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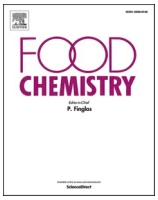

Analytical Methods

Per- and polyfluoroalkyl substances (PFAS) presence in food: Comparison among fresh, frozen and ready-to-eat vegetables

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ABSTRACT

There is a worldwide discussion to provide safety limits in food for per- and polyfluoroalkyl substances (PFAS), a group of persistent contaminants associated to human disease. Processed food is more at risk of containing increased amounts of PFAS as a consequence of intentionally or non-intentionally contamination during manipulation and packaging. Among food products, also vegetables can be submitted to industrial manipulation; therefore, a different PFAS content correlated to the level of vegetables processing is conceivable. This study assessed the amount and type of PFAS present in fresh, frozen and ready-to-eat vegetables. Differences have been observed between the three groups of samples in the average PFAS content; the difference between ready-to eat and frozen vegetables resulted statistically significative. Organic vegetables displayed a lower total amount of PFAS respect to the traditional counterpart. The impact of industrial manipulation remains to be cleared, but pesticides use during cultivation could be considered a source of PFAS contamination.

1. Introduction

The two main agencies for environment and human safety, USA Environmental Protection Agency (EPA) and European Food Safety Authority (EFSA) have recognised the problem of PFAS diffusion by establishing methods and limits in PFAS exposure. In particular EPA (US EPA, 2019) has established guidelines to detect, quantify and advisory levels for PFAS presence in drinking water (US EPA, 2016), while EFSA has established tolerable weekly intake (TWI) values for the sum of collectively-four PFAS: PFOA, PFNA, PFHxS and PFOS (EFSA, 2018). Furthermore, EFSA indicated as the most important contributions to the human diet products consisting of “fish meat”, “vegetal products” and “eggs and egg products” (Schrenk et al., 2020). Beside water, vegetal products may represent the first entry of these chemicals into the food chain up to humans. Plants such as spinach (*Spinacia oleracea*), tomato (*Lycopersicon esculentum*), lettuce (*Lactuca sativa*), strawberry (*Fragaria ananassa*) and corn (*Zea mays*) demonstrated both an uptake of PFAS through their roots from soil/nutrient solutions and an adsorption through their leaves directly from the atmosphere (Navarro et al., 2017; Liu et al., 2019). In fact, some plants have also been studied as a possible phytoremediation tool for soil and water contaminated sites due to their properties of PFAS hyperaccumulation (Huff et al., 2020). Currently, there are no regulatory limits for PFAS in food. However, regulations are under discussion worldwide, including in Europe, USA, and China. Recently (December 7, 2022), the EC Commission established the maximum levels of PFAS in eggs, fishery products and muscle meats (European Commission, 2022a). At the moment, vegetables products are not provided by specific limits, however the European Union is working to introduce monitoring guidelines and Maximum Limits (MRLs) in a wide range of food products, including vegetables. In the next years, an accurate monitoring of perfluoroalkyl substances will be realized to collect more comprehensive data on their presence in food. The recommendation considers not only EFSA’s four priority PFAS mentioned above (PFOS, PFOA, PFHxS and PFNA), but also a larger number of PFAS. The monitored foodstuffs should respond to consumption habits, including fruits, vegetables, starchy roots and tubers, mushrooms, seaweed, cereals, nuts, oilseeds, food for infants and young children, food of animal origin, non-alcoholic drinks, wine and beer. In this frame, attention limits for PFOS, PFOA, PFNA and PFHxS have been proposed in vegetables, above which further investigations of the causes

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of contamination should be carried out (European Commission, 2022b). Accordingly, PFOS and PFOA concentration in vegetables should not exceed 0.010 ng/g, PFNA should be below 0.005 ng/g and PFHxS should be below 0.015 ng/g.

Beside a first contamination during cultivation, vegetables could become contaminated also by subsequent industrial processes such as washing, chopping, packaging. In fact, industrial manipulation is known to be responsible for a certain amount of food contamination either by specific production or by using PFAS-containing packages. For example, in food packages, these substances are both intentionally used and non-intentionally added deriving from residues of recycled materials (Curtzwiler et al., 2021). Processed food products such as meat, fish and baby food have been investigated for their PFAS content (Genualdi et al., 2021) and increased levels of PFAS in newborns, whose mothers were higher consumers of ultra-processed foods, were found (Naspolini et al., 2021). On this frame, the hypothesis of different PFAS amounts correlated to the level of vegetables processing is expectable. Notwithstanding many analytical methods are present in literature on the determination of contaminants in vegetables, to the best of our knowledge, none took in consideration a comparison among vegetables undergoing a different grade of manufacturing process.

