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Effects of different cooking methods on fatty acid profiles in four freshwater fishes from the Laurentian Great Lakes region

Margaret R. Neff [a], Satyendra P. Bhavsar [a,b,*], Eric Braekevelt [c], Michael T. Arts [d,1]

[a] Ontario Ministry of the Environment, Sport Fish Contaminant Monitoring Program, Environmental Monitoring and Reporting Branch, 125 Resources Road, Toronto, Ontario M9P 3V6, Canada
[b] Centre for the Environment, University of Toronto, 33 Willcocks Street, Suite 1016V, Toronto, Ontario M5S 3E8, Canada
[c] Food Research Division, Health Canada, 251 Sir Frederick Banting Driveway, Tunney's Pasture, Ottawa, Ontario K1A 0K9, Canada
[d] Environment Canada, National Water Research Institute, 867 Lakeshore Road, PO Box 5050, Burlington, Ontario L7R 4A6, Canada

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ABSTRACT

Fish is often promoted as a healthy part of the human diet due its high content of long chain n-3 polyunsaturated fatty acids (LC-PUFA). Previous studies have shown that cooked fish can have different fatty acid profiles than raw fillets, depending on the cooking method and fish species. In this study, the fatty acid content of broiled, baked or fried skinless, boneless fillets of four fish species from the tributaries of the Great Lakes, or connecting rivers, was compared to fatty acid profiles in raw sections from the same fillet. Cooking treatments had little effect on n-3 fatty acid content; however, fried treatments generally had higher n-6 and MUFA content, which is likely a result of the cooking oil used (canola). Broiling or baking is generally the most healthy option presented in this study, as these methods result in lower levels of less-favourable fatty acids; however, the choice of cooking oil may also influence the overall fatty acid content in cooked fish.

1. Introduction

Freshwater and marine fish are often considered to be a healthy component of the human diet, due to relatively high ratios of polyunsaturated to saturated fatty acids (PUFA:SAFA) compared to other animal food sources (Health Canada, 2011). In particular, fish contain high concentrations of n-3 long-chain PUFA (LC-PUFA), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). These fatty acids have been identified as essential elements of the human diet (Arts, Ackman, & Holub, 2001) because they cannot be synthesized in amounts adequate for optimal health (Gerster, 1998; Pawlosky, Hibbeln, Novotny, & Salem, 2001). These essential n-3 fatty acids have been, and continue to be, investigated extensively in health studies, where the benefits of dietary consumption of n-3 LC-PUFA have been found in relation to cardiovascular disease, diabetes, inflammatory diseases, and neurological/neuropsychiatric disorders (Yashodhara et al., 2009).

Nutritional guidelines from various health agencies worldwide now provide recommendations for the dietary intake of EPA + DHA and/or n-3 fatty acids (e.g., European Food Safety Authority, 2012; Koletzko et al., 2008; Kris-Etherton, Harris, & Appel, 2002; Simopoulos, 1989; U.K Scientific Advisory Committee on Nutrition, 2004). In addition, diets with n-3:n-6 ratios close to 1 (Simopoulos, 2002, 2008) and PUFA:SAFA ratios >0.4 (Food and Agriculture Organization of the United Nations & World Health Organization, 1994) are also recommended for optimal health. Health Canada (2011) recommends the consumption of at least 150 g of cooked fish each week as part of a healthy diet.

In the Great Lakes region, ~4.2 million adults consume at least one Great Lakes sport fish meal over the course of a year (Imm, Knobeloch, & Anderson, 2005). Recently, Neff et al. (submitted for publication) analysed fatty acid content in several important sport fish species from Lake Erie and found that eight of the analysed 15 species had an EPA + DHA content which met the recommended daily intake of 250 mg (European Food Safety Authority, 2012; Koletzko et al., 2008). In addition, all species

* Corresponding author at: Ontario Ministry of the Environment, Sport Fish Contaminant Monitoring Program, Environmental Monitoring and Reporting Branch, 125 Resources Road, Toronto, Ontario M9P 3V6, Canada. Tel.: +1 416 327 5863; fax: +1 416 327 6519.
E-mail addresses: s.bhavsar@utoronto.ca, satyendra.bhavsar@ontario.ca (S.P. Bhavsar), eric.braekevelt@hc-sc.gc.ca (E. Braekevelt), michael.arts@ec.gc.ca, Michael.Arts@Ryerson.ca (M.T. Arts).
[1] Present address: Department of Chemistry and Biology, Ryerson University, 350 Victoria Street, Toronto, ON M5B 2K3, Canada.

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analysed had optimal n-3:n-6 and PUFA:SAFA ratios (Neff et al., submitted for publication). These results further corroborate the conclusion that freshwater fish are a healthy dietary choice for human consumers. However, previous studies have also highlighted that fish fatty acid content can vary with a variety of factors, such as species, season, fish size, and geographical location (e.g., Snyder & Hennessey, 2003; Wang, Miller, Perren, & Addis, 1990). In addition, the fatty acid content of raw, uncooked fish flesh may not be an accurate reflection of what is consumed by humans post-cooking.

