International Food Research Journal 21(6): 2147-2153 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Comparison of protein, total fat, and omega-3 fatty acids content in yellowtail catfish (*Pangasius pangasius*) and long tail shad (*Hilsa (clupea) macrura*) in raw and pressurized fish

*Asmah, R., Siti Sumaiyah, S. A. and Nurul, S. R.

Department of Nutrition and Health Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, 43400, Selangor, Malaysia

Article history

Received: 4 February 2014
Received in revised form: 3 May 2014
Accepted: 5 May 2014

Keywords

Yellowtail catfish
Long tail shad
Protein
Omega-3 fatty acids

Abstract

Omega-3 fatty acids have been shown to reduce the risk of chronic diseases like cardiovascular disease and cancer as well as promote brain development among infants and children. This study was carried out to compare total protein, fat and omega-3 fatty acids content of raw and pressurized fish of *P. pangasius* (yellowtail catfish) and *H. macrura* (long tail shad). The fish was cooked using pressure cooker for six minute to be pressurized. The protein content was determined by using Kjedahl method while total fat was determined using solvent extraction using chloroform and methanol. Fatty acid methyl esters (FAME) were prepared by a direct transesterification method, and quantified by gas chromatography using external standard. Results showed that marine fish *H. macrura* (long tail shad) had higher content ($p < 0.05$) of protein (18.30 ± 0.040 g/100 g), fat (10.965 ± 1.610 g/100 g), EPA (11.83 ± 0.02 g/100 g) and DHA (5.96 ± 0.31 g/100 g) compared to freshwater fish *P. pangasius* (yellowtail catfish). The protein content of pressurized fish was higher compare to raw fish, but there was no difference in total fat and omega-3 fatty acids content between raw and pressurized of freshwater fish *P. pangasius* and marine fish, *H. macrura*. In conclusion, marine fish are better source of protein, fat and omega-3 content, while pressurized fish shown to have comparable amount of protein, fat and omega-3 fatty acids content with raw fish. The result obtained assist the consumers to prepare a healthy menu in order to retain the protein and omega-3 fatty acids content of fish through healthy way of cooking.

Introduction

Fish can be classified into fresh water fish and marine fish. Yellowtail catfish (*Pangasius pangasius*) is a fresh water fish while long tail shad (*Hilsa (clupea) macrura*) is a marine fish. The main different of these two fish is according to their habitat. Fresh water fish spend some or all of their lives in freshwater like lakes, while marine fish derived from ocean. However, for some reason, marine fish is preferred compared to freshwater fish among Malaysians. Demand for freshwater fish has remained low in the past few years. Lack of information on the nutritional value of freshwater fish could be one of the main reasons why marine fish is preferred compare to freshwater fish.

Fish is rarely eaten raw but it is usually cooked in many different ways before consumption. Cooking method might affect the nutrient content of fish. During cooking, chemical and physical reactions take place that improve or impair the nutritional value. Bognar (1998) reported that digestibility of protein is increased due to protein denaturation in food but the content of thermolabile compounds, fat soluble vitamins or polyunsaturated fatty acids is often reduced. Cooking process will induces water loss in the food, that in turn increases its lipid content in most cases and only some fat is lost in the case of oiliest fish. However, this effect is also dependent on type of cooking method.

Previous studies showed that long chain n-3 polyunsaturated fatty acid in fish reduced CHD mortality (Wang *et al.*, 2006) and cardiovascular risk factors like serum triglyceride concentration, blood pressure, arrhythmias and inflammation (Calder, 2004). However, there are still limited studies on the effect of different types of fish in secondary prevention of CHD. Previous intervention trial, give more focus on lean fish oil effect rather than inclusion of fish in the diet. Moreover, this will give less accurate result as lean fish which known to have lower amounts of omega-3, eicosapentaenoic acid (EPA), and docosahexaenoic acid compare to fatty fish, that have been less investigated (Moore *et al.*, 2006).

From all the various components that affect quality of the edible portion of the fish, the lipid composition

*Corresponding author.
Email: *asmah@upm.edu.my*
Tel: +63 89472470; Fax +63 89426769

is the most important. Fish need polyunsaturated fatty acid (PUFAs) to provide tolerance to low water temperatures. Decreases in PUFA concentrations in lipids would therefore be expected in warmer waters (nearer the equator) like Malaysia. Although it is generally recognized that PUFA composition may vary among species of fish, little attention has been paid to the PUFA composition of different species when selecting fish for diets (Rahman *et al.*, 1995).