In this study samples of fresh, frozen and ready-to-eat vegetables purchased at street markets and at grocery stores located in three Italian districts have been analysed and results compared for the presence of PFAS in order to verify this hypothesis.

A specific LC-MS method for the determination of 22 PFAS and 3 PFAS precursors in vegetal food material has been developed and validated. To overcome the limit of the absence of real blank samples, isotopic dilution method was employed for sample quantification (Piva et al., 2022). Different types of vegetables have been considered by selecting products available both as fresh, frozen and ready-to-eat. Results are presented as type and amount of the detected PFAS in the three classes of vegetal food.

2. Materials and methods

2.1. Materials

Analytes and isotopically labelled standards (>98 purity) were purchased from Wellington Laboratories (Guelph, Canada). Native analytes included perfluoro-*n*-butanoic acid (PFBA); perfluoro-*n*-pentanoic acid (PFPeA); perfluoro-*n*-hexanoic acid (PFHxA); perfluoro-*n*-heptanoic (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); tetrafluoro-2-heptafluoropropoxy propanoic acid (HFPO-Da); perfluoro-3-methoxypropanoic acid (PFMPA); perfluoro-4-methoxybutanoic acid (PFMBA); nonafluoro-3,6-dioxaheptanoic acid (NFDHA); potassium perfluoro-1-butanesulfonate (PFBS); sodium perfluoro-1-pentanesulfonate (PFPeS); potassium perfluorohexanesulfonate (PFHxS); sodium perfluoro-1-heptanesulfonate (PFHpS); potassium perfluoro-1-octanesulfonate (PFOS); sodium dodecafluoro-3H-4,8-Dioxanonanoate (ADONA); 1H,1H, 2H,2H-perfluoro-1-hexanesufonate (4:2 FTS); 1H,1H, 2H,2H-perfluoro-1-octanesufonate (6:2 FTS); 1H,1H, 2H,2H-perfluoro-1-decanesufonate (8:2 FTS); potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS); potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdS); potassium perfluoro (2-ethoxyethane) sulfonate (PFEESA).

Isotopically labelled compounds, added to the sample before extraction in known amount (surrogate) were used. Since not all target PFAS have an isotopically labelled analogue, an alternate labelled compound is used as recommended in EPA-533 method.

Isotopically labelled standards were PFBA $^{13}C_4$ (MPFBA) used to quantify PFBA and PFMPA; PFPeA $^{13}C_5$ (M5PFPeA) used to quantify PFPeA and PFMBA; PFHxA $^{13}C_5$ (M5PFHxA) used to quantify PFHxA and NFDHA; PFHpA $^{13}C_4$ (M4PFHpA) used to quantify PFHpA and ADONA; PFOA $^{13}C_8$ (M8PFOA) used to quantify PFOA; PFNA $^{13}C_9$ (M9PFNA) used to quantify PFNA; $^{13}C_6$PFDA (M6PFDA) used to quantify PFDA, PFUnA $^{13}C_7$ (M7PFUnA) used to quantify PFUnA; PFDoA $^{13}C_2$ (MPFDoA) used to quantify PFDoA; HFPO-DA $^{13}C_3$ (M3HFPO-DA) used to quantify HFPO-DA, PFBS $^{13}C_3$ (M3PFBS) used to quantify PFBS and PFEESA; PFHxS $^{13}C_3$ (M3PFHxS) used to quantify PFHxS and PFPeS; PFOS $^{13}C_8$ (M8PFOS) used to quantify PFOS, 9Cl-PF3ONS, 11Cl-PF3OUdS and PFHpS; 4:2FTS $13C_2$ (M2-4:2FTS) used to quantify 4:2 FTS; 6:2 FTS $13C_2$ (M2-6:2FTS) used to quantify 6:2 FTS and 8:2 FTS $13C_2$ (M2-8:2FTS) used to quantify 8:2 FTS.

Mass-labelled perfluoro-n- [2,3,4 $^{13}C_3$] butanoic acid (M3PFBA); perfluoro-n-[1,2-$^{13}C_2$] octanoic acid (M2PFOA), and mass-labelled sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate (MPFOS) at chemical purities > 98 % and isotopic purities of ≥ 99 % were added to the purified extracts before injection and used as injection standards.

Target perfluoroalkyl analytes, surrogate mass-labelled compounds and injection standards were prepared in methanol at concentration of 50 ng/mL, 50 ng/mL, 100 ng/mL respectively.

