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Cooking fish is not effective in reducing exposure to perfluoroalkyl and polyfluoroalkyl substances

Satyendra P. Bhavsar [a,b,*], Xianming Zhang [b], Rui Guo [a], Eric Braekevelt [c], Steve Petro [a], Nilima Gandhi [b], Eric J. Reiner [a,b], Holly Lee [a], Roni Bronson [c], Sheryl A. Tittlemier [c,1]

[a] Ontario Ministry of the Environment, Toronto, ON M9P 3V6, Canada
[b] University of Toronto, Toronto, ON M5S 3E8, Canada
[c] Health Canada, Ottawa, ON K1A 0L2, Canada

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ABSTRACT

Consumption of fish is considered a part of a healthy diet; however, health risks from fish consumption exist due to potential exposure to various contaminants accumulated in fish. Cooking fish can reduce exposure to many organic chemicals in fish. Similar results have been presented for low levels of perfluoroalkyl and polyfluoroalkyl substances (PFASs), a class of contaminants of emerging concern, in grocery store fish. We examined the effectiveness of three cooking methods (i.e., baking, broiling, and frying) on reducing PFAS levels in four sport fish species. Samples of Chinook salmon, common carp, lake trout and walleye were collected from four rivers in Ontario, Canada and skin-off fillets were analyzed for regular groups of PFASs such as perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs), as well as perfluoroalkyl phosphonic acids (PFPAs), perfluoroalkyl phosphinic acids (PFPIAs) and polyfluoroalkyl phosphoric acid diesters (diPAPs), which are PFASs of emerging concern. Perfluorooctane sulfonate (PFOS) was the dominant PFAS detected and the concentrations were more than an order of magnitude higher than those reported for fish from grocery stores in Canada, Spain, and China. Although concentrations of PFOS in fish fillets generally increase after cooking, amounts of PFOS largely remain unchanged. Relatively minor differences in changes in the fish PFAS amounts after cooking depended on fish species and cooking method used. We conclude that cooking sport fish is generally not an effective approach to reduce dietary exposure to PFASs, especially PFOS.

1. Introduction

Fish consumption has been regarded as beneficial to human health due to the high-quality protein, minerals, antioxidants, vitamins and omega-3 polyunsaturated fatty acids (PUFAs) in fish (Domingo, 2007). Health benefits of fish consumption include optimal brain and retinal development and reduced risk of coronary heart disease (Egeland and Middaugh, 1997). Several health agencies such as the World Health Organization (WHO, 2002) and Health Canada (Health Canada, 2007a) recommend at least two servings of fish per week. While enjoying health benefits of fish consumption, humans are also subject to potential health risks from exposure to various contaminants accumulated in fish (Mozaffarian and Rimm, 2006). In order to minimize such health risks, various government agencies determine safe amounts of fish that can be consumed, and as necessary, issue fish consumption advisories (Bhavsar et al., 2011; Health Canada, 2007b; OMOE, 2011; U.S. EPA, 2010).

It has been demonstrated that cooking and removal of skin can reduce the concentrations of some organic contaminants, such as dioxins and polychlorinated biphenyls (PCBs) (Hori et al., 2005; Sherer and Price, 1993; Zhang et al., 2013). However, such practices have minimal impact on fish concentrations of some other contaminants, such as heavy metals. In fact, some studies have reported increases in concentrations of heavy metals after skin removal or cooking (Morgan et al., 1997; Perelló et al., 2008). The extent to which the concentration of a contaminant may be altered by cooking processes depends upon the type of tissue in which it accumulates. Neutral organic contaminants generally have a higher affinity for the fatty tissues of fish (Bertelsen et al., 1998), and loss of fat via skin removal or cooking is a major contributor to reducing concentrations of these contaminants (Bayen et al., 2005; Wilson et al., 1998). In contrast, heavy metals generally bind with tissue proteins (Hamza-Chaffai et al., 1995) and are less affected by fish processing methods.

Similar to heavy metals, binding to proteins is also considered to be the bioaccumulation mechanism for the ionizable perfluorinated compounds, a group of organic compounds with unique surface properties and low water and oil solubility (Conder et al., 2008; Haukas et al.,

* Corresponding author at: Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch, 125 Resources Road, Toronto, ON M9P 3V6, Canada. Tel.: +1 416 327 5863; fax: +1 416 327 6519.
E-mail addresses: satyendra.bhavsar@ontario.ca, s.bhavsar@utoronto.ca (S.P. Bhavsar).
[1] Current affiliation: Grain Research Laboratory, Canadian Grain Commission, Winnipeg, MB R3C 3G8, Canada.

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2007; Jones et al., 2003; Kelly et al., 2009). Historically, they have been used in diverse applications including surfactants and in aqueous film forming foam fire fighting agents (AFFF). The strong carbon-fluorine bonds of PFASs make them less susceptible to degradation and highly persistent in the environment (Lindstrom et al., 2011). The persistence makes it possible for PFASs to undergo long range transport to remote regions where no PFASs have been produced or used (Houde et al., 2011; Wania, 2007). Further, PFASs have been found in organisms at various trophic levels (Houde et al., 2011), and are considered endocrine disruptive, immunotoxic and tumorgenic in laboratory animals at relatively high doses (Betts, 2007). Because of their persistent nature, long range transport capability, bioaccumulation potential and toxicity, a group of PFASs including perfluorooctanesulfonic acid (also known as perfluorooctane sulfonate or PFOS), its salts and perfluorooctane sulfonyl fluoride were listed in Annex B of the United Nations Stockholm Convention on Persistent Organic Pollutants list in 2009 (UNEP, 2009).

