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Fatty Acid Composition of Three Different Marine Fish Under Different Culinary Process

*Perera SBST[1], Jayasinghe GDTM[2], De Silva MSW[1] and Jinadasa BKKK[2]**

[1]Department of Food Science and Technology, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila, Sri Lanka

[2]Analytical Chemistry Laboratory, National Aquatic Resources Research & Development Agency (NARA), Colombo-15, Sri Lanka

Abstract

The aim of this study was to compare the effect of three different culinary treatments (Chili curry, Coconut milk curry and Ambulthial) on the content of main Poly-Unsaturated Fatty Acids (PUFA) of three marine fish species namely, yellowfin tuna (*Thunnus albacares*), swordfish (*Xiphias gladius*) and spotted sardine (*Amblygaster sirm*). After culinary treatments, Gas Chromatography (GC) coupled with a Flame Ionization Detector (FID) and Bligh and Dyer fat extraction method was employed for fatty acid profile and total fat amount determination respectively. Higher amounts of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) were retained in Ambulthial compared with the other two treatments. However, cooking ingredients and the moisture level may also influence the overall EPA and DHA content in cooked fish.

Keywords: PUFA; Culinary treatments; Fish; Gas chromatography; Flame ionization detector

Introduction

Marine fish are very popular due to its easy access, availability, and limited ethnical or religious barriers. Fish are considered a healthy alternative for red meat because of its low-fat content, high availability of good fats and a high ratio of Poly-Unsaturated to Saturated Fatty Acids (PUFA:SFA) [1]. Fish lipids are dominated by saturated fatty acids such as palmitic (C16:0) and myristic (C14:0), Mono-Unsaturated Fatty Acids (MUFA) such as oleic (C18:1) and palmitoleic acids (C16:1) and also Poly-Unsaturated Fatty Acids (PUFA) like Eicosapentaenoic Acid (EPA, 20:5n-3) and Docosahexaenoic Acid (DHA, 22:6n-3) [2]. These long-chain n-3 fatty acids have been continuously studied comprehensively in health studies because of the benefits of dietary consumption of long-chain PUFA and its relation to cardiovascular diseases. EPA has been found to be particularly effective against certain mental conditions; especially depression [3].

International agencies worldwide provide difference recommendation for dietary intake of EPA, DHA and other omega 3 fatty acids. According to the American Heart Association (AHA) recommended the intake of EPA+DHA has been 2-4g/day for the patient [4]. In addition to that researchers made the recommendation for best Omega 6:3 ratios for good health condition [5], reported the ratio of 4:1 (omega 6:3) was associated with decreasing 70% of cardiovascular diseases, while the ratio of 2.5:1 reduced rectal cell proliferation in patients with colorectal cancer. In the same study, he mentioned that ratio of 2-3:1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5:1 had a beneficial effect on patients with asthma, whereas a ratio of 10:1 had adverse consequences. United States Food and Drug Administration (USFDA) recommended consuming 225-340g of fish per week as a part of a healthy diet [6].

There is a great difference in fish consumption can be seen between continent and countries. In Sri Lanka, per capita, fish consumption is about 15.8kg/year in the year of 2016 [7,8] analyzed several small pelagic fish species from Sri Lanka, and 36 fish species out of the 40 fish species showed a total amount of omega 3 PUFA contributed nearly 90% of total PUFA and it met the recommended average daily intake of DHA+EPA (250mg/day) [9]. These results highlighted that marine fish contributed to a healthy diet and life of humans. However, the fat and fatty acid profile of fish vary for a number of reasons; catch season and location, individual fish size, sex, fish physiology [10,11].

Moreover, fat and fatty acid profile of fresh fish do not reflect accurately what humans consume after cooking. The ultimate fatty acid content is influenced by cooking methods and culinary practices [12]. Cooking oil and additional ingredients such as coconut milk, spices also contributed

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Correspondence:
Jinadasa BKKK, Analytical Chemistry Laboratory, National Aquatic Resources Research & Development Agency (NARA), Colombo-15, Sri Lanka.
E-mail: *jinadasa76@gmail.com*

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to the final fatty acid content of cooked fish [13]. There are numerous studies regarding the fatty acid content of raw seafood, and limited studies available for the effects of cooking methods.

