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Assessment of perfluoroalkyl substances in food items at global scale

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ABSTRACT

This study assessed the levels of 21 perfluoroalkyl substances (PFASs) in 283 food items (38 from Brazil, 35 from Saudi Arabia, 174 from Spain and 36 from Serbia) among the most widely consumed foodstuffs in these geographical areas. These countries were chosen as representatives of the diet in South America, Western Asia, Mediterranean countries and South-Eastern Europe.

The analysis of foodstuffs was carried out by turbulent flow chromatography (TFC) combined with liquid chromatography with triple quadrupole mass spectrometry (LC-QqQ-MS) using electrospray ionization (ESI) in negative mode. The analytical method was validated for the analysis of different foodstuff classes (cereals, fish, fruit, milk, ready-to-eat foods, oil and meat). The analytical parameters of the method fulfill the requirements specified in the Commission Recommendation 2010/161/EU. Recovery rates were in the range between 70% and 120%. For all the selected matrices, the method limits of detection (MLOD) and the method limits of quantification (MLOQ) were in the range of 5 to 650 pg/g and 17 to 2000 pg/g, respectively.

In general trends, the concentrations of PFASs were in the pg/g or pg/mL levels. The more frequently detected compounds were perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorobutanoic acid (PFBA). The prevalence of the eight-carbon chain compounds in biota indicates the high stability and bioaccumulation potential of these compounds. But, at the same time, the high frequency of the shorter chain compounds is also an indication of the use of replacement compounds in the new fluorinated materials.

When comparing the compounds profile and their relative abundances in the samples from diverse origin, differences were identified. However, in absolute amounts of total PFASs no large differences were found between the studied countries. Fish and seafood were identified as the major PFASs contributors to the diet in all the countries. The total sum of PFASs in fresh fish and seafood was in the range from the MLOQ to 28 ng/g ww.

According to the FAO-WHO diets composition, the daily intake (DI) of PFASs was calculated for various age and gender groups in the different diets. The total PFASs food intake was estimated to be between 2300 and 3800 ng /person per day for the different diets.

Finally, the risk intake (RI) was calculated for selected relevant compounds. The results have indicated that by far in no case the tolerable daily intake (TDI) (150, 1500, 50,000, 1,000,000, 150, 1500 ng/kg body weight, for perfluorohexanesulfonate (PFHxS), fluorotelomer alcohol (FTOH), perfluorobutanesulfonic acid (PFBS), perfluorobutanoic acid (PFBA), PFOS and PFOA, respectively) was exceeded.

1. Introduction

Perfluoroalkyl substances (PFASs) are a group of chemicals characterized by their unique properties such as amphiphilicity and high resistance to degradation. Because of their unique

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features, PFASs are employed in a wide range of products and materials such as protective coatings for cloths and carpets, paper coatings, insecticides, paints, cosmetics and fire-fighting foams, among many others (Buck et al., 2011; Erik Kissa, 1994; Picó et al., 2011).

As a consequence of their continues use for more than 60 years, residues of PFASs are widely spread in the environment (Campo et al., 2014; De Silva et al., 2009; Delinsky et al., 2010; Giesy and Kannan, 2001; Lin et al., 2014; Llorca et al., 2012; Zhao et al., 2011). Some compounds can bioaccumulate and biomagnify in the food chain (Fang et al., 2014; Galatius et al., 2013; Jeon et al., 2010; Kannan et al., 2001; Vestergren et al., 2013; Xu et al., 2014; Yang et al., 2012), and have been detected in humans (Calafat et al., 2006; Llorca et al., 2010; Perez et al., 2012; Schecter et al., 2012).

Dietary intake is considered as one of the major routes of human exposure to PFASs (Schecter et al., 2010). Therefore, during the last years several studies have evaluated the occurrence of PFASs in food (D'Hollander et al., 2010; Domingo et al., 2012a; Ericson et al., 2008a; Eriksson et al., 2013; Haug et al., 2010a; Hlouskova et al., 2013; Jogsten et al., 2009; Ostertag et al., 2009), mainly PFOS and PFOA (Haug et al., 2010b). In these studies, high fish consumption rates were related to high PFASs exposure rates (D'Hollander et al., 2010; Haug et al., 2010b; Hlouskova et al., 2013).

PFASs are currently considered as emerging contaminants in the food chain. For this reason, the European Food Safety Authority (EFSA) has set the tolerable daily intakes (TDI) of PFOS and PFOA at150 ng/kg/day and 1500 ng/kg/day, respectively (EFSA, 2008), recommending as well the additional monitoring of PFASs of different chain lengths. On the other hand, in the Commission Recommendation 2010/161/EU urged to the Member States to monitor the occurrence of PFOS and PFOA, other PFAS with chain lengths between C4 and C15 and their precursors in food (EFSA, 2012).

Recent studies have indicated the prevalence of PFOS and PFOA and the presence of the longer chain homologs as well as the replacement compounds in food. For example, when analyzing samples from Faroe Islands, perfluoroundecanoic acid (PFUnDA) and PFOS were the most frequently detected in milk and drinking water, respectively (Eriksson et al., 2013).

However, the difficulties associated with the ultra-trace analysis of PFASs in food have hampered the study of the dietary exposure. As a result, few studies till now have evaluated the PFASs dietary exposure (Clarke et al., 2010; Domingo et al., 2012b; Ericson et al., 2008b; Kärrman et al., 2009; Noorlander et al., 2011; Tittlemier et al., 2007; Vestergren et al., 2012).

In one of the first studies in Canada (Tittlemier et al., 2007), the average of the dietary intake of total perfluorocarboxylates and PFOS was 250 ng/day. In 2008, (Ericson et al., 2008b) assessed the total PFASs daily intake in Catalonia (Spain). In this work, the average dietary intake for a standard adult man (70 kg of body weight) was found to be around 74.2 ng/day. In a more recent study by the same group, (Domingo et al., 2012a) this value has set around 1100 and 1700 ng/day fresh weight (fw), children being the most exposed population group. These rates corresponded to an amount of PFOA and PFOS between 290 and 450 ng/day.fw and between 80 and 150 ng/day.fw, respectively. Comparing these results with those from another study in the Netherlands (Noorlander et al., 2011), it can be seen that the total daily intake for PFOS was much higher in The Netherlands than in Spain due to a well-known higher consumption of dairy products in the Netherlands. In spite of that, it should be noted that in both cases the intake was below the tolerable intake values established by the EFSA.

