007 ng/g) and UPLC-MS/MS (TQS, 0.0005−0.003 ng/g)[32,34] (Table S3). With regard to specificity, no interfering peaks related to the characteristic m/z of the target PFCAs at the retention time were observed in procedure blank and each edible crop matrix blank, showing a high specificity of the HPLC-MS/MS determination (Figures S2 and S3).

Matrix Effect and Its Potential Influencing Factors. Although an optimized extraction and cleanup procedure was obtained, matrix effect (ME) occurred inevitably in the determination of trace organic analytes in complicated samples using HPLC-MS/MS.[37,45] ME is likely to exert great influence on the precision and accuracy of an analyte quantification, depending on the type and amount of matrices, analyte properties, instrument, etc.[37,38,46] So ME in each edible crop matrix for the target PFCAs was quantitated by comparing the slope ratios of standard calibration curves ($n = 3$) of each crop matrix and methanol. The equation applied to quantitate the MEs for each edible crop matrix was described as follows:

$$\text{ME} = \text{slope}_{\text{matrix}}/\text{slope}_{\text{methanol}} \quad (2)$$

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where $slope_{matrix}$ and $slope_{methanol}$ indicate the slope of standard calibration curves for matrix and methanol, respectively. According to previous studies, ME was categorized into seven levels, namely, strong suppression effect (ME < 0.5), medium suppression effect (0.5 < ME < 0.8), mild suppression effect (0.8 < ME < 0.9), negligible effect (0.9 < ME < 1.1), mild enhancement effect (1.1 < ME < 1.2), medium enhancement effect (1.2 < ME < 1.5), and strong enhancement effect (ME > 1.5).[37,46] As shown in Table 1, the ME for various target PFCAs varied greatly in different crop matrices. Specifically, strong suppression effect for PFHxA, PFHpA, and PFTeDA, medium suppression effect for PFNA and PFTeDA, and mild suppression effect for PFDA and PFDoA were observed in lettuce. Strong suppression effect for PFHxA, PFHpA, PFTrDA, and PFTeDA, medium suppression effect for PFDoA, mild suppression effect for PFDA, and mild enhancement effect for PFNA in pumpkin, as well as strong suppression effect for PFHxA and PFHpA, medium suppression effect for PFUnA, PFTrDA, and PFTeDA, and mild suppression effect for PFNA in carrot were found. As for grain, strong suppression effect for PFHxA, PFHpA, and PFTrDA, medium suppression effect for PFUnA and PFTeDA, mild suppression effect for PFDA and PFDoA, and medium enhancement effect for PFOA were observed.

ME for PFHpA, PFOA, and PFNA displayed significantly negative correlations with contents of chlorophyll a chlorophyll b, and protein (Table 4). The same correlations were observed on ME for PFOA and PFTeDA with contents of carotenoid, ME for PFHxA with contents of total dissolved sugar, and ME for PFNA and PFUnA with the contents of fiber. However, ME for PFHpA and PFDA with contents of total dissolved sugar, ME for PFHxA and PFOA with contents of fiber, and ME for PFTeDA with contents of protein presented significantly positive correlations. To further understand the relationships between the ME and the matrix constituents, their lineal regression equations were calculated and are listed in Table S4. It can be found that the main factors influencing ME for various PFCAs were protein (PFHpA, PFNA, and PFTrDA), carotenoid (PFOA and PFTeDA), fiber (PFHxA and PFUnA), and total dissolved sugar (PFDA), respectively, which may be linked to the affinity between the PFCAs and the proteins, and the hydrophobic interactions between the PFCAs and carotenoid, fiber, and total dissolved sugar.[20,27]

Additionally, the ME for PFCAs was also closely related to the carbochain lengths of the PFCAs. Remarkable suppression effect was observed in the PFCAs with shorter (C6−C7) or longer carbochain length (C13−C14), while generally mild suppression effect or mild enhancement effect was found in the PFCAs with moderate carbochain length (C8−C12). Considering that ME was inevitable, and matrix and PFCA variant dependent, the more realistic analysis results in the present study were obtained using matrix-matched standard calibrations referred to IS compounds.