Results from studies examining the effects of cooking on the fatty acid content of fish are variable, and overall suggest that both cooking method(s) and species influence whether an effect of cooking will be observed. Previous studies, covering a variety of fish species, have reported significantly lower EPA and DHA content after frying (e.g., Bakar, Rahimabadi, & Che Man, 2008; Candela, Astiasaran, & Bello, 1997, 1998; Gladyshev, Sushchik, Gubanenko, Demirchieva, & Kalachova, 2006; Stephen, Jeya Shakila, Jeyasekaran, & Sukumar, 2010; Türkkan, Cakli, & Kilinc, 2008). Additionally, some studies reported changes to the PUFA:SAFA and/or n-3:n-6 ratios after cooking (Candela et al., 1997, 1998). In contrast, a number of studies have reported no effects of various cooking methods on fish fatty acid composition (e.g., de Castro et al., 2007; Fajmonová, Zelenka, Komprda, Kladroba, & Sarmanová, 2003) or attributed observed treatment effects to the incorporation of cooking oil (e.g., Larsen, Quek, & Eyres, 2010; Weber, Bochi, Ribeiro, Victório, & Emanuelli, 2008). Notably, most studies to date have examined marine species, often from fish farms, and no studies, to our knowledge, have examined the effects of different cooking methods on freshwater fish species from the Laurentian Great Lakes region.

In this study, four commonly consumed sport fish were collected from southern Ontario Great Lakes tributaries for a comparative analysis of fatty acid content after the application of three different cooking treatments. In particular, we highlight changes in the n-3 LC-PUFA, EPA and DHA, as well as in n-3:n-6 and PUFA:SAFA ratios.

2. Materials and methods

2.1. Sample collection and preparation

A total of 21 individual fish were collected in 2010 and 2011 from southern Ontario river systems as a part of the Sport Fish Contaminant Monitoring Program of the Ontario Ministry of the Environment (Ontario Ministry of the Environment, 2013): Chinook salmon (*Oncorhynchus tshawytscha*, $n = 5$) from the Credit River, common carp (*Cyprinus carpio carpio*, $n = 5$) and white sucker (*Catostomus commersonii*, $n = 2$) from the Thames River, lake trout (*Salvelinus namaycush*, $n = 4$) from the Niagara River, and walleye (*Sander vitreus*, $n = 5$) from the Welland River. Fish were euthanized chemically by the anaesthetic Tricaine Methane Sulfonate or via a physical blow to the head, in accordance with the Animal Care Class Protocols developed by the OMNR Fisheries Animal Care Committee.

After collection, each fish sample was processed to yield two boneless, skinless fillets, which were then divided into 16 parts and then recombined to yield four sub-samples (Fig. S1). As the recombination of fillet sections inherently increases the surface area of each sub-sample, the individual sections were placed as adjacent as possible during the cooking procedure. This was done to reduce the influence of any potential variation in fatty acid content throughout the fillet (e.g., Wills & Hopkirk, 1976). One sub-sample was left raw and analysed for fatty acid content. The remaining three sub-samples were first subjected to one of three cooking treatments: broiling, baking, or frying. All sub-samples were frozen at −20 °C until the time of the cooking experiment.

2.2. Cooking details

Prior to cooking, frozen fish sub-samples were thawed naturally to room temperature. Empty aluminium cooking dishes were weighed (Table S1), and then brushed evenly with 10 g of canola oil (*Brassica napus* L.) and reweighed. Canola oil is a commonly available and a widely used vegetable oil in Canada (Canola Council of Canada, 2011). Each sub-sample for cooking was placed into an oiled dish, and weighed again.

For the frying treatment, an electric frying pan was set to 175 °C and allowed 10 min to reach the test temperature. The fish sub-samples in aluminium dishes were fried uncovered for 5 min, then flipped over and cooked for an additional 5 min. For the baking treatment, a small toaster oven was preheated to 200 °C, and the fish sub-samples in oiled aluminium dishes were baked uncovered for 15 min. For the broiling treatment, the same toaster oven was set to broil, and the fish sub-samples in oiled aluminium dishes were cooked uncovered for 10 min. An internal thermometer was used to record the broiling temperature, which was 300 °C. After cooking, the sub-samples were allowed to cool to room temperature, and then the total weight (dish + oil + fish) was measured and recorded (Table S1). Each cooked sub-sample was wrapped in aluminium foil and frozen at −20 °C for subsequent fatty acid analysis.

2.3. Fatty acid analysis

Wet muscle tissues were weighed, freeze-dried, then re-weighed on a Sartorious (model ME5) microbalance. Analysis involved three steps: gravimetric extraction, derivitization, and quantification on a gas chromatograph (GC). An internal standard (5 α-cholestane; Sigma–Aldrich; C8003) was added to the tissue before extraction to estimate percent recovery during the extraction procedure. Lipids were extracted (three times) in chloroform:methanol (2:1) (Folch, Lees, & Sloane-Stanley, 1957). The three extracts from each sample were combined into an acid-washed 15 ml centrifuge tube. This was followed by a salt wash (0.9% aqueous NaCl solution). After centrifugation (4000 rpm) to remove non-lipid material, the overlying lipid-containing solvent layer was collected and evaporated down to exactly 2 ml. From this volume, 200 μL aliquots of sample extract were pipetted into pre-weighed, seamless, tin cups (Elemental Microanalyses Ltd., catalogue No. D4057). The solvent in the tin cups was then evaporated at room temperature and the remaining lipid weighed on a Sartorious ME-5 microbalance to provide a gravimetric measure for percent of dry weight of total lipid content. The remaining lipid in the bulk extracts was stored at −85 °C for later analyses.