In the past decade, increasing surveillance data of PFAS concentrations in fish have become available worldwide. In some parts of North America, surveillance of sport fish for PFASs has resulted in the issuance of restrictive fish consumption advisories (Delinsky et al., 2010; OMOE, 2011). However, in contrast to neutral organic contaminants such as PCBs and dioxins, little information on the effects of different cooking methods on PFAS levels in fish is available. A recent comparison of PFAS concentrations in raw fish with the corresponding cooked fish by Del Gobbo et al. (2008) showed a decline in PFAS concentrations after cooking. These findings are, however, in contrast to the unchanged or even increased concentrations of heavy metals in fish after cooking even though both PFASs and heavy metals bind to protein. Since the fish samples utilized by Del Gobbo et al. (2008) were obtained from grocery stores and the PFAS concentrations were relatively low (e.g., PFOS ranging 0.21–1.68 ng/g ww) compared to those for sport fish, it is possible that analytical uncertainty might have contributed to the proposed decline in the concentrations after the cooking. Therefore, more data on how different cooking methods affect PFAS concentrations in fish are needed to refine exposure assessments and ensuing fish consumption advice.

The goal of this study is to gather information on the effectiveness of three cooking methods (i.e., baking, broiling, and frying) to reduce PFC levels in four fish species sampled from rivers in Ontario, Canada, rather than from a grocery store. We considered Chinook salmon (*Oncorhynchus tshawytscha*), common carp (*Cyprinus carpio*), lake trout (*Salvelinus namaycush*) and walleye (*Sander vitreus*). In addition to perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs), we also examined perfluoroalkyl phosphonic acids (PFPAs), perfluoroalkyl phosphinic acids (PFPIAs) and polyfluoroalkyl phosphoric acid diesters (diPAPs), which are PFASs of emerging concern. PFPAs and PFPIAs have been used as wetting and foam-dampening agents, while diPAPs have been used in food-contact paper products (Begley et al., 2005; Dupont, 2012; Mason Chemical Co., 2012). However, to date no submission has been made to the Food Directorate of Health Canada requesting the use of diPAPs in food packaging materials sold in Canada (Rulibikiye, Health Canada, personal communication).

2. Methods

2.1. Sample collection and preparation

Fish samples were collected in summer/fall of 2010/11 from four rivers in Ontario, Canada: Credit River (Chinook salmon, N = 5, 58–91 cm), Thames River (common carp, N = 5, 66–76 cm), Niagara River (lake trout, N = 4, 58–79 cm), and Welland River (walleye, N = 5, 46–62 cm). Relatively elevated concentrations of PFASs in fish from these locations were expected based on nearby industrial activities or previous monitoring work conducted by the Ontario Ministry of Environment (OMOE). The samples were measured for total length and weight, sexed and filleted (both sides, skin-off) in the field. The fillets were stored on ice and transported to the Ontario Ministry of the Environment's Sport Fish Contaminant Monitoring Program office in Toronto, Ontario, Canada where they were stored at −20 °C until further processing. The two fillets from each fish were partially thawed and segmented into 16 parts and classified into four groups (raw plus three cooking methods) as shown in the Supporting Information (SI) Fig. S1 in order to minimize influence of potentially varying PFAS levels in different parts of the fillets on the study results. Four subsamples from each fish were stored at −20 °C until further processing. Fillet samples for three cooking methods were then stored on ice and transported to the Health Canada laboratory in Ottawa, Ontario, Canada where they were stored at −20 °C until cooked. Raw and cooked fillets were later homogenized at the Toronto laboratory using a Buchi B-400 Mixer and stored again at −20 °C until chemical analysis.

2.2. Cooking details

Frozen fish fillets were allowed to warm to room temperature. The weights of large aluminum weighing dishes were measured, then 10 g canola oil was added to each dish and evenly distributed over the bottom of the dish using a silicone brush. We chose canola oil because it is typically used for cooking in Canada where the experiments were performed, and is the third most consumed vegetable oil in the world (Canola Council of Canada, 2013). The weight of the dish with the oil was measured and recorded. Each fish sample was then placed in its labeled weighing dish. The total weight (dish + oil + fish) was also measured.

2.2.1. Frying

An electric frying pan was set to 175 °C and given 10 min to reach test temperature. The aluminum dishes were placed in the frying pan and cooked uncovered. After 5 min, the fish fillets were carefully flipped with a plastic spatula and cooked for an additional 5 min.

2.2.2. Baking

A small toaster oven was preheated to 200 °C (measured using an oven thermometer). The aluminum dishes were placed in the oven and cooked uncovered for 15 min.

2.2.3. Broiling

The toaster oven was set to broil. The broiling temperature (measured using an oven thermometer) was set at 300 °C. The aluminum dishes were placed in the oven and cooked uncovered for 10 min.

2.2.4. Post-cooking

The samples were removed from heat and the internal temperature of the fish was immediately measured with a digital probe. The fish were allowed to cool before the total weight (dish + oil + fish) was measured. The fish was removed from its weighing dish, wrapped in aluminum foil, replaced in its original labeled bag and frozen to −20 °C for later analysis. The final weight of the dish with cooking juices and leftover canola oil was also measured. The weights of the cooking juices generated were calculated by subtracting pre-cooking weight of dish with oil from the final weight of the dish with juices and oil. Cooking juices and leftover canola oil were transferred to a polypropylene sample bottle and frozen for later analysis.