Acetonitrile and methanol (both LC-MS grade) were supplied by Carlo Erba Reagents (Milan, Italy), while formic acid (99–100 %) was supplied by VWR Chemicals (Fontenay-sous-Bois, France). Water for mobile phase was produced by Arium Ultrapure Water System (Sartorius, Goettingen, Germany). Activated carbon powder was obtained by Merck (Darmstadt, Germany). Ammonium acetate was provided by Sigma-Aldrich (St. Louis, USA). WAX polymer (150 mg, 6 mL) cartridges were manufactured from Agilent Technologies (Santa Clara, USA).

2.2. Sample collection

A total of 41 samples were collected as: fresh vegetables (n. 10: one package each of organic lettuce, red bell pepper, yellow pepper, organic carrots, onion, aubergine, green beans, courgette, tomato and valerian); frozen vegetables (n.8: one package each of broccoli, spinach, peas, zucchini, beans, carrots and two packages of mushrooms); and ready-to-eat vegetables (n. 23: three packages of lettuces, three packages of radishes, four packages of mixed salad, three packages of carrots, three packages of valerian, two packages of mushrooms, one package each of-organic lettuce, cabbage, spinach, rocket salad and organic rocket salad).

Fresh vegetables and ready-to-eat vegetables were stored at 4 °C while frozen vegetables were stored at −20 °C until sample preparation.

2.3. Sample preparation

Each product was sampled and analysed in triplicate. The procedure included fragmentation into small pieces using scissors and weighting 5 ± 0.10 g of each sample. Twelve μL of isotopically labelled standards solution (surrogate standards) were added into a 50 mL polypropylene tube to each sample.

Samples were extracted by using 10 mL of acetonitrile acidified with 150 μL of formic acid, 5 mL of water and 100 mg of activated carbon powder. Samples were homogenized under mechanical agitation for 40 min into a VIBA 330 shaker (Collomix GmbH, Gaimersheim, Germany). Debris were removed by centrifugation at 8000 g for 15 min.

Supernatants were collected and subsequently purified by weak anion exchange solid-phase extraction (SPE). Column conditioning was carried out by using 5 mL of methanol and 3 mL of formic acid 2 % in water. After sample loading, the cartridge was washed with 3 mL of formic acid 2 % in water and 3 mL of methanol. Cartridge was dried for 15 min and then a 2-step elution was performed with 2 mL of methanol / NH_4OH (90:10, v/v), twice. Extracts were taken to dryness under a stream of nitrogen at 45 °C. Dried extracts were reconstituted in 300 μL methanol / water (10:90, v/v) to which 2 μL of injection standard solution were added.

2.4. Instrumentation and analysis

Analyses were carried out in a LC-MS system composed of 1290 Infinity II coupled to an Ultivo triple quadrupole (Agilent Technologies, Santa Clara, USA).

Separations were achieved in a ZORBAX Eclipse Plus C18 2.1 × 50 mm, 1.8 μm (Agilent Technologies). The mobile phase consisted in 20 mM ammonium acetate in water (mobile phase A) and methanol (mobile phase B). The column temperature was set at 50 °C and flow rate was 0.250 mL/min. The optimized gradient was as follow: 10 %B at time 0 min; 30 %B at 2 min, 95 %B at 14 min, 100 %B at 14.50 min, 10 %B at 15.50, isocratic at 90 % for 1 min, post run up to 17 min. The injection volume was optimized at 5 μL. Additionally, a ZORBAX Eclipse Plus C18 3.0 × 50 mm, 1.8 μm (Agilent Technologies) was used as a delay column to trap any system related interferences.

Mass spectrometer operated in negative acquisition mode and source parameters were as follow: capillary Voltage 2500 V, gas temperature 320 °C, gas flow 8 L/min, Nebulizer 20 psi, sheath gas temperature 375 °C, sheath gas flow 12 L/min. All source parameters were optimized under LC conditions. Acquisition was performed in multiple reaction monitoring (MRM) by acquiring at least two transitions for each compound (except PFBA and PFPeA, due to their chemical structure). MRM transitions are reported in the supplementary material (Table 1 of supplementary material). Data analysis was carried out by MassHunter software (version 10.0, Agilent Technologies).

2.5. Method validation

The following parameters were tested during validation: selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, precision, recovery, matrix effect and stability. The obtained results were verified according to the recently published "Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed" (European Union Reference Laboratory, 2022). The selectivity of the method was evaluated in 12 non-fortified samples (lettuce, radish and peas) to check for possibly interfering compounds giving signals corresponding to any PFAS MRM transition.

The sensitivity of the method, expressed as LOD (limit of detection) and LOQ (limit of quantification) was calculated for each compound by signal to noise ratio (S/N) of the qualifier transition (when present). LOD and LOQ were defined as S/N equal to 3 and 10, respectively. Linear calibration curves were prepared using standard solution consisting of a concentration series of 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 ng/ml for each analyte. Origin was not included and a weight factor of 1/x was applied.

