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Fatty acids composition of two fish species in family Leiognathidae under different cooking methods

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Abstract
Analysis of fatty acid compositions in marine fish under different cooking methods is important to promote understanding of potential relationship between consumption of fish and human health from nutrition. This study was carried out to determine the fatty acid composition in two Leiognathus spp. *Gazza minuta* and *Leiognathus dussumieri* compared at three cooking methods, steaming, boiling and deep frying. It was noted that the steamed and boiled cooked fish muscle of *Leiognathus dussumieri* had low percentages of saturated fatty acids but *Gazza minuta* had high percentages. It was observed that after deep frying some fatty acids (C14:0 and C16:0) in *Gazza minuta* had high percentages and other fatty acids (C15:0, C17:0, C18:0) were significantly low and present study has shown that high percentages of monounsaturated fatty acids were found in the steamed and boiled cooked muscles of *Leiognathus dussumieri* and *Gazza minuta.* It was noted that high amount of polyunsaturated fatty acids, namely eicosapentaenoic (EPA) and Docosahexaenoic (DHA) in steamed and boiled cooked muscles of *Gazza minuta* and low amount in deep fried cooked muscle were recorded. Major n-6 fatty acid, arachidonic acid was found in high amount in steamed and boiled cooked samples of *Gazza minuta* but it was low in deep fried samples. Concentration of arachidonic acid and Docosahexaenoic acid in steamed and boiled samples were slightly lower in *Leiognathus dussumieri*. Finally, it can be suggested that from the health point of view fried fish is not a good source of omega-3 and omega -6 fatty acids and it reduces the health benefits.

Keywords: Cooking methods, omega -3 and omega-6 fatty acids, Leiognathidae, pony fishes

1. Introduction

Fish play an important role in human nutrition and health as they provide a good balance of proteins, lipids, vitamins, minerals and essential fatty acids. All over the world the significance of sea foods has gained attention because of high content of n-3 (or omega 3) polyunsaturated fatty acids (n-3 PUFAs) such as eicosapentaenoic acid (EPA; C20: 5 n-3) and docosahexaenoic acid (DHA, C22: 6 n-3) (Kocatepe et *al.* 2011) [12]. These fatty acids have beneficial effects on diseases such as coronary heart disease (Harris & Shacky, 2004) cancer (Gerber *et al.* 2005) [8] and inflammatory diseases (Billuzzi, 2001) [1].

Fish is usually cooked in different ways before consumption. Cooking (boiling, baking, roasting, frying and grilling) improves hygienic quality of the food by inactivation of pathogenic microorganisms and enhances digestibility and bio-availability of nutrients in the digestive tract (Kocatepe *et al.* 2011) [12]. During cooking, chemical and physical reactions take place which either improve or impair the food nutritional value (e.g. Digestibility is increased because of protein denaturation in food) but the content of thermolabile compounds, fat- soluble vitamins or polyunsaturated fatty acids is often reduced (Bognar, 1998) [2].

Leiognathids belong to family Leiognathidae. The Leiognathids are demersal fishes and widely distributed in coastal waters of Indo-West Pacific tropical and sub-tropical regions (James, 1984) [5]. This family of fishes is generally recognized by its downward protractile mouth and is commonly available in shallow coastal waters, estuaries and mangrove areas. They are known as pony fishes or slip mouths due to highly protractible mouth which protract either dorsally, rostrally or ventrorostrally (Kimura *et al*. 2003) [11].

Family Leiognathidae consists of 3 genera, Leiognathus, Secutor and Gazza. The species of Secutor and Gazza remain distinct within the respective genera and from species of the genus Leiognathus. Secutor exhibits certain specialized characters like the upwardly directed protrusible mouth and Gazza shows the development of prominent teeth.

In this study *Gazza minuta* and *Leiognathus dussumieri* were chosen due to good consumer acceptance and being the most available pony fish species in the west coast of Sri Lanka. In this study the effects of three forms of cooking methods commonly used by Sri Lankan consumers (steaming, boiling and deep frying) were examined on the fatty acid profiles in particular species.

2. Materials and Methods

The two pony fish species used in this study were purchased from a local fish markets in west coast of Sri Lanka. They were packed in ice and transported to the laboratory. Fish were prepared using common house hold practices, removing head region and guts and then washed with tap water. These samples were divided into four groups for applying different cooking methods.

The first group was uncooked. The other three groups were cooked in the steam (30 minutes), boiling (30 minutes) and deep fried in white coconut oil (140 ^{0}C, 3-5 minutes).

2.1 Frying Procedure

Adequate amount of white coconut oil was heated to 140 ^{0}C in a deep frying pan. Fish samples were cut into several pieces and fried for three to five minutes until the fish turned to golden brown colour.