2.2.5. Blanks

Canola oil (10 g) was added to an unused aluminum weighing dish and evenly distributed over the bottom of the dish using a silicone brush. The dish was then baked at 200 °C for 15 min. The dish was allowed to cool, then the oil was transferred to a polypropylene sample bottle.

2.3. Sample preparation for PFAS analysis

Complete details of sample preparation and PFAS analysis including the compounds analyzed are provided by Guo et al. (2012). Briefly, 3 mL of acetonitrile was added to a 1 g homogenized fillet sample and 1 mL of 60/40 acetonitrile/water was added to cooking juices or blank (canola oil). The resulting solution was shaken, centrifuged and transferred to a 15 mL polypropylene tube. The sample was extracted with a second aliquot of solvent and the supernatant was combined with the original solvent. Acetonitrile extracts were evaporated to about 1 mL using a gentle stream of nitrogen. The sample was then extracted twice with 5 mL methyl t-butyl ether (MTBE) following addition of 1 mL of 0.5 M tetrabutyl ammonium hydrogen sulphate (TBAS) at pH 4. The MTBE aliquots were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1 mL methanol. Prior to instrumental analysis, the extract was split. One fraction was spiked with mass-labeled PFCAs and PFSAs, and diluted with HPLC-grade water to make the sample extract solvent mixture 60% methanol and 40% water (V/V). The other fraction was spiked with mass-labelled diPAPs and was not diluted with water. Both fractions were filtered with a 0.2 μm syringeless polypropylene filter.

2.4. Instrumental analysis

Analysis was performed using an Agilent 1200LC liquid chromatograph with a Restek Ultra C18 column (50 × 2.1 mm, 3 μm; Restek, Bellefonte, PA, USA) coupled to a 4000 QTrap triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) in negative ion electrospray mode using multiple reaction monitoring (MRM). The mobile phase flow rate was 250 μL/min. Gradient elution was used to separate each group of analytes. For the PFCAs and PFSAs, the gradient started with 65% methanol with 10 mM ammonium acetate (Solvent A) and 35% HPLC-grade water with 10 mM ammonium acetate (Solvent B), followed by a linear gradient to 90% A in 6 min, kept for 1 min and then returned to the initial composition. The column temperature was 35 °C. For the PFPAs and PFPIAs, the gradient started with 20% A followed by a linear gradient to 65% A in 1 min, followed by a linear gradient of solvent A to 95% in 4 min. The final composition (95% A) was held for 2 min before being returned to initial composition. The column temperature was 30 °C. For the diPAPs, the gradient began with 70% A, the gradient was then changed to 95% A in 0.1 min, followed by linear gradient of solvent A to 100% in 1 min. This composition was kept for 4 min and then returned to the initial composition. The column temperature was held at 25 °C. Multiple reaction monitoring transitions and optimized mass spectrometer parameters for each target compounds are summarized by Guo et al. (2012).

PFCAs, PFSAs and diPAPs were quantified by multi-point (5–1000 fg/μL) calibration using mass-labeled internal standards, and transitions and conditions described by Guo et al. (2012). PFPIAs were quantified using external standard calibration as mass labeled internal standards were not available and matrix suppression/enhancement was not observed (Guo et al., 2012). PFPAs were quantified by standard addition as no internal standard was available and matrix effects were observed (Guo et al., 2012).

Two procedural blanks (HPLC-grade water) and a spiked fish blank (Captain High Liner, Alaskan Pollock) were analyzed with each set of 15 to 20 fish samples as quality control checks. Recoveries in spiked samples typically range between 80 and 120% (Guo et al., 2012). Values below limits of quantification (LOQ) were reported as not detected (ND). Each sample was extracted once and analyzed twice, and values were reported as arithmetic means of the two injections. Perfluorooctanoic acid (PFOA) and 6:2 diPAP were detected in procedural blanks with a mean concentration of 12 pg/g for PFOA and at lower than the LOQ for 6:2 diPAP. Values for PFOA were blank-corrected.

There are no certified reference materials available for cooked fish to ideally test if recoveries from cooked fish are the same as from raw. However, recoveries of internal standard added to raw and cooked fish and seafood in Del Gobbo et al. (2008) did not differ (unpublished data), indicating that the state of the fish (i.e., raw vs. cooked) does not affect fortified analyte recovery. In addition, Tittlemier et al. (2007) show that recoveries of PFASs for a variety of fortified food matrices including cooked chicken nuggets, organ meats, cured pork, and infant cereal were similar suggesting that there are no differences across these matrices.

