JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Impact of Industrial Production and Packaging Processes on the Concentration of Per- and Polyfluorinated Compounds in Milk and Dairy Products

Mona Still, Martin Schlummer,* Ludwig Gruber, Dominik Fiedler, and Gerd Wolz

Department of Product Safety and Analysis, Fraunhofer Institute Process Engineering and Packaging (IVV), Giggenhauser Strasse 35, D-85354 Freising, Germany

Supporting Information

ABSTRACT: Perfluorinated alkylated compounds (PFAA) have been identified in milk and dairy products at sub ppb levels, however, knowledge on the impact of industrial milk processing on PFAA levels is rare. This study examined industrial milk processing first by analytical screening of products of a cooperating dairy, which varied in kind and number of processing steps. Second, amounts of PFAA in raw milk, cream, skim milk, butter milk, and butter were mass balanced in industrial production. For migration testing, unpacked butter was sampled from the production and exposed to original packaging at 5 °C for 45 days. Screening identified dairy products with high fat contents to bear higher loads of PFAA. The mass balance of butter production revealed a significant impact of phase separation processes on concentrations in fat rich and aqueous phases. Storage of butter in packaging coated with a fluorinated polymer increased butter levels of both PFAA and FTOH.

KEYWORDS: *PFAS, milk, dairy, food processing, packaging, migration*

INTRODUCTION

Perfluorinated alkylated acids (PFAA) comprise of perfluorinated carboxylic acids (PFCA) and sulfonic acids (PFSA) and are the most widely investigated compounds within the class of per- and polyfluorinated alkyl substances (PFAS). PFAA are highly persistent compounds and have been found ubiquitously in the environment.[1−3] PFAA and their precursors, e.g., fluorotelomer alcohols (FTOH) have been detected in various industrial applications, for example, in fat- and greaseproof coatings of textiles or food packaging or as surface-active substances in firefighting foams.[4,5]

Because PFAA are found worldwide in human sera at ng/mL levels,[6,7] exploring routes of human exposure toward PFAS is an important issue. Food intake has been considered as a main contributor to human exposition,[8−10] and the screening of different food items showed highest concentrations of PFAA in fish, seafood, and meat with maximum levels in the upper ppb range.[11] PFAA were also detected in milk and cheese, but the sum of all PFAA homologues accounted for 36 ppt only. Nevertheless, a Dutch food study on PFAA in food computed a daily intake of PFOS from milk of 78.8 pg/kg (bw)/d, which accounted for 24.5% of the daily PFOS intake and identifies milk as a main contributor to human PFOS intake.[12] Ericson et al.[13] calculated a similar contribution of 20.9% from milk and dairy products in a Spain study for PFOS intake from food. Both studies merged raw or processed milk and dairy products, and thus the source of the detected PFAA levels remains unclear. Because of the ubiquitous occurrence of persistent PFAAs in the environment, a transfer into and an accumulation in aquatic and terrestrial food chains is expected and has been reported.[14,15] Application of PFAAs and their precursors in food contact materials poses a further source during production and storage of the food products.[16,17]

Food processes modify the shelf life or the structure of the food. Some of these processes can also change the PFAS concentration. Del Gobbo et al.[18] reported that different kinds of heat treatment led to a decrease of PFAA concentrations in food, but the mechanism could not be explained entirely. It is known that enzymatic degradation of polyfluorinated precursor substances may produce PFAAs, and thus microbial activity during the ripening of cheese and yogurt could increase PFAA loads in dairy products if precursors were present in raw milk. However, a recent Chinese study on milk and dairy products could not support this hypothesis.[19] Furthermore, packing processes are able to increase PFAA concentrations because PFAA were found in greaseproof coatings of food contact materials and shown to migrate into food or a simulant.[16,21] Only few literature data from laboratory cooking trials are available so far,[18] and there are no insights into the relevance and the dimensions in an industrial scale.

Food processes, which cause phase separation, alter PFAA concentrations in the products because the distribution between separated phases is dependent on their PFAA affinity. With increasing chain length, PFAAs show an increase of hydrophobicity[20] and exhibit a higher affinity for fat rich matrices. This has been shown by Sauer[21] in laboratory experiments on phase separation with milk and cream spiked to high ppb levels of PFAAs. Sauer observed the enrichment of longer chain PFAA congeners in the fat rich phase after separation of (a) skimmed milk and cream, (b) butter milk and butter, (c) cheese and whey, and (d) milk and milk skin.

Received: May 10, 2013
Revised: August 27, 2013
Accepted: September 3, 2013
Published: September 3, 2013

ACS Publications © 2013 American Chemical Society

However, it remains unclear if the observed PFAA separation is influenced by the high spiking levels.

The aim of this study was to assess the impacts of industrial food production on PFAA concentrations in food and to provide further knowledge about the contamination routes into food. Milk was chosen as model foodstuff because of its characteristics as homogeneous input material in contrast to fish and meat, where PFAA levels can vary a lot along different samples. All the above-discussed processes are used during the industrial production of milk and dairy products. In brief, *phase separation* occurs when raw milk is separated in cream and skim milk with a centrifuge-based separator or when cream is treated in the churning process, separating butter and buttermilk. *Enzymatic processes* occur in the production of cheese and yoghurt when enzymes are added to raw milk. Before trading, all milk and dairy products undergo a *thermal treatment* (e.g., pasteurization) and *packaging processes*. The study was separated into two phases. First, products of a cooperating dairy were subjected to screening analysis in order to identify process lines with significant impacts on PFAA levels. This process line, namely the production of butter, was examined closely and mass balanced in the second phase.

MATERIALS AND METHODS

Samples. For the method optimization, milk and dairy samples were purchased from local supermarkets. For product screening, milk and dairy products were selected due to the production processes that may have an impact on PFAA concentrations. Fourteen commercially available samples of various dairy products and raw milk were provided from a cooperating dairy.

PFAA mass balance experiments were performed at the same dairy in the second phase of the project, and samples were drawn during butter production by staff of the dairy. Samples were taken from the raw milk and after the main processing steps of butter production, the separator and the churning process, at sampling points designated for inline quality control. For this experiment, all samples were part of one batch.

Furthermore, seven pieces of butter were sampled directly after their production and packed in polypropylene (PP) vessels precleaned with methanol. The dairy provided a roll of packing material used for wrapping the butter. In our laboratories 250 g units of butter were packed in these wraps, whereas the contact area of butter and packaging accounted for 1 dm^2 per 23.5 g of butter.

Wrapped butter samples were stored at 5 °C for a whole of 45 days, and concentrations were determined at 0 and 45 days in both butter and butter wrap. For analytics, butter was sampled from the 5 mm top layer of the butter blocks because this was regarded as sufficient to assess the migration. Then 1 dm^2 of the butter wrap was cut from the center of the packaging material and used for analysis.

Upon sampling and transport, all samples were kept at temperatures below 4 °C and stored in our institute at 4 °C in a refrigerator until analysis.

Materials. *Chemicals.* Per analysis grades of formic acid, methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF), ammonium (NH_3), and hexane as well as HPLC-grade water were purchased from Fluka (Germany) and Merck (Germany).

Certified Standards. A mix of nine isotope labeled PFAA congeners, namely perfluorobutanoic, perfluorohexanoic, perfluorooctanoic, perfluorononanoic, perfluorodecanoic, perfluoroundecanoic, and perfluorododecanoic acid as well as perfluorohexane and perfluorooctane sulfonate, was purchased from Wellington Laboratories Inc., diluted with methanol to 0.2 μg/mL, and used as internal PFAA standard solution. Isotope labeled 2-perfluorobutyl ethanol (4:2 FTOH), 2-perfluorohexyl ethanol (6:2 FTOH), 2-perfluorooctyl ethanol (8:2 FTOH), and 2-perfluorodecyl ethanol (10:2 FTOH) were purchased from Wellington Laboratories Inc. (Ontario, Canada), diluted with methanol to 10 μg/mL, and used as internal FTOH standard solution. Certified solutions of individual native perfluoroalkyl acids (chain lengths C4 to C15 and C18), as well as K-perfluorobutane, K-perfluorohexane, K-perfluorooctane, and K-perfluorodecane sulfonate were also purchased from Wellington Laboratories Inc. and used to produce a native standard solutions concentrated from 0.1 to 200 ng/mL. Native FTOH standard solutions from 1 to 100 ng/mL were prepared from certified solutions of native 2-perfluorobutyl ethanol (4:2 FTOH), 2-perfluorohexyl ethanol (6:2 FTOH), 2-perfluorooctyl ethanol (8:2 FTOH), and 2-perfluorodecyl ethanol (10:2 FTOH) (Wellington Laboratories Inc.). Calibration standards were produced from these native and internal standard solutions.