The objective of this study was commonly consumed three marine fish species collected from Gampaha district, Sri Lanka, and comparative analysis of fatty acid content after three culinary treatments.

Materials and Methods

Sample collection

Fish samples were collected from local fish markets, supermarkets, and fish vendors on June-Dec 2016 as a part of the case study of the total dietary survey in Gampaha district, Sri Lanka conducted by 2016, covered the all divisional secretarial area of the district. Yellowfin tuna, (n=9)YFT (*Thunnus albacares*), swordfish (n=11), SF (*Xiphias gladius*) and spotted sardine, (n=8) SS (*Amblygaster sirm*) were selected for this study as they are the frequently consumed fish species. YFT and SS were sampled at approximately 1kg while SS was sampled 1kg in each. Samples were then immediately transported to the laboratory for further analysis.

Sample preparation

Fish samples were washed using clean running water in the laboratory kitchen. Then, fish samples were pre-prepared for cooking by removing the head, fins, scales, gut, and bones if necessary. After, each sample was divided into two subsamples as one for culinary treatment and one for raw analysis (stored in -20°C until analysis). Other portions of the sample were used to cook by three common culinary treatments.

Culinary treatments

One portion of the sample of each species was treated with 3 culinary treatments based on the ratio found in the survey; chili fish curry, fish curry with coconut milk and ambulthial. Cooking variables were randomly assigned among the fish samples were YFT (3:4:2), SS (3:3:2) and SF (3:5:3) ratios. The spices and cooking time were based on the survey results. The cooking time was between 15-25 min according to the survey results. Fish chili curry was prepared with powdered red chili as main spices (60g/kg fish). Milk curry was prepared using coconut milk as the main ingredient. Amubulthial is a traditional fish culinary method which is prepared with Garcinia paste and black pepper powder. Portions of samples were allowed to cool to room temperature and blended well using a domestic food blender. Then, the samples stored in the freezer for further analysis.

Fat analysis

Samples were thawed in the refrigerator (4°C) overnight. The total fat amount was analyzed using the Bligh and Dyer method know as a solvent extraction method [14]. Briefly, 10.0±0.1g of sample was weighed into a clean glass container followed by homogenization (DIAX 900, Heidolph, Germany) for 2 min with a mixture of water (5.0 ml), chloroform (15.0 ml) and methanol (30.0 ml). Then it was re-homogenized 30 seconds with additional chloroform (15.0 ml) and water (15.0 ml). Then the mixture was centrifuged at (K241, Centurion, UK) 3000 rpm, 10 min, and the chloroform layer was separated. Oil content was determined gravimetrically after evaporating a measured aliquot of the combined chloroform phase to dryness under oven (UFE-500, Memmert, UK). Each sample was analyzed triplicate.

Fatty acid analysis

The Fatty Acid Methyl Ester (FAME) was prepared based on the AOCS (American Oil Chemists' Society) method Ce 2-66 with a small modification. An Internal Standard (IS), heptadecanoic acid (17:0) from Sigma-Aldrich was added to measure the recovery and quantitative calculation. Weigh accurately correspondence amount (100 mg) of oil extract into the screw-capped tube and removed the methanol under the nitrogen purge. Next, added 2.0 ml of 0.5 ml methanolic sodium hydroxide and heated 5 min in boiling water bath. Then added 3.0 ml boron trifluoride reagent and 1.0 ml hydroquinone solution and again heated 5 min in boiling water bath. After cooling, added 10 ml saturated salt-solution and 5.0 ml n-heptane, shaked vigorously for 30 sec and let the tubes stand for a few minutes until separation of the phases. Then 1 ml of the organic phase was removed out and transferred to a 2 ml vial for Gas Chromatography (GC) analysis. GC-FID (Shimadzu, GC 2014, Japan) analysis was conducted using 1 µl split injections onto a 105m (Fused-Silica) DB wax column (Restak, PA). The temperature of the injector and detector was maintained at 225°C and 285°C respectively. Helium was used as the carrier gas and column flow was held at 1 ml/ min. A calibration curve was constructed using the Restek-35077 fatty acid FAME mix.