2.5. Data analysis

The changes in fish mass and PFOS concentrations/amounts were examined using two-factor (fish species and cooking method) ANOVA with multiple comparisons (Tukey HSD method). Statistical significance was set at $p < 0.05$. Self organizing map (SOM) was applied to the log-transformed concentration profiles of ten PFASs in five different fish species subjected to four processing methods (raw plus three types of cooking). SOM is a type of unsupervised artificial neural network which generates simple geometric relationships on a low (two-) dimensional display from a high-dimensional input space of the training samples (Kohonen, 2001). SOM has been widely used as a clustering and projection technique to extract features from large high-dimensional datasets for visualization and interpretation (Ericson et al., 2007; Mari et al., 2010). In order to generate the SOM, a value for each dimension of each member of samples is needed. Therefore, if a PFAS was not detected in all samples of a group (fish species, processing method), the PFAS was not included in construction of the SOM. In the case when a PFAS was detected only in a part of the samples within a group (fish species, processing method), half of the detection limit was assigned to the non-detected PFAS. After processes of training and self-learning from the input datasets, SOMs with an array of nodes (neurons) were generated using the SOM toolbox (version 2) (Alhoniemi et al., 2005). A weight vector of the same dimension of the input data (10 PFASs) was associated with each neuron. SOM can be separated into component planes which represent each dimension of the weight vector. Component planes can thus display the variable contribution to each neuron in the map.

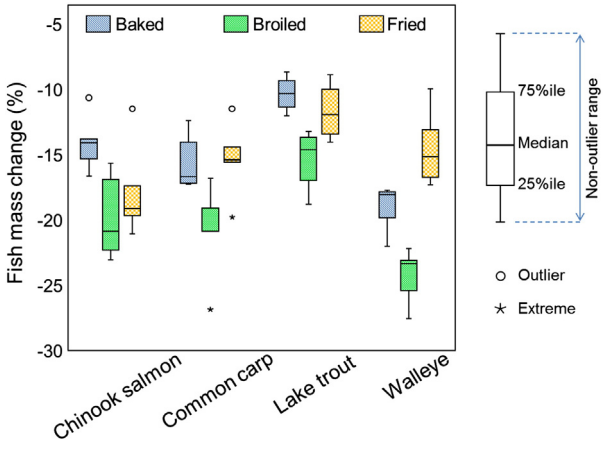

Fig. 1. Percent of fish mass change after baking, broiling and frying of the Chinook salmon, common carp, lake trout and walleye fillets. The results are presented as box–whisker plots, where the boxes represent the 25th–75th percentiles; the lines in the boxes represent the medians; the whiskers represent non-outlier ranges; and the hollow dots represent outliers and the stars represent extreme values.

3. Results and discussion

3.1. Changes in sample mass after cooking

The percentages of changes in mass for each fish species and cooking method are displayed using box–whisker plots (Fig. 1). Overall, cooking decreased the mass of the fish fillets by 10–25%. The mass declines differed significantly ($p < 0.01$) among species/cooking combinations; however, variations for the replicated samples of a species/cooking combination ranged only 3–10% (Fig. 1). There were significant differences ($p < 0.05$) in average declines of fish mass between lake trout (12.5%) and the other three species (17–19%) (Fig. S2, Table S1). For the three cooking methods, average fish mass decline due to broiling (21%) was statistically higher ($p < 0.01$) than those due to baking (16%) and frying (15%) (Table S2). The two-factor ANOVA also indicated that magnitude of fish mass change depends on both species and cooking method (Fig. S2).

The change in fish mass during baking, broiling and frying can be mainly attributed to the loss of moisture, which contributes approximately 70% to wet weight of a fish fillet. Previous studies (Gokoglu et al., 2004; Weber et al., 2008) observed less moisture loss by baking than grilling (equivalent to broiling of our study), which is consistent with the findings of this study (Fig. 1). However, reported higher dehydration of fish fillets due to frying compared to baking and grilling (Weber et al., 2008) is not supported by the lower mass reduction of fried fish samples in this study. This discrepancy is possibly due to fish fillets absorbing some oil while losing some moisture during frying.

3.2. PFAS concentrations and profiles

Five groups of PFASs, namely PFCAs, PFSAs, PFPAs, diPAPs and PFPIAs, were analyzed in raw, baked, broiled and fried fish fillets. Of the PFCAs analyzed, perfluoroheptanoic acid (PFHpA) was not detected in any sample and PFOA was detected in only 50% of the samples. PFPAs were not detected in any fish samples, and diPAPs were detected in <50% of the samples. Further, 8:8 PFPIA was detected in only 25% of the samples. These results are consistent with the recent findings of Guo et al. (2012) for Great Lakes lake trout. Therefore, in the cooking