Based on 21 measurements and consumption data for the general Norwegian population, a rough estimatimation of the TDI of PFASs was performed by Haugh et al. around 100 ng per day and PFOA and PFOS contributed to about 50% of the total intake (Haug et al., 2010a).

In the US, Schecter et al. (2010) studied the presence of PFAS among other persistent organic pollutants (POPs) in 31 types of samples collected in supermarkets from Dallas. PFOA was present in 17 of the 31 type of samples analyzed, ranging from 0.07 ng/g in potatoes to 1.80 ng/g in olive oil. In terms of dietary intake, PFOA was consumed at a higher level in comparison to other PFASs.

Recently, (Hlouskova et al., 2013) studied the occurrence of PFASs in 15 food commodities consumed in various European markets. In this study, PFOS followed by PFOA, PFBA, and PFNA were the most frequently detected compounds. Whilst PFHxA, PFHpA, PFHxDA were detected in only a few samples. About the different food commodities studied, seafood followed by pig and bovine liver were the samples that showed the highest levels.

In another recent study, (Herzke et al., 2013) the PFASs content in foods and vegetables collected in four countries (Belgium, Czech Republic, Italy and Norway), were studied. I20 different types of vegetables were sampled in Belgium, Czech Republic, Italy and Norway. Perfluorinated carboxylic acids were the main group of detected PFASs, with PFOA as the most abundant one in general followed by perfluorinated hexanoic acid and perfluorinated nonanoic acid. Dietary intake estimates for PFOA show only low human exposure due to vegetable consumption for adults and children, mostly governed by high intake of potatoes.

Should be pointed out that differences between different studies and countries can be attributed, at least in part, to the different analytical methods used by the different research groups. In addition, other factors contributing to these differences are the time between sampling and analysis (since the levels of some compounds can decrease along the time), conservation and temporal trends.

The main objectives of this study were (i) to expand market basket surveys and study the presence of 21 PFASs in common consumed food items in 283 food items (38 from Brazil, 35 from Saudi Arabia, 174 from Spain and 36 from Serbia); (ii) to assess the total daily PFASs intake in the diet of these countries, which have been selected as representatives of the diets in South America, Western Asia, the Mediterranean area and the South-Eastern Europe, and (iii) to assess the dietary risks associated with relevant PFASs in these diets.

2. Materials and methods

2.1. Sampling collection

Between September 2011 and February 2013, samples were purchased from different supermarkets and retail stores in representative cities of Brazil (Sao Paulo, Sao Sebastian, Pereque-Ilhabela, Porto Alegre), Saudi Arabia (Riyadh), Serbia (Belgrade and Novi Sad) and Spain (Barcelona, Girona and Madrid). The selected foodstuff samples in this work were among the most consumed in each country. The samples were collected as a regular consumer does. A total number of 283 food items (35 from Arabia, 38 from Brazil, 174 from Spain and 36 from Serbia) were studied. The 283 food items corresponded to 849 individual samples, 3 different individual samples for each food item. A summary of the samples is presented in Table S1 of the Supplemental Material.

Foodstuffs were pertaining to the following categories: (1) cereals, (2) pulses and starchy roots, (3) tree-nuts, oil crops and vegetable oils, (4) vegetables and fruits, (5) meat and meat products, (6) milk, animal fats, dairy products and eggs, (7) fish and seafood, and (8) other such as candies or coffee.

Immediately after sampling perishable samples were frozen at −80 °C before shipping on dry ice to the IDAEA-CSIC laboratory (Barcelona, Spain) for chemical analysis. Non-perishable samples were stored and shipped at room temperature. After reception at IDAEA-CSIC laboratory, individual units were melt, combined, homogenized and store in polypropylene tubes freeze at −20 °C until their analysis. The parts of the samples that were processed were those generally eaten by consumers.

2.2. Chemicals and standards

Analyses were performed using the isotope dilution method and all the analytical standards were from Wellington Laboratories (Guelph, ON, Canada). Labeled standards used were: [13C4] -perfluorobutanoic acid (MPFBA (13C4)), Ion [18O2]-perfluorohexanesulfonate (MPFHxS (18O2)), [13C2]-perfluorohexanoic acid (MPFHxA (13C2)), Ion [13C4]-perfluorooctanesulfonate (MPFOS (13C4)), [13C4]-perfluorooctanoic acid (MPFOA (13C4)), [13C5]-perfluorononanoic acid (MPFNA (13C5)), [13C2]-perfluorododecanoic acid (MPFDoA (13C2)), [13C2]-perfluorodecanoic acid (MPFDA (13C2)), [13C2]-perfluoroundecanoic acid (MPFUdA (13C2)), MFTA-MXA (>98%) [13C2]-perfluorohexylethanoic acid (MFHEA(13C2)), [13C2]-perfluorooctylethanoic acid (MFOEA(13C2)), [13C2]-perfluorodecylethanoic acid (MFDEA(13C2. Native standards used in this study were: perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoicacid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA), perfluorotetradecanoic acid (PFTeA, perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS),perfluorooctanesulfonate (PFOS), perfluorodecanesulfonate (PFDS), perfluorohexyl ethanoic acid (FHEA), perfluorooctyl ethanoic acid FOEA, perfluorodecyl ethanoic acid FDEA.

Water, methanol, and acetonitrile, Chromasolv Plus for HPLC, ammonium acetate (AcNH4; MW 77.08; 98%), and formic acid (HFo) were obtained from Sigma-Aldrich, Steinheim, Germany.

Table 1
Summary of body-weight for the different gender/age classes in the different countries.