Methods. *Analysis of Milk and Dairy Products. Extraction.* First, 10 g of milk or other dairy products (cream, yogurt, buttermilk, and a whey drink) were filled in 50 mL PP centrifugation vials and spiked with 10 μL of the PFAA internal standards mixture. The pH of 4 was adjusted with formic acid (10% in HPLC grade water) to disintegrate the milk proteins. The denatured proteins and the water phase were separated during a 10 min centrifugation at 10000 rpm (rpm, 11627*g*). The water was decanted and the precipitate washed twice with 1 mL of ACN. Again, the pH was adjusted to 4 if necessary.

As recoveries for this approach were unsatisfying, the extraction procedure was adapted for further analysis. After precipitation of milk proteins, the extraction was performed with 20 mL of ACN by 30 s mixing on Vortex, 15 min treatment in an ultrasonic bath, and 15 min shaking at 350 rpm. The mixture was centrifuged at 5500 rpm (5343*g*) if necessary, and the extract was decanted. The extraction procedure was repeated once. The extract volume was reduced to a volume of 1 mL under a gentle nitrogen stream at 40 °C.

Butter was extracted similar to milk without the adding of formic acid. Centrifugation was not required. Wrapped butter samples were additionally spiked with 10 μL of the internal FTOH standard mixture. Cheese samples required a more drastic disintegration, and 4 mL of acetic acid (50% in HPLC grade water), 10 mL of hexane, and 5 mL of H_2O were used for extraction, applying the same procedure as described above.

Clean Up. The extraction was followed by a cleanup with a weak anion exchange SPE column (Oasis WAX, 150 mg, Waters) and activated charcoal SPE column (ENVICarb, 500 mg, Supelco) as proposed by Ballesteros-Gomez et al.[22] The anion exchanger was preconditioned with 6 mL of MeOH and 6 mL of water (pH 4). ENVICarb columns were washed with 3 × 6 mL of 1% NH_3 in MeOH.

Volume reduced extracts were quantitatively transferred into a new PP centrifugation tube with 3 × 7.5 g of ACN/H_2O (pH 4) (1/1, v/v) and loaded onto the preconditioned anion exchanger SPE column. PP tubes were washed with 4 mL of water (pH 4), which were also rinsed over the anion exchanger. If the throughput velocity was inhibited by sample matrix, a weak vacuum was applied to keep up a speed of 1−2 drops per second. The SPE column was washed with 8 mL of ACN/THF (50/50, v/v). Before elution of the analytes the anion exchanger column was connected to the top of the preconditioned ENVICarb column, and the analytes were eluted from both columns with 6 mL of 1% NH_3 in MeOH. The volume of the eluate was reduced to dryness under a gentle stream of nitrogen and taken up in 300 μL of MeOH/H_2O (1/1, v/v) for LC-ESI-MS/MS measurement.

Wrapped butter samples were additionally analyzed for FTOH and required a slightly different clean up protocol. After loading the extracts onto the top of the anion exchange column, FTOH were eluted with 4 mL of MeOH before the ACN/THF rinse step. An aliquot of 1.5 mL was taken for GC-CI-MS measurement.

Analysis of Butter Wraps. Extraction. The print on the outside of the butter wrap was removed with acetone to minimize disturbance during further clean up. Then 1 dm^2 was used for analysis of PFAA and FTOH, and 10 μL of internal PFAA standard solution as well as 10 μL of internal FTOH standard solution were added. The extraction was performed in PP centrifuge tubes with 50 mL of MeOH, applying a 15 min ultrasonic treatment, a 90 min storage at 40 °C, and again a second 15 min ultrasonic treatment. The extract was diluted with the 4-fold amount of water and adjusted to pH 4 with formic acid (10%).

Clean Up. Oasis WAX columns were conditioned with 1% NH_3 in MeOH, MeOH, and with H_2O pH 4. The diluted extract was loaded onto the top of the column. FTOH were eluted with 4 mL of MeOH and an aliquot of 1 mL was used for GC-CI-MS analysis. Subsequently, PFAA were eluted with 5 mL of 1% NH_3 in MeOH, and the eluate was reduced to dryness under a nitrogen stream and redissolved in 300 μL of MeOH/H_2O (1/1, v/v) before analysis by LC-ESI-MS/MS.

Instrumental Analysis. PFAA Analysis by LC-ESI-MS/MS. Liquid chromatography was performed on a high-throughput-HPLC-system (Alliance 2795, Waters) using an injection volume of 10 μL. Analytes passed a precolumn (Phenomenex Luna C8(2), 3 μm, 100 Å, 20 mm × 4 mm) and a main column (Phenomenex Luna PFP(2), 5 μm, 100 Å, 150 mm × 3 mm) at 0.6 mL/min. Mass spectrometry was performed on a triple quadrupole MS (Quattro LC, Micromass) running in MRM (multiple reaction monitoring) mode. Electrospray ionization was done in negative mode at 300 V and a nitrogen stream. Identification of target analytes was based on retention times and mass ratios obtained for standard injections. For data processing, the software MassLynx V4.1 (Waters) was used. Mobile phases, gradient program, and mass transitions used for quantification and identification, respectively, are provided in the Supporting Information (Tables S1 and S2).

FTOH Analysis by GC-CI-MS. GC was performed on a HP 5890 Series II instrument equipped with a RTX 200 column (Restek, 30 m × 0.32 mm × 1.5 μm). A head pressure of 1.6 bar was applied, and the injector as well as the detector were heated to 250 °C. The injection volume was 2 μL, and the temperature of the GC oven started at 40 °C. After 5 min, the oven temperature was increased by 10 °C per min to 280 °C, which was then held for 5 min. Mass spectrometry was performed using a triple quadrupole mass spectrometer (TSQ 7000, Finnigan MAT) operated at an ionization energy of 50 eV with positive chemical ionization (PCI) using methane (Linde, Germany, purity 99.995%) as reagent gas. The filament emission was 100 μA, and the source was heated to 185 °C. Masses were monitored in the selected ion monitoring (SIM) mode and recorded the molecular ions and at least one fragment ion per analyte. Identification of target analytes was based on retention times and mass ratios obtained for standard injections. Table S3 in the Supporting Information lists the recorded mass-to-charge ratio (m/z) of target and qualifier fragments. XCalibur (Thermo Scientific) software was used for data processing. Quantification was based on an internal standard method via calibration with the aforementioned calibration standards.

Quality Assurance and Quality Control. Because of the lack of a second mass labeled internal standard, analytic recovery was assessed by the recovery of internal standards after sample preparation. They were computed by the area of the internal standard in the samples over the average area of internal standards measured in three method blank samples analyzed in the same batch.

Limits of detections (LOD) and limits of quantitation (LOQ), respectively, were determined sample specifically as the arithmetic mean concentration of three method blanks plus one, respectively, 10 times the standard deviation of these blank samples. Because of the extremely low concentrations, data between the LOD and the LOQ were marked ("[b]") to indicate the higher uncertainty of these results. In diagrams, data below the LOD were plotted as half the LOD value.

Method uncertainty was calculated as the relative standard deviation of analyte concentrations obtained by a 10-fold analysis of a homogenized milk sample (Table S4 in the Supporting Information).

RESULTS

Optimization and Performance of the Analytical Method. To provide information on any influence of industrial processing on PFAA concentrations, LODs in the low pg/g range are required. Thus, a minimum sample weight of 10 g was chosen to gain low LODs but still bearable recovery levels. However, extraction and cleanup of that sample amount turned out to be challenging. The initially applied method (method 1) separated water from a precipitated protein phase. The precipitate was washed twice with a total volume of 2 mL of ACN and added to the water phase. Figure 1 shows that

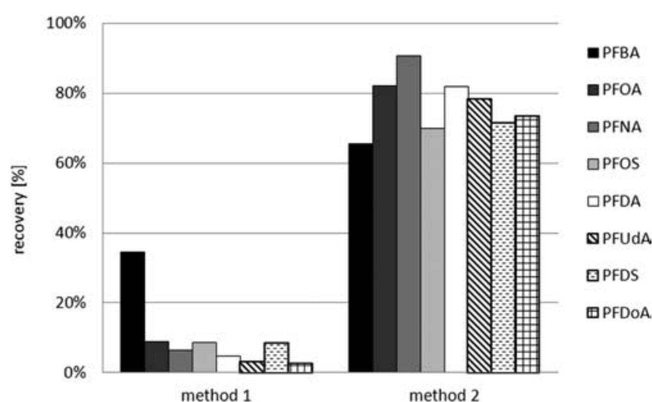

Figure 1. Comparison of average recoveries obtained by two different extraction methods applied to raw milk, fresh milk, low fat fresh milk, and cream.

recovery levels only exceeded 10% for PFBA. The optimized method 2 applied the 10-fold volume of ACN for the extraction of the precipitate, and all PFAA showed recoveries higher than 60%. No trends for the impact of chain length or acid group on the recovery were observed. This indicates that PFAA are found mainly in the protein phase. Furthermore, the increase in recovery lowered the LOD from a medium LOD of 9 pg/g in "method 1" to 3 pg/g in "method 2".