Accuracy and Precision. Accuracy and precision of the developed method were evaluated using recovery tests conducted by the four crop matrix blanks spiked with target PFCAs at four concentrations, i.e., 0.5, 10, 25, and 50 ng/g. All recoveries for the developed method are described in Table 3. All the recoveries obtained for the target PFCAs in the four crop matrices ranged from 70% to 114% with RSD lower than 12%, meeting the requirements of DG SANCO/12459/2011 guidelines with recovery from 70% to 120% and RSD lower than 20%. Furthermore, compared with the recent analytical methods on the target PFCAs in crops (recoveries 48%−155%, RSDs ≤ 30),[2,24,32−35] the present study showed comparable or even better precision and accuracy (recoveries 70%−114%, RSDs ≤ 12), indicating high precision and accuracy of the developed method (Table S3).

Method Application. In order to evaluate its practicability, a total of 91 real crop samples including lettuce (n = 7), mustard leaf (n = 9), pakchoi (n = 3), celery (n = 3), pumpkin (n = 9), cucumber (n = 9), eggplant (n = 10), bitter dish (n = 12), *Lactuca sativa* (n = 20), and loofah (n = 9) were determined using the developed method. These samples were collected from several farms close to various fluoride factories that produced tetrafluoroethylene (TFE), polytetrafluoroethylene (PTFE), etc., in south China's Pearl River Delta area. Matrix-matched standard calibrations and quality control at 0.5 ng/g were carried out for each crop sample in determination procedures with recoveries in the range of 71%−112% and RSDs lower than 10% (Table S5). Data in Table 5 show that all the target PFCAs except for PFTeDA were detected in the collected samples with average total concentrations ranging from 0.22 to 15 ng/g (dw). PFOA was a dominant pollutant that was detected in all the samples with average concentrations ranging from 0.12 to 14 ng/g (dw), much higher than the other PFCAs (0.10−1.4 ng/g, dw). This might be attributed to the common use of PFOA as dispersant in the production process of TFE and PTFE.[47] More than five PFCAs were detected in lettuce, pakchoi, eggplant, bitter dish, and *Lactuca sativa* with detection rate and average concentrations ranging from 10% to 100% and 0.10 to 8.7 ng/g (dw), respectively (Table 5, Table S6), while only one compound (PFOA) was detected in cucumber and loofah. PFCA concentrations in crops were species and pollution level dependent.[17,20] The average total PFCA concentrations (0.22−15 ng/g, dw, i.e., 0.01−0.67 ng/g, fw) in crops in the present study were generally higher than those in vegetables (0.011−0.038 ng/g, fw) from retail stores in Belgium, Czech Republic, Italy, and Norway,[26,27] and in the more fermented teas (0.95−7.0 ng/g, dw) from China, but lower than in the less fermented teas (2.6−43 ng/g, dw) from China.[48] Higher PFCA concentrations detected in the less fermented teas could be related to long exposure to environmental PFCAs, while lower PFCA concentrations in more fermented teas might be attributed to the PFCA degradation in fermentation.[48]

In summary, a reliable, precise, and sensitive method was achieved for simultaneous analysis of nine trace PFCAs (C6−C14) in various edible crop matrices including cereal, root vegetable, leafy vegetable, and melon vegetable using ultrasonic extraction followed by SPE and common HPLC-MS/MS (Q-Trap), nearing the sensitivity of superior equipment at pg/g level (fw), e.g., UPLC-MS/MS and HPLC-QTOF-HRMS. The successful application of the developed method to determine PFCAs in various types of crops sampled from several farms demonstrated its practicability for regular monitoring of PFCAs in real crops, which is of great importance to assess the health risk of human exposure to PFCAs via crop origin food consumption.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b02677.

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HPLC-MS/MS, methods, regression equations, recoveries, and detection rate of target PFCAs (PDF)

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Notes

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