In-house quality controls (QC) were prepared in lettuce at the concentration of 1 and 5 ng/ml and analysed (n = 9) for three non-consecutive days for accuracy and precision data. Recovery of the extraction procedure was calculated on surrogate compounds.

Matrix-matched calibration was not required due to the isotope-labelled surrogate used as internal standard for each target analyte. Matrix-effect was evaluated through isotope-labelled surrogate performance. Stability of the processed samples was studied by analysing samples stored at room temperature for 48 h against a freshly prepared calibration curve. Prepared procedural blanks were processed in parallel with the samples to evaluate contamination throughout the analytical procedure.

3. Results and discussion

3.1. Method validation

Optimization of the extraction procedure was preliminary tested on lettuce and verified on more pigmented matrices (i.e. radish). Different extraction solvents were evaluated among methanol, acetonitrile and a mixture of both but the best results in terms of absolute recovery were obtained in acetonitrile. Recovery improved after the introduction of water and formic acid to the extraction solution as also confirmed by previous studies (Genualdi et al., 2021). One hundred mg of activated carbon powder showed suitable to clean the samples from the pigments (Powley et al., 2005).

Method validation data obtained under the described analytical parameters proved suitable for most of the analytes. Importantly, results were in good agreement with values considered as minimal requirements for the official control of PFAS in food and feed. In particular, limit of detection and limit of quantification were in the range 0.003 – 0.03 ng/g and 0.05–0.5 ng/g, respectively. LOQs were routinely re-examined throughout the method development and analysis to ensure that the reported limits would be representative of the system under operational conditions. Accuracy results were in the range 1–30 % for all PFAS at both QC levels, except for four compounds (PFMBA, NFDHA, 6:2 FTS, 11Cl-PF3OUdS), while precision resulted 1–24 %, except for three compounds (PFMBA, NFDHA, 6:2 FTS). Recovery was in the range 53–118 %; these results were in accordance with the guidelines used for the determination of PFAS in food (30–140 %). Matrix-effect was in the range 80–110 % for all PFAS. Stability of processed samples was in the range of 55–96 %. Procedural blanks did not report any PFAS above LOQ. All data from validation procedure are reported in tables 2–3 and Fig. 1 of the Supplementary Material.

3.2. Samples analysis

Fourty-one vegetal specimen undergoing a different grade of industrial manipulation were analysed in triplicate for the presence of PFAS. Fresh, frozen and ready-to-eat vegetable products were considered for this study and results were reported as mean values. As a general result, all samples confirmed the presence of at least one PFAS, 13 PFAS were detected above the LOD and 12 were above LOQ. Total PFAS content in the analysed samples, expressed as the sum of all quantified PFAS in one sample, ranged from 0.007 to 0.391 ng/g. Total PFAS content for each food category varied between 0.014 and 0.391 ng/g for the ready-to-eat group, from 0.009 to 0.166 ng/g for frozen vegetables and from 0.007 to 0.296 ng/g for fresh vegetables (Table 1).

Notwithstanding the limited number of samples, some differences have been observed between the three groups of vegetables. Average PFAS content in ready-to eat group (n. 23) was 0.13 ng/g (SD 0.09), while in fresh (n. 10) and frozen (n. 8) groups were 0.07 ng/g (SD 0.07) and 0.06 ng/g (SD 0.05), respectively. The difference between ready-to eat and frozen vegetables resulted slightly statistically significative ($p < 0.0469$), while the difference was not statistically significative between ready-to-eat and fresh samples. Four samples (2 lettuce, carrots, rocket salad) derived from organic cultivation and although the small number of samples hampers any statistical evaluation, it is noteworthy that in these samples the sum of PFAS was lower than their traditional counterpart (Fig. 1).

PFOA was detected in the 82.9 % of cases, with 100 % frequency in frozen product, 90 % in fresh material and 74 % in ready-to-eat vegetables. Other compounds which exhibited a high detection frequency were PFBA, PFHpA, PFPeA, PFOS and PPFxA. PFBA was detected in 32 of the 41 analysed samples (78 %). It was present in 87 % of the ready-to-eat group, in 62.5 % of frozen group and in 70 % of fresh vegetables. PFHpA was detected at the frequency of almost 71 % of all samples; in particular 69.5 % of ready-to-eat, 100 % of frozen vegetables and 50 % of fresh vegetables were found positive to PFHpA. High detection frequency was also found for PFPeA (58.5 %) and PFHxA (46.3 %). PFOS was detected in 56.1 % of total cases. Its frequency was 47.8 % in ready-to-eat products, 62.5 % in frozen and 70 % in fresh vegetables. Lower detection frequencies were detected for PFNA (24.3 %), PFBS (19.5 %), PFUnA (17 %), PFDA (14.6 %), and PFHxS/4:2FTS (2.4 %). Among the 12 quantified PFAS, 8 belonged to the perfluoroalkyl carboxylic acids (PFCA) and 4 to the perfluoroalkyl sulphonic acids (PFSA). Among the PFCAs, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA were

Table 1
Results of PFAS determination in the analysed samples.