Prior to analyses, fatty acids were methylated to fatty acid methyl esters (FAME) using the sulphuric acid in methanol method (Christie, 1989). Three individual pure fatty acid standards (20:2n-6, 20:5n-3, and 22:6n3; Sigma catalogue #s E3127, E7006 and D2534, respectively), were used to estimate the derivitization efficiency (mean efficiency = 100%). FAME concentrations were quantified on an Agilent 6890 GC with the following configuration: splitless injection; column = Supelco (SP-2560 column); oven = 70 °C (hold 1 min) to 140 °C at 20 °C min^{-1} and hold for 5 min; 170 °C at 4 °C min^{-1}, then to the final temperature of 240 °C at 2 °C min^{-1} and hold for 12 min, carrier gas = helium, 20 cm s^{-1}; detector = FID @ 250 °C; injector = 250 °C; total run time = 64 min $sample^{-1}$. A 37-component FAME standard (Supelco #47885-U) was used to identity and quantify (4-point calibration curves) FAME in the samples (unknowns), by comparing their retention times to those of the FAME standard. Results are reported as μg FAME mg dry weight $tissue^{-1}$ and also as % contribution of each FAME to total quantified FAME.

2.4. Statistical analysis

The change in the amount of a fatty acid (mg) in a fish sample after cooking was assessed by calculating the percent change in fatty acid content from the initial raw sample, by the following steps:

$$FAC_r * W_{un}/100 = FA_{un} \quad (1)$$

$$FAC_c * W_c/100 = FA_c \quad (2)$$

$$(FA_c - FA_{un})/FA_{un} * 100 \quad (3)$$

where FAC_r is the fatty acid content (mg/100 g wet weight or WW) of the raw sub-sample of a fish, W_{un} is the uncooked weight (g) of a treatment sub-sample of the same fish, FAC_c is the post-cooking fatty acid content (mg/100 g WW) of the treatment sub-sample, and W_c is the post-cooking weight (g) of that treatment sample. FA_{un} and FA_c thus equal the amount (mg) of fatty acid in a treatment sub-sample, pre- and post-cooking. These values are used to calculate the percent change in the fatty acid (mg) due to cooking (i.e., Eq. (3)). This was calculated a total of three times for each fish sample (fried, baked and broiled portions), for EPA, DHA, n-3, n-6, SAFA, MUFA, PUFA and ∑FA values.

In these calculations, the fatty acid content of the raw portion of the fish sample (i.e., FAC_r) is used to estimate the amount of fatty acid (mg) in the aliquots of fish, prior to cooking. Fatty acid content was not measured in the actual treatment aliquots of the fish sample prior to cooking. Thus, it was assumed that measurements of fatty acids in the raw portion of the fish were applicable to pre-cooked portions sampled from that same fish.

Differences in fatty acid content among cooking treatments for each species were assessed using repeated measures ANOVA. This technique is similar to a paired *t*-test, which tests for significant differences in the effect of a cooking treatment on the fatty acid content within a species, by examining the changes in each individual sample. This method removes the influence of any differences due to the individual samples in each species group by examining the within-sample change across the treatments, rather than the overall before and after values for each treatment for the group as a whole. Differences among treatments in EPA, DHA, n-3, n-6, and SAFA, MUFA and PUFA were compared for each species, measured as wet weight content (mg/100 g WW), dry weight content (mg/100 g DW) and proportion of total fatty acids (%). This analysis was not done for white sucker, due to insufficient sample size ($n = 2$).

Fatty acids are often reported on a wet weight basis in fish fatty acid studies, because this most accurately reflects the fatty acid content ingested upon consumption. However, fatty acid content measured on a dry weight basis may be more appropriate when making comparisons among cooking treatments. This is because differential water loss due to evaporation (i.e. when different cooking methods are used) can erroneously suggest differences in fatty acid content when the results are expressed solely on a wet weight basis. In addition, we provide fatty acid content measured as a proportion of all measured fatty acids, because this reporting method is useful for comparisons of patterns among species which differ in size and total lipid content.

Statistical analysis was performed in R (R Core Team, 2013). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Fatty acid content in raw fish

Fatty acid content (mg/100 g WW) in raw fillets varied by species. On a dry weight basis, lake trout had the highest content of EPA, DHA, n-3, n-6, SAFA, MUFA and PUFA, while walleye had the lowest EPA, n-6, SAFA, MUFA and PUFA content, and common carp the lowest DHA and n-3 content (Table S2). On a proportional basis, raw common carp fillets had the lowest beneficial fatty acids (e.g., EPA and DHA, as well as n-3 and PUFA), and the highest n-6, SAFA and MUFA (Table S3). Walleye had the highest proportion of EPA (6%), while Chinook salmon had the highest proportion of DHA (25%), n-3 (40%) and PUFA (51%).

3.2. Fatty acid content among treatments

Cooked samples had a lower moisture content than raw samples for all four species, but % moisture was generally similar for each cooking treatment, with lake trout having the largest differences among treatments (Fig. S2). Loss of moisture during the cooking process may have been exacerbated by the sample preparation protocol, as each sub-sample was four small pieces, cooked as one.