Body weight average (kg).				
	Children 6–9 years	Adolescents 10–19 years	Adults 20–60 years	Seniors >60 years
Brazil				
Male	31	62	77.2	67
Female	26	56	62.5	65
Saudi Arabia				
Male	32.5	65	80	70
Female	27.5	59	65.5	68
Serbia				
Male	32	55.5	70.5	65.5
Female	27	49.5	65	70
Spain				
Male	30	56	70	65
Female	25	53	55	60

Table 2
No-observed-adverse-effect level (NOAEL) and the lower confidence limit on the benchmark dose (BMDL) for TDI calculation of PFBA, PFBS and PFHxS, and tolerable daily intake (TDI) values for PFOS and PFOA set by the EFSA.

Compounds	Noael	Ref	Tdi ng/kg body weight
PFHxS (Noael)	10 mg/kg/day	(Lau et al., 2007)	150
FTOH (Noael)	200 mg/kg/day	(Lau et al., 2007)	1500
PFBS (Noael)	60 mg/kg/day	(Division, 2011)	50,000
PFBA (Bmdl)	3.01 mg/kg/day	(Division, 2011)	1,000,000
PFOS (Tdi)		(EFSA, 2008)	150 ng/kg body weight
PFOA (Tdi)		(EFSA, 2008)	1500 ng/kg body weight

2.3. Sample preparation

The preparation of the samples prior to analysis was as follows:

Milk, dairy products and solid matrices were extracted by alkaline digestion using a previously protocol (Llorca et al., 2012). Very briefly, 2 g of sample were weighed into 20 ml polypropylene (PP) tube and 250 μl of surrogate internal solution mixture (400 μg/l in methanol) was added to obtain a final concentration of 10 μg/L (100 ng) of each standard. After 20 min of equilibration, at room temperature, 10 mL of sodium hydroxide solution (20 mM in methanol) was added. Then the samples were digested at room temperature in an orbital shaker during 4 h at 125 rpm. After this time, the samples were centrifuged at 4000 rpm during 15 min. Finally a supernatant aliquot of 200 mL was transferred into vial, which was stored at 4 °C until the on-line purification and analysis. Liquid samples as soups and juices were just filtered prior purification and analysis.

2.4. Online extraction procedure

Purification was carried out by turbulent flow chromatography (TFC) using the Aria TLX-1 system (Thermo Fisher Scientific, Franklin, MA, USA), which comprised a PAL auto sampler (CTC Analytics, Zwingen, Switzerland), two mixing binary pumps (eluting pump and loading pump), and a three-valve switching device unit with six-port valve. The entire system was controlled via Aria software, version 1.6. The purification of the target analytes was achieved using two extraction columns C_{18} XL (50 mm × 0.5 mm, 60 μm particle size, 60 Å pore size)) and Cyclone (50 mm × 0.5 mm, 60 μm particle size, 60 Å pore size) connected in tandem. Liquid samples and extracts were loaded into the enrichment columns using ultrapure water acidified at pH 4.5 with formic acid.

Table 3
Method limits of detection (MLOD) and method limits of quantification (MLOQ) achieved during validation study on spiked materials.

	Cereals mixture (rice+corn+wheat)		**Fish** (Sardines pool)		**Juice** (Natural filtered orange juice)		**Milk (UHT)**		**Oil (Olive oil)**		**Meat** (Pork loin)		**Lyophilized home made soup**	
	MLOD (pg/g)	MLOQ (pg/g)	MLOD (pg/g)	MLOQ (pg/g)	MLOD (pg/mL)	MLOQ (pg/mL)	MLOD (pg/mL)	MLOQ (pg/mL)	MLOD (pg/mL)	MLOQ (pg/mL)	MLOD (pg/g)	MLOQ (pg/g)	MLOD (pg/g)	MLOQ (pg/g)
PFBA	97	325	64	214	89	298	111	371	49	166	43	143	106	353
PFBS	31	103	58	192	60	198	56	185	15	50	46	151	109	364
PFPeA	94	313	92	307	92	307	107	355	47	163	51	162	14	46
PFHxA	48	158	50	167	54	179	32	108	24	78	122	379	138	463
PFHxS	100	333	107	356	157	522	118	393	51	161	121	365	64	215
PFHpA	101	338	59	196	73	244	132	440	50	152	53	158	102	341
FHEA	48	158	50	167	536	1788	32	108	24	75	81	252	79	264
PFOA	50	168	81	269	83	277	12	40	25	75	53	163	62	206
PFOS	73	242	21	70	38	128	14	48	36	108	12	37	14	47
PFOSA	32	101	7	22	68	227	5	17	15	49	21	63	42	140
FOEA	158	525	104	347	58	194	59	197	79	253	203	651	194	648
PFNA	124	412	96	321	39	131	79	263	62	195	56	168	99	332
PFDA	24	79	98	328	104	345	12	40	12	36	91	278	41	133
PFDS	26	85	98	328	41	137	28	94	13	34	30	98	70	233
PFUdA	156	521	209	697	211	704	148	493	78	243	89	267	13	43
PFDoA	77	255	123	410	117	389	84	281	38	118	124	411	130	435
FDEA	56	185	103	345	157	522	166	554	28	93	168	549	168	560
PFTrA	76	253	141	469	613	2043	60	201	38	142	67	205	46	153
PFTeDA	104	346	75	250	205	683	112	374	52	160	80	273	79	264
PFHxDA	121	402	162	541	97	325	71	238	60	185	93	283	79	265
PFODA	101	335	81	270	452	1508	207	689	50	169	144	433	232	769

2.5. Instrumental analysis and quality assurance

After the purification, the analytes were directly transferred to the analytical column a Hypersil GOLD PFP (50 × 3) (Thermo Fisher Scientific, Franklin, MA) for their chromatographic separation. The injection volume was 20 μL and the flow rate was set at 400 μl/min. The gradients used for the purification and chromatographic separations are summarized in Table S2 of the Supplemental Material.

LC was coupled to a triple quadrupole mass spectrometer Thermo Scientific TSQ Vantage (Thermo Fisher Scientific, San Jose, CA), equipped with a Turbo Ion Spray source, employed in the negative electrospray ionization (ESI (-)) mode. Acquisition was performed in selected reaction monitoring mode (SRM) to obtain enough identification points (IP) for confirmation of each analyte (European Commission Decision 2002/657/EC). Capillary and vaporizer temperatures were 270 °C and 300 °C, respectively. The scan time and width were set at 0.02 s and 0.02 m/z. Data was processed by the Xcalibur software version 1.4. Precursor ions and transitions used for quantification and confirmation, as well as, retention times of the target compounds are listed in Table S3 of the Supplemental Material.