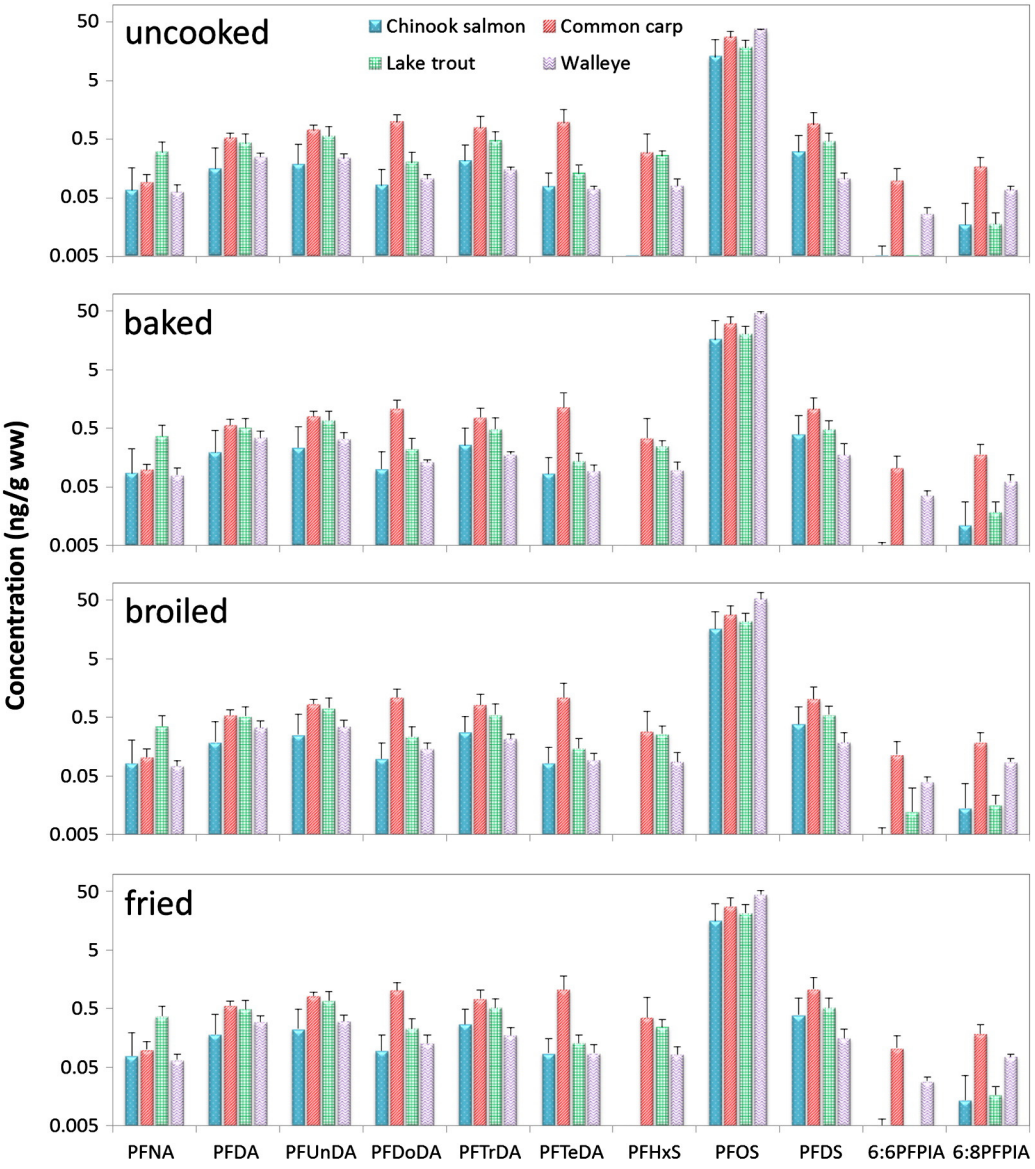

Fig. 2. Concentrations (ng/g wet weight) of perfluorinated compounds in raw, baked, broiled and fried Chinook salmon, common carp, lake trout and walleye fillets. Error bars indicate the standard deviation of replicated samples.

effect analysis, PFHpA, PFOA, PFPAs, diPAPs and 8:8 PFPIA were excluded. The PFASs that were detected in >60% of the samples included perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexane sulfonic acid (PFHxS), PFOS, perfluorodecane sulfonic acid (PFDS), 6:6 PFPIA and 6:8 PFPIA (Table S3).

Of the PFASs analyzed, PFOS concentrations (16–53 ng/g ww) were 1–2 orders of magnitudes higher than those of the other PFASs (Fig. 2, Table S3), which is consistent with the PFAS profiles in fish measured by other studies (Awad et al., 2011; Del Gobbo et al., 2008; Zhang et al., 2011). PFOS concentrations in fish analyzed in this study were compared with other studies which measured PFOS in fish muscle or edible portion. Fish PFOS levels measured in this study were within the ranges of <6–300 ng/g ww for fish from Michigan waters (Giesy and Kannan, 2001), 1–2000 ng/g ww for fish from Minnesota lakes and rivers (Delinsky et al., 2010), and <1–100 ng/g ww for fish from the Great Lakes (De Silva et al., 2010); however, they were more than an order of magnitude higher than those for fish collected from retail markets in Canada (Del Gobbo et al., 2008; Tittlemier et al., 2007), Spain (Ericson et al., 2008), and China (Gulkowska et al., 2006). As such, this study addresses the current knowledge gap on the effects of cooking on environmentally relevant fish PFOS levels, especially for the Great Lakes area.

Concentrations of $\sum$ PFCAs varied between fish species and cooking methods, and contributed only 2–20% of $\sum$ PFASs (Table S3). Unlike the measurements of PFNA and PFUnDA as the two dominant PFCAs by Del Gobbo et al. (2008), PFNA in this study was lower than the longer chain PFCAs for species other than lake trout. PFUnDA was at higher concentrations than PFNA, but depending on fish species, PFUnDA could be lower than PFDoDA and PFTeDA. Previous studies indicated PFCAs with an odd number of perfluoroalkyl carbons had higher concentrations than the corresponding shorter, even number PFCAs in fish (Del Gobbo et al., 2008; Martin et al., 2004). In this study, we found that the pattern of relative abundance of PFASs with odd–even number of carbons varies among the fish species, and PFCAs in only lake trout and walleye followed such a pattern.

