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The perfluoroalkyl substance (PFAS) contamination level in milk and milk products in Poland

Katarzyna Sznajder-Katarzyńska [a], Magdalena Surma [a, *], Wiesław Wiczkowski [b], Ewa Cieślik [a]

[a] Malopolska Centre of Food Monitoring, Faculty of Food Technology, University of Agriculture in Krakow, Balicka St. 122, 30-149 Krakow, Poland
[b] Department of Chemistry and Biodynamics of Food, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, Tuwima St.10, 10-748 Olsztyn, Poland

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ABSTRACT

Cross-sectional studies concerning the contamination of milk and milk products by perfluoroalkyl substances (PFASs) are presented and the extent of contamination that may occur in food samples, collected in Poland in 2016 is evaluated; not only milk, cottage cheese, natural yoghurt and butter but also previously untested foods, including kefir, sour cream and Camembert-type cheese. Levels of 7 perfluoroalkyl carboxylic acids (PFCAs) and 3 perfluoroalkane sulfonates (PFSAs) were analysed using the QuEChERS extraction method followed by micro-HPLC-MS/MS. All PFASs were detected with an RSD of lower than 10%. The most commonly detected was perfluorooctanoic acid, followed by perfluorobutanoic acid and perfluorohexane sulfonate, on par with perfluorooctane sulfonate. The largest contributor to the total PFAS concentration in the investigated food samples was perfluorobutanoic acid, and its summary concentration within the group was estimated to be 13.34 ng g^{-1}. The results for 43.4% of samples analysed were greater than LOQ (limit of quantification).

1. Introduction

Poly- and perfluoroalkyl substances (PFASs) constitute numerous chemicals of anthropogenic origin, which are persistent and thus globally widespread in the environment. PFASs were first put on the market in the 1940s (Wang, Dewitt, Higgins, & Cousins, 2017). Large numbers of hydrocarbon structures forming the group of perfluoroalkyl substances are resistant to physical, chemical and biological degradation. Consequently, some of them have been identified as persistent organic pollutants (POPs). Perfluorooctanesulfonate (PFOS), its salts and perfluorooctanesulfonylfluoride (PFOSF) were included under the Stockholm Convention as new POPs in May 2009 (Stockholm Convention, 2009).

Due to their thermal and chemical stability, in addition to their amphiphilic nature, they have been applied in a wide range of industrial, commercial and domestic products. Because of their persistence and ability to bioaccumulate in the environment, many pathways for human exposure to PFASs are well known, e.g., drinking water, food of vegetable and animal origin, air and dust, contact with every-day, PFASs-containing materials (Cousins, 2015; Inoue et al., 2012; Krafft & Riess, 2015). In nature, especially in living organisms, high absorption level and low elimination rates of perfluoroalkyl substances have been observed (Geueke, 2016). These substances enter the body through the digestive and respiratory system and the skin; moreover, they are not metabolised and instead accumulate in the body (Giesy & Kannan, 2002; Wolny & Krupa, 2012).

General toxicological findings associated with exposure to PFASs include hepatotoxicity (Malinverno, Colombo, & Visca, 2005), hepatomegaly (Elcombe et al., 2012), hepatocellular adenoma (Butenhoff, Chang, Olsen, & Thomford, 2012), peroxisomal proliferation (Berthiaume & Wallace, 2002), congestion and thickened epithelial walls in lungs (Cui, Zhou, Liao, Fu, & Jiang, 2009), reproductive toxicity (Luebker, York, Hansen, Moore, & Butenhoff, 2005), immunotoxicity (Fair et al., 2011; Grandjean et al., 2012; Hu, Strynar, & Dewitt, 2010; Keil, Mehlmann, Butterworth, & Peden-Adams, 2008; Peden-Adams et al., 2008), and neurotoxicity (Johansson, Fredriksson, & Eriksson, 2008; Mariussen, 2012). In vitro studies show that they exert cytotoxic effects on hepatoma

* Corresponding author. Tel.: +48 12 6624827.
E-mail address: m.surma@ur.krakow.pl (M. Surma).

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HepG2 cells (Florentin, Deblonde, Diguio, Hautemaniere, & Hartemann, 2011). These compounds also exhibit a capacity to interfere with hepatic enzyme activity (Narimatsu, Nakanishi, Hanioka, Saito, & Kataoka, 2011) and to exert anti-inflammatory effects, thus modulating secretion of pro-inflammatory cytokines in blood cells (Brieger, Bienefeld, Hasan, Goerlich, & Haase, 2011; Corsini et al., 2011). In view of these facts, several studies have attempted to estimate the dietary intake of PFASs (Domingo et al., 2012; Haug et al., 2010; Noorlander, van Leeuwen, Te Biesebeek, Mengelers, & Zeilmaker, 2011).

Biomonitoring of perfluoroalkyl substances in the human body started in 2000. Many PFASs have been detected in human matrices, most commonly in blood samples (Góralczyk et al., 2015; Lau et al., 2007). In recent years, a number of papers confirmed the occurrence of these compounds in breast milk (Kärrman et al., 2007), seminal plasma (Guruge et al., 2005), umbilical cord blood (Inoue et al., 2004) and liver (Domingo et al., 2012). However, in contrast to most other POPs, they do not tend to accumulate in fat tissues but rather bind to serum albumin and other cytosolic proteins and accumulate mainly in the liver, kidneys, and bile secretion (Jones, Hu, De Coen, Newsted, & Giesy, 2003; Pérez et al., 2013).

Milk and its derivatives are food products of animal origin commonly consumed by humans. Milk and milk products are found to be rich in bone-building nutrients, especially calcium, selenium, magnesium and vitamins (B2, B5, B12, and D). One of the fundamental ingredients of milk is protein, and milk protein is regarded as high-quality protein consisting of the essential amino acids needed by humans (FAO, 2013). Intake of high-protein food based on milk and milk products may be a likely route for human exposure to perfluoroalkyl substances because of their high affinity to proteins (binding to β-lactoglobulin) and tendency to concentrate along the trophic chains (Fromme, Tittlemier, Völkel, Wilhelm, & Twardella, 2009; Ropers et al., 2009). It is therefore appropriate to estimate the amounts of the most commonly occurring PFASs to define the food contamination level. However, there are still few cross-sectional data available regarding contamination of milk and milk products by perfluoroalkyl substances. According to an EFSA Scientific Report (EFSA, 2011), results of monitoring of perfluoroalkylated substances in food in the period from 2000 to 2009 showed that out of 251 dairy product samples, only two sheep milk samples were found to be positive for PFOS, with concentrations of 0.14 and 0.26 μg L^{-1}.

Factors that affect raw milk contamination by chemicals include health of the animal, hygiene of the staff, safety levels of the animal's feed, and more advanced levels of contamination that occur during transportation, preservation or processing (Khaniki, 2007). A number of studies have reported that some packaging materials, such as fluorochemical-treated grease-, water- or oil-resistant paper and plastic containers, which are commonly used in the fast-food and dairy industries, could be a source of PFAS contamination (Surma, Wiczkowski, Zieliński, & Cieślik, 2015c; Wang, Shi, Pan, & Cai, 2010; Wu et al., 2010).

Regulation (EC) No 178/2002 of the European Parliament and of the Council on January 28, 2002, which outlines the general principles and requirements of food laws, and the Act of August 25, 2006, regarding food and nutrition safety, are the two main documents, underlying food regulations (Act of August 25, 2006; EC, 2002). Because pollution by PFASs tends to be a global and particularly complex issue, EFSA's Scientific Panel on Contaminants in the Food Chain made recommendations about an in-depth assessment of the levels of PFASs in both food and humans (EFSA, 2008). Acting in accordance with the Commission recommendation (2010/161/EU) of March 17, 2010 regarding the monitoring of perfluoroalkylated substances in food, the method of analysis applied should give reliable and reproducible results (Commission Recommendation, 2010). Currently, developing sample clean-up methods consistent with the principles of green chemistry while maintaining the expected sensitivity and efficiency are still a challenge.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), method, developed by Anastassiades, Lehotay, Štajnbaher, and Schenck (2003), represents one of the most prominent but simultaneously simple sample preparation approaches. Application of ENV SPE Bulk Sorbent (Agilent Technologies, Santa Clara, CA, USA) in the sample treatment step for the determination of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonates (PFSAs) has been used successfully to clean up different food matrices (Surma, Piskuła, Wiczkowski, & Zieliński, 2017; Surma, Wiczkowski, Cieślik, & Zieliński, 2015b). Their analysis is a challenging task, not only because the low concentration levels expected for these compounds in food samples, but also because of the complexity of matrices, so efficient sample preparation procedures and very sensitive determination technique are needed. Methods employed for sample preparation usually consist of one of three approaches: liquid extraction (Eriksson, Kärrman, Rotander, Mikkelsen, & Dam, 2013; Wang et al., 2010; Xing et al., 2016), alkaline digestion (Haug et al., 2010) or enzymatic hydrolysis (Kowalczyk et al., 2013). These are followed by solid-phase extraction (SPE), usually with weak anion exchanges (WAX) (Domingo et al., 2012; Eriksson et al., 2013; Kowalczyk et al., 2013; Wang et al., 2010), hydrophilic-lipophilic balanced sorbent (HLB) (Wang et al., 2010; Xing et al., 2016) or graphitised non-porous carbon (ENVI-carb) (Eriksson et al., 2013; Haug et al., 2010; Wang et al., 2010) cartridges.

Currently, through wide access to advanced equipment and technologies, it is essential to apply such a method of analysis to reach the recovery rates of measured analytes in the range of 70–120%, with a limit of quantification (LOQ) of 1 μg kg^{-1} according to the Commission Recommendation (2010). The LC–MS/MS technique with MRM ratios is a generally accepted technique for qualitative and quantitative determination of PFASs in complex matrices. The high selectivity and sensitivity that distinguish this technique allow, for determination of perfluoroalkyl substances concentrations at very low, or even trace, levels (Guillarme, Ruta, Rudaz, & Veuthey, 2010).