Examination of the difference in the actual amount of fatty acids (mg) between the raw portion of a fish sample and the corresponding three cooked portions indicated small changes in EPA, DHA, n-3, SAFA, with the majority of samples within ±50% of uncooked portions (Fig. 1). Differences in fatty acid content (mg/100 g) between cooked and uncooked treatments were greater for n-6 and MUFA, where changes were generally positive, with the majority of samples +50% (Fig. 2; note the change in scale of the *y*-axis from Fig. 1). In some cases, percent change values were quite large – for example, one fried walleye sample had 1229 mg MUFA compared to 122 mg in the raw portion (904% increase). This difference in MUFA content is within the expected range, however, given that all cooked samples were treated with 10 g of canola oil. As canola oil is comprised of ~64% MUFA, 10 g of canola oil is roughly equivalent to 6400 mg MUFA. The influence of canola oil retention in the cooked treatments will be discussed further below.

Differences in fatty acid content (mg/100 g) among treatments varied by species, fatty acid and cooking method (Tables 1, S2 and S3). EPA and DHA content (mg/100 g DW) did not significantly differ between cooked and uncooked fillets in any of the species examined ($p > 0.05$, Table 1). The following discussion is based on wet weight measurements (Table 1). Total n-3 content did not statistically differ between cooked and uncooked fillets for Chinook salmon and common carp ($p > 0.05$, Table 1). Fried samples of all four species were generally statistically higher in n-6 and MUFA content compared to other cooking treatments (Table 1). SAFA was also generally statistically higher in fried samples, but with less distinction from other treatments (Table 1). Differences in PUFA content between cooked and raw fillets were less consistent across the species, but generally fried samples had the highest PUFA content and raw samples the lowest (Table 1).

Cooked and uncooked fillets had significantly different n-3:n-6 and PUFA:SAFA ratios for some species (Table 1). Fried Chinook salmon n-3:n-6 ratios were 2.5× lower than raw samples, and 1.8× lower than broiled samples; however, there was no significant difference among cooking treatments in the ratios of ∑PUFA:SAFA. In contrast, for common carp, there were no significant differences between raw and cooked n-3:n-6 ratio, while fried PUFA:SAFA ratio was 2.1× greater than those for broiled fillets. Fried and baked lake trout n-3:n-6 ratios were 1.3× and 1.1× lower, respectively, than raw samples, while fried PUFA:SAFA ratio was 1.1× greater than uncooked and broiled samples. For walleye, baked, broiled and fried n-3:n-6 ratios were all significantly lower (1.5×, 1.7× and 3×, respectively) than raw samples, while fried samples were ~1.3× greater than all other treatments in PUFA:SAFA ratio.

There were also significant differences in the relative proportions of various fatty acids between cooked and raw fillets

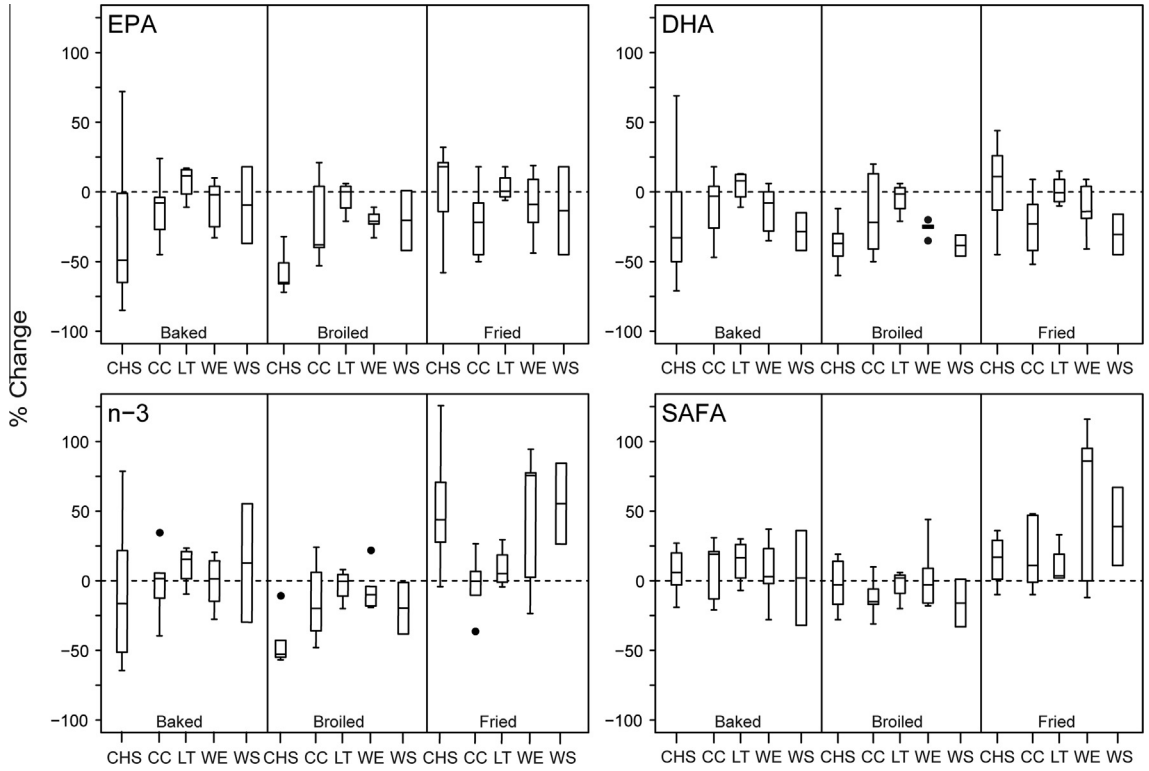

Fig. 1. Percent change in the amount of fatty acid (mg) between raw and cooked (baked, broiled or fried) fillets for (a) EPA, (b) DHA, (c) n-3 and (d) SAFA, for Chinook salmon (CHS), common carp (CC), lake trout (LT), walleye (WE) and white sucker (WS).