2.2 Fat extraction

Fat extraction was done by using Bligh and Dyer method. About 100g of fish sample was homogenized with 200 ml of methanol and then 100 ml of chloroform for about 2 minutes. Another 100 ml of chloroform was then added into it and homogenized for another 1 minute. Next, it was filtered under suction and the chloroform layer was separated by using the separating funnel. Separated chloroform layer was then evaporated in the water bath and in a oven for 1 hour at 70 ^{0}C. Extracted fat was then flushed with nitrogen and stored at -20 ^{0}C until analysis were done.

2.3 Fatty acid analysis

Preparation of n-3 fatty acids methyl ester was carried out according to Jham, Teles and Campos (1982) method. The fatty acids (25 g) were hydrolyzed with 500 ml of KOH in methanol (0.5M) at 100 ^{0}C for 5 min. in tightly capped glass bottles. After hydrolysis, the mixture was esterified with 200 ml of HCl in methanol (4:1 vol/vol) and then heated in an oil bath for 15 min. at 100 ^{0}C. The mixture was cooled and then 100ml of distilled water was added. The fatty acid methyl ester was extracted with 1000 ml of hexane. The hexane layer was dried quickly over anhydrous sodium sulphate and the solvent was removed using a vacuum rotary evaporator at 40 ^{0}C. The fatty acids methyl ester was stored at -20 ^{0}C in nitrogen until use.

The methyl esters of fatty acid were analysed by gas chromatography on the gas chromatograph (Supelco wax 10), the temperature of injector and detector 250 ^{0}C and 250 ^{0}C, the oven was first maintained at 190 ^{0}C and then programmed to 220 ^{0}C at the rate of 5 ^{0}C/ minute.

3. Statistical analysis

The data were analyzed using One-way ANOVA and two sampled t-test at significance level of 5% (Minitab Version 14).

4. Results & Discussion

Fatty acid profiles were studied in the most available two pony fish species *Gazza minuta* and *Leiognathus dussumieri* under different cooking processes such as steamed boiled and deep fried. In the present research, figure 1 shows the total fatty acid profiles (SFA, MUFA & PUFA) of the two pony fish species under different cooking processes and a significant difference ($p<0.05$) between the total saturated and polyunsaturated fatty acids were recorded.

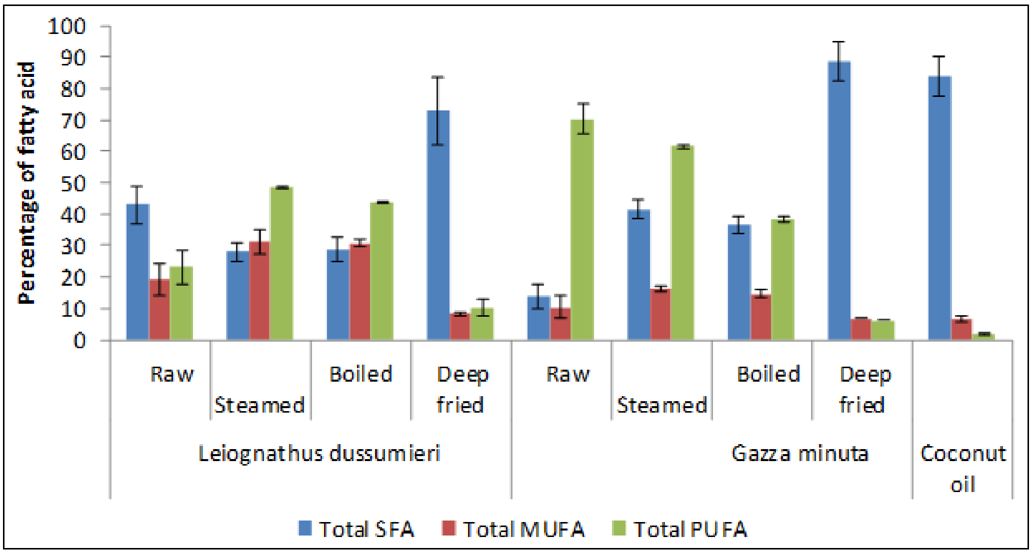

Fig 1: Fatty acid profile of raw and cooked fish muscles of *Gazza minuta* and *Leiognathus dussumieri*

The saturated fatty acid composition of cooked fish muscles from the two species are given in Table 1, along with the saturated fatty acid composition of the white coconut oil used for deep frying. Fresh white coconut oil was used for deep frying the fish (steak cross sections) from the two species, but the oil was not analysed after cooking.

It was noted that the steamed and boiled cooked fish muscle of *Leiognathus dussumieri* had low percentage of C14:0, C15:0, C17:0 and C18:0. The saturated fatty acid composition of fish muscle of *Leiognathus dussumieri* was greatly changed after deep frying, with high percentage of C14:0 fatty acid and low percentage of C15:0, C16:0, C17:0 and C18:0 fatty acids.