To the best of our knowledge, existing literature provides little information about the determination of PFASs in milk and its derivatives and to date, the QuEChERS methodology has only been used by Yu et al. (2015). Consequently, the goal of this study was to investigate the presence of 10 PFASs in common consumed products derived from milk and milk products. Seven PFCAs, including perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA), in addition to three PFSAs, including perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS), were analysed. Thirty-five different food items were examined, including milk, cottage cheese, natural yoghurt, kefir (bonny clabber), sour cream, Camembert-type cheese and butter. Samples were analysed using a micro-HPLC-MS/MS system after being subjected to a modified QuEChERS method with dispersive solid-phase extraction (Surma, Giżejewski, & Zieliński, 2015a).

Despite the numerous studies that have been conducted, at present, there is no EU legislation specifying maximum PFASs levels in foodstuffs, including milk and related food matrices. The novelty of this paper is its cross-sectional study of food contamination of milk and milk products by perfluoroalkyl substances and an attempt to evaluate, for the first time, the extent of contamination that may occur in kefir, sour cream and Camembert-type cheese;

the results of this study may be important for further EFSA recommendations regarding the health risks of these compounds to humans.

2. Materials and methods

2.1. Chemicals and reagents

Native standards of PFASs containing three perfluorosulfonates (PFBS, PFHxS, and PFOS) and seven perfluoroalkyl carboxylic acids (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, and PFDA) in methanol, with a chemical purity of >98% each; perfluoro-n-[$^{13}C_8$] octanoic acid (L-PFOA) in methanol, with a chemical purity of >98%, and sodium perfluoro-1-[$^{13}C_8$] octanesulfonate (L-PFOS) in methanol, with a chemical purity of >98%, were obtained from Wellington Laboratories (Guelph, ON, Canada). Reagents of MS grade including acetonitrile (MeCN), methanol (MeOH), water (H_2O) and formic acid (FA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Stock, intermediate and working standard solutions of native PFASs, L-PFOA (IS_1 – internal standard) and L-PFOS (IS_2) were prepared in acetonitrile. Stock solution of PFASs at a concentration of 5 μg mL^{-1}, L-PFOA at a concentration of 49 μg mL^{-1} and L-PFOS at a concentration 50 μg mL^{-1} were purchased as a solution prepared in methanol. Intermediate (100 ng mL^{-1}) and working (1 ng mL^{-1}) standard solutions of native PFASs and IS were prepared in 20% MeOH (v/v) with the addition of 1% (v/v) of formic acid.

Acetonitrile (HPLC grade) for extraction was purchased from Merck KGaA (Darmstadt, Germany). Magnesium sulphate anhydrous p.a. and sodium chloride p.a. were purchased from POCh SA (Gliwice, Poland). ENV (styrene-divinylbenzene) SPE Bulk Sorbent was obtained from Agilent Technologies (Santa Clara, CA, USA). Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

As a matrix-matched calibration curve, a series of standard solutions in corresponding blank sample extracts were used. They were prepared via addition of known volumes of standard mixture solution to the corresponding blank sample extracts at concentrations between 1 and 20 ng mL^{-1}, being in good agreement with Commission Recommendation (2010). Blank samples were prepared in acetonitrile. 20 μL of the L-PFOA and L-PFOS solution (2.5 μg mL^{-1}) were added to each standard solution. A series of standard solutions were prepared in triplicate.

2.2. Material

Milk and milk products (dairy products) were purchased in Polish markets in 2017. This publication is a part of project grant focused on the analysis of the occurrence of selected perfluoroalkyl substances in food groups that are part of the model daily food intake and are fundamental elements of the proper nutritional pyramid. That is why material was divided into seven groups: milk with a fat content of 1.5%, semi-skimmed cottage cheese, natural yoghurt, kefir (bonny clabber), sour cream with a fat content of 12%, Camembert-type cheese and butter. The Samples were collected in accordance with PN-EN ISO 707:2009, “Milk and milk products: Guidance on sampling”. The contents of intact and unopened containers of size greater than the minimum sample size constituted the samples. If the size of a single unopened retail container did not meet the minimum sample size, a composite from multiple unopened retail containers constituted the sample. If a sample was collected from retail containers, it was preheated beforehand. For milk and liquid milk products (sour cream), the minimum sample size was 100 mL or 100 g; for semi-solid and solid milk products except for butter and cheese, it was 100 g; for butter and related products, it was 50 g; and for cheese, it was 100 g.

Commercially available samples of each product were obtained from 5 different suppliers; consequently, 35 different food items were studied. Samples were taken from the product produced in the highest volume in each category. The foods were selected carefully and were among the most-consumed in Poland. Five samples of each were examined in triplicate. Sampling was performed in such a manner as to obtain representative samples of the product. The samples were analysed in their original form in amounts of 10 mL and 10 g for liquid and semi-fluid/solid samples, respectively.

2.3. Equipment

The target substances were analysed via micro-high-performance liquid chromatography/tandem mass spectrometry (micro-HPLC/MS/MS) with negative ion electrospray ionisation (ESI) and multiple reaction monitoring (MRM) mode of operation for qualitative and quantitative analysis. The micro-HPLC system LC200 (Eksigent, AB SCIEX, Concord, Canada) is composed of multichannel pump, autosampler set to 4 °C, column oven and system controller. The mass spectrometer QTRAP 5500 (AB SCIEX, Concord, ON, Canada) consists of a triple quadrupole, ion trap and ion source. The system is controlled using the Analyst 1.5.1 software package (AB SCIEX). Chromatographic determinations were performed with the use of HALO C_{18} column with dimensions 50 mm × 0.5 mm × 2.7 μm (Eksigent) under the following conditions: at 45 °C with the flow rate of 20 μL min^{-1}. The gradient elution system composed of water/formic acid (99.0/1.0, phase A) and acetonitrile/formic acid (99.0/1.0, phase B) was used in the chromatographic separation of complex mixtures of analytes. The gradient was established as follows: 40% B (0–0.5 min), 40–90% B (0.5–3.0 min), 90% B (3.0–4.0 min), 90–40% B (4.0–4.2 min) and 40% B (4.2–5.0 min). The optimal conditions for PFAS identifications were as follows: curtain gas flow: 25 L min^{-1}, collision gas flow: 9 L min^{-1}, ionspray voltage: −4500 V, temperature: 350 °C, 1 ion source gas flow: 30 L min^{-1}, 2 ion source gas flow: 35 L min^{-1}, declustering potential range: −30 to −85 V, entrance potential: −10 V, collision energy range: −10 to −65 eV, collision cell exit potential range: −10 to −38 V (Surma et al., 2015a). An MPW 352R Centrifuge (MPW Med. Instruments, Warsaw, Poland) was used for sample preparation. A Vacuum Concentrator Plus (Eppendorf AG, Hamburg, Germany) was used for extract concentration.

2.4. Sample preparation method for PFAS determination

In this study, concentrations of perfluoroalkyl substances in the milk and milk products were determined via the QuEChERS method with the d-SPE sample clean-up step prior to micro-HPLC-MS/MS analysis. These tests were performed using the methodology evaluated and validated by Surma et al. (2015b) in a previous study. Because of the high fat content of the analysed foodstuffs, which ranged from 1.5 to 83%, based on previous experiments, an additional fat freezing-out step was applied to reduce the matrix effect (Surma et al., 2015a). Liquid or semi-liquid samples were analysed as supplied. Solid samples were shredded before analysis. The QuEChERS method includes a salting-out extraction process followed by dispersive solid phase extraction. In the first stage of the extraction process, 10 g or 10 mL, i.e., a representative portion, of each sample were transferred into a 25-mL centrifuge tube. An adequate amount of sample was spiked with 10 μL ISs (internal standards) solution with a concentration of 2.5 μg mL^{-1}. Afterwards, the milk and milk-related samples were extracted with 10 mL MeCN with the addition of 150 μL FA by sonication and vigorous mechanical agitation for 2.5 min and 1 min, respectively.

Then, 1 g NaCl and 4 g $MgSO_4$ were added to the tube, and it was shaken vigorously for 1 min. For phase separation, the samples were centrifuged for 15 min at 8693 ×*g*. Then, 6 mL of the acetonitrile phase obtained by separation of supernatant into organic and inorganic layers were added to a PP (polypropylene) 15-mL tube containing 0.15 g ENV SPE Bulk Sorbent, used as a dispersive cleanup kit, and 0.90 g $MgSO_4$. Tubes were immediately shaken for 30 s and centrifuged for 5 min at 8693 ×*g*. Then, 6 mL of the acetonitrile phase obtained by separation of the supernatant into organic and inorganic layers were added to a PP 15-mL tube containing 0.15 g ENV SPE Bulk Sorbent, used as a dispersive cleanup kit, and 0.90 g $MgSO_4$. The tubes were immediately shaken for 30 s and centrifuged for 5 min at 8693 ×*g*. Four mL of supernatant were moved into a 4-mL tube, followed by placing the tube in freezer storage at a temperature of −12 °C for 20 h to remove residual fat. After this step, samples were evaporated to dryness in a vacuum concentrator at 40 °C, just after filtration through a paper filter. Immediately before micro-HPLC-MS/MS analysis, residues were dissolved in 1 mL MeOH and subsequently diluted fivefold in water via addition of 1% (v/v) FA. The same procedure was employed to prepare blank samples and reagent blanks. Each sample for the assay was prepared in triplicate.