For identification purposes, retention times of PFASs in the standards and in the samples were compared at a tolerance of ±2.5%. Moreover, in accordance with the Decision 2002/657/EC (Decision 2002), the relative ion intensities (ratios between the areas of the most intense transitions) of a compound in a sample or in a matrix were compared with the same ratios in the calibration curve, with a tolerance of ±2 SD. The method was validated in accordance with the criteria described in EU Commission with special attention to linearity, recovery, matrix effects, precision, limits of detection (LOD) and quantification (LOQ).

Quantification was achieved using internal calibration with isotopically labeled standards added as surrogates in the beginning of the sample treatment, thus correcting quantification errors due to matrix interferences and extraction and/or MS acquisition discrepancies. The ratio of the most intense transition peak area to the corresponding surrogate peak area was graph represented toward concentration.

Calibration series were prepared in the different blank matrices (fish, cereals mixture, fruit juice, milk, lyophilized home made vegetables and chicken soup, oil and meat) to calculate recoveries, address possible matrix ionization suppression or enhancement effects, linearity and the method limits of detection and quantification in each type of sample. In order to ensure homogeneous distribution of the analytes in the matrix, after fortification, the samples were mixed on a rotary mixer for 10 min at room temperature.

Linearity range was defined by plotting the peak area ratio of the PFAS to the internal standard versus PFAS concentration. The following criteria for linearity range were applied: linear regression through zero with a correlation coefficient better than 0.990, bias from the calibration line less than 25% for all individual calibration points, and the average percentage relative standard deviation (RSD) of four replicates less than 25%. The lower limit of the linear range was at MLOQ.

Recoveries and precision were calculated according to the 2002/657/EC Decision. Since no certified reference materials were available for the analytes and matrices of interest, the recovery from fortified blank samples was measured as an alternative to trueness. Briefly, each blank type of samples was spiked in quintuplicate as previously described at three different levels (MLOQ, 10.0, 100.0 ng/g). Precision, expressed as repeatability, was calculated by repeated analyses on the same sample sets as used for recovery tests, with the only difference that independent samples were re-extracted and analyzed on two other occasions for calculating inter-day repeatability.

Table 4
Summary of the concentrations of target compounds (pg/g) in positive samples.

Compounds	Frequency (%)	Min (pg/g)	Max (pg/g)	Mean (pg/g)	Median (pg/g)
Brazil					
PFBA	20.9	234	834	567	523
PFBS	4.5	478	486	482	482
PFHxA	6.0	157	388	270	265
PFHxS	4.5	179	523	351	351
PFOA	22.4	26,5	750	200	102
PFOS	14.9	39,9	234	115	65
PFOSA	3.0	228	228	228	228
PFDA	4.5	111	228	170	170
Saudi Arabia					
PFBA	8.5	327	422	370	366
PFBS	8.5	296	490	436	479
PFPeA	2.1	153	153	153	153
PFHxA	21.3	120	5512	931	192
PFOA	23.4	35	1245	312	202
PFOS	17.0	65	15,000	2028	90
PFOSA	8.5	22	580	192	83
PFNA	2.1	430	430	430	430
PFDoA	8.5	140	375	236	215
Serbia					
PFBA	19.0	120	1400	575	500
PFBS	5.2	166	460	273	192
PFPeA	5.2	360	776	517	415
FHEA	8.6	125	946	476	268
PFOA	17.2	45	700	355	375
PFOS	18.9	54	2700	629	387
PFOSA	10.3	53	2100	711	315
PFNA	10.3	28	430	208	185
PFDA	3.4	43	490	267	267
PFDS	1.7	205	205	205	205
PFDoA	19.0	120	1400	575	500
Spain					
PFBA	7.7	317	5700	1093	531
PFBS	3.2	49	13,000	1744	401
PFPeA	2.5	280	28,000	7782	1500
PFHxA	13.3	130	2900	418	225
PFHpA	0.4	240	240	240	240
FHEA	0.7	1700	7000	4350	4350
PFOA	16.1	40	8000	835	365
PFOS	25.0	25	14,500	1143	340
PFOSA	14.0	15	5300	1152	820
FOEA	0.4	79	79	79	79
PFNA	13.3	39	13,000	1175	430
PFDA	2.1	60	2200	772	385
PFDS	0.7	154	250	202	202

2.6. Intake of PFASs

The total daily intake (total-DI) was calculated as the sum of the DI of PFASs for the different groups of foodstuffs. The individual DI for each foodstuff class was calculated as the sum of the mean concentrations of each compound in an individual food class, multiplied by the consumption and divided by the average body weight, according to the following expression:

$$\text{Daily intake(DI)} = \frac{\Sigma\,(\text{Compound concentration} \times \text{Consumption})}{\text{Body Weight}}$$

According to the FAO-WHO data, the body-weight (Table 1) for different age/gender groups of the different geographical areas was estimated.

When a concentration was under the MLOQ, DI were calculated assuming to be one-half of that MLOQ, and when the values were below the MLOD then was assumed to be one-half of that MLOD.

The tolerable daily intakes (TDI) for PFOS and PFOA were established by the EFSA at 150 ng/kg b.w. per day and 1500 ng/kg b.w. per day, respectively. These values were set considering the no-observed-adverse-effect level (NOAEL) or the lower confidence limit on the benchmark dose (BMDL) and an overall uncertainty factor (UF) of 200 according to the following expression:

$$\text{TDI(tolerable daily intake)} = \frac{\text{NOAEL or BMDL}}{\text{UF}}$$

In this work, using the same criteria as the EFSA and the NOAEL or BMDL values from previous studies, the TDI values for the more representative short chain compounds (PFBA, PFBS and PFHxS) were estimated and summarized in Table 2.