PFPIAs differed among fish species with the average concentration in common carp being about an order of magnitude higher than the other fish species (Table S3). 6:6 PFPIA and 6:8 PFPIA were detected in 70–75% of all the fish samples, while 8:8 PFPIA was detected only in common carp. 6:8 PFPIA was dominant in all five fish species with concentrations ranging from 0.01 to 0.3 ng/g ww in raw samples. As a new class of PFASs, PFPIAs have been reported in few fish studies. The concentrations of 6:6 PFPIA and 6:8 PFPIA observed for raw fish in this study (Table S3) were either comparable or higher than those for Great Lakes lake trout recently reported by Guo et al. (2012). Our finding of higher 6:8 PFPIA concentrations than 6:6 PFPIA is also consistent with those for Great Lakes lake trout (Guo et al., 2012) and human sera (Lee and Mabury, 2011), and could reflect differences in emissions and/or bioaccumulation potential between these two PFPIAs.

3.3. PFAS patterns and clustering

In order to investigate how different cooking methods affect PFAS concentrations in the four fish species, the four dimensional datasets (cooking methods, fish species, chemicals, concentrations) were analyzed using the self-organizing map (SOM), an unsupervised neural network technique to visualize high dimensional data and to establish pattern similarities (Kohonen, 2001). The color of a neuron in the U-matrix of SOM indicates the average distance between the neuron and its closest neighbors. Therefore, cluster of the dataset can be represented by the color groups in the U-Matrix. The U-matrix and Labels of the SOM for the four dimensional datasets clustered the values by fish species and not by cooking methods (Fig. 3), indicating that baking, broiling and frying are not unique from each other in changing PFAS

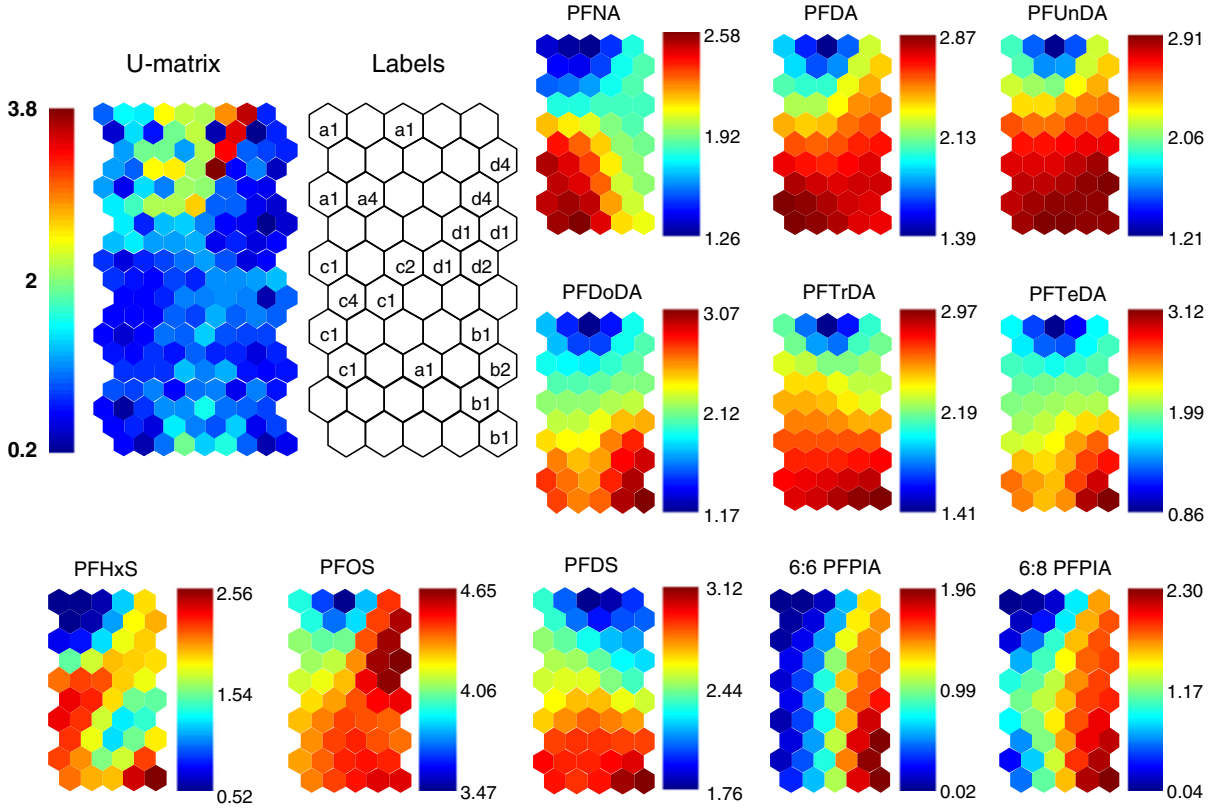

Fig. 3. U-matrix, unit labels and component planes of self-organizing maps for the logarithm transformed PFAS concentrations in (1) baked, (2) broiled, (3) fried, and (4) raw samples of (a) Chinook salmon, (b) common carp, (c) lake trout and (d) walleye.

concentrations in fish. Component planes (c-planes) represent the contribution of each variable to each neurons of the SOM. The c-planes can identify the PFASs with similar contribution to the data clustering. As such, similar color distributions in the c-planes of PFDA and PFUnDA as well as 6:6 PFPIA and 6:8 PFPIA indicate positive correlations between the PFASs (Fig. 3). The c-planes and unit labels suggest that PFPIAs contributed mainly to the clustered neurons representing common carp (bottom right of the SOM) while PFOS contributed mainly to the clustered neurons representing walleye (near top right of the SOM). Such information indicates that PFPIAs were higher in common carp and PFOS was higher in walleye compared to the other species.