	PFBA (ng/g)	PFPeA (ng/g)	PFHxA (ng/g)	PFHpA (ng/g)	PFOA (ng/g)	PFNA (ng/g)	PFDA (ng/g)	PFUnA (ng/g)	PFBS (ng/g)	PFHxS (ng/g)	PFOS (ng/g)	4:2FTS (ng/g)	ΣPFAS (ng/g)
RADISH (ready-to-eat)	0.008	<LOQ		0.019	0.009				0.010				0.046
RADISH (ready-to-eat)	0.008			<LOQ	0.032*								0.04
RADISH (ready-to-eat)	0.012			0.02	0.05*	<LOQ							0.082
LETTUCE (ready-to-eat)	0.048	0.027		<LOQ	<LOQ	<LOQ			0.008				0.083
LETTUCE (ready-to-eat)	0.159	0.017		<LOQ									0.176
LETTUCE (ready-to-eat)	0.110	0.005		<LOQ	<LOQ	<LOQ					<LOQ		0.115
MIXED SALAD (ready-to-eat)	0.008				0.077*		<LOQ				<LOQ		0.085
MIXED SALAD (ready-to-eat)	0.200	0.174	0.017		<LOQ	<LOQ					<LOQ		0.391
MIXED SALAD (ready-to-eat)	0.024			<LOQ	0.064*	0.008*					0.019*		0.115
VALERIAN (ready-to-eat)	0.225	0.043	0.005	<LOQ							<LOQ		0.273
VALERIAN (ready-to-eat)	0.145	0.012		<LOQ				0.004	0.016		<LOQ	0.062	0.239
VALERIAN (ready-to-eat)	0.158	0.018	0.003										0.179
CARROTS (ready-to-eat)	0.048			<LOQ	0.064*								0.112
CARROTS (ready-to-eat)				<LOQ	0.048*								0.048
CARROTS (ready-to-eat)	0.060			<LOQ									0.060
ORGANIC LETTUCE (ready-to-eat)		<LOQ	<LOQ	<LOQ	0.003	0.007*		<LOQ			0.004		0.014
CABBAGE (ready-to-eat)	0.028	0.007	0.004	<LOQ	0.011*						0.007		0.057
SPINACH (ready-to-eat)	0.058						<LOQ		0.006				0.064
MUSHROOMS (ready-to-eat)		<LOQ	<LOQ	<LOQ	0.018*	0.005	<LOQ				0.005		0.028
MUSHROOMS (ready-to-eat)	0.064	0.013	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ				0.005		0.082
MIXED SALAD (ready-to-eat)	0.273				<LOQ				0.008				0.281
ROCKET SALAD (ready-to-eat)	0.174	0.034	0.011		<LOQ	<LOQ			0.009				0.228
ORGANIC ROCKET SALAD (ready-to-eat)	0.082	0.009			<LOQ						0.004		0.095
BROCCOLI (frozen)	0.077	0.044	0.016	0.026	0.003						<LOQ		0.166
SPINACH (frozen)	0.037			0.006	<LOQ						<LOQ		0.043
PEAS (frozen)		0.003		0.006	<LOQ						<LOQ		0.009
COURGETTE (frozen)	0.048		<LOQ	0.004	<LOQ								0.052
MUSHROOMS (frozen)		0.004	0.003	0.006	0.004						0.007		0.024
MUSHROOMS (frozen)	0.019	0.004		0.006	0.005		0.004				0.006		0.044
BEANS (frozen)			0.088	0.005	<LOQ								0.093
CARROTS (frozen)	<LOQ	0.005	<LOQ	0.006	0.004								0.015
ORGANIC LETTUCE (fresh)	0.030		<LOQ	0.004	0.003	0.003		0.007	0.004		0.007		0.058
ORGANIC CARROTS (fresh)		0.004	0.004	0.005	0.008		0.005	0.014					0.040
RED BELL PEPPER (fresh)		<LOQ	<LOQ										/
YELLOW PEPPER (fresh)		<LOQ	<LOQ		0.014*								0.014
AUBERGINE (fresh)	0.024				<LOQ						<LOQ		0.024
ONION (fresh)	<LOQ			0.004	0.003						<LOQ		0.007
VALERIAN (fresh)	0.091	0.04	0.034	0.014	0.010			0.004	0.008	0.004	0.017*		0.222