Finally, according to the following equation, the risk indexes for the different diets were calculated.

$$\text{Total RI} = \sum_{n\,=\,\text{each items, PFAS}}^{\infty} \left(\frac{\text{DI}}{\text{TDI}}\right)$$

3. Results and discussion

An analytical method that agrees with the Commission Recommendation in 2010/161/EU for the analysis of 21 PFASs was validated for different food matrices.

The method was based on a pre-treatment step, followed by on-line TFC coupled with LC-QqQ-MS using electrospray ionization (ESI) in negative mode. It was validated for cereals, fish, fruit, milk, ready-to-eat foods, oil and meat. The MLOQs were in general at the pg/g level and pg/mL in solid samples and beverages (Table 3), respectively. Recovery rates were in the range between 50% and 120%, and the method presented the adequate precision

and inter-day repeatability. For quantification purposes labeled internal standard addition was used.

The method was applied to the analysis of 283 food items (38 from Brazil, 35 from Saudi Arabia, 174 from Spain and 36 from Serbia). A summary of the average concentrations of all target compounds in positive samples in the analyzed food items is presented in Table 4.

In this study, PFOS and PFOA followed by PFBA, PFHxA, PFOSA and PFNA were the most frequently found. In general, migration of compounds from food packaging material can results in PFCAs with chain length of C4–C18 (Begley et al., 2008). Therefore, the presence of PFHxA and PFBA, can be attributed to migration from materials used for packaging (Jogsten et al., 2009; Zafeiraki et al., 2014). Whilst PFDoA and PFDA were detected in some few food items, and FDEA, PFTrDA, PFTeDA, and PFODA could not be detected in any sample. As in other studies (Hlouskova et al., 2013), differences in the compounds profiles and their relative abundances were found between different countries.

Fish and seafood were major contributors of PFASs to the diet in all cases, as expected (Fig. 1). The occurrence of PFOS, PFOA, PFOSA and PFNA in fresh fish and seafood can be related to bioaccumulation through the aquatic food chain (Xu et al., 2014). However, the presence of shorter chain compounds can also be related to cross contamination from materials in contact with the fish during its transport and manipulation (Still et al., 2013). In particular, in the case of PFHxA with a low bioaccumulation potential, the trace concentrations of this compound in fish can be related to contamination from the food-wrapping paper used by retail stores or packaging.

In Figure S1 of the Supporting Information, the per capita food supply figures according to the FAO (2009) database 2014, are presented. As can be seen, in Western Asia and South America the fish and seafood consumption constitutes around a 1% of the total average of food supply. Due to that reason, no fresh fish samples from Saudi Arabia were included in this study. Just a brand of canned tuna was analyzed but the results were not considered for the daily intake calculations. On the other hand, due to the difficulties in transport, no fresh fish samples from Brazil were analyzed. Thus, the values presented here and used for daily intake calculations were estimated from previous data in Atlantic fish.

In general trends, PFOS, PFOA, PFBA, PFPeA and PFHxA were the most representative compounds detected in milk, dairy products and eggs. As in fish, the occurrence of eight-carbon chain compounds is consequence of their environmental prevalence and high bioaccumulation potential. In agreement with their relative lipophilicity, PFOA was mainly found in skimmed milk, soft cheese creams and desserts; whereas PFOS was present in whole milk and fat cheese.

In meat and meat-processed foods, PFOA, PFOS and PFBA were the most frequently detected compounds. However, in Saudi Arabia and Spain PFOSA was also detected in some of the samples and in the European samples PFPeA was also detected.

To explore the differences between the trends of PFASs contamination in packed foods, a principal component analysis (PCA) was applied. The predominance of longer chain compounds (8C or more), in front of the occurrence of shorter chain compounds with or without PFHxA was studied. The predominance of longer chain compounds is related to environmental contamination, and in general, to classic perflorocarbons chemistry. While, the occurrence of shorter chain compounds is consequence of the perfluorocarbon chemistry replacement, in particular in the case of PFHxA,