However the knowledge of lipid content and fatty acid composition in freshwater fish is still limited to a few species. Moreover, lipid may deteriorative through processing and storage which can affect flavor, odor, color and texture (Nurhan, 2007). The same thing goes to protein, studies suggest that, the digestibility of protein could be reduced as a result of chemical reaction that taking place when food is treated at high temperature (Usydus *et al*., 2008). Therefore, this study was conducted to compare protein content, total fat and long chain omega-3 fatty acid composition in different type of fish (fresh water fish and marine fish) for raw and pressurized fish.

This comparative study will produce data on protein, total fat and omega-3 content of freshwater and marine fish. Somehow these kinds of data are still limited and yet there is no comparison study on raw and pressurized fish have been made. Thus, this information will provides health professionals and consumers with options in food choices and meal planning with the goal towards achieving the recommended intakes for omega-3 fatty acids. Furthermore, the result obtained can help the consumers in preparing a healthy menu in order to retain the protein and omega-3 content of fish through healthy type of cooking.

Materials and Methods

Sample

In this study, sampling process was based on convenient sampling method. Fish samples that have been used were yellowtail catfish (*P. pangasius*) and long tail shad (*H. macrura*). A simple survey on the availability of the sample has been carried out in order to get the sample. The freshness of the fish was taken into consideration while purchasing a sample such as the brightness of the skin, the translucent corners of the eye, the firm flesh, the fresh aroma and the stiffness of the fish. Then the sample was homogenized and only the edible portions were taken for experiment. For raw fish, the fish was stored in the freezer (-20ºC) until processed in lab. For pressurized fish, fish was cooked first in pressure cooker for six minutes before experiment.

Determination of protein content using Kjedahl method

Firstly, the edible portion of fish (freshwater and marine fish) was weighed accurately (1g) into a digestion tube. Then, two Kjeltabs Cu 3.5 (alternatively 7 g K_2SO_4 and 0.8 g $CuSO_4$ x 5 H_2O) were added to the flask. Twelve mL of concentrated H_2SO_4 was added and shake vigorously to homogenous the sample. The exhaust system is attached to the digestion tube in the rack and set the water aspirator to full effect. The lack was load with exhaust into a preheated digestion block (420ºC). After 5 minutes the water aspirator was turn off. The digesting was continued until all samples are clear with a blue/green solution. After that, the rack of tubes was removed with exhaust still in place and allowed to cool for 10-20 minutes. Next, 80 ml deionized water was added to the tubes. Then 25 – 30 ml of receiver solution was added to a conical flask, placed into the distillation unit and raises the platform so that the distillate outlet is submerged in the receiver solution. The digestion tube was placed in the distillation unit and the safety door was closed. The 50 ml of 40% NaOH will be dispensing into the tube. Next the steam valve was opened on the Kjeltec 2200 and distilled for approximately 4 minutes. At about 90% of distillation time the distillate platform was lowered on the Kjeltec 2200. Finally, the distillate will be titrated with standardized HCI (0.1 N OR 0.2 N) until blue/grey end point is achieved (AOAC, 1989).

Formula of protein determination:

$$\% N = \frac{(T-B) \times N \times 14{,}007 \times 100}{\text{Weight}_{\text{sample}} \text{ (mg)}}$$

% Protein = N x F (6.25)
N = Nitrogen
T = Titration
B = Blank
F= Factor

Extraction of fat

The total lipid content of food is commonly determined by organic solvent extraction methods. The accuracy of these methods greatly depends on the solubility of the lipid in the solvent use and the ability to separate lipids from complexes with other macromolecules. The lipid content of a food determined by extraction with one solvent may be quite different from the content determined with another solvent of different polarity. However, according to Kinsella *et al*. (1977) for fish, the most suitable method for fat extraction is solvent extraction using chloroform and methanol.