Also, application of additional sorbent (C_{18}) for sample clean-up was conducted. Furthermore, hexane (hx, 5 mL) and acetonitrile were added in the extraction step to selected samples. Four variants of the method were tested: 1 (ENV), 2 (ENV + C_{18}), 3 (hx + ENV), and 4 (hx + ENV + C_{18}). In method 1 as a dispersive cleanup kit 0.15 g ENV SPE Bulk Sorbent was used. In method 2 besides 0.15 g ENV, plus 0.3 g of additional C_{18} was used. Method 3 based on use only 0.15 g ENV as a dispersive cleanup kit as a in method 1 but with 5 mL of additional hexane (apart from 10 mL of acetonitrile) was used in the extraction step. Similarly, method 4 consisted of using a dispersive cleanup kit as in method 2 (0.15 g ENV and 0.3 g C_{18} plus, additionally, 5 mL hexane (apart from 10 mL acetonitrile) in extraction step.

Because milk and related food matrices with a specified and certified concentrations of PFASs are still not commercially available, recovery studies were conducted. The efficiency of the methods used in this study was established on the basis on the recovery ratio of analysed samples according to the analysis of samples that had been previously fortified. Milk samples were selected as test samples and then fortified with standard solution mixture containing the ten analysed PFASs to the fortification level of 0.005 mg kg^{-1}. Samples were analysed in accordance with the procedure described above except that at the beginning, approximately 15 min before extraction, native standard solution of PFASs was added to the sample (62.5 μL, 100 ng mL^{-1}), and additional sorbent or solvent was applied. Calibration curves for each perfluoroalkyl substance determined in this study were set up established by defining the ratio of the particular peak areas, divided by the peak area of the L-PFOA (for PFCAs) and L-PFOS (for PFSAs), against the concentration of the analyte. In the peak areas of the analytes of fortified samples, the peak areas of the analytes from the blank matrix were taken into account.

Linearity, selectivity, recovery, repeatability, limit of detection (LOD), and limit of quantification (LOQ) were verified to confirm the analytical performance of the QuEChERS method for PFAS determination.

2.5. Statistical analysis

At the beginning of statistical analysis, the Shapiro–Wilk test was performed to evaluate the normal distribution of the variables (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS and PFOS) within each tested group. The test showed that assumption of normality was not met for all the variables. Levene's test was used to test the homogeneity of variances. The analysis of the scores indicated a heterogeneous distribution of variances ($p < 0.05$). Based on prior tests, a Kruskall-Wallis H non-parametric test was performed to test whether there were any differences among groups (milk, cottage cheese, natural yoghurt, kefir/bonny clabber, sour cream, Camembert-type cheese, and butter) for each of the 10 variables. A *p*-value of $p < 0.05$ was adopted as statistically significant. The Statistica 12.5.192.7 software package (Statsoft Inc., Tulsa, OK, USA) for Windows was used.

For PFPeA, the results of the Kruskal–Wallis H test ($H(6,35) = 11.36571$, $p > 0.05$) showed that the groups were not significantly different. For PFHxA, PFHpA, PFNA, PFDA, and PFBS, the Kruskal–Wallis test results were statistically significant, but multiple comparison tests revealed no significant differences among the variables in any of the pairs. For this reason, the groups for which the given variable had an average value of 0.00 were eliminated from the test. Repeated Kruskal–Wallis tests, conducted for each variable, were not statistically significant ($p > 0.05$). For two perfluoroalkyl carboxylic acids, namely PFBA and PFOA, and two perfluoroalkane sulfonates, namely PFHxS and PFOS, the Kruskal–Wallis test results were statistically significant, and multiple comparison tests showed significant differences among the variables within pairs being compared.

3. Results and discussion

3.1. Analytical performance of the method

Specificity of the analytical procedure was performed based the MRM ion pairs and were summarised and described in previous work (Surma et al., 2015a). Method accuracy expressed as recovery values for selected perfluoroalkyl substances for all tested methods are presented in Fig. 1. The best recovery ratio (70–120%, according to the Commission Recommendation, 2010) within RSD lower than 10% for all PFASs was obtained for method 1, which used only acetonitrile as the extraction solvent and ENV sorbent for extract clean-up. Moreover, there was no difference in the purity of extracts prepared with different methods. Therefore, this method was used for further PFASs determinations and was verified to confirm the analytical performance. For all target analytes, the repeatability, expressed as the relative standard deviation (RSD) parameter, was less than 10%. Due to insufficient data regarding allowed RSD value for recovery ratios for PFASs determination in Commission Recommendation (2010), good laboratory practices and validation guidelines recommend adopting the value of 10%. There are several different approaches to establishing limits of detection (LOD) and quantification (LOQ) (Glaser, Foerst, McKee, Quave, & Budde, 1981). In this case limit of detection (LOD) value was computed based on the slope of the calibration curve and standard deviation of the results received for the series of blank samples according to the equation

$$LOD = 3.3 * s/a$$

where

a – slope of the calibration curve.

s – standard deviation of the results received for the series of blank samples (Konieczka et al., 2004).

The limit of quantification (LOQ) was calculated as three times of LOD value. For the investigated analytes, LOQ values changed from 0.010 ng kg^{-1} for PFHS to 0.027 ng kg^{-1} for PFPeA. The obtained results are in good agreement with the value (1 μg kg^{-1}) specified in the Commission Recommendation (2010). Sensitivity of assay was determined as the calibration slope coefficient. Highest

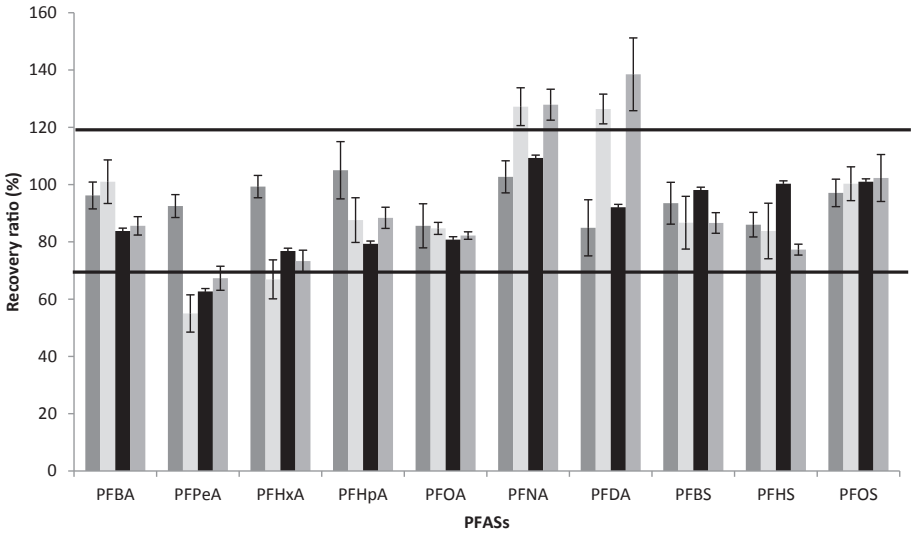

Fig. 1. PFASs recovery ratio for all tested methods: ■, method 1, ENV; ■, method 2, ENV + C_{18}; ■, method 3, hx + ENV; ■, method 4, hx + ENV + C_{18}.

sensitivity was calculated for PFBS and the lowest for PFDA. To establish the equations of the calibration curves ($y = ax + b$), the least-squares method was involved. Linearity was observed in a range of concentrations between 1 and 20 ng mL^{-1} (Table 1).

3.2. Analysis of real samples

In this study, we attempted to estimate the concentrations of several PFASs in milk and milk-related products, which constitute one of the most important groups of food products in the human diet. Although there are a number of literature reports regarding the concentrations of perfluoroalkyl substances in milk and milk products, none of the previous studies included such a broad group of products and number of analysed PFASs, simultaneously. Hence, determination of the concentration of selected PFASs in milk with a fat content of 1.5% (SM), semi-skimmed cottage cheese (SCC), natural yoghurt (Y), kefir (bonny clabber) (K), cream with a fat content of 12% (C), Camembert-type cheese (CTC) and butter (B) was addressed. Seven perfluoroalkyl carboxylic acids (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, and PFDA) and three perfluoroalkane sulfonates (PFBS, PFHxS, and PFOS) were quantitatively analysed. To the best of our knowledge, no information concerning perfluoroalkyl substance contamination levels of kefir/bonny clabber, Camembert-type cheese or sour cream with 12% fat content has been presented in the literature.

PFCAs were detected in all analysed milk and milk product samples in a range from 1.81 ng g^{-1} for cottage cheese to 7.27 ng g^{-1} for natural yoghurt. PFSAs were found within the range 0.09 ng g^{-1} and 1.00 ng g^{-1} for kefir/bonny clabber and sour cream, respectively. The values outlined above refer to the sum of 10 analysed compounds assessed for each individual product type. The results for PFCAs and PFSAs are presented in Fig. 2 and Fig. 3, respectively. The detailed results of the LC-MS/MS analysis of milk and milk products involved in this study are summarised in Table 2 and Table 3 for PFCAs and PFSAs, respectively. Fig. 4 and Fig. 5, concerning PFCAs and PFSAs, respectively, summarise the median concentrations and interquartile ranges for all analytical groups.

The per cent values of samples (%) in which particular PFCAs were found increased in the following order: PFPeA (17.1)/PFDA (25.7)/PFHxA and PFHpA (34.3)/PFNA (45.7)/PFBA (71.4)/PFOA (100.0). Perfluorooctanoic acid was identified in all of the samples analysed, but its concentration was not very high. The concentration of PFOA ranged from 0.05 ng g^{-1} for butter to 0.50 ng g^{-1} for Camembert-type cheese. The highest concentration was observed for sour cream and Camembert-type cheese, with median levels of 0.33 ng g^{-1} and 0.49 ng g^{-1}, respectively (see Fig. 4).