3.4. Effect of cooking on PFASs in fish

Because PFOS is generally the most abundant PFAS reported in fish (e.g., Fig. 2) and the presence of which has required restrictive fish consumption advisories in at least some parts of North America (Delinsky et al., 2010; OMOE, 2011), below we present detailed analysis of the changes in the fish PFOS concentrations and amounts after baking, broiling and frying.

PFOS concentrations in all fish species increased significantly ($p < 0.05$) after cooking except for broiling and frying of common carp, which had no significant changes ($p > 0.05$) in PFOS concentrations (Fig. 4a). This is in contrast to reported 54–100% decline in low PFAS concentrations after cooking fish obtained from a grocery store

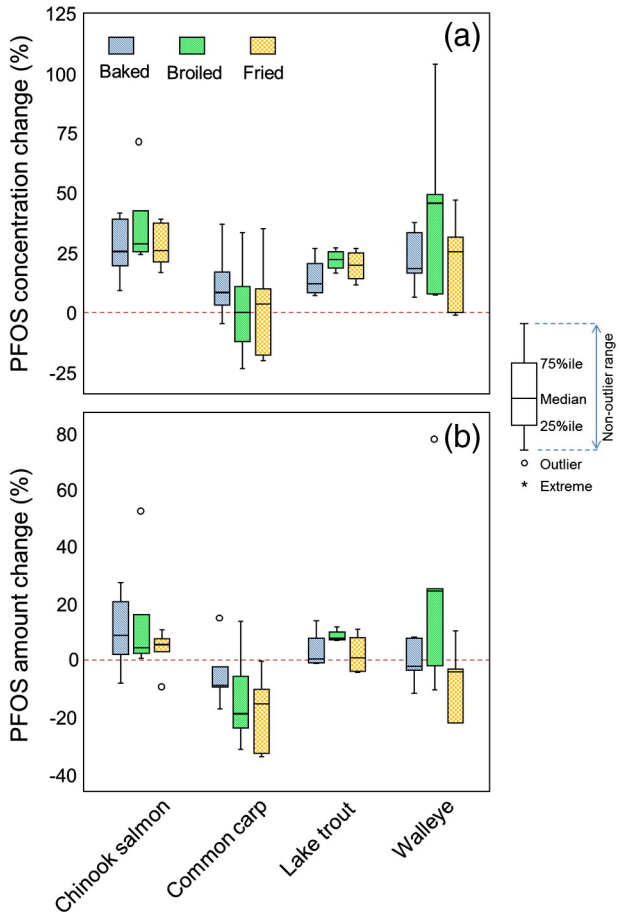

Fig. 4. Relative changes (in percentage) of PFOS (a) concentrations and (b) amounts after baking, broiling and frying of the Chinook salmon, common carp, lake trout and walleye fillets compared to the corresponding raw fish values. The results are presented as box–whisker plots, where the boxes represent the 25th–75th percentiles; the lines in the boxes represent the medians; the whiskers represent non-outlier ranges; and the hollow dots represent outliers.

(Del Gobbo et al., 2008). As mentioned above, since the fish samples utilized by Del Gobbo et al. (2008) were obtained from grocery stores and the PFAS concentrations were relatively low (e.g., PFOS ranging 0.21–1.68 ng/g ww) compared to those for sport fish, it is possible that analytical uncertainty might have contributed to the proposed decline in the concentrations after the cooking. Concentrations of PFASs in the current study were approximately 10× higher than those in the samples analyzed by Del Gobbo et al. (2008). We are unsure on the mechanism of loss during cooking; however, it is possible that the higher initial concentrations in this study obscured any small loss of PFASs that were noticeable in the Del Gobbo et al. (2008) study. In addition, some of the differences in the results from this study and those reported by Del Gobbo et al. (2008) can be attributed to differences in the cooking methods. For example, Del Gobbo et al. (2008) geared fish selection and cooking methods to include those common in Asian–Canadian populations. In the study by Del Gobbo et al. (2008), baked fish was marinated in rice wine, and fried fish was cooked in sesame oil 5 min longer than in the current study. Further, differences in the ratios of fish to oil and the surface areas of fish (e.g., small pieces versus larger portions affecting moisture/fat loss) may have contributed to the differences in the results.

The findings of this study are also in contrast to the observations for most neutral organic contaminants in fish, but are in agreement with the observations for some heavy metals (Bayen et al., 2005; Morgan et al., 1997; Perelló et al., 2008; Wilson et al., 1998). Neutral organic compounds are mainly associated with the fatty tissue of fish and are removed from fish fillet via dripping oil while cooking (Domingo, 2011). The heat applied to cook fish can also accelerate removal of neutral organic compounds from the muscle via evaporation to air (Domingo, 2011). Therefore, even though some moisture in fish fillet is lost during cooking, the wet weight based concentrations of neutral organic compounds in fish fillet generally decrease after cooking. In contrast, PFASs and heavy metals are generally associated with proteins of biota and are less likely to be removed via cooking. As mass of fish fillet decreases due to loss of moisture during cooking (Fig. 3), the wet weight based concentrations are likely to remain unchanged or increase after cooking.