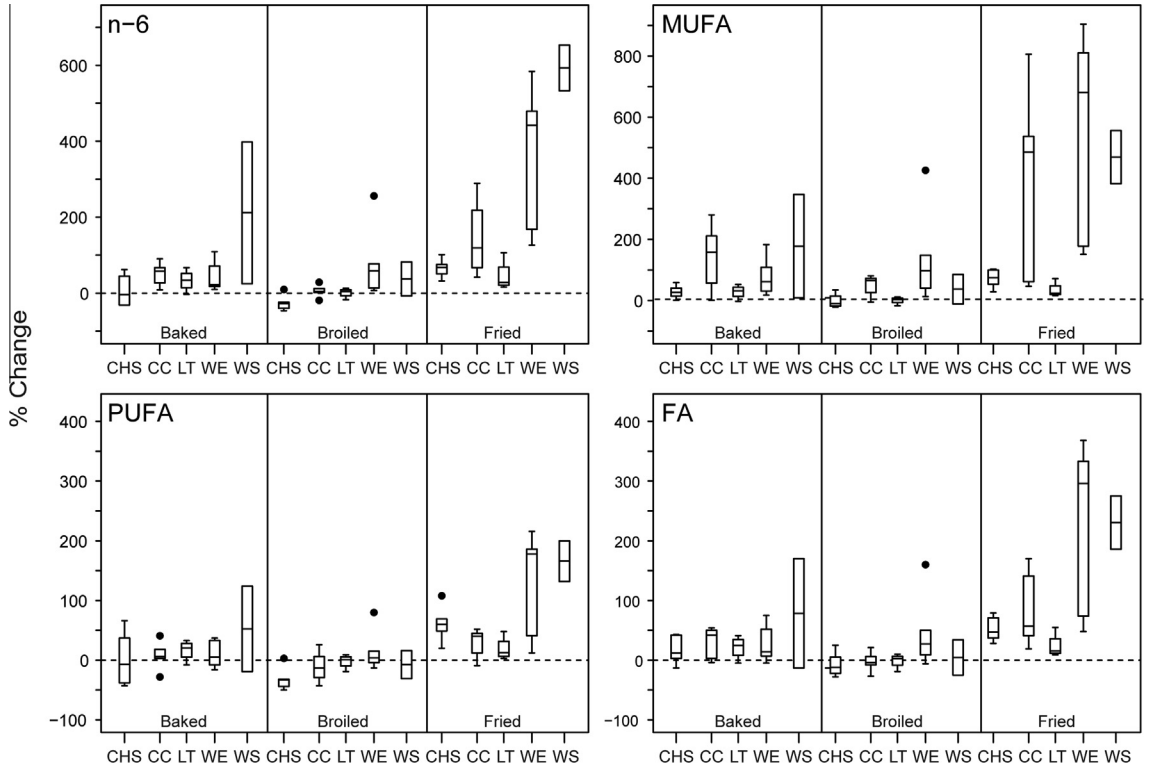

Fig. 2. Percent change in the amount of fatty acid (mg) between raw and cooked (baked, broiled or fried) fillets for (a) n-6, (b) MUFA, (c) PUFA and (d) FA, for Chinook salmon (CHS), common carp (CC), lake trout (LT), walleye (WE) and white sucker (WS). Note that the *y*-axis is on a different scale from Fig. 1.

(Table S3). There were significant differences in %EPA and %DHA among cooked and raw fillets of Chinook salmon, lake trout and walleye, which was likely a result of the relative increase or decrease of other fatty acids across cooking treatments (Table S3). In addition, the relative proportion of MUFA was significantly greater in fried fillets compared to all other treatments for

Table 1
Summary of mean values ± standard deviation (mg/100 g WW) of ALA, LIN, EPA, DHA, n-3, n-6, SAFA, MUFA, PUFA and total FA, as well as n-3:n-6 and PUFA:SAFA, for all five species. Superscripts indicate results of repeated measure ANOVA for comparison of each of the four treatments, where significant differences among pairs of treatments are indicated using different letters. Significant pairwise comparisons were found using the Holm–Sidak method (RM ANOVA) or Tukey test (RM ANOVA on Ranks). Fatty acids denoted with † are those analyses which were conducted using an RM ANOVA on Ranks.