Nevertheless, during further sample analyses the recovery of PFSA dropped occasionally to very low levels (<4%) although recovery for PFCA exceeded 60% for all homologues. However, no relation between these findings and changes of extraction parameters could be found. In those cases, samples were analyzed again until a recovery level higher than 30% could be reached.

For the stability assessment of the final method, a homogeneous milk sample was analyzed 10 times and the standard deviation was calculated for all PFAA congeners that gave a peak signal-to-noise greater than 3. For all congeners, the standard deviation was below 15%, so 15% were regarded as uncertainty of the applied method.

The extraction method for butter showed good recoveries and low LODs throughout the whole study. The disintegration and extraction of cheese showed also good recoveries in the pretests. However, during the screening of different cheese products, low recoveries of PFSA occurred and this issue could not be solved entirely. So levels for PFSA in cheese samples are missing.

Screening of Milk and Dairy Products. All PFAA congeners were measured, but Table 1 lists only those congeners showing levels above the LOD in at least one sample. LOD levels can vary between butter, cheese, and the remaining dairy products because different matrices cause different interferences in the chromatogram.

Concentrations in the lower ppt range were detected in raw milk. As shown in Table 1, PFAA concentrations in processed milk samples and yoghurts did not differ significantly from raw milk. Concentrations in cheese samples exhibit slightly increased levels of longer chain PFCA (PFNA to PFDoA).

In the group of other dairy products, however, significantly increased levels of PFOS and PFDS occur in products with increased fat content like butter, cream, and cream yoghurt.

Table 1. PFAA Concentrations in Dairy Products in pg/g and Their Different Impact Processes[a]

products	product details	PFBA	PFOA	PFNA	PFOS	PFDA	PFUdA	PFDS	PFDoA
raw milk	~3.5% fat	6.5	6.2[b]	<1.6	<7.4	<3	5.1	<4.8	3.5
Milk Products									
fresh milk	1.5% fat	5.1	6.5[b]	<1.6	<7.4	4.2[b]	8.7[b]	6.4[b]	<2.5
fresh whole milk	3.8% fat	10.1	2.3	<1.6	8.5[b]	<3	9[b]	<4.8	<2.5
UHT milk	3.5% fat	4.7	1.6[b]	3.4	5.2	2.1	<7.8	<4.8	4.6
Yoghurt Low Fat									
yogurt	0.1% fat	7.8	5	<1.6	9.4	<3	<7.8	<4.8	<2.5
yogurt	3.8% fat	5.2	5.5[b]	<1.6	10.3	2.3	<7.8	<4.8	<2.5
Cheese									
semihard cheese	30% fat, polymer packaging	5	7.2	9.4	nd[e]	9.6	8.2[b]	nd	11.1[b]
semisoft cheese	50% fat, polymer packaging	6.9	4.8	6.2	nd	5.5	16	nd	12.1[b]
soft cheese	50% fat, paper wrap[c]	11.3	<1.9	8.1	nd	9.8	<6.7	nd	<3.7
Other Dairy Products									
whey drink	0.1% fat	3.2	6.4[b]	<1.6	<7.4	4.3[b]	<7.8	14.8	<2.5
butter milk	1% fat	4.9	6.9[b]	<1.6	<7.4	3.5	10.8[b]	<4.8	<2.5
cream yogurt	10% fat	3.6	4.8[b]	<1.6	19.7	<3	<7.8	18.3	9.4
cream	32% fat	4.8	2.9	<1.6	18.9	<3	<7.8	<4.8	9.8
butter	82% fat paper wrap[d]	8.2	13.4	4.7[b]	14.6	7.4	7.6[b]	6.2[b]	11.4

[a]Entries marked with "<" indicate levels below LOD. [b]Levels between LOD and LOQ. [c]Tests with X-ray fluorescence did not identify fluorine traces. [d]Tests with X-ray fluorescence proved the presence of fluorine. [e]nd, levels were not calculated due to very low recovery of internal standards.

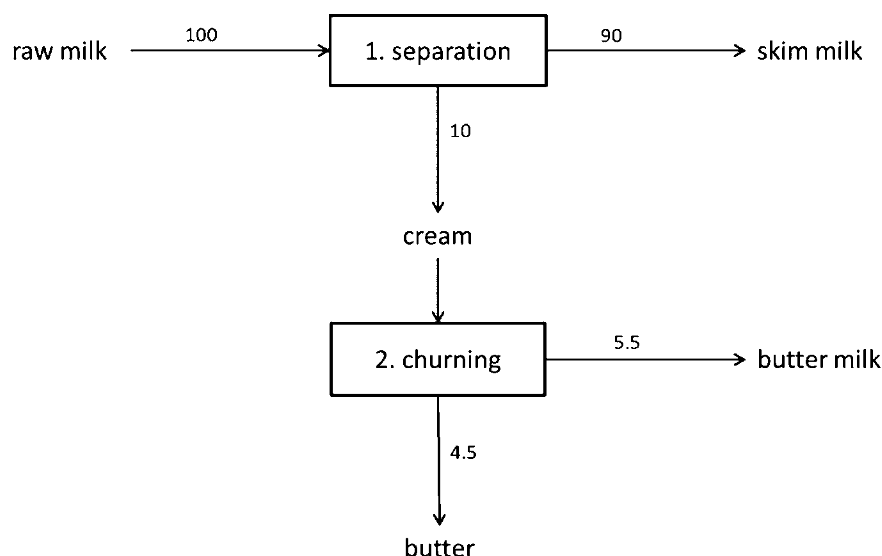

Figure 2. Main processes and mass flow of the products during butter production.

This most significant increase of PFAA levels is visible in the butter sample, where all detected PFAA concentrations increase those measured in raw milk. Levels of PFOA and PFDoA doubled or increased by a factor of 3, and comparable high levels of PFOS and PFDA occur which have not been detected in raw milk and are a factor of 2 lower in processed milk. Thus, the process line producing butter from raw milk was selected for a more detailed mass balancing trial.

Results from Industrial Mass Balance Trial. PFAA levels were measured during the production process of butter in all educts, intermediate, and final products, i.e., raw milk, cream, skim milk, butter milk, and butter. All analyzed products were part of one raw milk batch, therefore no individual high PFAA levels from unusually high loaded raw milk batches could occur.

A percentage mass flow of intermediate and final products was given by the cooperating dairy and is displayed in Figure 2. One hundred mass units of raw milk are divided in the separator in 90 mass units of skim milk and 10 mass units of cream. Cream undergoes a mechanical treatment during churning until the oil-in-water emulsion is reversed into a water-in-oil emulsion, namely 4.5 mass units of butter. The residual water is collected as 5.5 mass units of buttermilk.

Concentrations in the low pg/g range were detected in all samples for PFBA, PFOA, PFNA, PFDA, PFUdA, PFDoA, and PFOS. In some cases, the levels were nearby the calculated LODs. To enable generating a complete mass balance, these concentrations were taken into account if the signal-to-noise ratio of the native mass traces of the individual LC-MS/MS exceeded 3.

The ratios of concentrations in intermediate and final products over levels of raw milk are shown in Figure 3. For PFBA, no significant change of concentrations was monitored. For the other PFAAs with chain lengths over C7, a significant increase of levels is obtained in butter and to a lower extent in cream. For PFOS, a 1.5- to 3-fold increase of levels is visible in cream and butter, respectively. There is only a slight increase in