An increased wet weight based PFOS concentration after cooking, however, cannot singularly be interpreted as increased risk of human exposure to PFOS because exposure/risk depends on the total amount of PFOS in the fish (rather than concentration). Overall, changes in the fish PFOS amount after cooking (Fig. 4b, SI Table S4) differed significantly ($p < 0.01$) among fish species and cooking methods after allowing effects of each other; however, the interaction effect between fish species and cooking methods was not significant ($p = 0.25$) (i.e., the effect of cooking on PFOS amount was not significantly affected by fish species). Declines in common carp PFOS amounts were significantly greater ($p < 0.05$) than those for Chinook salmon and walleye (Fig. 4b, SI Table S5). Among the cooking methods, differences existed between broiling and frying (Fig. 4b, SI Table S6).

The amount of PFOS declined significantly ($p < 0.05$) in common carp after baking and frying, and increased significantly ($p < 0.05$) in lake trout after broiling (SI Tables S4 and S7). All other fish/cooking combinations showed no significant changes ($p > 0.05$) in PFOS amount (SI Tables S4 and S7). Although, frying seems to be more effective than baking and broiling in removing PFOS from the fish samples (SI Fig. S3), the reductions of PFOS amount were generally not substantial or statistically significant except for common carp ($p > 0.05$, SI Tables S4 and S7). The increases in fish PFOS amounts observed for a number of species/cooking combinations (Fig. 4b, SI Table S4) were also generally not significant ($p > 0.05$, SI Tables S4 and S7). Further, these increases (generally <20%) as well as wide ranges of change in PFOS amount for replicates (Fig. 4b) could be a result of ±10–15% uncertainty in both before and after cooking measurements.

Similar to PFOS, other PFASs analyzed generally showed little statistically significant change in their amount after cooking (SI Tables S4 and

S7). Chinook salmon fillets lost 14–20% of PFHxS and 25–28% of 6:8 PFPIA after cooking (SI Tables S4 and S7). Common carp also lost 4–20% of PFDA, PFUnDA, PFDoDA, PFTrDA, PFHxS, PFOS, 6:6 PFPIA and 6:8 PFPIA depending on the cooking method applied (SI Tables S4 and S7). Most of the statistically significant ($p < 0.05$) changes seen in lake trout PFAS amounts were minor (<15%) increases (SI Tables S4 and S7). Walleye lost 24 and 13% of 6:8 PFPIA when baked and fried, respectively (SI Tables S4 and S7).

There are no certified reference materials available for cooked fish to ideally test if recoveries from cooked fish are the same as from raw. However, recoveries of internal standard added to raw and cooked fish and seafood in Del Gobbo et al.(2008) did not differ (unpublished data, S. Tittlemier), indicating that the state of the fish (i.e., raw vs. cooked) does not affect fortified analyte recovery. In addition, Tittlemier et al. (2007) show that recoveries of PFASs for a variety of fortified food matrices including cooked chicken nuggets, organ meats, cured pork, and infant cereal were similar suggesting that there are no differences across these matrices.

The study was designed to minimize influence of potentially varying PFAS levels in different parts of the fillets on the study results (Fig. S1), cooked portions were not compared to identical position on the fish body for the raw portion. Although this could have contributed to the variability observed in the effects of the cooking methods, no systematic differences in fish PFOS amounts after cooking across all the species indicate that the subsampling method did not have any major influence on the study results.

3.5. PFASs in blank canola oil and leftover juices

All PFAS measurements in blank canola oil samples were below the detection limits (results not shown). Concentrations of PFASs in cooking juices were mostly below the detection limits except for some measurements of PFOA (18% of the samples, ranged ND–0.03 ng/g ww), PFOS (93% of the samples, ND–1.3 ng/g), 6:2 diPAP (9% of the samples, ND–0.09 ng/g ww), and 8:2 diPAPs (34% of the samples, ND–2.4 ng/g ww). PFOS concentrations and amounts (absolute value and as percentages of the amounts in the corresponding raw fish) in the juices collected after cooking are shown in SI Table S8. The amount of PFOS loss through the leftover cooking juices were <0.01% of the amount in the raw fish. Overall, the results show that PFAS losses to the cooking juices were negligible.

4. Conclusions

A cooking experiment was conducted on skin-off fillets of Chinook salmon, common carp, lake trout and walleye collected from four rivers in Ontario, Canada to investigate the effectiveness of three cooking methods (baking, broiling, and frying) on reducing PFC levels in four fish species. In addition to PFASs such as PFCAs and PFSAs, PFASs of emerging concern PFPAs, PFPIAs and diPAPs were also considered. PFOS was the dominant PFAS detected and the concentrations were more than an order of magnitude higher than those collected from grocery stores in Canada, Spain, and China. This indicates that sport fish harvested near sites with a history of PFAS contamination could represent a point source of dietary exposure to PFOS for recreational or subsistence fish consumers. Concentrations of PFOS in fish fillets generally increase after cooking; however, the amount of PFOS largely remains unchanged. Although changes in the PFAS amounts after cooking depended on fish species and cooking method used, cooking is generally not an effective approach to reduce dietary exposure to PFASs, especially PFOS.

Conflict of interest

The authors declare that they have no competing financial interests.

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Appendix A. Supplementary data

Supplementary data (8 tables and 3 figures) to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.01.024.

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