The PFOA levels in the analysed group of food products are shown in Fig. 6a. Statistical differences in PFOA levels were observed in milk and milk products between Camembert-type cheese and milk ($p = 0.0213$), Camembert-type cheese and cottage cheese ($p = 0.0311$) and Camembert-type cheese and butter ($p = 0.0279$). Perfluorobutanoic acid was detected in 5 of 7 products analysed and ranged from 0.11 ng g^{-1} for cottage cheese to 2.56 ng g^{-1} for natural yoghurt. The value 2.56 ng g^{-1} indicated for PFBA in natural yoghurt represented the highest amount determined among the analysed PFASs. Moreover, within the group of natural yoghurts, the largest dispersion in the total concentration of PFCAs was observed (see Fig. 2). Sour cream and Camembert-type cheese were free from PFBA contamination. Significant differences in the concentration PFBA between natural yoghurt and sour cream ($p = 0.0029$), natural yoghurt and Camembert-type cheese ($p = 0.0029$), sour cream and butter ($p = 0.0328$) and butter and Camembert-type cheese ($p = 0.0328$) were found. The PFBA levels in milk and related milk products are shown in Fig. 6b. Similar to the case for PFBA, perfluorononanoic acid was also identified in 5 groups of products, but it was not detected in Camembert cheese and butter samples. Its concentration ranged from 0.03 to 0.10 ng g^{-1}. The maximum concentrations found for perfluorodecanoic acid and perfluorohexanoic acid did not exceed

Table 1
Data for quantification of the measured perfluoroalkyl substances.[a]

Perfluoroalkyl substance	a	R^2	LOD (ng g^{-1})	LOQ (ng g^{-1})
PFBA	1.547	0.999	0.008	0.025
PFPeA	1.859	0.998	0.009	0.027
PFHxA	1.614	0.999	0.007	0.021
PFHpA	1.307	0.998	0.006	0.016
PFOA	1.863	0.996	0.005	0.015
PFNA	0.801	0.998	0.006	0.019
PFDA	0.559	0.998	0.004	0.013
PFBS	6.893	0.999	0.004	0.012
PFHS	3.964	0.999	0.003	0.010
PFOS	1.405	0.997	0.004	0.012

[a] Abbreviations are: a, calibration slope; R^2, coefficients of determination; LOD, limit of detection; LOQ, limit of quantification.

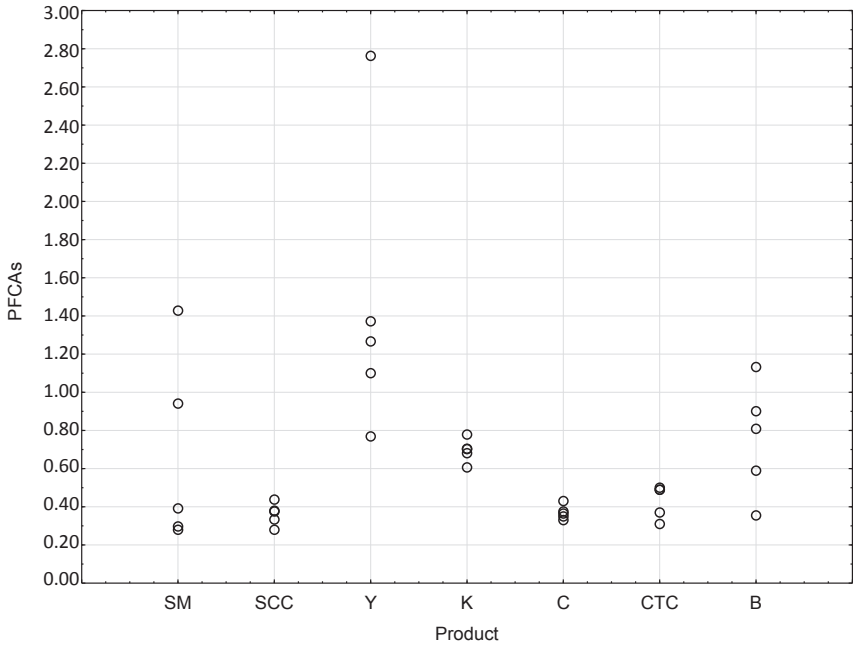

Fig. 2. The total content of PFCAs in the investigated products: SM, milk; SCC, semi-skimmed cottage cheese; Y, natural yoghurt; K, kefir/bonny clabber; C, sour cream; CTC, Camembert-type cheese; B, butter.

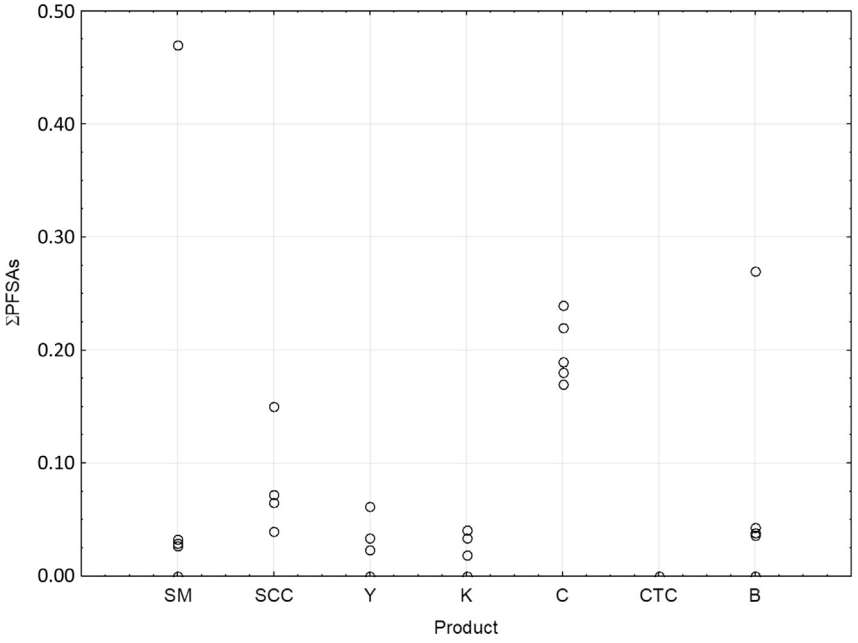

Fig. 3. The total content of PFSAs in the investigated products: SM, milk; SCC, semi-skimmed cottage cheese; Y, natural yoghurt; K, kefir/bonny clabber; C, sour cream; CTC, Camembert-type cheese; B, butter.

0.08 g g^{-1}. Perfluoroheptanoic acid was mostly detected in a few samples of milk, cottage cheese, natural yoghurt and kefir/bonny clabber. The least frequently identified perfluoroalkyl substance was perfluoropentanoic acid, which resulted in the lowest total concentration within the group of milk and milk products, 0.43 ng g^{-1}. In contrast, the highest total concentration was found for PFBS, 13.34 ng g^{-1}. All 7 analysed PFCAs were identified in natural yoghurt samples, in contrast to Camembert-type cheese, in which only the presence of PFOA was found. Fig. 4 shows that the highest median among all analysed PFCAs was calculated for PFBA in a yoghurt sample (0.91 ng g^{-1}, with an interquartile range 0.82–0.92); moreover, for this compound, the highest dispersion in the results (especially for milk and butter samples) within tested groups was observed.

Among the three PFSAs compounds considered, PFHxS and PFOS were detected with the same high frequency, in 48.6% of samples. PFBS was relatively low: its occurrence was confirmed in 14.3% of the analysed samples, but the results obtained for two samples of milk were below the LOQ. PFBS was identified only in butter in the range between 0.01 and 0.02 ng g^{-1}. Except for two products, sour

Table 2
Content of selected perfluoroalkyl carboxylic acids (PFCAs) in milk and milk products analysed.[a]

Parameter	Milk	Cottage cheese	Natural yoghurt	Kefir/Bonny clabber	Sour cream	Camembert type cheese	Butter
Perfluorobutanoic acid (PFBA)							
Range	0.12–0.98 (5)	0.11–0.18 (5)	0.37–2.56 (5)	0.23–0.56 (5)	–	–	0.20–1.00 (5)
CV (%)	88.79	22.76	75.14	31.83	–	–	59.76
∑ PFBA	13.34						
Perfluoropentanoic acid (PFPeA)							
Range	–	–	0.07 (1)	0.07–0.16 (2)	–	–	0.04–0.06 (3)
CV (%)	–	–	223.61	151.42	–	–	94.74
∑ PFPeA	0.43						
Perfluorohexanoic acid (PFHxA)							
Range	–	0.02–0.03 (3)	0.06–0.08 (3)	0.06–0.07 (3)	–	–	0.05–0.07 (3)
CV (%)	–	92.47	94.08	92.51	–	–	94.32
∑ PFHxA	0.67						
Perfluoroheptanoic acid (PFHpA)							
Range	0.02–0.25 (3)	0.02–0.03 (3)	0.06–0.10 (3)	0.05–0.07 (3)	–	–	–
CV (%)	180.67	91.62	98.60	93.66	–	–	–
∑ PFHpA	0.76						
Perfluorooctanoic acid (PFOA)							
Range	0.07–0.32 (5)	0.08–0.17 (5)	0.14–0.20 (5)	0.12–0.18 (5)	0.32–0.35 (5)	0.31–0.50 (5)	0.05–0.34 (5)
CV (%)	81.15	36.40	16.60	16.46	3.30	20.07	98.82
∑ PFOA	7.20						
Perfluorononanoic acid (PFNA)							
Range	0.04–0.09 (4)	0.03–0.06 (5)	0.07–0.10 (3)	0.07 (1)	0.03–0.05 (3)	–	–
CV (%)	76.70	28.24	93.77	223.61	94.79	–	–
∑ PFNA	0.83						
Perfluorodecanoic acid (PFDA)							
Range	0.02–0.04 (3)	0.03–0.08 (4)	0.06 (2)	–	0.07 (1)	–	–
CV (%)	95.09	78.60	137.01	–	223.61	–	–
∑ PFDA	0.47						
∑ PFCAs	3.34	1.81	7.27	3.47	1.85	2.16	3.79

[a] Content given in ng g^{-1}; number of samples given in parentheses. Abbreviation: CV, coefficient of variation.

cream and Camembert-type cheese, perfluorohexane sulfonate was detected at concentrations between 0.01 ng g^{-1} for kefir/bonny clabber and 0.05 ng g^{-1} for cottage cheese. Detailed data regarding the PFHxS levels are shown in Fig. 6c. In terms of the PFHxS levels, statistically significant differences between cottage cheese and sour cream ($p = 0.0060$) and between Camembert-type cheese and cottage cheese ($p = 0.0060$) were observed. The PFOS concentrations in milk and milk products ranged from 0.02 to 0.47 ng g^{-1} (both results identified for milk samples). The biggest dispersion in total concentrations of analysed PFSAs was observed for milk samples (see Fig. 3). Fig. 5 shows the median concentrations of detected PFSAs, with the interquartile range in parentheses; the highest, 0.19 ng g^{-1} (0.18–0.22) was for PFOS in the sour cream sample. The greatest variation in results can be observed for PFOS determined in samples of white cheese and sour cream. The mean concentration estimated for PFOS was highest among the particular PFSAs analysed and was calculated to be 2.13 ng g^{-1} (see Table 3). There was a statistically significant difference in terms of the PFOS level between two groups: Camembert-type cheese and sour cream, with a p-value equal to 0.0191. The PFOS levels in the analysed group of food products are shown in Fig. 6d.