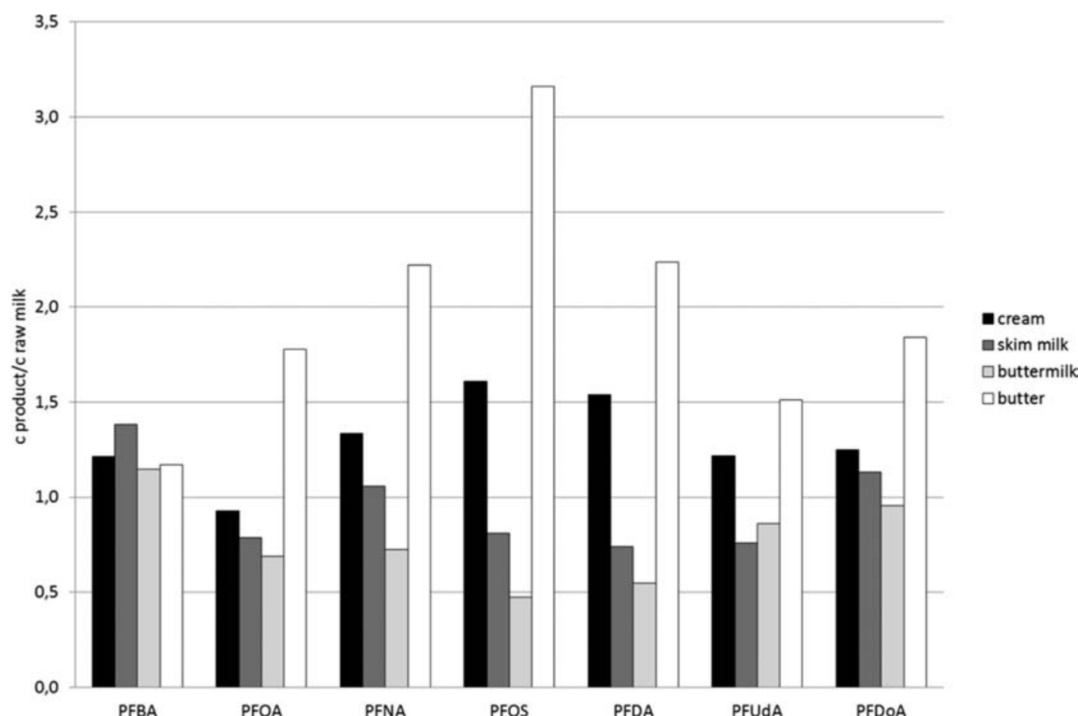

Figure 3. PFAA concentration ratios from the different products and the raw milk.

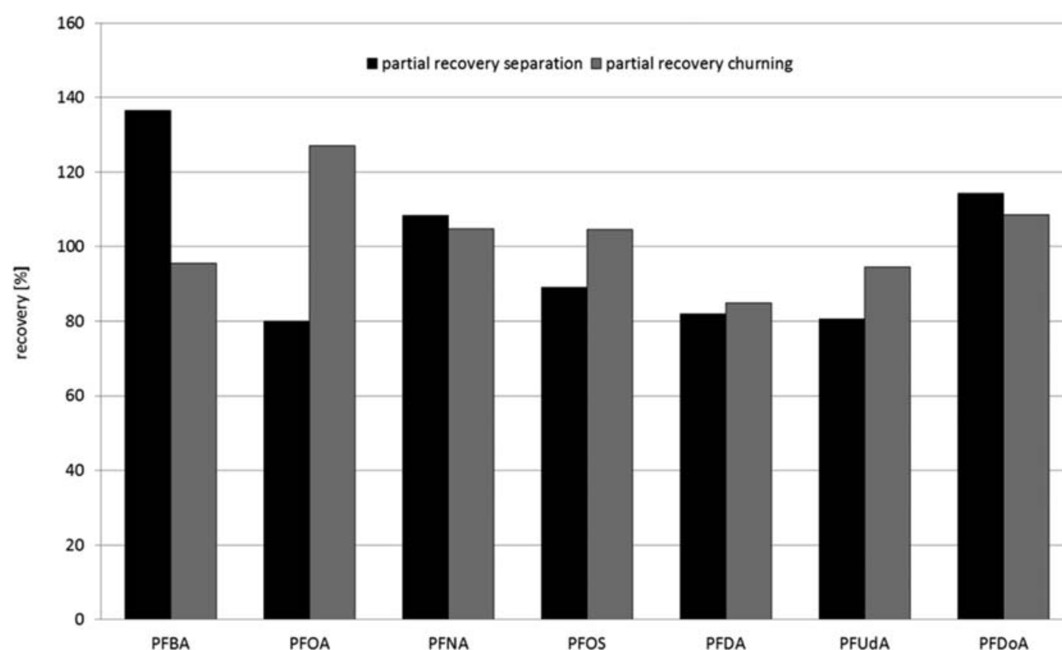

Figure 4. Recoveries of different PFAA during the partial processes separation and churning for butter production.

cream for some substances that is nevertheless present in all substances except for PFBA.

PFAA masses in products were calculated by multiplication of PFAA levels and mass units of the respective product given in Figure 2. The percent PFAA recovery of the separator process was calculated by dividing PFAA masses in skim milk and cream by PFAA masses of the raw milk, whereas the percentage recovery of the churning process was calculated by dividing PFAA masses in butter milk and butter by PFAA masses of cream. Both recoveries are presented in Figure 4 and range between 77 and 137%. Considering the extremely low concentration ranges, these recovery levels are considered conclusive and indicate at a complete coverage of the PFAA mass flows throughout the production of butter. The recovery of the whole process is not displayed because it is almost the same as for the recovery during separation. This is due to the fact that PFAA masses in skim milk with a flow of 90 mass units are dominating the recovery calculations.

Migration of PFAS from Packaging Wraps into Butter. In Figure 5, the initial PFAS concentrations in the butter wraps are displayed. Those levels in the low ng/g range stayed virtually constant during the time of storage. PFAA with a straight number of carbons are dominating the PFAA profile found in higher concentrations. The lack of sulfonic acids is

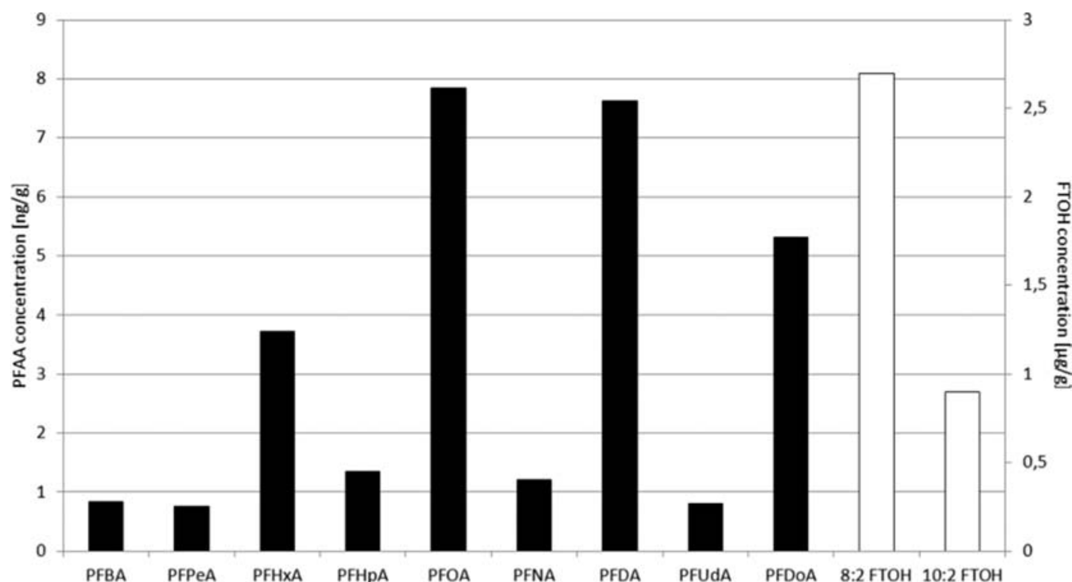

Figure 5. Initial PFAS concentrations in the butter wrap before the migration experiment.

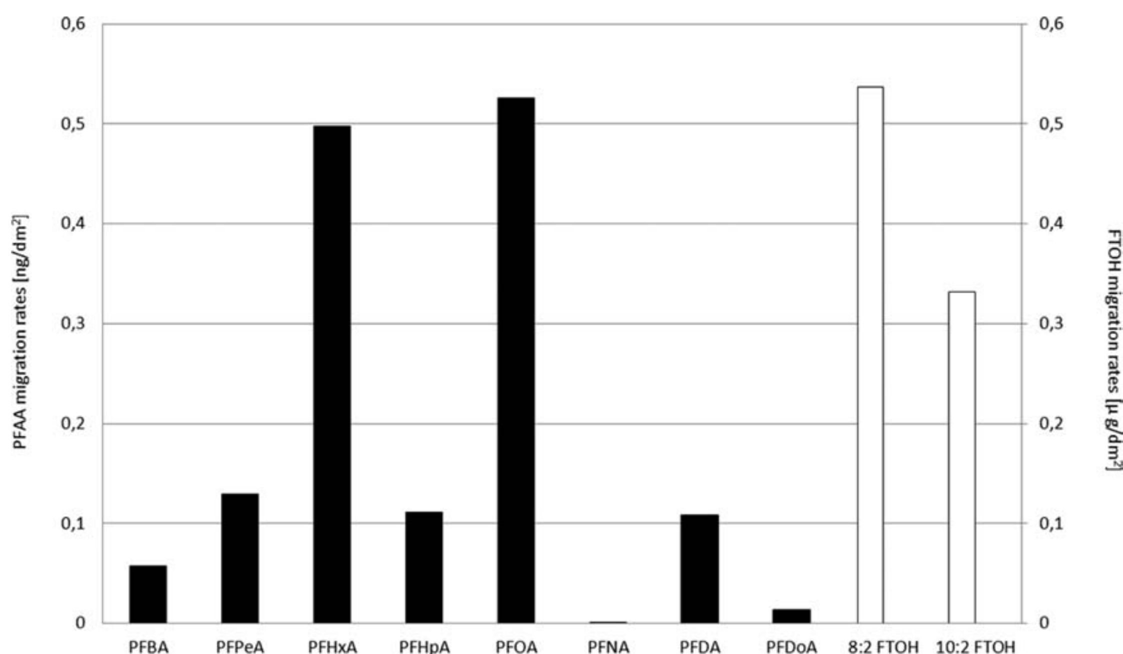

Figure 6. Migration of PFAS after the 45 days storage at 5 °C.

conspicuous. PFSA concentrations were under the LOD or present in very low concentrations. FTOH concentrations exceed PFAA levels by a factor of about 1000.