Solvent extraction method

The tissue (30 g) was homogenized in 60 ml methanol and 30 ml chloroform (2: 1) according to the method of Kinsella *et al.* (1977). Representative samples of fish (30 g) were homogenized in Warring blender for 2 min with a mixture of methanol (60 ml) and chloroform (30 ml). One volume of chloroform (30 ml) was added to the mixture and after blending for an additional 30 sec distilled water (30 ml) was added. The homogenate was stirred with a glass rod and filtered through Whatman No. 1 filter paper on a Buchner funnel with slight suction. The filtrate was transferred to a separatory funnel. The lower clear phase was drained into a 250 ml round-bottom flask and concentrated with a rotary evaporator at 40ºC. The concentrated lipid extract was quantitatively transferred to a vial and made up to a final volume of 20 ml with chloroform. Aliquots (2 ml each) were evaporated in tared vials to constant weight under nitrogen to determine the lipid content. Butylated hydroxytoluene (BHT) at a concentration of 0.05% (of the lipid) was added to the remaining lipid extract, and the extract was stored at -40ºC for further analysis.

$$\text{Fat (\%)} = \frac{W - W_0 \text{ X } 100}{S}$$

S = Weight (g) of sample before drying
W_0 = Weight (g) of flask without fat
W = Weight (g) of flask with fat

Methylation preparation

During FAME preparation, pre-test was done to identify the suitable method for mehylation process. The pre-test includes sodium methoxide method, potassium hydroxide method and boron trifluoride (BF_3) method. Finally boron trifuoride (BF_3) was chosen while comparing the peak.

Firstly 0.125 g of fish oil was put in a test tube. Second, 0.5 ml of boron trifluoride (BF_3) in MeOH (14%) was added to the test tube. Next the test tube containing the fish oil and boron trifluoride (BF3) in MeOH (14%) was incubated using incubator shaker in 55ºC for 1.5 hour. Then, 0.5 ml of saturated sodium hydrogen carbonate ($NaCHO_3$) and 0.75 ml of n-hexane was then added to the test tube. The mixture was mixed and shake well using vortex for about 30 second. Then, the mixture was store for 5 minutes under room temperature so that it will form two layers. Lastly, 0.5 ml of upper layer contain hexane was carefully pipette off and insert into vial for Gas Chromatography (GC) analysis (Kinsella *et al*., 1977).

Identification of FAMEs standard

To determine each type of fatty acid, standards of FAMEs 37 was used. Fatty acids of the sample were determined by comparing the retention time of sample with those of the standards FAMEs 37 that were used as external standard in this study for each chromatography peak of each fatty acid. Comparing chromatography peak with external standard is called identification process, while comparing chromatography peak with internal standard is called quantification process. Since there is no internal standard used in this study due to some limitation, so quantification cannot be made.

Gas Chromatography (GC) analysis

Omega-3 fatty acids composition of fish samples were analyzed using gas chromatography (GC) model 6890 (USA Agilent Technology) equipped with split-splitness injector, detector Hewlett-Packard EL-980 flame ionization detection (FID) system to separate and quantify each FAMEs components. FAMEs were separated using DB-23 column (60 m x 0.25 mm LD, 0.15 µm polyethylene glycol film) (Agilent, J&W Scientific GC Column , USA). Chromatography data were recorded and integrated using Chemstations software (version 6.0). Oven temperature was held at 50ºC, 1 min, then increased to 175ºC at 4ºC/ min and lastly increased to 230ºC, held for 5 min. Temperatures for injector and detector were set at 250ºC and 280ºC, respectively. 1 µl of sample volume was injected with split ratio of 0:50 at column temperature 110ºC. Carrier gases that were used for the system are helium gas, 1.0 ml/min controlled at 103.4 kPa, hydrogen and air used for FID was held at 275.6 kPa (David *et al*., 2002).

Statistical analysis

The results were analyzed by using Scientific Package of Social Science (SPSS) version 17.0. Two different set of statistics, which is descriptive and analytical statistics was applied. The descriptive statistic was used to analyze mean, standard deviation (SD) and coefficient variation (CV) whereby analytical statistics, ANOVA was used to compare the means of the protein, fat and omega-3 content of freshwater fish and marine fish for raw and pressurized fish. The confidence interval of statistic is 95% and the significant value is set at $P < 0.05$.