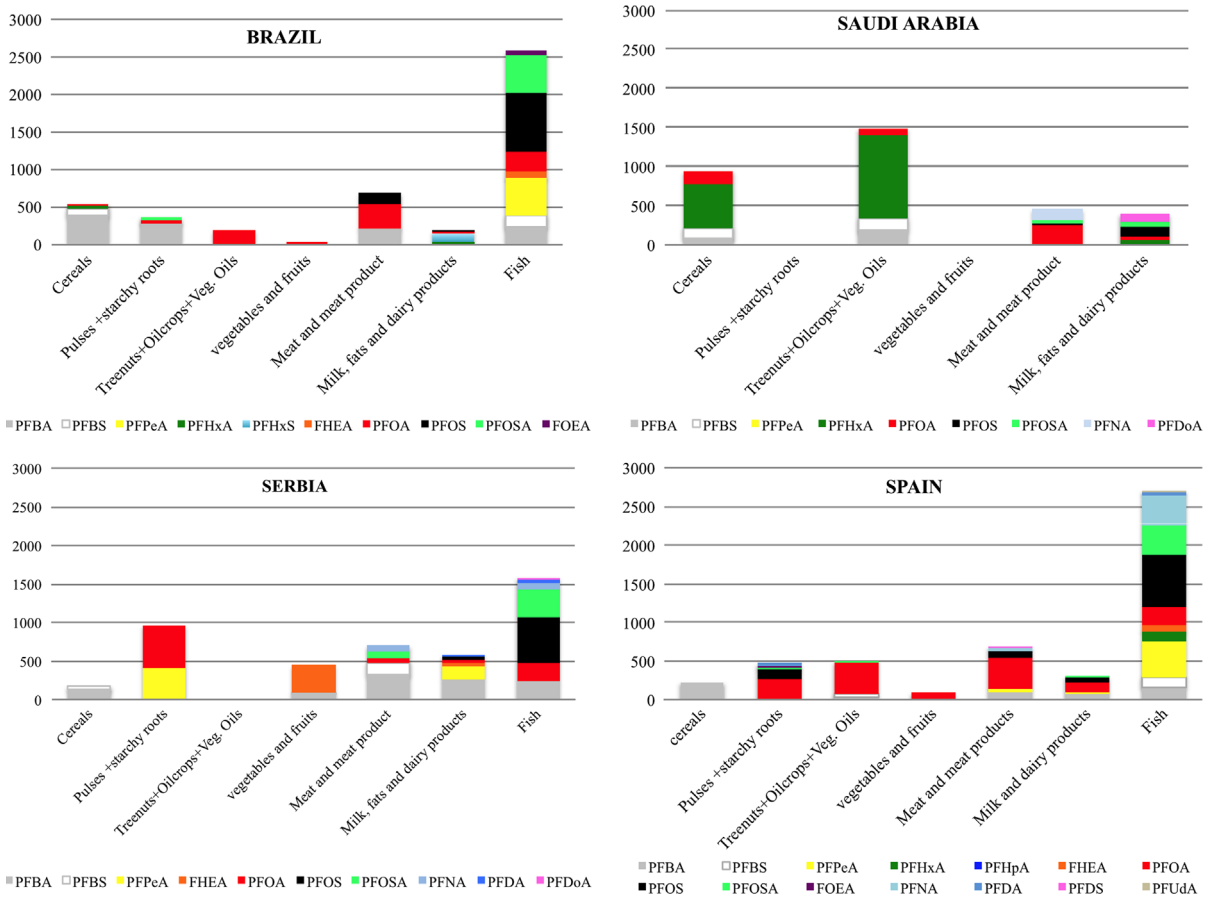

Fig. 1. Concentration in pg/g for the compounds detected in the different food groups from each country.

could be related to the transference from packaging or materials used during food processes as cited earlier. The mean values and the total concentrations were calculated according to the Directive 2009/90/EC (Commission of the European Communities, 2009). Therefore, concentrations below the MLOD were assimilated to zero and concentrations below the MLOQ were considered the MLOQ/2. In all cases, a 95% confidence interval was considered.

The results of the PCA showed that the PC1 and PC2 explained 99.4% of the total variance. In Fig. 2, the loadings of PC1 and PC2 are presented. As can be seen, the occurrence PFOS, PFOA, PFOSA and PFNA can be coincident with PFBA or PFBS. However, the predominance of short chain compounds including PFHxA follows an inverse relation with the presence the longer chain compounds (PFOS, PFOA, PFOSA and PFNA). The score plots of PC1 and PC2 for

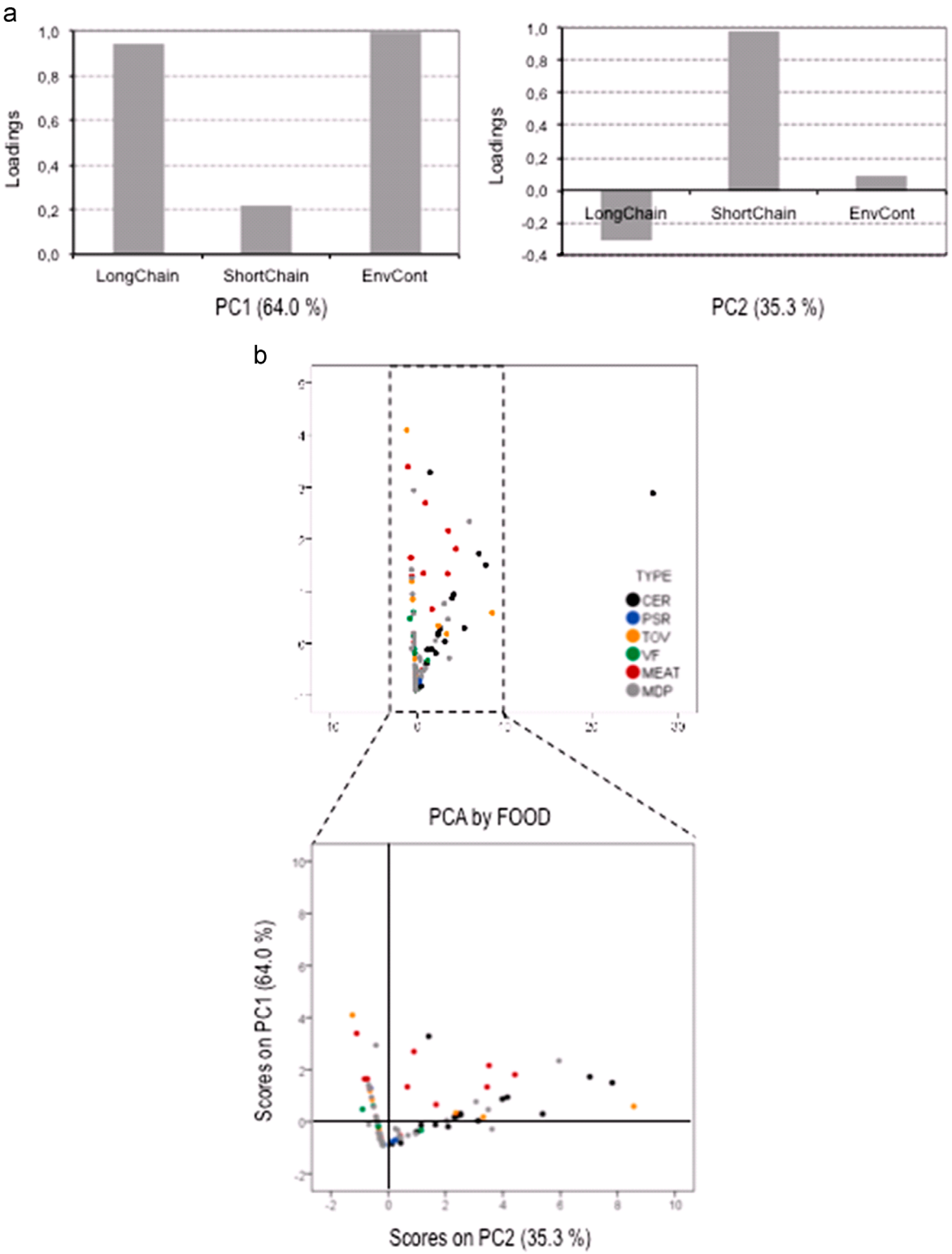

Fig. 2. Principal component analysis (PCA) of more frequently found compounds grouped by the number of carbon chains in 3 groups: long chain compounds (with 8 or more C), Short chain (less than 8 C) and all frequent compounds with exception of PFHxA. (a) PC1 and PC2 loadings. (b) Score presentation of PC1 and PC2.