The migration experiment was carried out at 5 °C for a whole of 45 days, which are standard conditions of the cooperating the dairy with respect to storage related quality parameters. Migration is calculated by the concentration increase of PFAS in butter during storage because initial PFAS levels (c_0) in the butter are not zero. Thus c_0 concentrations are subtracted from the final concentration after 45 days storage (c_{45}), and this sheer increase is multiplied with the mass of the 5 mm thick top layer of the butter m_{bt}. Finally, it is divided by the contact area between the butter and the butter wrap A_c to give the migrated concentrations per dm^2 in contact with the butter.

$$\text{migration}\ [\text{ng/dm}^2] = (c_{45} - c_0) \times m_{bt} \times A_c^{-1} \quad (1)$$

As displayed in Figure 6, concentrations increased during storage for almost all PFAA congeners except for PFOS and PFUdA and these concentration increases are higher than the 15% uncertainty of the method. PFHxA and PFOA are clearly dominating the profile of migrated congeners. The migration of 8:2 FTOH exceeds the migration 10:2 FTOH, which is consistent with the higher initial concentration of 8:2 FTOH in the butter wrap.

DISCUSSION

Analytical Performance. Because of high concentration factors and good cleanup efficiency, the chosen analytical approach enabled a sensitive and sufficiently precise analysis of milk and dairy products. Despite levels below 10 pg/g, a 10-fold

analysis of milk resulted in relative standard deviations below 15% for all reported PFAA congeners and proved the good reproducibility of the method. LODs were determined sample specifically and were low compared to LODs reported elsewhere. In the literature, LODs varied strongly, which is probably due to the different approaches on how to determine a LOD. Young et al.[23] are found with a median LOD of 293 pg/g, which is strongly above other literature levels and of this work. Nine samples were spiked, analyzed, and the LODs for every single substance were calculated from that variance and standard deviation. The method reported by Ericson et al.[13] reached a median LOD of 18 pg/g, calculated with the response at the expected retention times or with the blank concentration if it accounted for at least 50% of the signal found in the sample. Similar levels are produced by Wang et al.[21] with a median LOD of 14.5 pg/g, which was calculated as the 3-fold standard deviation of the median blank. The median LOD in this work achieved with the improved method "2" is 3.3 pg/g and far below the LODs by Young et al. and still 5 or 4 times lower than by Ericson et al. and Wang et al.

Upon optimizing the extraction procedure, a high affinity of PFAA to milk proteins has been observed which caused an insufficient extraction of PFAA in method 1. Here samples were adjusted to pH 4 right after the adding of the internal standard, and the precipitated protein was separated from the water phase were. Despite the short residence time of the internal standard in the sample, recoveries in the water phase were below 10% for PFAA with more than seven carbon atoms. Recoveries increased when the precipitated proteins were extracted with increased amounts of ACN and increased extraction times. Furthermore, there are hints that binding to milk proteins is dependent on the acid group. Partly arising problems with the recovery were mainly observed for PFSA. As in these cases, all PFSA were concerned; this is most likely not due to interferences in the LC-MS method and not due to separation issues of the SPE cleanup. PFSA seem to be bound more strongly to milk proteins, which makes the extraction more difficult and vulnerable.

Comparison of Screening Results of Milk and Dairy Products with Literature Data. Literature data on PFAA concentrations in milk and dairy products are quite inconsistent except for the fact that concentrations are in the ppt range. Wang et al.[21] screened various milk and dairy product samples and described PFHpA, PFOA, and PFNA as the most frequently found substances. PFOS and PFOA, respectively, were found in median concentrations of 24 and 26 pg/g, respectively. In this study, lower concentrations were reported with 14 pg/g PFOS and 4 pg/g PFOA. PFHpA could not be found at all above the LOD, and PFNA could only be detected in one milk sample with a concentration of 3 pg/g. These not matching findings can occur due to different regional loads of PFAS in China and Germany and also due to different packing materials, which were suspected by Wang et al. to increase PFAS concentrations in some samples. Furthermore, they screened 84 samples with high differences in the determined concentrations which increased the median concentrations.

A Spanish study on milk and dairy products detected only PFOS, PFOA, and PFHpA at levels above the LOD.[13] In a mixture containing different dairy products like cheese and yogurt, PFOS and PFOA were found in median concentrations of 121 and 56 pg/g. This is not consistent with the findings of this study because PFHpA levels were below LOD and PFOS and PFOA concentrations did not reach such high concentrations in any screened product. This might be due to the fact that Ericson et al.[13] found PFAA concentrations only in some samples, and these concentrations varied, for example, for PFOS between <14 and 820 pg/g. Thus, single increased loads could strongly affect median concentrations.

A study from the U.S. FDA investigated 10 perfluorinated compounds, including PFOA and PFOS, in 12 raw and 49 retail milk samples from across the United States. With the exception of a single raw milk sample (with a PFOS value of 160 pg/g) obtained from a dairy farm that had applied PFAA containing biosolids to its fields, there were no milk samples containing PFAAs.[23]

In another study by Noorlander et al.,[12] PFOS and PFOA were detected in milk about with 10 and 1 pg/g and in butter with about 33 and 16 pg/g, respectively. Those concentrations are similar to our results (compare Table 1) and may represent current levels in Middle Europe.

Impact of Industrial Food Processing on PFAA Levels. There are different ways how food processing may influence PFAA concentrations. One is the uptake from contaminated water as reported by Xiao et al.[24] This uptake route could be excluded as a possible contamination source because in the collaborating dairy, the heat treatment was carried out by indirect heat transfer (heat exchangers). However, uptake from water may be an impact factor if steam pasteurization is applied. This might be the reason for partly increased milk levels observed in prior studies[13,21] (compare Comparison of Screening Results of Milk and Dairy Products with Literature Data).

Another hypothesis is that heat can act as an impact factor. Del Gobbo et al.[18] stated that all heating processes like cooking, baking, or frying decreased PFAA concentrations. Mass losses were similar for all three heating processes, and no correlation was apparent between those and concentration changes of PFAA. So they assumed that PFAA are not being removed with the issuing water or fat. As PFAA are reported to bind to serum albumin,[25] the authors suggested these interactions or bonds may be destroyed during heating and PFAA can be removed from the food. Anyway, a loss of PFAA upon heating could not be confirmed in this study but it has to be considered that the heating of milk is performed without any intake or loss from or into the process environment. Furthermore, no trend in concentration changes was observed in our study.

Milk processes like centrifugation in a separator or churning cause a phase separation and initiate a distribution of PFAA between both phases. This has been studied in a diploma thesis by Sauer[21] in lab scale experiments with milk and cream spiked to 20 ng/g. Milk was separated (a) to cream and skim milk by centrifugation, (b) to cheese and whey by addition of lab-ferment, and (c) milk and milk skin by cooking, and (d) cream to butter and butter milk. Sauer realized significantly higher concentrations of PFAA in fat rich phases, especially for longer chain congeners (>C6). Data of our analytical screening of milk and dairy products support the findings of Sauer, as we observed slightly increased levels in products with higher fat content. However, increases in our data set were less significant compared to these laboratory scale experiments with highly spiked samples. This may be due to the fact that products included in our screening study have been produced from different raw milk charges.

Enzymatic processes were considered as a possible way of increasing the PFAA content because it was shown that per- and polyfluorinated precursors were degraded into PFAA in a

microbial system.[26] So it might be feasible for heterofermentative organisms to decarboxylate and oxidize FTOH to PFAA during the ripening of cheese and yogurt. However, data of our study do not support that assumption. Levels in yoghurt were well comparable to raw milk and indicate a negligible effect of enzymatic process. Cheese levels of PFNA, PFDA, PFUdA, and PFDoA were higher than levels in raw milk. However, enzymatic processing to cheese initiates a precipitation, i.e., a phase separation, and thus, the enzymatic effect, cannot be studied separately. Increased PFAA levels in cheese were therefore more likely caused by its higher fat content, as discussed above.