Results and Discussion

Protein content

The Kjeldahl method is the most common reference method for the determination of protein

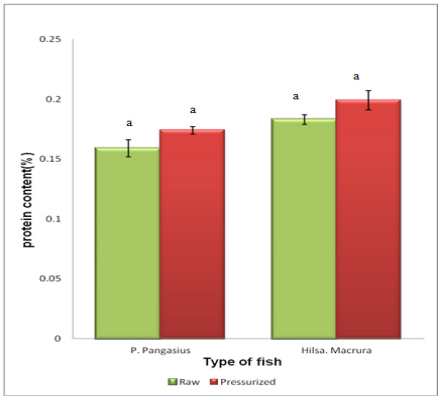

Figure 1. Graph on total protein content of yellowtail catfish (*P. pangasius*) and marine fish, long tail shad (*H. macrura*) in raw and pressurized fish.

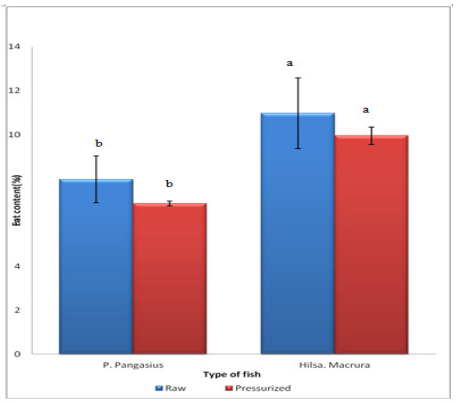

Figure 2. Graph on total fat content of yellowtail catfish (*P. pangasius*) and marine fish, long tail shad (*H. macrura*) in raw and pressurized fish

in fish. Result showed that the protein content was highest in marine fish, long tail shad (*H. macrura*) with 18.30% ± 0.04 compared to yellowtail catfish (*P. pangasius*), 15.90% ± 0.01 respectively which is in agreement with the data from Tee *et al.* (1997) who found 16.60% of total protein content for yellowtail catfish (*P. pangasius*) and 18.40% for long tail shad (*H. macrura*) (Figure 1). On the other hand, pressurized yellowtail catfish (*P. pangasius*) had 17.40% ± 0.03 of protein compared to 15.90% ± 0.01 for raw fish. Similarly, pressurized long tail shad (*H. macrura*) had 19.90% ± 0.01of total protein compared to 15.90% ± 0.01 of protein for raw fish. It was reported previously that freshwater known to have a good potential of protein ranged from 19.9% to 23.0% (Zuraini *et al*., 2006). Despite of that, the present study found that marine fish long tail shad (*H. macrura*) had higher protein content compared to freshwater fish yellowtail catfish (*P. pangasius*). The differences in protein content could be due to the difference in species studied which influences the variability in protein content of fresh water and marine fish. It was shown in Figure 1 that pressurized fish had higher protein content compared to raw fish. Similarly, Garcia-Arias *et al.* (2003) reported that

Table 1. Fatty acid profiles (% total fatty acids) of of yellowtail catfish (*P. pangasius*) and marine fish, long tail shad (*H. macrura*) in raw and pressurized fish

Species	*P.pangasius* Raw	*H.macrura* Raw	*P.pangasius* Pressurized	*H.macrura* Pressurized
C14:0	1.17±0.43	4.80±0.98	1.06±0.08	3.74±0.43
C16:0	24.08±1.52	22.60±7.66	20.22±1.18	15.78±3.82
C18:0	6.6±1.09	17.42±2.40	11.66±0.65	25.60±4.59
C20:0	0.38±0.16	5.97±3.68	0.33±0.02	7.45±0.21
C22:0	1.23±0.43	1.60±1.24	1.17±0.07	0.90±0.11
C23:0	0.71±0.15	3.53±2.68	0.36±0.02	0.73±0.64
C24:0	0.68±0.17	2.01±3.65	0.55±0.03	1.54±1.34
ΣSFA	**34.85**	**57.93**	**35.35**	**55.74**
C24:1	0.28±0.03	0.99±0.43	0.93±0.05	0.33±0.31
C18:1n9t	5.08±0.32	1.43±0.56	5.01±0.29	2.15±0.13
C18:1n9c	21.73±1.2	11.37±1.34	19.43±1.15	14.37±1.90
ΣMUFA	**27.09**	**13.79**	**25.37**	**16.85**
C18:2n6t	1.19±0.37	2.39±0.48	1.14±0.06	1.50±0.06
C18:2n6c	7.09±0.42	1.69±0.58	7.03±0.41	3.40±0.65
C18:3n3	1.13±0.34	5.95±4.75	1.03±0.06	0.39±0.35
C20:5n3	2.45±1.74	11.83±0.02	2.38±0.13	11.49±2.08
C22:6n3	0.23±0.12	5.96±0.31	0.18±0.01	5.16±2.18
ΣSFA	**34.85**	**57.93**	**35.35**	**55.74**
ΣMUFA	**27.09**	**13.79**	**25.37**	**16.85**
ΣPUFA	**12.09**	**27.82**	**11.76**	**21.94**
Σ *n3*	**2.68**	**17.79**	**2.56**	**16.65**