From the range of different products under investigation, only Camembert-type cheese was free from PFSAs contamination. Only in butter were all PFSAs (PFBS, PFHxS and PFOS) found during the experiment in concentrations above the LOQ.

Among the ten PFASs compounds analysed, PFOA, PFBA, and PFNA from the group of PFCAs and PFHxS and PFOS from the group of PFSAs were detected with the highest frequencies (from 100% to almost 50% of analysed samples). PFPeA and PFBS were found occasionally, and their detection frequencies were less than 18%. To conclude, 43.4% of results obtained for the samples analysed were above the LOQ. The total concentration of the 10 perfluoroalkyl substances analysed was estimated to be 23.69 ng g^{-1}, 2.64 ng g^{-1} and 26.32 ng g^{-1} for PFCAs, PFSAs and PFASs, respectively. There was no significant difference between the other results.

Due to the high persistence, bioaccumulation potential and toxicity of PFASs, there is a need for monitoring of their concentrations in food items to assess the potential impact on human health. Consumption of contaminated food represents one of the most significant pathways of exposure to PFASs, due to their ability to bioaccumulate in trophic chains and indirectly through their migration from packaging (Jogsten et al., 2009; Wu et al., 2010). Begley et al. (2005) have shown that perfluorochemicals have the capacity to migrate into food matrices from food-paper contact. In their study, the migration into oil from microwave popcorn bags was estimated to be 300 ng g^{-1} for PFOA. Wang et al. (2010) studied total PFAS concentration in milk due to the packaging materials used; they reported significant differences ($p < 0.001$) among three types of containers. Moreover, some recent reports suggest the ability of PFASs to bind to serum albumin and other cytosolic proteins and consequently accumulate in protein-rich tissue (Jones et al., 2003; Li et al., 2017; Sheng, Li, Liu, Zhang, & Dai, 2016). Therefore, there is a need to consider that the diversity and relatively high concentrations of several PFASs may occur as a result of this phenomenon.

There are number of publications suggesting the occurrence of PFASs in breast milk during lactation, confirmation of a trend of accumulation and distribution of these substances within protein-rich matrices (Fromme et al., 2010; Lee et al., 2018). Total concentrations of PFASs in the breast milk of Korean lactating mothers reported by Lee et al. (2018) ranged from 31.7 to 1004 ng L^{-1} (n = 293; 16 PFASs were analysed). In Table 4, the PFAS concentrations in milk and milk products obtained by different authors are compared. Selected results of the current study have also been included in the comparison. PFOA was detected in almost all of the analysed samples, and the remaining PFASs were detected less frequently, in good agreement with our study. Only raw milk, examined by Xing et al. (2016), was free from PFOA contamination, and the contamination by PFOS was lower than in commercial milk. Similarly, previous studies reported that unprocessed foods were

Table 3
Content of selected perfluoroalkane sulfonates (PFSAs) in milk and milk products analysed.[a]

Parameter	Milk	Cottage cheese	Natural yoghurt	Kefir/Bonny clabber	Sour cream	Camembert type cheese	Butter
Perfluorobutane (PFBS)							
Range	–	–	–	–	–	–	0.01–0.02 (3)
CV (%)	–	–	–	–	–	–	91.78
∑ PFBS	0.04						
Perfluorohexane (PFHxS)							
Range	0.01 (3)	0.04–0.05 (5)	0.01–0.02 (3)	0.01–0.02 (3)	–	–	0.02–0.03 (3)
CV (%)	91.29	26.44	97.27	96.43	–	–	92.32
∑ PFHxS	0.47						
Perfluorooctane (PFOS)							
Range	0.02–0.47 (4)	0.03–0.10 (3)	0.02–0.04 (2)	0.02–0.03 (2)	0.17–0.24 (5)	–	0.27 (1)
CV (%)	195.43	113.13	147.59	137.13	14.58	–	223.61
∑ PFOS	2.13						
∑ PFSAs	0.57	0.48	0.12	0.09	1.00	0.00	0.39

[a] Content given in ng g^{-1}; number of samples given in parentheses. Abbreviation: CV, coefficient of variation.

free from PFASs in general and thus production and processing steps are a potential source of contamination (Still, Schlummer, Gruber, Fiedler, & Wolz, 2013).

The PFASs levels in milk and milk related products identified and summarised in this report are similar to those from previous studies listed in Table 4. This is particularly clear when considering the concentrations obtained for PFOA and PFOS, which are similar to or lower than those that have been observed in other independent reports. However, in the present study, compared with previous studies performed around the world, PFBA was detected much more frequently and was a major contributor to the total PFAS concentration within the milk and milk products group (>50% of the total). This may be due to changes in industrial trends for replacing long-chain PFASs, especially C8-based perfluoroalkyl chemicals such as PFOS and PFOA, to alternative, more environmentally friendly chemicals or implementation of non-chemical techniques (Stockholm Convention, 2011; 2012). Although long-chain PFASs are being phased out and transitions to alternative product-based technologies are being progressively implemented and improved, PFBA, PFBS and PFHxA are still primary substitutes for perfluorooctane-based compounds, in addition to typical by-products of degradation processes of replacement substances and product-associated impurities that may reach the environment (Renner, 2006; Wang, Cousins, Scheringer, & Hungerbühler, 2013). However, it is worth pointing out that there are some concerns about the long-term impact of alternatives on human health and environment due to their chemical and physical similarities to long-chain PFASs (Lindstrom, Strynar, & Libelo, 2011; Scheringer et al., 2014).

Nevertheless, there are still no systematic, cross-sectional studies concerning the occurrence of different PFASs in commercially available food items on which to base a far-reaching dietary exposure assessment. In this paper, we outline horizontal research that can contribute a more detailed view to the prevalence of perfluoroalkyl substances in the environment. The average monthly consumption of several dairy products per capita in households in Poland is reported in the Statistical Yearbook of Agriculture 2016 (Central Statistical Office, 2016). The highest

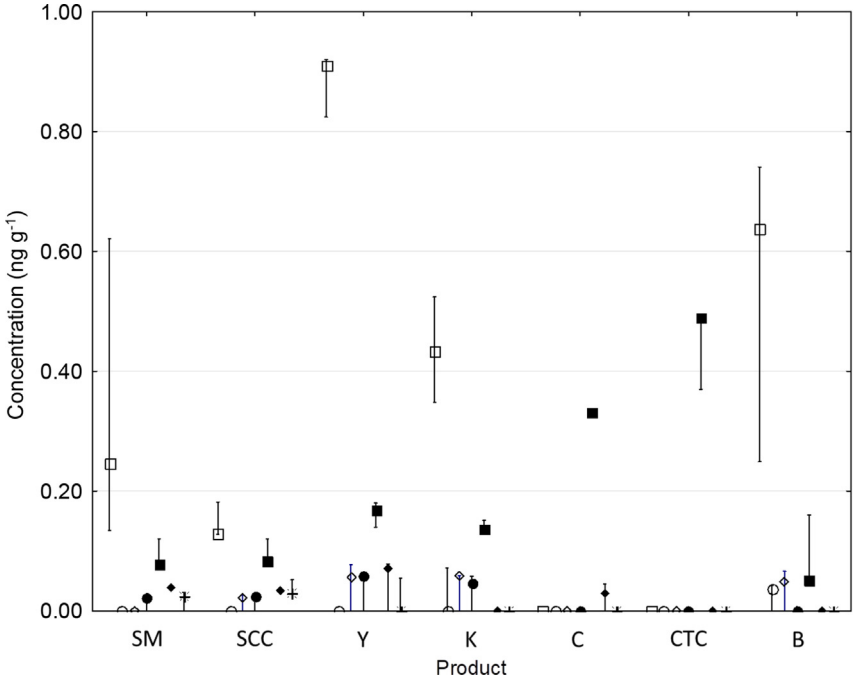

Fig. 4. Median (symbol) and interquartile (whisker: 25%–75%) concentration ranges for PFCAs (□, PFBA; ○, PFPeA; ◇, PFHxA; ●, PFHpA; ■, PFOA; ◆, PFNA; ✳, PFDA)for all analytical groups: SM, milk; SCC, semi-skimmed cottage cheese; Y, natural yoghurt; K, kefir/bonny clabber; C, sour cream; CTC, Camembert-type cheese; B, butter.