Finally, paper-based greaseproof packaging materials, which are coated with fluoropolymers and applied for products with higher fat content, could contribute to the PFAA load of dairy products. With respect to products of the collaborating dairy, that effect could only be studied with butter wrapped in such packaging. Other products of that dairy with higher fat content were packed in polymer packaging. In our study, butter exhibited the highest levels of PFAA. As discussed above, the increase of PFAA levels was at least partly attributed to two phase separation processes that butter was subjected to. However, PFAS levels identified in butter wraps (Figure 5) indicates a high source of PFCA with a straight number of carbon atoms and with 8:2 and 10:2 FTOH of precursors of PFOA and PFDA.

Migration of PFAS from packaging into food has been studied by several authors. Sinclair et al.[27] showed that PFOA could be released from microwave popcorn bags in concentrations from 5 to 34 ng and FTOH with up to 258 ng/bag. Trier et al.[28] found di- and triPAPs in microwave popcorn bags which can be degraded to PFAA just like FTOH.[29] Begley et al.[16] could prove that FTOH could migrate from packaging into a food. On the basis of these results, it is expected that parts of the PFAS concentrations found in the packaging material could migrate into the food and increase the PFAA concentration either through direct migration of PFAA or through migration and following degradation of precursors.

All in all, heating and enzymatic processes do not seem to cause significant changes in concentration to indicate an influence. There are single small concentration changes, but the most distinct trend is apparent in high fat products obtained from raw milk by phase separation processes. For butter, an additional input from the coated packaging has to be taken into consideration. So for further in-depth investigations, the process line of butter production was chosen because it includes two phase separation steps, produces a high fat content product, and applies greaseproof packaging.

Monitoring PFAA Levels along an Industrial Food Processing Chain. *Impact of Milk Processing.* PFAA levels were monitored along the industrial production line of unpacked butter in the cooperating dairy (Figure 2). Impact of packaging was investigated separately. Despite very low PFAA concentrations, the recovery study displayed in Figure 4 indicate a reasonably complete tracking of PFAA congeners along both processes. As shown in Figure 3, concentrations of longer chain PFAA in both products with higher fat content, i.e., cream and butter, were found to increase levels in raw milk. For all longer chain PFAA, butter levels were higher than cream levels and indicate a stepwise rise. The separation process increased PFAA concentrations by a factor of 1.2−1.6 during the production of cream,and the churning process affected a further increase by a factor of 1.2−2.1 upon butter production by churning.

Enrichment of PFAA in in fat-rich phases is not self-explanatory because it seems to contradict the above-mentioned high affinity of PFAA toward proteins, which are highly concentrated in the water phases. On the other hand, hydrophobicity of PFAA increases with increasing chain length and may explain their affinity for fatty phases.[19] Distribution of PFOS and PFOA between water, oil, and protein was investigated by Ropers et al.[30] In an oil-in-water emulsion, 89−100% of PFOS and 92−100% of PFOA were present in the water phase. By adding of whey protein, the distribution of PFOS and PFOA was shifted in favor of the oil and 43% PFOS and 73% PFOA remained in the water phase. The authors suggest electrostatic interactions between proteins and the acid groups of PFOS and PFOA and also hydrophobic interactions during the orientation of the carbon chain into the oil droplet. With a higher initial PFAA concentration, the equilibrium state is reached more quickly and with higher chain length the binding affinity was increased due to higher hydrophobic interactions. Also, the acid group seems to have an impact on the binding because sulfonic acids have higher binding affinities than carbonic acids with the same chain length. This shows that the binding affinity of PFAAs cannot be explained by using octanol−water partition coefficients (K_{ow}).[31] Jeon et al.[32] described hydrophobic and electrostatic interactions for charged molecules as main interactions between PFAAs and clay particles. By changing the pH value, they varied the protonation state of charged molecules and discovered a change in their binding affinity.

A deeper look into the processes involved in butter production helps to understand the distribution behavior: Milk fat droplets are emulsified in milk by an outer membrane consisting mainly of proteins and phospho- and glycolipids. During separation, the main part of milk proteins, casein and whey proteins, is separated into the skim milk. Churning breaks the membrane of the fat droplets, and membrane proteins as well as residual milk proteins are separated into the butter milk. So butter is very poor in proteins. PFAAs prefer binding to proteins,[33] which suggests higher PFAA concentrations in skim milk or butter milk. The mechanism proposed by Ropers et al.,[30] however, poses a possible explanation for higher concentrations in cream. PFAA bind to membrane proteins of fat globules and orient themselves into the membrane. As membrane proteins are separated into the cream and cream accounts for only 10 wt % of the raw milk, the concentration in cream exceeds raw milk levels if more than 10% of the initial PFAA amount goes with membrane proteins.

During the churning, the membrane of the fat globules is broken and free fat is released, which begins to form a continuous phase around little dispersed water droplets. Residual amounts of milk proteins and proteins released from the membrane of the fat globules are washed out with the issuing water into butter milk. Amphiphilic PFAA congeners, however, might arrange themselves at interfaces between fat and dispersed water droplets, and with increasing chain length and therefor higher hydrophobicity,[30] they tend to orient themselves into the continuous fat phase. This might then prevent them from an effective extraction into butter milk.

Amphiphilic gangliosides naturally present in raw milk behave similarly. They concentrate in cream during the separation process and are scarcely found in skim milk. But during churning, they are released from the membrane, and due

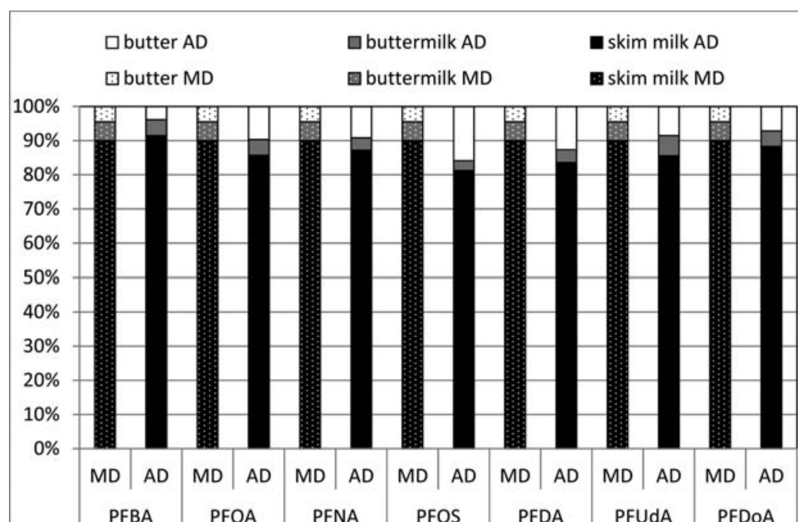

Figure 7. Mass distribution (MD) of phases separated during butter production compared to the distribution of the amounts of PFAA (AD) observed in the products.

to their strongly polar character, they are almost completely washed into the buttermilk. In contrast to PFAA, gangliosides have a much larger polar and a smaller lipophilic moiety. Therefore, it is expected that they accumulate at the interface between the continuous fat phase and water droplets like PFAA, but they orient themselves into the polar water phase, which facilitates their removal from the butter.

Concentrations in skim milk and butter milk were often below the LOD, but in order to create a complete mass balance, these concentrations were also used for calculation. Despite very low PFAA concentrations, the recovery study displayed in Figure 4 indicates a reasonably complete tracking of PFAA congeners along both processes. Furthermore, this is a strong indication that there is neither a significant loss of PFAAs by adsorption to surfaces of process equipment nor a release from these surfaces into dairy products.

Comparing the distribution of the amount of PFAS congeners (AD) with the mass distribution (MD) of the separated phases occurring during butter production (Figure 7), it is apparent that the main part of PFAAs is still present in the protein rich skim milk, as the slight concentration increases do not compensate for the high mass share (90%) of skim milk. PFAA amounts found in the butter are higher than the 4.5% expected from the mass distribution due to the concentration increase discussed above. This has also been reported by Sauer[21] in samples spiked with native substances. Despite the above-discussed interaction with membrane proteins, these results indicate a competitive interaction of PFAAs with milk proteins present in the skim and butter milk phases. This study, however, revealed that besides the protein binding, hydrophobic interactions play an important role in the mechanism of arranging PFAA at interfaces.

Impact of Packing. Results displayed in Figures 5 and 6 clearly indicate the sources of PFAS in the applied butter wrap and a transfer of these compounds into the butter. In butter wraps, the highest PFAA levels were found for PFCA, especially for congeners with a straight number of carbons in the chain. The profile and the absence of significant amounts of PFSA indicate a FTOH source for these loadings because they contain only even numbers of carbon atoms in the chain due to their manufacturing process. FTOH concentrations in the butter wraps were about a factor 1000 higher than those of PFAA. The PFAS concentrations in the butter wraps stayed nearly constant during the whole 45 days, which hints at a state where the source was not used up yet or less likely to a continuous production of PFAS from precursors.