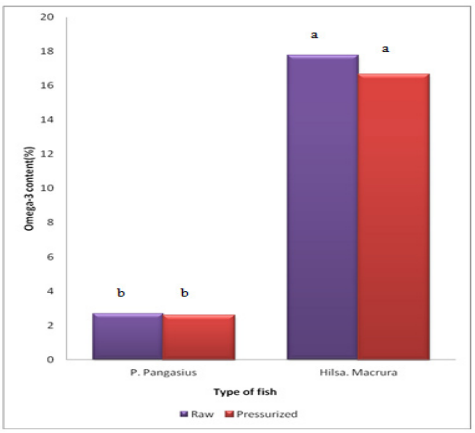

Figure 3. Graph on total omega-3 content of yellowtail catfish (*P. pangasius*) and marine fish, long tail shad (*H. macrura*) in raw and pressurized fish

the protein content was significantly higher protein content in cooked fish compared to raw fish. During cooking, the chemical and physical reactions that take place improve or impair the food nutritional value which the digestibility is increased due to protein denaturation. In other study, protein content of cooked fish was increased due to loss of moisture content of fish after cooking (Ersoy, 2011).

Total fat content

Figure 2 shows that fat content is higher in marine fish, long tail shad (*H. macrura*) with 10.97% ± 1.61 compared to yellowtail catfish (*P. pangasius*), 7.96% ± 1.06, respectively. Tee *et al.* (1997) reported slightly lower fat content for both yellowtail catfish (*P. pangasius*) and long tail shad (*H. macrura*) which is 4.20% and 11.04% of total fat, respectively. In addition, pressurized yellowtail catfish (*P. pangasius*) and long tail shad (*H. macrura*)

had lower total fat content (6.86% ± 0.11 and 9.95% ± 0.40, respectively) in comparison with raw fish (7.96% ± 1.06 and 10.97% ± 1.61, respectively). The decrease in fat content of pressurize fish might be due to melting of fat.

Total fat content has been shown to be influenced by species, diet, gender and habitat of the fish (Rasoarahona *et al*., 2005). Marine fish lives in sea which exposed to different types of soil, while fresh water fish lives in pond and mostly was feed by farmer. Also, the body composition of fresh water fish is high in water; hence tend to have lower fat content (5-10%) (Osman *et al*., 2001). This was confirmed by Larsen *et al*. (2010) that found inverse relationship between moisture content in fish and total extractable lipid content.

Omega-3 fatty acids content

Table 1 presents fatty acid composition of raw and pressurized yellowtail catfish (*P. pangasius*) and long tail shad (*H. macrura*) fish. Gas chromatography analysis of fatty acid methyl esters from the lipids of fish of those samples revealed the presence of 16 fatty acids.

Total MUFA, PUFA and SFA

Figure 3 shows mean values of 15 fatty acids for seawater fish and freshwater fish, respectively. The fatty acid composition of yellowtail catfish (*P. pangasius*) were found to be 34.85% saturated (SFA), 27.09% monounsaturated (MUFAs) and 12.09% polyunsaturated (PUFA), whereas the fatty acid composition of long tail shad (*H. macrura*) consisted of 57.93% saturated (SFA), 13.79% monounsaturated (MUFAs) and 27.82% polyunsaturated (PUFA). Among those fatty acids, palmitic acid (C16:0) (24.08%) and oleic acid (C18: 1n9c) (21.73%) accounted the highest proportions of fatty acid in freshwater. While, the major fatty acid in marine fish species were palmitic acid (C16:0) (22.60%), stearic acid (C18:0) (17.42%), oleic acid (C18: 1n9c) (11.37%) and eicosapentanoic acid (C20:5n3) (11.83%).