Present study has shown that the steamed and boiled cooked fish muscle of *Gazza minuta* had high percentages of C14:0, C15:0, C16:0 C17:0 and C18:0 fatty acids compared with those of raw muscle. C14:0 and C16:0 fatty acids in deep fried cooked *Gazza minuta* fish muscle showed high percentages, while the other fatty acids C15:0, C17:0 and C18:0 were significantly low compared with raw muscle.

In addition, major fatty acids of white coconut oil (C12:0 and C14:0) were significantly high in deep fried fish muscles of *Gazza minuta* and Leiognathus *dussumieri*. Parallel results were reported for deep fried grouper fillets in soybean oil by Gall *et al.* (1983) [6]. It has been shown that the major fatty acids present in the soybean oil must have been absorbed by the grouper fillets during the frying process. Due to absorption of these exogenous saturated fatty acids, relative concentration of the major fatty acids which were originally contained in the grouper fillets must have decreased. Results of the present study may also be explained the same way.

Monounsaturated fatty acid composition in cooked fish muscles in *Leiognathus dussumieri* and *Gazza minuta* is given in Table 2. Significantly high percentages of C16:1, C18:1, C20:1 and C22:1 fatty acids were found in the steamed and boiled cooked muscles of *Leiognathus dussumieri* and *Gazza minuta* when compared with those of raw muscles of the same species. However, deep fried cooked muscles of the same species contained significantly lower levels of the three fatty acids; C16:1, C20:1 and C22:1 and high percentage of C18:1. Absorption of significantly high amount of C18:1 fatty acid from white coconut oil cooking medium could be the reason for elevated percentage of C18:1 and decrease percentages of C16:1, C20:1 and C22:1 fatty acids in deep fried cooked muscles of the same species.

Table 1: Saturated fatty acid composition of cooked fish muscles of *Gazza minuta* and *Leiognathus dussumieri* and white coconut oil (as percentages of fatty acid methyl esters)

Leiognathus dussumieri					***Gazza minuta***				
Fatty acid	**Raw**	**Steamed**	**Boiled**	**Deep fried**	**Raw**	**Steamed**	**Boiled**	**Deep fried**	**Coconut Oil**
C10: 0		ND	ND	5.8		ND	ND	6.5±1.71	5.4±0.12
C12: 0		0.1±.001	0.1±0.002	45±2.31		0.2±0.03	0.1±0.005	49.8±2.64	47±2.14
C14: 0	5.3±0.5	4.5±0.15	4.1±1.12	18.3±2.11	1.0±0.25	4.0±0.2	3.5±0.06	19.7±1.51	19.7±1.31
C15: 0	1.0±0.12	0.6±0.05	0.6±0.04	0.1±0.002	0.2±0.22	0.9±0.001	0.8±0.001	0.1±0.002	
C16: 0	22.6±3.7	15.9±1.23	16.3±2.11	0.2±0.001	8.7±2.3	24.6±1.31	20.4±2.12	9.4±0.53	8.9±2.11
C17: 0	1.8±0.17	0.7±0.02	0.8±0.03	0.1±0.004	0.3±0.05	0.8±0.04	1.0±0.001	ND	
C18: 0	12.2±1.4	6.1±1.31	6.9±0.51	3.5± 0.51	3.5±0.92	11.1±1.31	10.7±0.31	3.1±0.12	2.8±0.71

Table 2: Monounsaturated fatty acid composition of cooked fish muscle of *Gazza minuta* and *Leiognathus dussumieri* and white coconut oil (as percentages of fatty acid methyl esters)

Leiognathus dussumieri					***Gazza minuta***				
Fatty acid	**Raw**	**Steamed**	**Boiled**	**Deep fried**	**Raw**	**Steamed**	**Boiled**	**Deep fried**	**Coconut oil**
C16: 1	6±1.34	12.5±1.50	12.9±0.71	0.8±0.004	1.3±0.8	4.2±0.11	3.6±0.11	0.4±0.001	
C18: 1	11.1±3.5	15.1±2.31	16.6±0.53	7.2±0.71	3.9±2.1	10.8±0.84	9.9±1.12	6.3±0.12	6.5±1.11
C20: 1	1.7±0.1	0.8±0.004	1.3±0.01	0.1±0,003	0	0.7±0.005	0.5±0.002	0.1±0.002	
C22: 1	0.5±0.12	2.9±0.05	ND	ND	5.2±0.91	0.3±0.001	0.5±0.002	ND	

Results are mean ± standard deviation of three replicates and calculated on a wet weight basis

Table 3: Fatty acid composition of n-3 and n-6 in cooked fish muscles of *Gazza minuta* and *Leiognathus dussumieri* and white coconut oil. (as percentages of total fatty acid methyl esters) (wet weight basis)