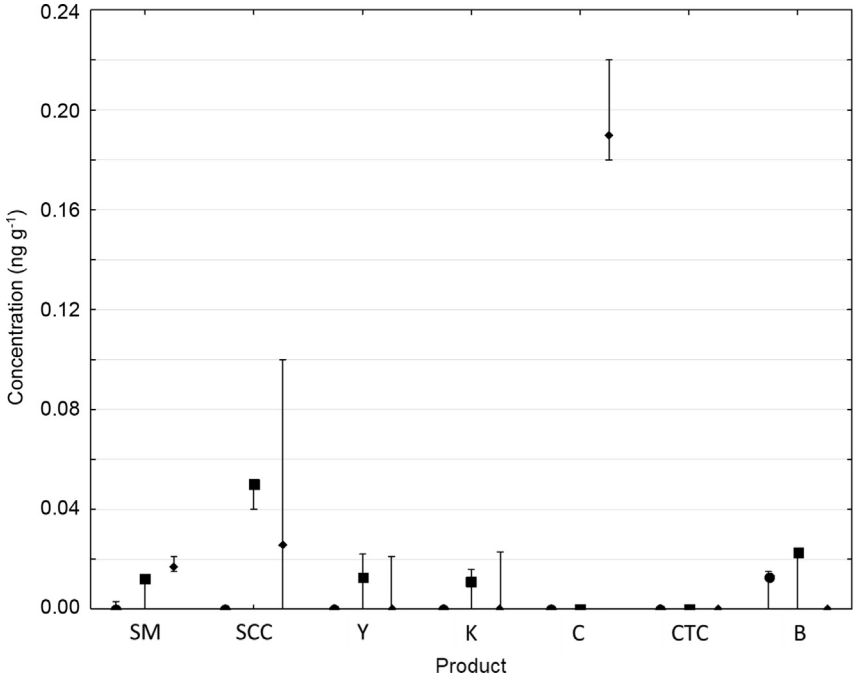

Fig. 5. Median (symbol) and interquartile (whisker: 25%–75%) concentration ranges for PFSAs (●, PFBS; ■, PFHxS; ◆, PFOS) for all analytical groups: SM, milk; SCC, semi-skimmed cottage cheese; Y, natural yoghurt; K, kefir/bonny clabber; C, sour cream; CTC, Camembert-type cheese; B, butter.

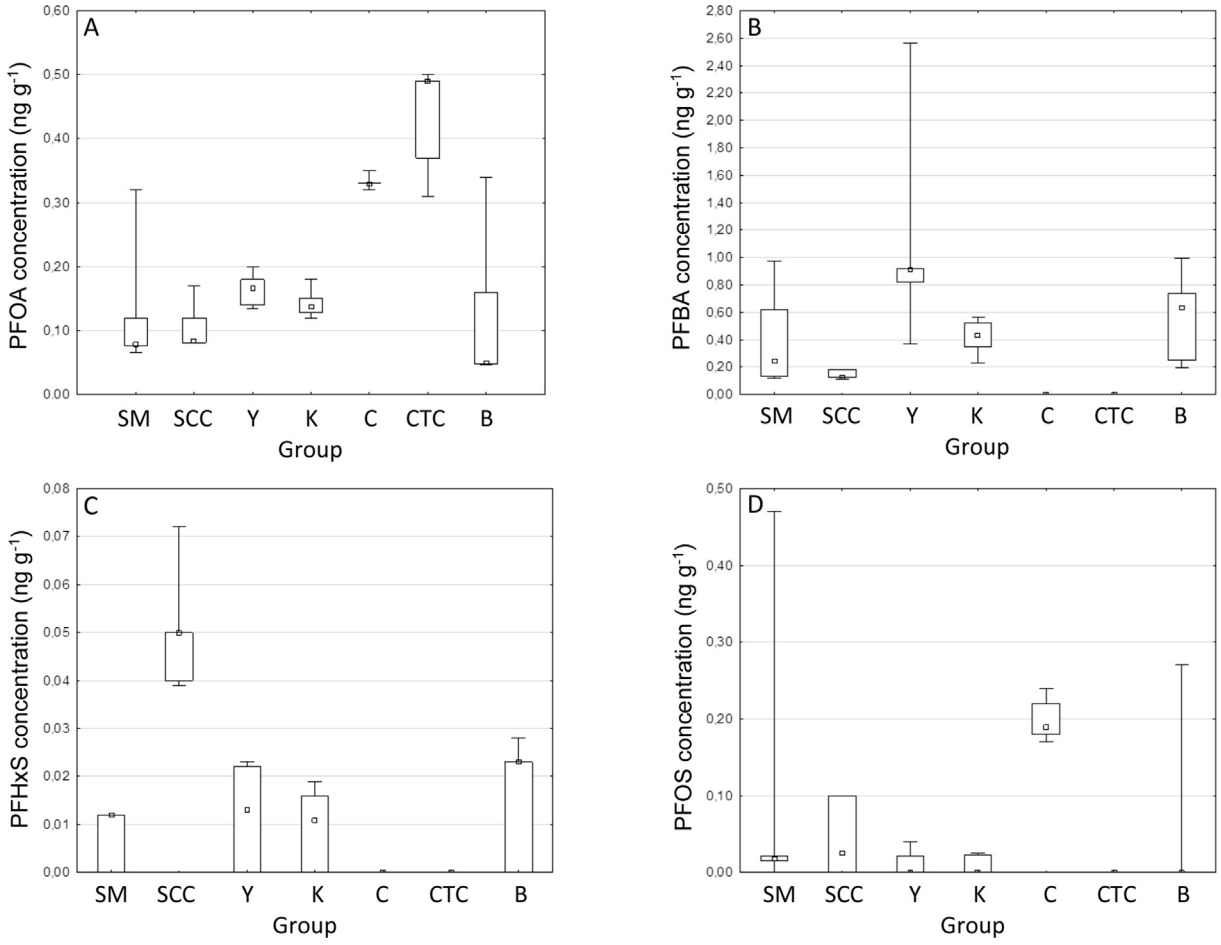

Fig. 6. Box plot of concentration of PFASs (A, PFOA; B, PFBA; C, PFHxS; D, PFOS) in milk and milk products (small box, median; large box, interquartile 25%–75%; bar, minimum–maximum) on the basis of different products: SM, milk; SCC, semi-skimmed cottage cheese; Y, natural yoghurt; K, kefir/bonny clabber; C, sour cream; CTC, Camembert-type cheese; B, butter.

Table 4
Comparison of perfluoroalkyl carboxylic acids and perfluoroalkane sulfonate contents in milk and milk products determined by different authors.[a]

Food sample	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	Unit	Year	Location	Reference
Milk 1.5%	0.12–0.98	–	–	n.d.-0.25	0.07–0.32	n.d.-0.09	n.d.-0.04	<0.01	n.d.-0.01	n.d.-0.47	ng g^{-1}	2016	Poland	Current study
Cottage cheese	0.11–0.18	–	n.d.-0.02	n.d.-0.03	0.08–0.17	0.03–0.06	n.d.-0.08	–	0.04–0.05	n.d.-0.10				
Natural yoghurt	0.37–2.56	n.d.-0.07	n.d.-0.08	n.d.-0.10	0.14–0.20	n.d.-0.10	n.d.-0.06	–	n.d.-0.02	n.d.-0.04				
Butter	0.20–1.00	n.d.-0.06	n.d.-0.07	–	0.05–0.34	–	–	n.d.-0.02	n.d.-0.03	n.d.- 0.27				
Retail milk	–	–	–	–	<5.0–151.8	–	–	–	–	<10.0–172.9	ng L^{-1}	2014–2015	China	Xing et al. (2016)
Raw milk	–	–	–	–	–	–	–	–	–	<10.0–25.1				
Yoghurt	–	–	–	–	<5.0–279.9	–	–	–	–	<10.0–200.6				
Milk	n.d.-0.34	n.d.-0.043	n.d.-0.028	n.d.-0.041	n.d.-0.11	n.d.-0.37	n.d.-0.043	–	n.d.-0.092	n.d.-0.12	μg L^{-1}	2015	China	Yu et al. (2015)
Cows' milk, group 1	–	–	–	–	0.07	–	–	0.016	1.86	9.06	μg L^{-1}	2013	Germany	Kowalczyk et al. (2013)
Cows' milk, group 2	–	–	–	–	n.d.	–	–	n.d.	1.75	33.09				
Milk 0.5%	–	–	<19.0	–	<67.0	51.0	16.0	17.0	–	–	pg g^{-1} ww	2012	Faroe Islands	Eriksson et al. (2013)
Milk 1.5%	–	–	<19.0	–	<67.0	57.0	13.0	<17.0	–	–				
Yoghurt 3.4%	–	–	<8.5	–	<55.0	<14.0	<2.9	<1.6	–	–				
Yoghurt 0.9%	–	–	<7.2	–	<47.0	<12.0	<2.5	<1.4	–	–				
Creme fraiche 18%	–	–	11.0	–	<57	<14	<3.1	<1.7	–	–				
Milk (W and SS)	<150.0	<20.0	<50.0	240.0	390.0	<130.0	<13.0	<4.1	<2.6	<6.9	pg g^{-1} fw	2011	Spain	Domingo et al. (2012)
Milk	–	–	<6.0	<3.0	1.0	<1.0	1.0	<4.0	<2.0	10.0	pg g^{-1} fw	2009	Netherlands	Noorlander et al. (2011)
Cheese	–	–	<9.0	7.0	<19.0	7.0	8.0	<12.0	<25.0	<85.0				
Butter	–	–	20.0	5.0	16.0	1.0	6.0	<3.0	16.0	33.0				
Milk	–	–	1.5	<0.87	4.7	<2.1	4.0	<0.24	<0.11	7.0	pg g^{-1} fw	2008	Norway	Haug et al. (2010)
Cheese	–	–	<7.7	7.4	13.0	16.0	6.6	<1.5	<0.65	12.0				
Milk	–	–	–	<13.0–312.0	<18.0–178.0	<27.0–476.0	<15.0–44.0	–	–	<5.0–695.0	pg g^{-1} ww	2008–2009	China	Wang et al. (2010)
Milk powder	–	–	–	<26.0	<36.0–482.0	<54.0–192.0	<30.0	–	–	<10.0–175.0				
Yoghurt	–	–	–	<13.0–106.0	<18.0–229.0	<27.0–256.0	<15.0–100.0	–	–	<5.0–32.0				