Trend of MUFA, PUFA and SFA content in pressurized fish

The fatty acid content in pressurized fish tends to be lower compare to raw fish. This effect must be primarily due to fat loss produced by this process. However, the observed changes were not homogeneous for the different fatty acid because some fatty acid decreased, and some increased. Thus, fatty acid changes have to be a consequence of the action of heat. Mainly, favouring the loss of the more accessible fatty acids. Nevertheless, ANOVA result shows that there is no statistically difference between fatty acid content of raw and pressurizes fish for both marine fish, long tail shad (*H. macrura*) and freshwater yellowtail catfish (*P. pangasius*) at $p < 0.05$ value. This result is in agreement with study done by Garcia-Arias *et al.* (2003) which reported that fatty acid composition tend to be lower in cooked fish compare to raw fish.

Content of omega 3 fatty acids in fish sample

Two of the important groups of PUFA in human nutrition are the omega-6 and omega-3 fatty acids. For omega-3 fatty acids, the precursor of its family is ALA. Many studies have shown ALA, EPA and DHA are beneficial to our heart system and have protective effects on related fish been being investigated. The value of omega-3 fatty acids content calculated was expressed as the sum of the amount of ALA, EPA and DHA that exits in the fish.

Result showed that marine fish, long tail shad (*H. macrura*) have the higher content of eicosapentanoic acid (EPA) and docohexaenoic acid (DHA) which is 11.83% ± 0.02 (EPA) and 5.96% ± 0.31 (DHA) compare to freshwater yellowtail catfish (*P. pangasius*) which is only 2.45% ± 1.74 (EPA) and 0.23% ± 0.12 (DHA). Briefly, omega-3 content is 2.68% in freshwater yellowtail catfish (*P. pangasius*) and 17.79% in marine fish, long tail shad (*H. macrura*). This study has shown that marine fish were richer in omega-3 fatty acids content. Therefore, the consumption of marine fish can give benefits to cardiovascular patient (Shahidi and Miraliakbari, 2004).

When comparison was made between raw fish and pressurized fish, EPA and DHA content tend to be lower in pressurized fish compare to raw fish. In yellowtail catfish (*P. pangasius*) EPA content was 2.45% ± 1.74 in raw fish and 2.38% ± 0.13 in pressurized fish. While for DHA content were 0.23% ± 0.12 and 0.18% ± 0.01 in raw and pressurized fish, respectively. On the other hand, in long tail shad (*H. macrura*), EPA content was 11.83% ± 0.02 in raw fish and 11.49% ± 2.08 in pressurized fish whereas DHA content was 5.96% ± 0.31 and in 5.16% ± 2.18 in raw and pressurized fish, respectively.

However, when the comparison was made between four types of sample, ANOVA result of omega-3 content shows statistically different between raw fish yellowtail catfish (*P. pangasius*) and long tail shad (*H. macrura*), at $p = 0.036$ and also between pressurized fish of yellowtail catfish (*P. pangasius*) and marine fish long tail shad (*H. macrura*) at $p = 0.030$. This result had shown a similar result with the

comparative studies of freshwater fish and seawater fish of turkey species done by Ozogul *et al.* (2007) which this study shown that the omega-3 content of marine fish has a higher content compare to freshwater fish at significant value ($p < 0.05$). But the result show no statistically significant at $p < 0.05$ when the comparison was made solely between raw fish and pressurized fish of yellowtail catfish (*P. pangasius*). Similar result also shown that there is n statistically significant between raw fish and pressurized fish of long tail shad (*H. macrura*) at $p < 0.05$. This result had shown a similar result with the previous study which shown that the omega-3 content of cooked fish tend to decrease compare to raw fish but the result are less significant at p (<0.05) (Garcia-Arias *et al*., 2003). This finding is very important as it show that the pressurized fish can be good source of omega-3 as it able to retain the omega-3 content. However there are still some study that shown the increase of omega-3 in cooked fish compare to raw fish at significant value ($p < 0.05$) (Ersoy, 2011). This might due to different type of fish and method of cooking involved in that study.

Conclusion

The marine fish long tail shad (*H. macrura*) was better source of protein, total fat and omega-3 content compare to freshwater fish, yellowtail catfish us (*P. pangasius*). Pressurized fish can be applied in human daily dietary intake as an alternative way of retaining or maintaining nutrient (protein, total fat and omega-3) as it contain same nutrient as raw fish.

Acknowledgement

The authors would like to acknowledge the financial support from Universiti Putra Malaysia.