packed food showed that some groups as milk and milk products present common trend, in this case the occurrence of long chain compounds with low concentrations of PFBA and PFBS predominates. While, in the case of cereals, the influence of PC2 was higher and other food items, such as meat and meat products did not presented a clear tendency. One of main factors influencing PFASs contamination in food items in general can be attributed to food packaging and paper wrapping. Comparing the different countries, strong economies and emerging countries, such as Saudi Arabia and Brazil, showed a high levels of short chain compounds, whereas in Europe the concentrations of PFOA continues being high as a result of the environmental occurrence of this compound and the slower replacement of PFOA by new less persistent compounds during the last years.

The mean concentrations of PFASs, the foodstuff consumption according to the FAO/WHO (Figure S1 of the Supporting Information) and the mean body weight for different age/gender classes (Table 1) were used to calculate the total DI, (summarized in Fig. 3). The average food consumption was assumed for adolescents, adults and seniors; while 2/3 of the average food consumption was considered for children.

The total PFASs intake were estimated to be 3778, 3218, 2635 and 2295 ng/person per day in Mediterranean countries, South-Eastern Europe, Western Asia and South America, respectively. Children are the most exposed population group, Fig. 3. Using these data and the tolerable daily intake (TDI), the risk index (RI) for each diet was assessed (Fig. 4). The diet composition is one of the more influential factors in terms of intake (Fig. 4) and, the risk is proportional to the toxic compounds. Therefore, PFOS followed by PFOA were the compounds explaining most of the risk associated with PFASs consumption through the diet. In spite of high concentrations from packaging of the rest of the short chain compounds, their risk is lower than PFOS and PFOA due to their low RI. According to that, the highest risk was associated with the Mediterranean diet due to the higher fish consumption (around a 4% of total diet according to the FAO). As can be seen in Fig. 4 the risk index was as follows: Mediterranean diet > South-Eastern Europe > Western Asia > South America. The diets showing the mayor risks were also those with a higher fish and seafood consumption. However, the risk indexes in all cases were much lower than 1. Therefore, no imminent health damages produced by PFASs consumption through the diet can be considered in any case.

However, it should be pointed out that TDIs were estimated according to the EFSA using the NOAELs or BMDLs. These values were obtained by acute toxicity test and the effects of chronic exposure are not considered. There is an ongoing discussion about the relevance of the TDIs set by EFSA. Some studies indicate that these levels should be 100-folds lowered (Grandjean and Budtz-Jørgensen, 2013).

In comparison with other studies the results reported here were found in the same or in slightly lower levels of contamination for

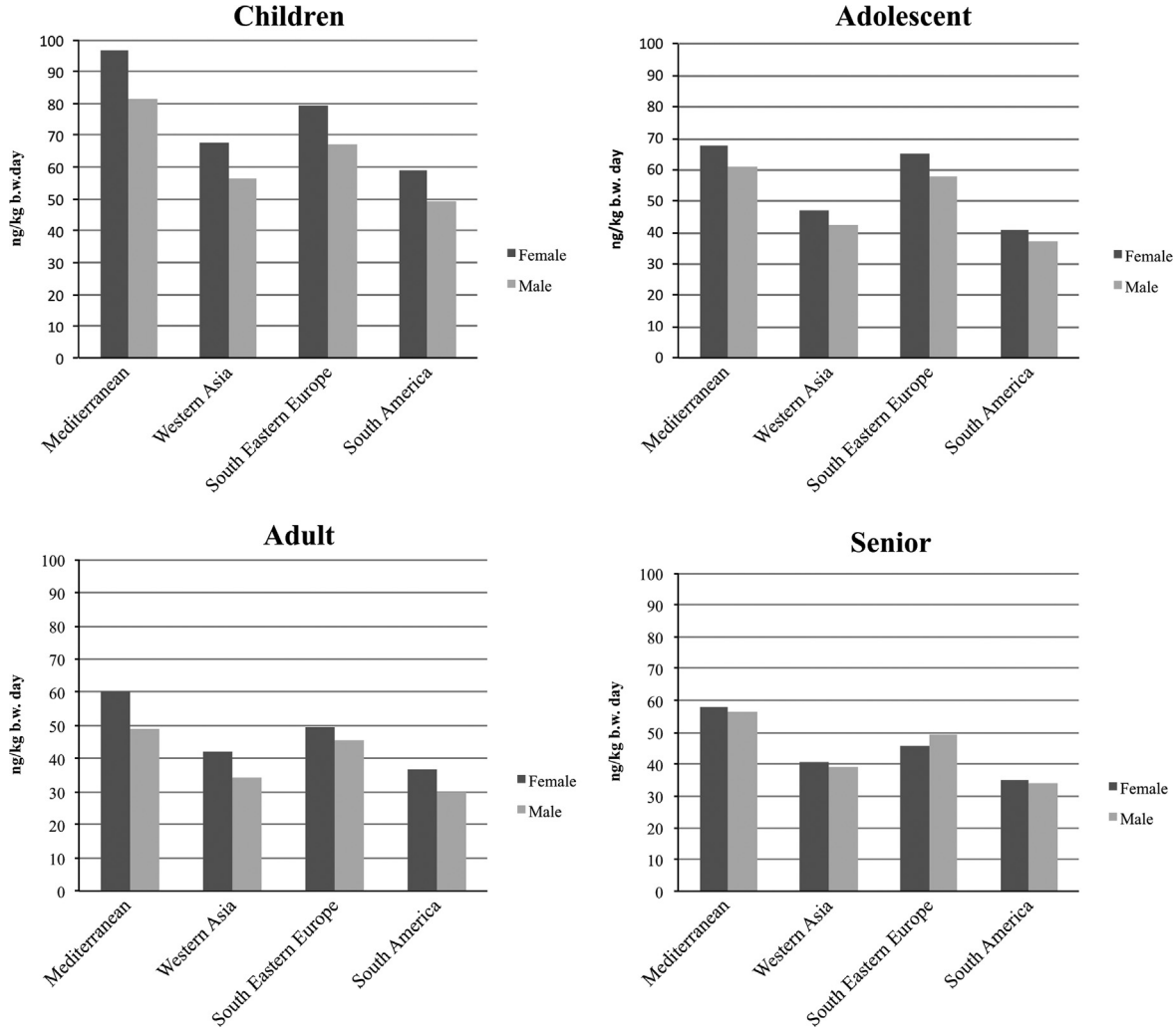

Fig. 3. Total daily intake in the different countries and gender/age groups.