Species	FA	N	Raw	Baked	Broiled	Fried
Chinook salmon	ALA	5	60 ± 62^{a}	96 ± 72^{c}	82 ± 77^{b}	150 ± 86^{d}
	LIN	5	66 ± 70^{a}	139 ± 87^{c}	107 ± 93^{b}	256 ± 110^{d}
	EPA	5	55 ± 34	60 ± 45	61 ± 55	58 ± 47
	DHA†	5	231 ± 106	250 ± 159	260 ± 199	226 ± 151
	n-3	5	429 ± 278	507 ± 371	503 ± 440	529 ± 381
	n-6	5	147 ± 128^{a}	232 ± 161^{b}	200 ± 178^{a,b}	344 ± 178^{c}
	n-3:n-6†	5	3.6 ± 1.1^{a}	2.2 ± 0.2^{a,b}	2.7 ± 0.4^{a}	1.4 ± 0.3^{b}
	SAFA	5	355 ± 353^{a}	407 ± 345^{a,b}	399 ± 388^{a,b}	455 ± 381^{b}
	MUFA	5	445 ± 524^{a}	750 ± 596^{b}	629 ± 615^{a,b}	1188 ± 696^{c}
	PUFA	5	576 ± 405^{a}	739 ± 531^{a,b}	702 ± 616^{a,b}	873 ± 556^{b}
	PUFA:SAFA†	5	2.2 ± 0.8	2.1 ± 0.5	2.1 ± 0.5	2.3 ± 0.6
	FA	5	1376 ± 1276^{a}	1896 ± 1462^{b}	1730 ± 1612^{a,b}	2516 ± 1625^{c}
Common carp	ALA	5	85 ± 69^{a}	130 ± 167^{a,b}	60 ± 47^{a}	217 ± 131^{b}
	LIN	5	335 ± 252^{a}	501 ± 516^{a,b}	321 ± 243^{a}	723 ± 432^{b}
	EPA†	5	58 ± 36	50 ± 66	30 ± 24	75 ± 64
	DHA†	5	21 ± 6	21 ± 19	16 ± 5	26 ± 14
	O-3	5	203 ± 124	239 ± 285	135 ± 80	370 ± 234
	O-6	5	455 ± 284^{a}	615 ± 590^{a,b}	415 ± 268^{a}	873 ± 493^{b}
	O-3:O-6	5	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
	SAFA	5	1058 ± 439^{a}	1372 ± 700^{b}	1338 ± 694^{a,b}	1426 ± 598^{b}
	MUFA	5	1891 ± 1019^{a}	2869 ± 1596^{a}	2404 ± 1423^{a}	3574 ± 1368^{b}
	PUFA	5	658 ± 403^{a,b}	854 ± 874^{a,b}	551 ± 340^{a}	1242 ± 718^{b}
	PUFA:SAFA	5	0.6 ± 0.2^{a,b}	0.6 ± 0.3^{a,b}	0.4 ± 0.1^{a}	0.8 ± 0.3^{b}
	FA	5	3607 ± 1843^{a}	5094 ± 3050^{b,c}	4293 ± 2443^{a,b}	6242 ± 2642^{c}
Lake trout	ALA	4	263 ± 117^{a}	385 ± 95^{c}	306 ± 109^{b}	417 ± 83^{d}
	LIN	4	268 ± 121^{a}	472 ± 113^{c}	332 ± 117^{b}	560 ± 70^{d}
	EPA	4	322 ± 115	374 ± 82	355 ± 82	365 ± 91
	DHA	4	785 ± 189	897 ± 124	870 ± 139	879 ± 105
	O-3	4	1828 ± 609^{a}	2188 ± 458^{b}	2040 ± 493^{a,b}	2183 ± 431^{b}
	O-6	4	703 ± 271^{a}	981 ± 245^{b}	817 ± 261^{a}	1057 ± 190^{b}
	O-3:O-6	4	2.7 ± 0.4^{a}	2.3 ± 0.2^{b,c}	2.6 ± 0.3^{a,b}	2.1 ± 0.1^{c}
	SAFA	4	1588 ± 475^{a}	1960 ± 368^{b}	1790 ± 416^{a,b}	1926 ± 359^{b}
	MUFA	4	3005 ± 774^{a}	4173 ± 632^{b}	3512 ± 720^{a,b}	4406 ± 409^{b}
	PUFA	4	2532 ± 875^{a}	3169 ± 689^{b}	2857 ± 751^{a,b}	3258 ± 642^{b}
	PUFA:SAFA	4	1.6 ± 0.1^{a}	1.6 ± 0.1^{a,b}	1.6 ± 0.1^{a}	1.7 ± 0.1^{b}
	FA	4	7125 ± 2114^{a}	9302 ± 1630^{b}	8159 ± 1856^{a,b}	9590 ± 1403^{b}
Walleye	ALA	5	48 ± 34^{a}	76 ± 37^{b}	98 ± 47^{c}	185 ± 44^{d}
	LIN	5	24 ± 17^{a}	82 ± 33^{b}	129 ± 79^{c}	345 ± 75^{d}
	EPA	5	47 ± 25	52 ± 29	48 ± 23	46 ± 20
	DHA	5	144 ± 80	156 ± 95	139 ± 72	140 ± 67
	O-3	5	285 ± 168^{a}	337 ± 189^{a,b}	335 ± 158^{a,b}	414 ± 144^{b}
	O-6	5	91 ± 45^{a}	155 ± 59^{a}	195 ± 87^{a}	412 ± 91^{b}
	O-3:O-6	5	3 ± 0.4^{a}	2.1 ± 0.5^{b}	1.8 ± 0.6^{b}	1 ± 0.2^{c}
	SAFA	5	222 ± 131^{a}	273 ± 131^{a,b}	277 ± 118^{a,b}	342 ± 97^{b}
	MUFA	5	253 ± 171^{a}	485 ± 199^{a,b}	645 ± 306^{b}	1370 ± 306^{c}
	PUFA	5	376 ± 214^{a}	492 ± 245^{a}	530 ± 225^{a}	826 ± 224^{b}
	PUFA:SAFA	5	1.7 ± 0^{a}	1.8 ± 0.1^{a}	1.9 ± 0.2^{a}	2.4 ± 0.2^{b}
	FA	5	852 ± 515^{a}	1249 ± 568^{a}	1453 ± 614^{a}	2538 ± 607^{b}
White sucker	ALA	2	13 ± 2	116 ± 106	42 ± 18	265 ± 3
	LIN	2	33 ± 3	242 ± 215	94 ± 40	554 ± 8
	EPA	2	92 ± 14	104 ± 35	95 ± 20	92 ± 32
	DHA	2	136 ± 2	125 ± 40	112 ± 18	113 ± 32
	O-3	2	277 ± 19	397 ± 203	294 ± 69	513 ± 85
	O-6	2	71 ± 7	279 ± 228	128 ± 45	590 ± 0
	O-3:O-6	2	3.9 ± 0.1	1.7 ± 0.6	2.3 ± 0.3	0.9 ± 0.1
	SAFA	2	272 ± 25	351 ± 150	300 ± 52	446 ± 72
	MUFA	2	318 ± 59	1064 ± 814	557 ± 173	2129 ± 4
	PUFA	2	348 ± 26	676 ± 430	422 ± 114	1103 ± 85
	PUFA:SAFA	2	1.3 ± 0	1.8 ± 0.4	1.4 ± 0.1	2.5 ± 0.2
	FA	2	938 ± 109	2092 ± 1394	1280 ± 339	3677 ± 161