References

AOAC. 1989. Official method of analysis for nutrition labeling, 16th edition. Gaithersburg, Maryland, USA: AOAC International.

Bognár, A.1998. Comparative study of frying to other cooking techniques. Influence on the nutritive value. Grasas y Aceites 49:3-4.

Calder, P. C. 2004. n-3 fatty acids and cardiovascular disease: Evidence explained and mechanisms explored. Clinical Science 107(1):1-11.

David, F., Sandra, P. and Wylie, P. L. 2002. Improving the analysis of fatty acid methyl esters using retention time locked methods and retention time databases. Agilent Technologies Application Note 1-5.

Ersoy, B. 2011. Effects of cooking methods on the proximate, mineral and fatty acid composition of european eel (*Anguilla anguilla*). International Journal of Food Science and Technology 46(3):522-527.

Garcıa-Arias, M., Álvarez Pontes, E., Garcıa-Linares, M., Garcıa-Fernandez, M. and Sanchez-Muniz, F. 2003. Cooking–freezing–reheating (CFR) of sardine (*Sardina pilchardus*) fillets. effect of different cooking and reheating procedures on the proximate and fatty acid compositions. Food Chemistry 83(3):349-356.

Kinsella, J., Shimp, J., Mai, J. and Weihrauch, J. 1977. Fatty acid content and composition of freshwater finfish. Journal of the American Oil Chemists' Society 54(10):424-429.

Larsen, D., Quek, S. Y. and Eyres, L. 2010. Effect of cooking method on the fatty acid profile of new zealand king salmon (*Oncorhynchus tshawytscha*). Food Chemistry 119(2):785-790.

Moore, C. S., Bryant, S. P., Mishra, G. D., Krebs, J. D., Browning, L. M., Miller, G. J. and Jebb, S. A. 2006. Oily fish reduces plasma triacylglycerols: A primary prevention study in overweight men and women. Nutrition 22(10):1012-1024.

Nurhan, U. 2007. Change in proximate, amino acid and fatty acid contents in muscle tissue of rainbow trout (*Oncorhynchus mykiss*) after cooking. International Journal of Food Science & Technology 42(9):1087-1093.

Osman, H., Suriah, A. and Law, E. 2001. Fatty acid composition and cholesterol content of selected marine fish in malaysian waters. Food Chemistry 73(1):55-60.

Ozogul, Y., Ozogul, F. and Alagoz, S. 2007. Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of turkey: A comparative study. Food Chemistry 103(1):217-223.

Rahman, S. A., Huah, T. S., Nassan, O., and Daud, N. M. 1995. Fatty acid composition of some Malaysian freshwater fish. Food Chemistry 54(1):45-49.

Rasoarahona, J. R. E., Barnathan, G., Bianchini, J. P. and Gaydou, E. M. 2005. Influence of season on the lipid content and fatty acid profiles of three tilapia species (*Oreochromis niloticus*, *O. macrochir* and *Tilapia rendalli*) from Madagascar. Food Chemistry 91(4):683-694. .

Shahidi, F. and Miraliakbari, H. 2004. Omega-3 (n-3) fatty acids in health and disease: Part 1-cardiovascular disease and cancer. Journal of Medicinal Food 7(4):387-401.

Tee, E. 1999. School-administered weekly iron-folate supplements improve hemoglobin and ferritin concentrations in Malaysian adolescent girls. The American Journal of Clinical Nutrition 69(6):1249-1256.

Usydus, Z., Szlinder-Richert, J., Polak-Juszczak, L., Kanderska, J., Adamczyk, M., Malesa-Ciecwierz, M. and Ruczynska, W. 2008. Food of marine origin: Between benefits and potential risks. part I. canned fish on the polish market. Food Chemistry 111(3):556-563.

Wang, C., Harris, W. S., Chung, M., Lichtenstein, A. H., Balk, E. M., Kupelnick, B., Jordan, H. S. and Lau, J.

2006. n-3 fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary-and secondary-prevention studies: A systematic review. American Journal of Clinical Nutrition 84(1):5-17.

Zuraini, A., Somchit, M., Solihah, M., Goh, Y., Arifah, A., Zakaria, M. and Mat Jais, A. 2006. Fatty acid and amino acid composition of three local Malaysian *Channa* spp. fish. Food Chemistry 97(4): 674-678.