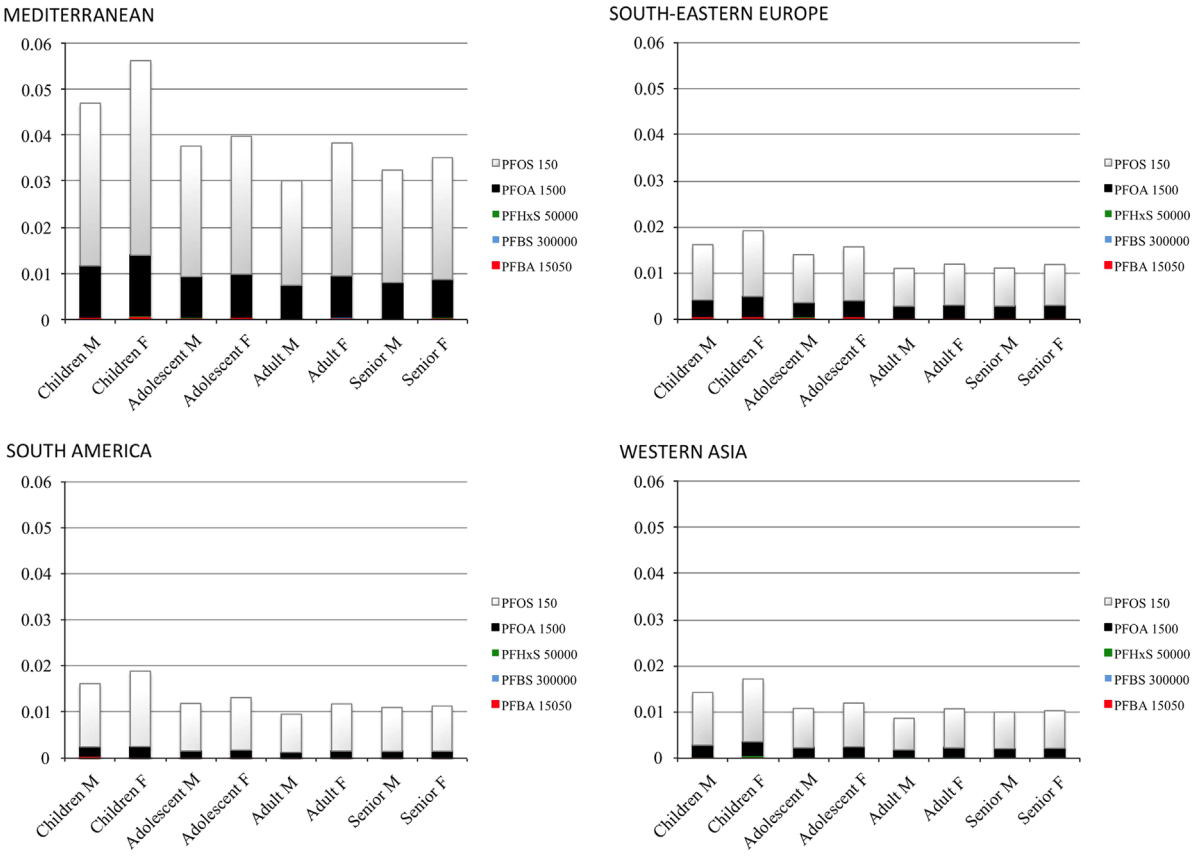

Fig. 4. Risk Index calculated for each diet and gender/age groups.

some compounds, such as PFOS (mean concentration between 115-2028 pg/g w.w. for the different diets). For example Clarke et al., studied 252 individual food items in UK reporting the highest concentration of 59 ng/g of PFOS in fish (Clarke et al., 2010). More recently, Hlouskova et al., studied the PFASs content in 50 pooled samples for different European countries. In this study, PFOS was the more frequently detected compound and it was found in the range of 0.98-2600 pg/g w.w. (Hlouskova et al., 2013).

The progressive reduction of the PFOS content in animal foods could reflect the slow reduction of PFOS in the environment since its stop of production in Europe and America but not in China.

Finally, this work contributes to enlarge the information about shorter chain compounds and the total PFASs amount in countries not studied before as Brazil, Saudi Arabia and Serbia.

4. Conclusions

A sensitive and robust analytical method in compliance with the Commission Recommendation 2010/161/EU for different food matrices was validated. The method was applied to the analysis of 283 food items among the most widely consumed foodstuffs in Brazil, Saudi Arabia, Serbia and Spain. These countries were chosen as representatives of the diet in South-Eastern Europe, the Mediterranean countries, the Western Asia and South America.

The analytical method presented here integrates the sample enrichment step with the instrumental determination. Therefore, sample manipulation is minimized as well as chances of contamination, analytes losses and time of analysis. In addition, small sample sizes are required.

Differences in the compounds profile and concentrations were found between the same groups of food items from different countries. In particular these differences were found in packed foods and can be attributed to food processing or/and packaging migration and different packaging materials.

In term of contamination from the food web, PFOS and PFOA were the more relevant and frequently detected compounds.

As it was expected, fish and seafood were the major contributors to PFASs to the diet. When the different diets were compared, the highest intake was recorded for the Mediterranean and South-Eastern European diets. But the highest risk index was identified for the Mediterranean diet due to the highest fish consumption.

However, for all the studied diets the RI were by far lower than the maximum tolerable limits.

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Appendix A. Supporting information

Supplementary data associated with this paper can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.08.004.

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