(*continued on next page*)

Table 1 (*continued*)

	PFBA (ng/g)	PFPeA (ng/g)	PFHxA (ng/g)	PFHpA (ng/g)	PFOA (ng/g)	PFNA (ng/g)	PFDA (ng/g)	PFUnA (ng/g)	PFBS (ng/g)	PFHxS (ng/g)	PFOS (ng/g)	4:2FTS (ng/g)	ΣPFAS (ng/g)
GREEN BEANS (fresh)	0.013				0.003			0.004			<LOQ		0.020
COURGETTE (fresh)	0.135				0.003			0.005			<LOQ		0.143
TOMATO (fresh)	0.115	0.119	0.031	0.003	0.028*						<LOQ		0.296

*Values exceeding the attention limits recommended by European Commission.

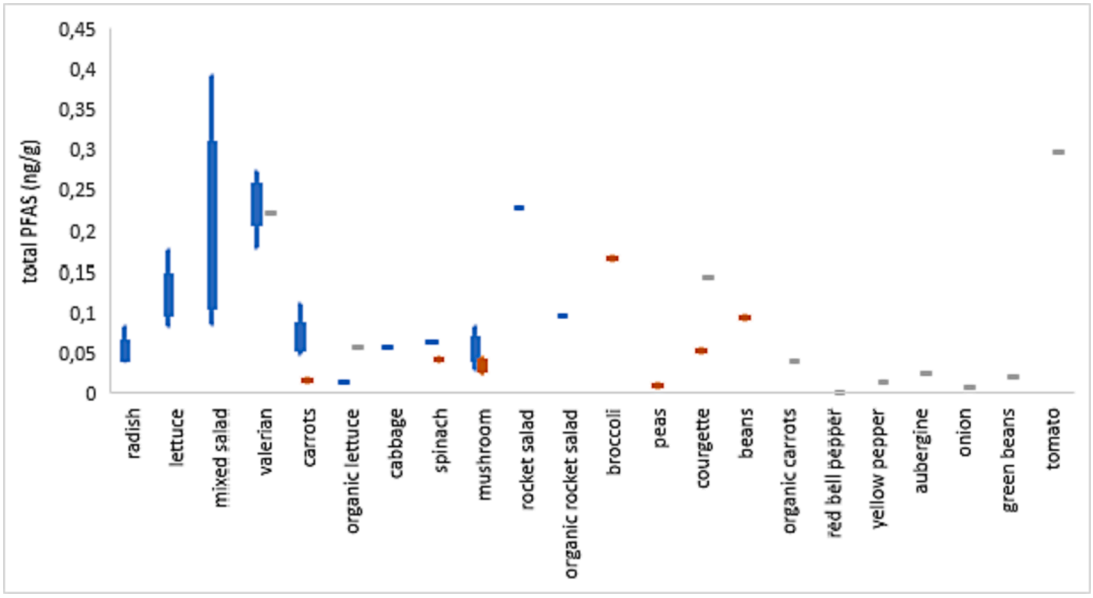

Fig. 1. Schematic representation of total PFAS detected in each sample. Blu lines: ready-to-eat vegetables; orange lines: frozen vegetables; grey line: fresh vegetables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

quantified; while within the PFSAs, PFBS, PFHxS, PFOS and 4:2 FTS were quantified. ADONA was detected but below the LOQ (Fig. 2).

Among all, PFBA was the compound detected at the highest concentrations. Its concentration reached up to 0.273 ng/g in ready-to-eat vegetables, 0.07 ng/g in fresh products and 0.05 ng/g in frozen food. Furthermore, its concentration varied significantly both within the individual groups and between the different groups of analysed samples. High levels of PFBA were present mostly in leafy vegetables, such as