Statistical analysis

The data analysis was performed using SPSS software (version 17.0). One-way ANOVA analysis method was used to analyze the differences in fatty acid profile for three different culinary methods. Fatty acid profiles were reported on a wet weight basis because this most precisely reflects the fatty acid content ingested upon consumption. In addition, fatty acid content given as a proportion of all measured fatty acids, because this reporting method is suitable for evaluations of patterns among species which vary in size and total fat content.

Results

Fat content in raw and cooked fish

Table 1 shows the mean total fat % in raw samples and cooked samples with the culinary treatments. Each value represents Mean ± SD. The highest mean total fat % was observed in coconut milk curry treatment. Two other cooking methods showed lower values for mean total fat % compared to the samples cooked by coconut milk method.

Fatty acid composition in the raw and cooked fish sample

Though 37 fatty acids could be identified by using the food industry FAME mix (Restek-35077), nutritionally important 4 fatty acids were mainly considered to compression which are, C18:2 (Linoleic acid), C18:3 (α- Linolenic acid), C20:5 (Eicosapentaenoic acid, EPA) and C22:6 (Docosahexaenoic acid, DHA). Meanwhile, the selected fatty acid % of raw samples and cooked samples are listed in Table 2 for three fish species separately. Each value represents a Mean ± SD of three replicates (n=3).

Table 1: Total fat % (w/w) values of SW, YFT,and SS before and after culinary treatments.

Fish species	Fresh	Culinary treatment		
		Ambulthial	Chilli curry	Coconut milk curry
SF	2.26±0.37	2.15±0.45	1.73±0.45	5.88±1.17
YFT	3.23±0.42	0.96±0.14	2.40±0.35	7.96±0.49
SS	3.70±0.39	3.63±0.34	4.16±0.19	5.98±0.60

Table 2: Changes of PUFA percentage in three fish species before and after culinary treatments.

Species	Treatment	C18:2		C18:3		C20:5		C22:6	
		Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
SF	AT	0.15±0.03	0.14±0.05	0.46±0.17	0.77±0.12	3.43±1.36	3.45±0.57	0.23±0.02	0.34±0.10
	CC	0.18±0.08	16.67±0.86	0.23±0.07	0.20±0.08	2.62±1.03	2.10±0.37	0.39±0.10	0.17±0.10
	CM	0.11±0.03	3.56±0.63	0.43±0.07	0.22±0.07	4.97±1.00	2.09±0.64	0.20±0.05	0.07±0.02
SS	AT	1.74±0.25	1.88±0.07	0.16±0.04	0.19±0.04	1.71±0.20	1.97±0.34	0.09±0.02	0.14±0.02
	CC	1.34±0.36	5.73±0.64	0.17±0.01	0.12±0.02	1.45±0.46	1.19±0.43	2.20±0.22	1.56±0.18
	CM	1.42±0.42	3.32±0.77	0.18±0.02	0.05±0.01	1.67±0.34	0.61±0.07	1.77±0.35	0.22±0.02
YFT	AT	0.06±0.01	0.37±0.08	0.02±0.01	0.55±0.05	1.27±0.12	1.48±0.12	0.83±0.27	1.01±0.23
	CC	0.33±0.08	10.43±1.01	1.12±0.18	0.91±0.28	0.99±0.18	0.47±0.19	0.37±0.12	0.26±0.27
	CM	0.16±0.07	1.98±0.24	0.36±0.10	0.29±0.09	0.32±0.06	0.12±0.05	1.12±0.21	0.48±0.09

AT- Ambulthial, CC-Chili Curry, CM-Coconut Milk curry

When considering differences in the mean fatty acid % between raw and cooked samples in all three fish species, the highest difference found in linoleic acid. It observed in the samples cooked with chili curry method followed by coconut milk curry for all three fish species. Other three selected fatty acids; α-Linolenic acid, EPA and DHA showed the highest mean difference % values in the samples cooked by ambulthial method for all three fish species compared to the mean difference of α-Linolenic, EPA and DHA in the samples cooked by other two culinary treatments methods.