[a] Abbreviations are PFBA, perfluorobutanoic acid; PFPeA, perfluoropentanoic acid; PFHxA, perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFBS, perfluorobutane; PFHxS, perfluorohexane; PFOS, perfluorooctane; W and SS, whole and semi-skimmed; n.d., not detected; ww, wet weight; fw, fresh weight.

consumption level was estimated for milk, 3.15 L per capita, and it was smaller for cheeses and yoghurt, with quantities of 0.83 and 0.50 kg, respectively. In the dairy product consumption data, cream and butter were shown to be eaten in the lowest amounts, 0.35 and 0.26 kg per capita, respectively. For milk and butter, a trend towards greater consumption starting from the year 2005 has been observed. According to the World Health Organisation's GEMS database, consumption of milk in Poland is 233 g per day, and other dairy products account for an additional 53 g per day. The Food and Agriculture Organisation of the United Nations predicted a global increase in milk and milk related products consumption (OECD/FAO, 2016). The expected consumption of fresh dairy products will increase from 52% to 54% within the next 10 years in developing countries. Consumption of dairy products in developing countries is likely to increase on average for fresh dairy products, butter, whole milk powder, skim milk powder and cheese by 2.9%, 1.0%, 1.1%, 1.5% and 0.8% annually. On the basis of data from the Statistical Yearbook of Agriculture 2016 and the OECD/FAO report, it can be demonstrated that milk and milk products constitute one of the most important components of the human diet, and consumption of these products is increasing consistently. Therefore, further research must be performed to verify the real intake of PFASs with diet containing the investigated products and to establish the real risk associated with PFAS contamination.

4. Conclusions

The results of the analytical survey reported in this study indicate that the dispersive solid phase extraction (d-SPE) and micro-HPLC-MS/MS detection are highly applicable for determination of selected perfluoroalkyl carboxylic acids and perfluoroalkane sulfonates in milk and milk products. Use of the polymer-based sorbent ENV for efficient PFAS extraction from milk and milk products based on the QuEChERS method can be recommended. This paper presents a cross-sectional study of contamination of milk and milk products by 10 perfluoroalkyl substances and tries to evaluate, for the first time, the extent of contamination that may occur in food samples that are most popular among Polish consumers, including not only milk, cottage cheese, natural yoghurt and butter but also the not previously tested substances kefir, sour cream and Camembert-type cheese. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS and PFOS were found and their concentrations quantified. The PFASs levels in the investigated products, identified and summarised in this report are similar to those found in previous studies performed worldwide. Nevertheless, attention should be drawn to short-chain PFASs, which are increasingly used in industrial applications, and their long-term effects on the environment and humans remains poorly understood. Because an upward trend in milk and milk product consumption is observed, a wide range of PFASs were identified and more than 43% of results obtained for the samples analysed were above the LOQ, there is a need to perform further studies to assess the real risk arising from intake of PFASs via this type of food.

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References

Act of August 25. (2006). *Food and nutrition safety Act of 25 August 2006, 171*. Dzienniku Ustaw. item 1225, with amendments.

Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*, 412–431.

Begley, T. H., White, K., Honigfort, P., Twaroski, M. L., Neches, R., & Walker, R. A. (2005). Perfluorochemicals: Potential sources of and migration from food packaging. *Food Additives & Contaminants, 22*, 1023–1031.

Berthiaume, J., & Wallace, K. B. (2002). Perfluorooctanoate, perfluorooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. *Toxicology Letters, 129*, 23–32.

Brieger, A., Bienefeld, N., Hasan, R., Goerlich, R., & Haase, H. (2011). Impact of perfluorooctanesulfonate and perfluorooctanoic acid on human peripheral leukocytes. *Toxicology In Vitro, 25*, 960–968.

Butenhoff, J. L., Chang, S. C., Olsen, G. W., & Thomford, P. J. (2012). Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in Sprague Dawley rats. *Toxicology, 293*, 1–15. https://doi.org/10.1016/j.tox.2012.01.003.

Central Statistical Office. (2016). *Statistical yearbook of agriculture 2016*. Warsaw, Poland: Central Statistical Office.

Commission Recommendation. (2010). Commission recommendation (2010/161/EU) of 17 March 2010 on the monitoring of perfluoroalkylated substances in food. *Official Journal of the European Union, 53*, 22–23. L68/2.

Corsini, E., Avogadro, A., Galbiati, V., dell'Agli, M., Marinovich, M., Galli, C. L., et al. (2011). In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). *Toxicology and Applied Pharmacology, 250*, 108–116.

Cousins, I. T. (2015). Per- and polyfluoroalkyl substances in materials, humans and the environment. *Chemosphere, 129*, 1–3. https://doi.org/10.1016/j.chemosphere.2014.08.036.

Cui, L., Zhou, Q. F., Liao, C. Y., Fu, J. J., & Jiang, G. B. (2009). Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. *Archives of Environmental Contamination and Toxicology, 56*, 338–349.

Domingo, J. L., Jogsten, I. E., Eriksson, U., Martorell, I., Perelló, G., Nadal, M., et al. (2012). Human dietary exposure to perfluoroalkyl substances in Catalonia, Spain. Temporal trend. *Food Chemistry, 135*, 1575–1582.

EC. (2002). Regulation (EC) No 178/2002 of the European parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities, 45*, 1–24. L 31/1.

EFSA. (2008). Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific opinion of the Panel on contaminants in the food chain. *EFSA Journal, 653*, 1–131.

EFSA. (2011). Scientific report of ESFA. Results of the monitoring of perfluoroalkylated substances in food in the period 2000 – 2009. *EFSA Journal, 9*, 1–34.

Elcombe, C. R., Elcombe, B. M., Foster, J. R., Chang, S. C., Ehresman, D. J., & Butenhoff, J. L. (2012). Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARα and CAR/PXR. *Toxicology, 293*, 16–29.

Eriksson, U., Kärrman, A., Rotander, A., Mikkelsen, B., & Dam, M. (2013). Perfluoroalkyl substances (PFASs) in food and water from Faroe Islands. *Environmental Science and Pollution Research, 20*, 7940–7948.

Fair, P. A., Driscoll, E., Mollenhauer, M. A. M., Bradshaw, S. G., Yun, S. H., Kannan, K., et al. (2011). Effects of environmentally-relevant levels of perfluorooctane sulfonate on clinical parameters and immunological functions in B6C3F1 mice. *Journal of Immunotoxicology, 8*, 17–29.

FAO. (2013). *Milk and dairy products in human nutrition. Milk and Dairy Products in Human nutrition*. Rome, Italy: Food and Agriculture Organisation of the United Nations.

Florentin, A., Deblonde, T., Diguio, N., Hautemaniere, A., & Hartemann, P. (2011). Impacts of two perfluorinated compounds (PFOS and PFOA) on human hepatoma cells: Cytotoxicity but no genotoxicity? *International Journal of Hygiene and Environmental Health, 214*, 493–499.

Fromme, H., Mosch, C., Morovitz, M., Alba-Alejandre, I., Boehmer, S., Kiranoglu, M., et al. (2010). Pre- and postnatal exposure to perfluorinated compounds (PFCs). *Environmental Science & Technology, 44*, 7123–7129.

Fromme, H., Tittlemier, S. A., Völkel, W., Wilhelm, M., & Twardella, D. (2009). Perfluorinated compounds - exposure assessment for the general population in western countries. *International Journal of Hygiene and Environmental Health, 212*, 239–270.

Geueke, B. (2016). Dossier – per- and polyfluoroalkyl substances (PFASs). In *Food Packaging Forum, July* (pp. 1–13).

Giesy, J. P., & Kannan, K. (2002). Perfluorochemical surfactants in the environment. *Environmental Science and Technology, 36*, 146A–152A.

Glaser, J. A., Foerst, D. L., McKee, G. D., Quave, S. A., & Budde, W. L. (1981). Trace analyses for wastewaters. *Environmental Science and Technology, 15*, 1426–1435.

Góralczyk, K., Pachocki, K. A., Hernik, A., Struciński, P., Czaja, K., Lindh, C. H., et al. (2015). Perfluorinated chemicals in blood serum of inhabitants in central Poland in relation to gender and age. *Science of the Total Environment, 532*, 548–555.

Grandjean, P., Andersen, E. W., Budtz-Jørgensen, E., Nielsen, F., Mølbak, K., Weihe, P., et al. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *Journal of the American Medical Association, 307*, 391–397.

Guillarme, D., Ruta, J., Rudaz, S., & Veuthey, J. L. (2010). New trends in fast and high-resolution liquid chromatography: A critical comparison of existing approaches. *Analytical and Bioanalytical Chemistry, 397*, 1069–1082.

Guruge, K. S., Taniyasu, S., Yamashita, N., Wijeratna, S., Mohotti, K. M., Seneviratne, H. R., et al. (2005). Perfluorinated organic compounds in human blood serum and seminal plasma: A study of urban and rural tea worker populations in Sri Lanka. *Journal of Environmental Monitoring, 7*, 371–377.

Haug, L. S., Salihovic, S., Jogsten, I. E., Thomsen, C., van Bavel, B., Lindström, G., et al. (2010). Levels in food and beverages and daily intake of perfluorinated compounds in Norway. *Chemosphere, 80*, 1137–1143.

Hu, Q., Strynar, M. J., & Dewitt, J. C. (2010). Are developmentally exposed C57BL/6 mice insensitive to suppression of TDAR by PFOA? *Journal of Immunotoxicology, 7*, 344–349.