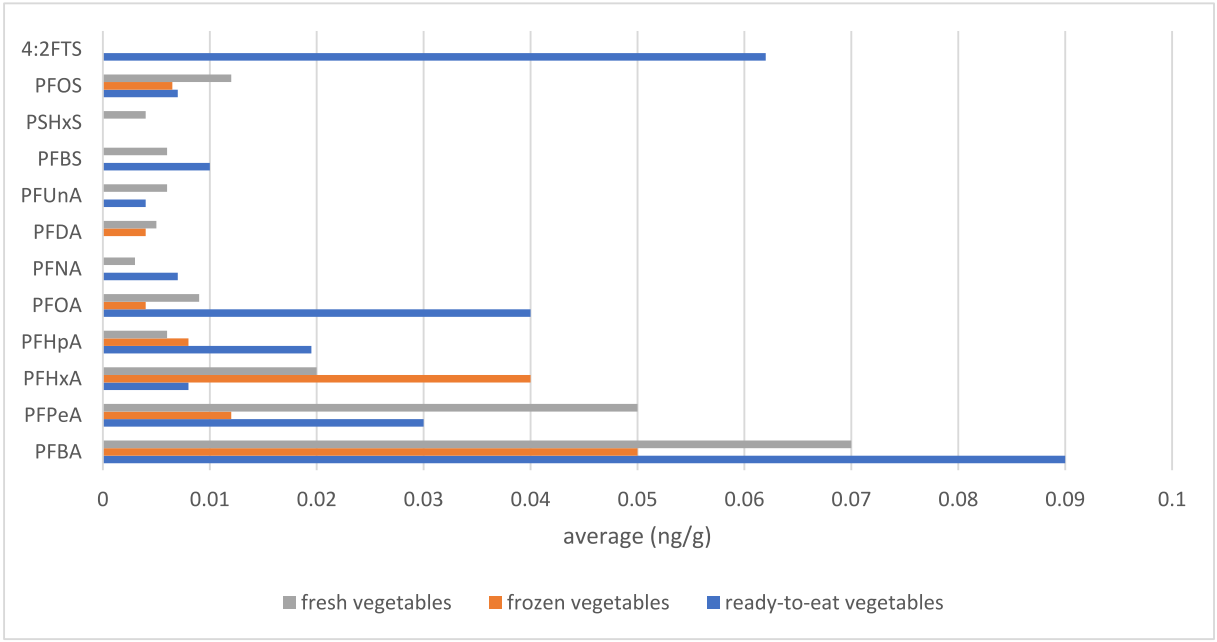

Fig. 2. Schematic representation of the average amounts of quantified PFAS for each food category.

lettuce (0.159 ng/g), mixed salad (0.200 and 0.273 ng/g), valerian (0.225 and 0.158 ng/g) and rocket salad (0.174 ng/g).

PFOA was found very frequently in all samples but at relatively low concentrations (0.003–0.077 ng/g), in many cases concentrations were below LOQ. Highest levels of PFOA were found in ready-to-eat vegetables and in particular in two samples of mixed salad (0.077 and 0.064 ng/g, respectively) and in carrots (0.064 ng/g). PFOA total amount in ready-to-eat products was 0.376 ng/g while the total amount of PFOA was 0.016 ng/g and 0.072 ng/g in frozen and fresh vegetables, respectively. PFHxS, 4:2FTS, PFBS, PFUnA and PFNA were not found in any of the frozen specimen but were present either in fresh and ready-to-eat vegetables or both. 4:2 FTS was found in only one sample of ready-to-eat valerian at the concentration of 0.062 ng/g.

In consideration of the attention limits in vegetables recommended by the European Commission for PFOS, PFOA, PFNA and PFHxS, 2 samples exceeded the 0.010 ng/g limit for PFOS, 10 samples exceed the 0.010 ng/g limit for PFOA, 2 samples exceeded the 0.005 ng/g limit for PFNA. Any sample displayed PFHxS values above the 0.015 ng/g limit. Nine samples exceeding the recommended limits belonged to the ready-to-eat group and three belonged to the fresh vegetables group; frozen vegetables were in line with the recommended limits.