Discussion

The above mentioned culinary treatments are the main fish processing method in Sri Lanka and raw fish consumption is a very rare condition. Heating is affected to enhance the fish flavor, taste, inactivate pathogenic microorganisms and increase shelf life [15]. Fish prepared as chili curry and ambulthial method does not appreciably affect the level of selected fatty acid, while all fish species treated with coconut milk for curry increased the mean total fatty acid content. Coconut milk constituted 11-18% fat and the majority were Saturated Fatty Acids (SFA). Total SFA content of coconut milk is about 16.5g/100g. Predominant saturated fatty acid in coconut milk is lauric acid (C12:0) it accounts for half of the total saturated fatty acids (8.8g/100g). Other major saturated fatty acids in coconut milk are myristic acid (3.8g/100g) and palmitic acid (2g/100g) [16]. According to that information, incorporation of coconut milk with fish causes SFA content to increase in cooked fish. SFA has a very low potential to oxidize due to thermal energy during the cooking process [17], hence total fatty acids % in the samples cooked using coconut milk method shows the highest increase (Table 1& 2) in the mean total fatty acid %.

Linoleic acid and α-linolenic acid EPA, and DHA are known as essential fatty acids for humans, which the human body cannot synthesize on its own [18]. Therefore, these two fatty acids should be taken from the individual's the diet. Previous studies reported EPA and DHA are important for proper fetal development, including neuronal, retinal, and immune function. EPA and DHA may also affect many aspects of cardiovascular functions including inflammation, peripheral artery disease, major coronary events, and anti coagulation [19]. According to the results, all three fish species show the highest differences of linoleic acid, which is observed in the samples, cooked as chili curry. It might be due to the high amount of chili as a spice in this cooking method.

According to the results, all three fish species have the lowest difference of α-linolenic acid.EPA and DHA were observed in ambulthial treatment compared to difference % values of those three fatty acids in the samples cooked the by other two cooking methods. These three fatty acids are not considerably available in the cooking ingredients used in the ambulthial cooking method. The reason for the increase of these three fatty acids in the ambulthial method can depend on two factors such as evaporation of moisture and incorporation of antioxidants from cooking ingredients. Antioxidant potential of spices used in the ambulthial method is high compared to the other two cooking methods. In the ambulthial cooking method, spices such as garcinia, chili, lemon juice, garlic, and turmeric powder were used [20,21]. Capsaicin from chili, [22], ascorbic acid and hesperetin, naringin, and lemon juice [23] are the main vitamins and main antioxidant substances in the ingredients.

When cooking fish, following three cooking methods, the external fatty acids are incorporated through cooking ingredients such as chili, pepper, garlic etc. Total fatty acid % of those spices are very low compared to coconut milk. Total fatty acid % of chili, pepper, and garlic is about 17.2%, 3.26% and 0.50% respectively (USDA 2016). These spices contain a high amount of polyunsaturated fatty acids compared to the saturated fatty acid content. An increase of total fatty acid % due to the addition of external fatty acids from spices during the cooking process is very low in chili curry and ambulthial treatment. For this reason, it showed lowest difference values for mean total fatty acid % between raw and cooked samples of chili and ambulthial curry compared to the mean difference values of total fatty acid % in the samples cooked with coconut milk method.

In general, cooked samples should have lower moisture content than raw samples [24] but moisture % was generally similar for each cooking treatment due to adding some amount of water for boiling the fish and other ingredients. There are some additional factors affect the fatty acid profile of fish species such as sex, the maturity level of fish etc [25]. Hence, to reduce the variation within the samples, each fish filleted and recombined with other subsamples.

Conclusion

Fatty acids composition of the selected three species varied with the type of culinary treatment. Hence, further research needs to be confirmed the fatty acids in spices that are used in culinary treatment.

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