The highest concentrated PFAS in the butter wrap were expected to have the highest migration rates due to Fick's law. A comparison of concentrations profiles in butter wraps and butter reveals, however, that migration decreases with chain length. Whereas levels of PFOA and PFDA dominate the profile in the wrap and PFHxA shows only half that level, the migration profile is dominated by PFHxA and PFOA and PFDA reaches only one-fifth of the PFOA level. These findings indicate that migration is possibly inhibited by longer chain lengths as reported earlier.[39] 8:2 FTOH has a higher migration rate as expected from the initial concentration in the butter wrap, which was higher than for 10:2 FTOH. 6:2 FTOH could not be detected in the butter.

With 5 °C, the experiment was carried out at a very low temperature compared to the storage parameters reported in the literature so far. Begley et al.[16] described PFOA migrating from a PTFE based sealant into the food simulant Miglyol at 100 °C for 2 h. At a temperature of 175 °C, 7 times the PFOA amount can migrate, which is nevertheless just 17% of the whole content of PFOA in the sealant. Dinglasan-Panlilio and Mabury[34] showed that coatings with fluorinated surfactants or fluoropolymers bear up to 3.8% unbound FTOH. They suspected that those free FTOH do not bind to the polymer during the manufacturing process and remain as residues within the product from where they can be released. From popcorn bags as 2.1 ± 0.9 mg/g or 7 μg/dm^2 FTOH migrate into Miglyol at heating for 2 min at 200 °C in a microwave. Jogsten et al.[35] found higher PFOS loads in packed salad compared to not packed salad. FTOHs were found to migrate from FCM into food and food simulants,[36] which can also be degraded to PFAA.[37] At higher temperatures, migration rates over 100% were found with the minimum migration rate of 281%. A deliberation of FTOHs from side chains of the polymer was suspected. Sinclair et al.[38] found a PFOA and FTOH release during heating of microwave popcorn bags and with higher contents in the packaging after the heat treatment. This was

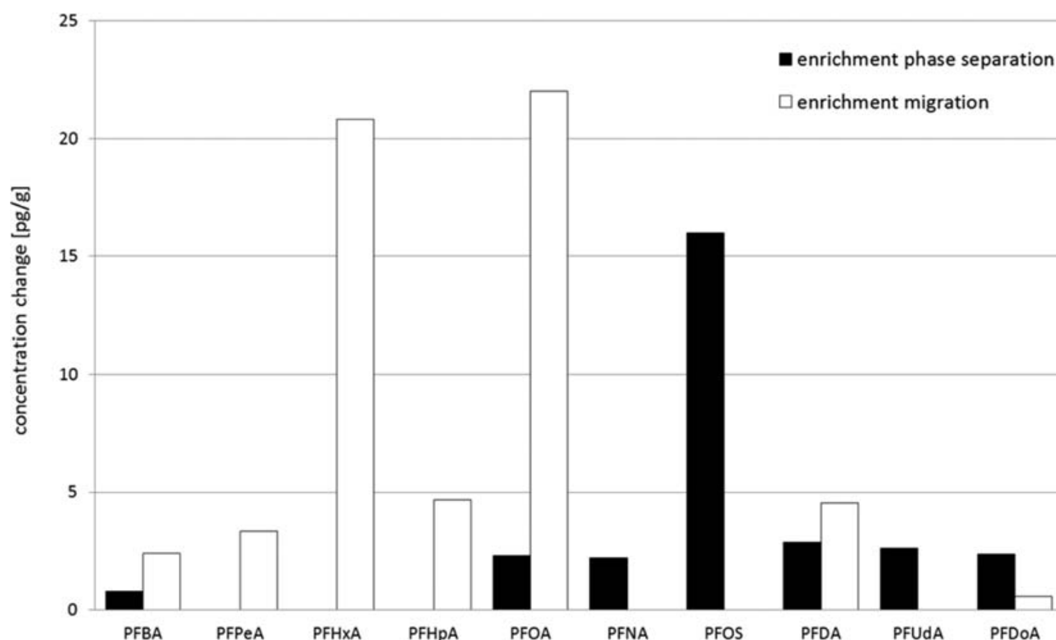

Figure 8. Total increase of PFAA concentration during the production of butter and the 45 days storage at 5 °C.

again interpreted as a hint to a release of FTOHs from precursor compounds.

The study concept did not allow distinguishing between sources of PFCA levels in butter. Thus it remains unclear whether increased levels of PFHxA, PFOA, and PFDA originate from migration of PFCA or from migration of PFCA precursors. As there is no evidence for a strong microbial activity, however, the contribution of FTOH to PFCA levels in butter is probably low.

Comparison of the Impacts of Processing and Packaging. In the cooperating dairy, two industrial processes were identified that have a major impact on PFAS levels in milk and dairy products, namely phase separating processes (separator and churning) and packaging. However, these processes have an impact on different PFAS congeners. Thus, concentration changes attributed to processing and packing were calculated and compared in Figure 8.

In general, the increase of PFCA with a chain length up to eight carbons is caused mainly by the migration during storage. Except for PFDA, processing is the main increase way for PFAA with a chain length higher than eight carbons. All in all, there is a concentration increase in butter from storage of about 0.061 ng/g for PFAAs and 36.4 ng/g for FTOHs, which could be avoided rather easily by using a packing material without fluoropolymer coating. Fiedler et al.[40] showed that indeed also fluorine free butterwraps are being used in the market. So this would be an easy way to reduce PFAA loads on food items. The concentration during the processing is just a reallocation of compounds, thus, levels can only be decreased by decreasing the exposure of dairy cows.

ASSOCIATED CONTENT

Supporting Information

HPLC conditions, MS/MS transitions monitored for PFAA analysis by ESI-MS/MS, ions monitored for FTOH analysis by CI-MS, repeatability of the analytical approach expressed in terms of relative standard deviations of PFAA obtained by a 10-fold analysis of a homogenized milk sample. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: ++49 (0) 8161/491-750. E-mail: martin.schlummer@ivv.fraunhofer.de.

Funding

The study was financed by the EU project PERFOOD (KBBE-227525), and the financial support of the European Union is gratefully acknowledged.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Stock, N. L.; Furdui, V. I.; Muir, D. C. G.; Mabury, S. A. Perfluoroalkyl contaminants in the Canadian Arctic: evidence of atmospheric transport and local contamination. *Environ. Sci. Technol.* **2007**, *41*, 3529−3536.

(2) Hemat, H.; Wilhelm, M.; Völkel, W.; Mosch, C.; Fromme, H.; Wittsiepe, J. Low serum levels of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) in children and adults from Afghanistan. *Sci. Total Environ.* **2010**, *408*, 3493−3495.

(3) Jin, Y. H.; Liu, W.; Sato, I.; Nakayama, S. F.; Sasaki, K.; Saito, N.; Tsuda, S. PFOS and PFOA in environmental and tap water in China. *Chemosphere* **2009**, *77*, 605−611.

(4) Herzke, D.; Olsson, E.; Posner, S. Perfluoroalkyl and polyfluoroalkyl substances (PFASs) in consumer products in Norway—a pilot study. *Chemosphere* **2012**, *88*, 980−987.

(5) Trier, X. *Polyfluorinated Surfactants in Food Packaging of Paper and Board*; University Kopenhagen: Kopenhagen, 2011.

(6) D'Eon, J. C.; Mabury, S. A. Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? *Environ. Sci. Technol.* **2011**, *45*, 7974−7984.

(7) Haug, L. S.; Thomsen, C.; Bechert, G. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. *Environ. Sci. Technol.* **2009**, *43*, 2131−2136.