Inoue, Y., Hashizume, N., Yakata, N., Murakami, H., Suzuki, Y., Kikushima, E., et al. (2012). Unique physicochemical properties of perfluorinated compounds and their bioconcentration in common carp cyprinus carpio L. *Archives of Environmental Contamination and Toxicology, 62*, 672–680.

Inoue, K., Okada, F., Ito, R., Kato, S., Sasaki, S., Nakajima, S., et al. (2004). Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: Assessment of PFOS exposure in a susceptible population during pregnancy. *Environmental Health Perspectives, 112*, 1204–1207.

Jogsten, I. E., Perelló, G., Llebaria, X., Bigas, E., Martí-Cid, R., Kärrman, A., et al. (2009). Exposure to perfluorinated compounds in Catalonia, Spain, through consumption of various raw and cooked foodstuffs, including packaged food. *Food and Chemical Toxicology, 47*, 1577–1583.

Johansson, N., Fredriksson, A., & Eriksson, P. (2008). Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. *Neurotoxicology, 29*, 160–169.

Jones, P. D., Hu, W., De Coen, W., Newsted, J. L., & Giesy, J. P. (2003). Binding of perfluorinated fatty acids to serum proteins. *Environmental Toxicology & Chemistry, 22*, 2639–2649.

Kärrman, A., Ericson, I., van Bavel, B., Darnerud, P. O., Aune, M., Glynn, A., et al. (2007). Exposure of perfluorinated chemicals through lactation: Levels of matched human milk and serum and a temporal trend, 1996–2004, in Sweden. *Environmental Health Perspectives, 115*, 226–230.

Keil, D. E., Mehlmann, T., Butterworth, L., & Peden-Adams, M. M. (2008). Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicological Sciences, 103*, 77–85.

Khaniki, G. R. J. (2007). Chemical contaminants in milk and public health concerns: A review. *International Journal of Dairy Science, 2*, 104–115.

Konieczka, P., Namieśnik, J., Zygmunt, B., Bulska, E., Świtaj-Zawadka, A., Naganowska, A., et al. (2004). Ocena i kontrola jakości wyników analitycznych *(Chapt. 8.7.* Gdańsk, Poland: CEEAM.

Kowalczyk, J., Ehlers, S., Oberhausen, A., Tischer, M., Fürst, P., Schafft, H., et al. (2013). Absorption, distribution and milk secretion of the perfluoroalkyl acids PFBS, PFHxS, PFOS, and PFOA by dairy cows fed naturally contaminated feed. *Journal of Agricultural and Food Chemistry, 61*, 2903–2912.

Krafft, M. P., & Riess, J. G. (2015). Selected physicochemical aspects of poly- and perfluoroalkylated substances relevant to performance, environment and sustainability-Part one. *Chemosphere, 129*, 4–19.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., & Seed, J. (2007). Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicological Sciences, 99*, 366–394.

Lee, S., Kim, S., Park, J., Kim, H. J., Choi, G., Choi, S., et al. (2018). Perfluoroalkyl substances (PFASs) in breast milk from Korea: Time-course trends, influencing factors, and infant exposure. *Science of the Total Environment, 612*, 286–292.

Li, K., Gao, P., Xiang, P., Zhang, X., Cui, X., & Ma, L. Q. (2017). Molecular mechanisms of PFOA-induced toxicity in animals and humans: Implications for health risks. *Environment International, 99*, 43–54.

Lindstrom, A. B., Strynar, M. J., & Libelo, E. L. (2011). Polyfluorinated compounds: Past, present, and future. *Environmental Science and Technology, 45*, 7954–7961.

Luebker, D. J., York, R. G., Hansen, K. J., Moore, J. A., & Butenhoff, J. L. (2005). Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in sprague-dawley rats: Dose-response, and biochemical and pharmacokinetic parameters. *Toxicology, 215*, 149–169.

Malinverno, G., Colombo, I., & Visca, M. (2005). Toxicological profile of hydrofluoropolyethers. *Regulatory Toxicology and Pharmacology, 41*, 228–239.

Mariussen, E. (2012). Neurotoxic effects of perfluoroalkylated compounds: Mechanisms of action and environmental relevance. *Archives of Toxicology, 86*, 1349–1367.

Narimatsu, S., Nakanishi, R., Hanioka, N., Saito, K., & Kataoka, H. (2011). Characterization of inhibitory effects of perfluorooctane sulfonate on human hepatic cytochrome P450 isoenzymes: Focusing on CYP2A6. *Chemico-Biological Interactions, 194*, 120–126.

Noorlander, C. W., van Leeuwen, S. P., Te Biesebeek, J. D., Mengelers, M. J., & Zeilmaker, M. J. (2011). Levels of perfluorinated compounds in food and dietary intake of PFOS and PFOA in The Netherlands. *Journal of Agricultural and Food Chemistry, 59*, 7496–7505.

OECD/FAO. (2016). Dairy and dairy products. In *OECD-FAO agricultural outlook 2016-2025*. Paris: OECD Publishing.

Peden-Adams, M. M., Keller, J. M., Eudaly, J. G., Berger, J., Gilkeson, G. S., & Keil, D. E. (2008). Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicological Sciences, 104*, 144–154.

Pérez, F., Nadal, M., Navarro-Ortega, A., Fàbrega, F., Domingo, J. L., Barceló, D., et al. (2013). Accumulation of perfluoroalkyl substances in human tissues. *Environment International, 59*, 354–362.

Renner, R. (2006). The long and the short of perfluorinated replacements. *Environmental Science and Technology, 40*, 12–13.

Ropers, M. H., Durand, S., Veyrand, B., Beaumal, V., Marchand, P., Anton, M., et al. (2009). Contamination of food by fluorinated surfactants - distribution in emulsions and impact on the interfacial protein behaviour. *Food Hydrocolloids, 23*, 1149–1155.

Scheringer, M., Trier, X., Cousins, I. T., de Voogt, P., Fletcher, T., Wang, Z., et al. (2014). Helsingør Statement on poly- and perfluorinated alkyl substances (PFASs). *Chemosphere, 114*, 337–339.

Sheng, N., Li, J., Liu, H., Zhang, A., & Dai, J. (2016). Interaction of perfluoroalkyl acids with human liver fatty acid-binding protein. *Archives of Toxicology, 90*, 217–227.

Still, M., Schlummer, M., Gruber, L., Fiedler, D., & Wolz, G. (2013). Impact of industrial production and packaging processes on the concentration of per- and polyfluorinated compounds in milk and dairy products. *Journal of Agricultural and Food Chemistry, 61*, 9052–9062.

Stockholm Convention on Persistent Organic Pollutants. (2009). *Report of the conference of the parties of the Stockholm convention on persistent organic pollutants on the work of its fourth meeting, Geneva, Switzerland* (pp. 4–8).

Stockholm Convention on Persistent Organic Pollutants. (2011). *Guidance on alternatives to perfluorooctane sulfonic acid and its derivatives.* UNEP/POPS/POPRC.6/13/Add.3/Rev.1.

Stockholm Convention on Persistent Organic Pollutants. (2012). *Technical paper on the identification and assessment of alternatives to the use of perfluorooctane sulfonic acid in open applications*. UNEP/POPS/POPRC.8/INF/17.

Surma, M., Giżejewski, Z., & Zieliński, H. (2015a). Determination of perfluorinated sulfonate and perfluorinated acids in tissues of free-living European beaver (castor fiber L.) by d-SPE/micro-UHPLC-MS/MS. *Ecotoxicology and Environmental Safety, 120*, 436–444.

Surma, M., Piskuła, M., Wiczkowski, W., & Zieliński, H. (2017). The perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonates (PFSAs) contamination level in spices. *European Food Research and Technology, 243*, 297–307.

Surma, M., Wiczkowski, W., Cieślik, E., & Zieliński, H. (2015b). Method development for the determination of PFOA and PFOS in honey based on the dispersive solid phase extraction (d-SPE) with micro-UHPLC-MS/MS system. *Microchemical Journal, 121*, 150–156.

Surma, M., Wiczkowski, W., Zieliński, H., & Cieślik, E. (2015c). Determination of selected perfluorinated acids (PFCAs) and perfluorinated sulfonates (PFASs) in food contact materials using LC-MS/MS. *Packaging Technology and Science, 28*, 789–799.

Wang, Z., Cousins, I. T., Scheringer, M., & Hungerbühler, K. (2013). Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their potential precursors. *Environment International, 60*, 242–248.

Wang, Z., Dewitt, J. C., Higgins, C. P., & Cousins, I. T. (2017). A never-ending story of per- and polyfluoroalkyl substances (PFASs)? *Environmental Science and Technology, 51*, 2508–2518.

Wang, J. M., Shi, Y. L., Pan, Y. Y., & Cai, Y. Q. (2010). Perfluorinated compounds in milk, milk powder and yoghurt purchased from markets in China. *Chinese Science Bulletin, 55*, 1020–1025.

Wolny, A., & Krupa, K. (2012). Narażenie populacji na perfluorowane związki alifatyczne - świadomość zagrożeń. *Journal of Ecology and Health, 16*, 10–15.

Wu, Y.-M., Hu, C.-Y., Wang, L., Cheng, J., Wang, Z.-W., & Zhu, Y. (2010). Migration of contaminants from packaging materials into dairy products. In *17th IAPRI World Conference on Packaging 2010* (pp. 207–211).

Xing, Z., Lu, J., Liu, Z., Li, S., Wang, G., & Wang, X. (2016). Occurrence of perfluorooctanoic acid and perfluorooctane sulfonate in milk and yogurt and their risk assessment. *International Journal of Environmental Research and Public Health, 13*, 1037.

Yu, Y., Xu, D., Lu, M., Zhou, S., Peng, T., Yue, Z., et al. (2015). QuEChERs combined with online interference trapping LC-MS/MS method for the simultaneous determination of 20 polyfluoroalkane substances in dietary milk. *Journal of Agricultural and Food Chemistry, 63*, 4080–4086.