Chinook salmon, common carp and walleye, while patterns in %SAFA and %PUFA were more variable by species (Table S3).

3.3. Fatty acid content and health guidelines

Researchers and health organizations worldwide suggest that the human diet should consist of a n-3:n-6 ratio $\geqslant 1$ (Simopoulos, 2002, 2008), a PUFA:SAFA ratio >0.4 (Food and Agriculture Organization of the United Nations & World Health Organization, 1994) and a daily intake of EPA + DHA of at least 250–450 mg for the general adult population (European Food Safety Authority, 2012; Health Canada, 2007; Koletzko et al., 2008; Simopoulos, 1989; U.K Scientific Advisory Committee on Nutrition, 2004). It should be noted that requirements for specific subsets of the population may vary – an average daily intake of 200–300 mg DHA is recommended for pregnant and lactating women, and 500 mg/day EPA + DHA for primary prevention of cardiovascular disease (Kris-Etherton, Grieger, & Etherton, 2009).

Mean values for wet weight EPA and DHA content in each cooking treatment, as well as mean n-3:n-6 and PUFA:SAFA ratios were compared to recommended dietary guidelines (Table 2). All species that had n-3:n-6 ratios ⩾1 and PUFA:SAFA ratios >0.4 in raw fillets maintained similar ratios in cooked fillets.

4. Discussion

Frying, baking or broiling does not appreciably affect levels of EPA and DHA, or total n-3 fatty acids, in Chinook salmon, common carp, lake trout or walleye (except for DHA in walleye). From a human health perspective, this is important as cooking fish does not reduce the amount of beneficial fatty acids consumed compared to what is contained in a raw fillet. However, there were differences in n-6, SAFA, MUFA and PUFA content, which could be important from a human health standpoint, particularly with respect to n-3:n-6 and PUFA:SAFA ratios.

Fried samples generally had the highest content (mg/100 g) of n-6, SAFA and MUFA, on both a dry and wet weight basis relative to the raw, broiled and baked fillets. For this experiment, 10 g of canola oil was added to each treatment prior to cooking, but was not added to the raw samples. Canola oil generally contains ~64% MUFA, 28.5% PUFA and 7.5% SAFA, with n-6 fatty acids dominating the PUFA profile (Canola Council of Canada, 2011). Thus, when canola oil is retained in cooked fish, it would be expected to raise the amount total fatty acids in the fillet (and particularly MUFA and n-6 PUFA content), as well as lower n-3:n-6 and raise PUFA:SAFA ratios. Thus, the fatty acid profile of canola oil, and its retention in the fillet post-cooking, explains some of the observed differences in fatty acid content between cooked and uncooked treatments, as well as the variation among different cooking methods. For example, fried fillets – a cooking method that is expected to retain the greatest amount of oil – had the greatest total fatty acid content out of all cooking treatments, and often demonstrated a greater total fatty acid content than the uncooked samples. More specifically, increases in n-6, SAFA, MUFA and PUFA were observed in cooked treatments, as well as lower n-3:n-6 ratios (Chinook salmon, lake trout, walleye) and higher PUFA:SAFA ratios (common carp, lake trout, walleye) in fried samples. However, it should be noted that canola oil contains neither EPA nor DHA; its n-3 fatty acid content is solely comprised of ALA (α-linolenic acid). Therefore, any difference in EPA and DHA content across treatments is not due to the addition of canola oil. Overall, these results further highlight the importance of oil selection when cooking fish (Agren & Hanninen, 1993). Canola oil is one of the most healthy options of potential cooking oils, and utilisation of a different oil may worsen the fatty acid profile from a human dietary perspective.