(8) Fromme, H.; Schlummer, M.; Möller, A.; Gruber, L.; Wolz, G.; Ungewiß, J.; Boehmer, S.; Dekant, W.; Mayer, R.; Liebl, B.; Twardella, D. Exposure of an adult population to perfluorinated substances using duplicate diet portions and biomonitoring data. *Environ. Sci. Technol.* **2007**, *41*, 7928−7933.
(9) Trudel, D.; Horowitz, L.; Wormuth, M.; Scheringer, M.; Cousins, I. T.; Hungerbühler, K. Estimating consumer exposure to PFOS and PFOA. *Risk Anal.* **2008**, *28*, 251−269.
(10) Vestergren, R.; Cousins, I. T.; Trudel, D.; Wormuth, M.; Scheringer, M. Estimating the contribution of precursor compounds in consumer exposure to PFOS and PFOA. *Chemosphere* **2008**, *73*, 1617−1624.
(11) Haug, L. S.; Salihovic, S.; Jogsten, I. E.; Thomsen, C.; van Bavel, B.; Lindström, G.; Becher, G. Levels in food and beverages and daily intake of perfluorinated compounds in Norway. *Chemosphere* **2010**, *80*, 1137−1143.
(12) Noorlander, C. W.; van Leeuwen, S. P. J.; Biesebeek, J. D. T.; Mengelers, M. J. B.; Zeilmaker, M. J. Levels of perfluorinated compounds in food and dietary intake of PFOS and PFOA in The Netherlands. *J. Agric. Food Chem.* **2011**, *59*, 7496−7505.
(13) Ericson, I.; Marti-Cid, R.; Nadal, M.; Van Bavel, B.; Lindström, G.; Domingo, J. L. Human exposure to perfluorinated chemicals through the diet: intake of perfluorinated compounds in foods from the Catalan (Spain) Market. *J. Agric. Food Chem.* **2008**, *56*, 1787−1794.
(14) Loi, E. I. H.; Yeung, L. W. Y.; Taniyasu, S.; Lam, P. K. S.; Kannan, K.; Yamashita, N. Trophic magnification of poly- and perfluorinated compounds in a subtropical food web. *Environ. Sci. Technol.* **2011**, *45*, 5506−5513.
(15) Tomy, G. T.; Budakowski, W.; Halldorson, T.; Helm, P. A.; Stern, G. A.; Friesen, K.; Pepper, K.; Tittlemier, S. A.; Fisk, A. T. Fluorinated organic compounds in an eastern Arctic marine food web. *Environ. Sci. Technol.* **2004**, *38*, 6475−6481.
(16) Begley, T. H.; White, K.; Honigfort, P.; Twaroski, M. L.; Neches, R.; Walker, R. A. Perfluorochemicals: potential sources of and migration from food packaging. *Food Addit. Contam.* **2005**, *22*, 1023−1031.
(17) Trier, X.; Granby, K.; Christensen, J. H. Polyfluorinated surfactants (PFS) in paper and board coatings for food packaging. *Environ. Sci. Pollut. Res.* **2011**, *18*, 1108−1120.
(18) Del Gobbo, L.; Tittlemier, S.; Diamond, M.; Pepper, K.; Tague, B.; Yeudall, F.; Vanderlinden, L. Cooking decreases observed perfluorinated compound concentrations in fish. *J. Agric. Food Chem.* **2008**, *56*, 7551−7559.
(19) Wang, J. M.; Shi, Y. L.; Pan, Y. Y.; Cai, Y. Q. Perfluorinated compounds in milk, milk powder and yoghurt purchased from markets in China. *Chin. Sci. Bull.* **2010**, *55*, 1020−1025.
(20) Sundstrom, M.; Bogdanska, J.; Pham, H. V.; Athanasios, V.; Nobel, S.; McAlees, A.; Eriksson, J.; DePierre, J. W.; Bergman, A. Radiosynthesis of perfluorooctanesulfonate (PFOS) and perfluorobutanesulfonate (PFBS), including solubility, partition and adhesion studies. *Chemosphere* **2012**, *87*, 865−871.
(21) Sauer, M. Perfluorierte Verbindungen in Lebensmitteln: Einfluss von Koch und Verrbeitungsprozesses. Diploma thesis, Fresenius University of Applied Science, Idstein, Germany, 2011; 88 pp.
(22) Ballesteros-Gomez, A.; Rubio, S.; van Leeuwen, S. Tetrahydrofuran-water extraction, in-line clean-up and selective liquid chromatography/tandem mass spectrometry for the quantitation of perfluorinated compounds in food at the low picogram per gram level. *J. Chromatogr., A* **2010**, *1217*, 5913−5921.
(23) Young, W. M.; South, P.; Begley, T. H.; Diachenko, G. W.; Noonan, G. O. Determination of perfluorochemicals in cow's milk using liquid chromatography−tandem mass spectrometry. *J. Agric. Food Chem.* **2012**, *60*, 1652−1658.
(24) Xiao, F.; Simcik, M. F.; Gulliver, J. S. Partitioning characteristics of perfluorooctane sulfonate between water and foods. *Arch. Environ. Contam. Toxicol.* **2012**, *62*, 42−48.
(25) Messina, P.; Prieto, G.; Dodero, V.; Cabrerizo-Vilchez, M. A.; Maldonado-Valderrama, J.; Ruso, J. M.; Sarmiento, F. Surface characterization of human serum albumin and sodium perfluorooctanoate mixed solutions by pendant drop tensiometry and circular dichroism. *Biopolymers* **2006**, *82*, 261−271.
(26) Dinglasan, M. J. A.; Ye, Y.; Edwards, E. A.; Mabury, S. A. Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. *Environ. Sci. Technol.* **2004**, *38*, 2857−2864.
(27) Sinclair, E.; Kim, S. K.; Akinleye, H. B.; Kannan, K. Quantitation of gas-phase perfluoroalkyl surfactants and fluorotelomer alcohols released from nonstick cookware and microwave popcorn bags. *Environ. Sci. Technol.* **2007**, *41*, 1180−1185.
(28) Trier, X.; Nielsen, N. J.; Christensen, J. H. Structural isomers of polyfluorinated di- and tri-alkylated phosphate ester surfactants present in industrial blends and in microwave popcorn bags. *Environ. Sci. Pollut. Res.* **2011**, *18*, 1422−1432.
(29) D'eon, J. C.; Mabury, S. A. Exploring indirect sources of human exposure to perfluoroalkyl carboxylates (PFCAs): evaluating uptake, elimination, and biotransformation of polyfluoroalkyl phosphate esters (PAPs) in the rat. *Environ. Health Perspect.* **2011**, *119*, 344−350.
(30) Ropers, M. H.; Durand, S.; Veyrand, B.; Beaumal, V.; Marchand, P.; Anton, M.; Le Bizec, B. Contamination of food by fluorinated surfactants—distribution in emulsions and impact on the interfacial protein behaviour. *Food Hydrocolloids* **2009**, *23*, 1149−1155.
(31) Liu, C. H.; Gin, K. Y. H.; Chang, V. W. C.; Goh, B. P. L.; Reinhard, M. Novel perspectives on the bioaccumulation of PFCs—the concentration dependency. *Environ. Sci. Technol.* **2011**, *45*, 9758−9764.
(32) Jeon, J.; Kannan, K.; Lim, B. J.; An, K. G.; Kim, S. D. Effects of salinity and organic matter on the partitioning of perfluoroalkyl acid (PFAs) to clay particles. *J. Environ. Monit.* **2011**, *13*, 1803−1810.
(33) Sabín, J.; Prieto, G.; González-Pérez, A.; Ruso, J. M.; Sarmiento, F. Effects of fluorinated and hydrogenated surfactants on human serum albumin at different pHs. *Biomacromolecules* **2006**, *7*, 176−182.
(34) Dinglasan-Panlilio, M. J. A.; Mabury, S. A. Significant residual fluorinated alcohols present in various fluorinated materials. *Environ. Sci. Technol.* **2006**, *40*, 1447−1453.
(35) Jogsten, I. E.; Perello, G.; Llebaria, X.; Bigas, E.; Marti-Cid, R.; Kärrman, A.; Domingo, J. L. Exposure to perfluorinated compounds in Catalonia, Spain, through consumption of various raw and cooked foodstuffs, including packaged food. *Food Chem. Toxicol.* **2009**, *47*, 1577−1583.
(36) Fengler, R.; Schlummer, M.; Wolz, G.; Gruber, L.; Franz, R. Data on migration of poly- and perfluorinated compounds from Food Contact Materials into Food and Food simulants. In 4th International Workshop Per- and Polyfluorinated Alkyl Substances—PFASs, Idstein, Germany, 2012.
(37) Dinglasan, J.; Mabury, S. A. Residual Telomer Alcohols a Probable Source of Perfluorinated Acids in the Environment. In 25th International Symposium on Halogenated Persistent Organic Pollutants—DIOXIN 2005, Toronto, 2005.
(38) Sinclair, E.; Kim, S. K.; Akinleye, H. B.; Kannan, K. Quantitation of gas-phase perfluoroalkyl surfactants and fluorotelomer alcohols released from nonstick cookware and microwave popcorn bags. *Environ. Sci. Technol.* **2007**, *41*, 1180−1185.
(39) de Fatima Poças, M.; Oliveira, J. C.; Pereira, J. R.; Brandsch, R.; Hogg, T. Modelling migration from paper into a food simulant. *Food Control* **2011**, *22*, 303−312.
(40) Fiedler, D.; Schlummer, M.; Kizlauskas, M.; Gruber, L. Update on the occurrence of fluorinated compounds in European food packaging items. In 3rd International Workshop Anthropogenic Perfluorinated Compounds, Amsterdam, 2011.