4. Discussion

In recent years, consumers are becoming increasingly aware of the healthy aspects of foodstuffs, paying more attention to their safety (Caioni et al., 2022). One of main safety problems is associated to the presence of persistent contaminants, such as PFAS, and their accumulation throughout the food chain up to humans (Lu et al., 2015; Pabel et al., 2017). Food analysis demonstrated to be of pivotal importance in explaining the variation in PFAS plasma concentrations associated to different dietary patterns in humans (Menzel et al., 2021). A correlation was found among the plasma presence of some PFAS and a frequent consumption of packaged foods (Shravanthi et al., 2021). Vegetable products also accumulate PFAS, either by adsorption from the environment through their roots or through their leaves (Gu et al., 2022) or through the exposure to pesticides of their edible parts during cultivation (EPA, 2021). Finally, another potential source of PFAS contamination is represented by industrial processing and packaging (Ramirez Carnero et al., 2021). The purpose of our work was to evaluate the presence of PFAS in vegetables undergoing a different grade of industrial manipulation. Fresh vegetables were thus compared to frozen and ready-to-eat products purchased in different Italian local stores. Ready-to-eat vegetable products have been included in this research for their widespread consumption; they are defined as “fruits and vegetables that undergone procedures such as washing, sorting, trimming, peeling, slicing or chopping that do not affect their fresh life quality” (Kader and Gil, 2008). On this frame a specific sample pre-treatment protocol and a LC-MS/MS method exploiting the isotopic dilution protocol has been developed and validated. The method allowed the detection of a total of 25 PFAS, 21 of which satisfying the requirements described in the recent Guidelines (European Union Reference Laboratory, 2022). PFMBA, NFDHA, 6:2 FTS and 11Cl-PF3ONS did not fitted the requirements of precision and accuracy limits and thus their analysis had to be considered semi-quantitative. In literature, PFAS quantification was performed mostly on seafood, meat and eggs but also raw vegetables have been analysed (Domingo et al., 2012). For example, Sznajder-Katarzyńska and colleagues (Sznajder-Katarzynska et al., 2018), detected PFOS and PFOA in carrots, tomatoes, and white cabbage. PFOA was found in carrots and tomatoes at the concentrations of 0.0049 ng/g and 0.501 ng/g, respectively; PFOS was detected only in white cabbage at variable concentrations of 0.017–2.141 ng/g. The recent paper by Zhou and colleagues evaluating the risk assessment of PFAS in vegetables reviewed 112 papers (Zhou et al., 2021). Their results revealed concentrations PFOA > PFOS in most studies and when present, PFBA exceeded PFOS and PFOA amounts. Our data, even in consideration of the different groups of vegetables confirmed the trend of concentrations PFBA > PFOA > PFOS for the studied vegetables. Unfortunately, a comprehensive comparison among literature is difficult because of the variety of existing and detected compounds in each study. Likewise, temporal trends are discrepant due degradation products burden. However, as a general consideration, the detected amounts of PFAS were found negligible in comparison of home-produced vegetables cultivated in residential gardens around fluorochemical industrial park, which displayed higher levels of total PFAS (1.7–85 ng/g) with neat prevalence of PFBA and PFBS (Bao et al., 2019). Also, greenhouse tomato and greenhouse cucumber cultivated in in the same area confirmed a higher total of PFAS content (22–105 ng/g) with prevalence of PFBA and PFBS (Bao et al., 2020). In both studies the calculated bioaccumulation efficiencies in plants were observed to be negatively associated with the carbon chain length of PFAS (Bao et al., 2020). This could be observed also in our case, confirming higher amounts for C4 and C5 carbon chain molecules such as PFBA and PFPeA.

Our data revealed that ready-to-eat products contained higher PFAS concentrations respect to fresh and frozen vegetables, with a statistically significant difference between ready-to-eat and frozen vegetables. In most cases, frozen products contained absolute lower amounts of contaminants respect the other two groups. Among ready-to-eat vegetables, leafy products contained higher amounts of contaminants. The recommended attention limits proposed by European Commission were exceeded mostly in the ready-to-eat group of samples, while frozen vegetables were totally in compliance with the limits. Because of the small number of samples, only a general remark on the observed lower amount of PFAS in ready-to-eat organic vegetables than the traditional counterpart can be proposed. This fact could find an explanation in the ban of use of pesticides in these cases, but further studies are needed to understand if organic vegetables may really contain lower concentrations, independently from packaging and industrial manipulation.

To the best of the authors’ knowledge, this is the first study assessing different PFAS content in vegetable food products and in reason of the limited number of samples, more studies are needed to confirm our results.

5. Conclusions

This research examined the PFAS content in fresh, frozen and ready-to-eat vegetables. PFOA, PFBA, PFHpA, PFPeA, PFOS and PPFxA were frequently detected in all the analysed vegetables. PFOS, PFOA and PFNA concentrations were found above the attention limits recommended by the European Commission for vegetables. Frozen products contained the lowest amounts of PFAS. Ready-to eat group showed the highest values for PFAS, but samples deriving from organic cultivation showed lower amounts than their traditional counterpart. The impact of industrial manipulation on PFAS burden in vegetables for human consumption remains to be cleared as well as the use of pesticides as a source of PFAS contamination not removed by industrial processes.

CRediT authorship contribution statement

Elena Piva: Conceptualization, Methodology, Data curation, Writing – original draft. **Paolo Fais:** Supervision. **Pasquale Ioime:** Conceptualization, Methodology. **Giovanni Cecchetto:** Funding acquisition, Resources. **Guido Viel:** Funding acquisition, Resources. **Mattia Forcato:** Data curation, Writing – original draft. **Jennifer P. Pascali:** Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.135415.

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