Aside from the addition and retention of canola oil, there are additional factors which may have influenced the variation in fatty acid content between treatments – for example, variation in fatty acid content within an individual fish (e.g., Wills & Hopkirk, 1976). To reduce the influence of within-sample variation, in the fatty acid content, two fillets from each fish sample were first divided into 16 sections and then recombined to make four sub-samples, and subsequent data analyses considered the fatty acid content of the raw portion to be representative of the fatty acid content of the treatment portions prior to cooking. Although this method would help in minimizing variation in fatty acid content among sub-samples prior to cooking, some differences in the content for the raw and pre-cooked portion could be expected.

Based on the observed differences in fatty acid content between raw and cooked fillets, as well as on how the fatty acid content of these four species compares to current dietary guidelines for EPA + DHA and n-3:n-6 and PUFA:SAFA ratios, it appears that lake trout and Chinook salmon are healthy food choices. These species, whether baked, broiled, or fried, contain high EPA and DHA levels, and very favourable n-3:n-6 and PUFA:SAFA ratios compared to the other two species analysed in this study. However, frying and baking fillets in canola oil increased n-6 and MUFA content compared to uncooked and broiled samples.

It should be noted, that fatty acid content and cooking method are not the only health considerations to make when selecting a fish species for consumption. Many freshwater systems, including the Laurentian Great Lakes region from where fish samples were collected for this study, currently have restrictive fish consumption advisories due to harmful levels of environmental contaminants (e.g., mercury and PCBs) in the fish tissue. Recently, there has been interest in estimating the relative risk in consuming fish due to contaminant concentrations with the relative benefit of obtaining essential fatty acids (e.g., Dewailly, Ayotte, Lucas, & Blanchet, 2007; Foran et al., 2005; Turyk et al., 2012; Neff et al., submitted for publication). This trade-off can have important implications for human health. For example, while broiled lake trout samples in this study had, overall, the most healthy 'among-species' and

Table 2
Assessment of each species according to three nutritional criteria: PUFA:SAFA, n-3:n-6, and the amount of EPA + DHA in a 227 g fish meal. According to multiple sources, favourable values for optimal human nutrition for the general population are PUFA:SAFA >0.4, n-3:n-6 >1.0, and EPA + DHA intake of approximately 250–450 mg/day (Simopoulos 2002, 2008; FAO/WHO 1994; European Food Safety Authority, 2012; Koletzko et al. 2008; U.K Scientific Advisory Committee on Nutrition, 2004). For each species, mean values for all three criteria were calculated, for each cooking method. Grey boxes indicate values which meet recommendations; bolded values under EPA + DHA indicate values which meet the 450 mg/day guideline.

Species	Nutritional criteria favourable values cooking method	PUFA:SAFA ⩾ 0.4	n-3:n-6 ⩾ 1.0	EPA + DHA (mg/227 g meal) >250–450 mg/day
Chinook salmon	Raw	2.2	3.6	**650.4**
	Baked	2.1	2.2	**702.4**
	Broiled	2.1	2.7	**728.4**
	Fried	2.3	1.4	**644.1**
Common carp	Raw	0.6	0.5	178.5
	Baked	0.6	0.4	161.0
	Broiled	0.4	0.3	103.7
	Fried	0.8	0.4	229.7
Lake trout	Raw	1.6	2.7	**2513.0**
	Baked	1.6	2.3	**2883.5**
	Broiled	1.6	2.6	**2781.0**
	Fried	1.7	2.1	**2824.5**
Walleye	Raw	1.7	3.0	433.2
	Baked	1.8	2.1	**472.6**
	Broiled	1.9	1.8	422.7
	Fried	2.4	1.0	420.5

'within-treatment' fatty acid profile, lake trout from the sampled location also have very restrictive consumption advisories, where the general population is advised to consume only one 227 g fish meal per month for fish between 55 and 70 cm, and 0 fish per month if >70 cm (Table S4, Ontario Ministry of the Environment, 2013). Also, members of the sensitive population (i.e., children and women of child-bearing age) are advised to not to consume lake trout from the Niagara River or Chinook salmon from the Credit River (Table S4). Thus, despite favourable EPA + DHA content, n-3:n-6 and PUFA:SAFA ratios, lake trout and Chinook salmon from these locations are not likely to be a healthy way of obtaining these dietary fatty acid recommendations. In contrast, walleye in this study were collected from a location with much less restrictive consumption advisories (Table S4). While not as fatty as other species, EPA + DHA content for walleye ranged from 421 to 473 mg per 227 g fish meal, and when consumed according to consumption advisories, would be a healthy way to obtain EPA + DHA in the diet. However, Philibert, Vanier, Abdelouahab, Chan, and Mergler (2006) has shown that the assimilation of EPA and DHA from fish tissue into human blood plasma upon ingestion can vary by fish species, particularly among lean and fatty fish.

In conclusion, we demonstrate that broiling, baking or frying fish likely has no appreciable effect on the EPA or DHA content of Chinook salmon, common carp, lake trout or walleye (except for DHA) collected from Great Lakes connecting channels or tributaries in southern Ontario, Canada. However, broiling or baking is overall healthier than frying because fish cooked by these two methods were generally lower in the amounts fatty acids such as n-6 which are currently already present in high amounts in the typical western diet.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.04.104.

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