Leiognathus dussumieri					***Gazza minuta***				
Fatty acid	**Raw**	**Steamed**	**Boiled**	**Deep fried**	**Raw**	**Steamed**	**Boiled**	**Deep fried**	**Coconut oil**
C18: 3n-3	0.3±0.1	ND	0.2±0.005	ND	0.2±0.1	0.3±0.001	0.4±0.002	ND	
C18: 4n-3	0.8±0.1	ND	ND	ND	0	0.1±0.002	ND	ND	
C20: 3n-3	0	1.4±0.01	1.5± 0.01	ND	0.2±0.1	0.2±0.001	0.1±0.002	ND	
C20: 4n-3	0	ND	0.1±0.001	0.1±0.001	0.1±0.02	0.4±0.003	0.4±0.013	ND	
C20: 5n-3	8.2±1.4	3.1±0.001	3.5±0.13	0.7±0.002	1.5±0.8	3.8±0.14	4.3±0.15	0.2±0.031	0.1±0.001
C21: 3n-3		2.3±0.002	1.9 ± 0.01	0.5±0.001		0.9±0.004	1.8±0.002	ND	
C21: 5n-3	0.4±0.1	ND	ND	ND	51.9±7.9	ND	ND	ND	
C22: 5n-3	2.8±0.91	1.9±0.004	1.7±0.01	0.4±0.001	2.8±0.9	1.7±0.12	2.2±0.011	0.1±0.001	
C22: 6n-3	8.8±2.2	5.1±0.19	4.9±0.12	0.8±0.002	6±1.2	17.7±0.31	20.7±0.67	0.8±0.11	
Total n-3		13.8±.052	8.9±.048	2.5±.0005		25.1±.088	29.9±.165	1.1±.041	
C18: 2n-6	0.4±0.3	6.6±0.13	6.9±0.14	2.0±0.001	0.4±0.1	1.0±0.001	1.1±0.001	1.8 ±0.005	1.6±0.12
C20: 2n-6	0.5±.02	ND	0.5±0.001	ND	5.9±1.4	0.3±0.002	0.3±0.021	ND	
C20: 3n-6	0.4±0.1	ND	0.1±0.001	ND	0	0.1±0.001	0.2±0.011	ND	
4n-6	0.9±0.1	2.8±0.01	1.8±0.002	0.3±0.001	0	1.5±0.01	2.5±0.003	0.1±0.011	
C20: 4n-6	2.6±1.1				1.3±0.2				
C22: 5n-6	0	ND	ND	ND	0	1.2±0.002	0.2±0.021	ND	
Total n-6	23.5	11.6±.06	11.7±.052	2.7	70.3	7.3±.003	8.6±.008	2.1±.003	
Total unknown fatty acids		14.3	14.0	3.5		9.2	9.5	1.5	

ND- Not detected

Composition of polyunsaturated fatty acid contents of raw and cooked fish mussels of *Gazza minuta* and *Leiognathus dussumieri* is given in Table 3. Present results indicated that the concentration of Linoleic acid (C18:2n-6) increased in all cooked samples of both species investigated. It was reported that the predominant fatty acid in deep fried cooked samples of *Gazza minuta* and *Leiognathus dussumieri* was Linoleic acid. Similar results were reported by Xiao and Joanne (2007) [17] for Australian Bass Strait scallops and *Pecten fumatus.* Marchamy *et al.* (2009) [14] also indicated the same pattern for Indian mackerel and *Rastrelliger kanagurta*.

Linoleic acid is the most abundant PUFA in the human skin. Along with other components, it plays vital role in preserving our epidermal water barriers (Marichamy *et al.* 2009) [14]. Present results revealed the presence of high amount of linoleic acid in fried fish. GC analysis of the white coconut oil used in frying processes indicated that the main unsaturated fatty acids found were C18:2n-6 and C20:5n-3. Therefore, concentration of C18:2n-6 from the cooking oil also contributed to high concentration of C18:2n-6 in fried fish muscles. Simillar results were reported by Xiao Q Su and Joanne (2007) [17] for fried scallop muscles.

Fish oils are rich in polyunsaturated fatty acids namely eicosapentaenoic (EPA) and Docosahexaenoic (DHA), which are found to reduce the risk of cardio vascular diseases (Cadler, 2004) [3]. Present results have shown that the high amount of these two fatty acids in steamed and boiled cooked fish muscles of *Gazza minuta* when compared to those of raw muscles of the same species. However, percentage of EPA and DHA were recorded as lowest in deep fried cooked samples of the same species. Arachidonic acid (C20:4n-6) is one of the major n-6 fatty acid in fish oil and it was found in high amounts in steamed and boiled cooked samples of *Gazza minuta* but it was low in deep fried sample. Concentration of arachidonic acid and Docosahexaenoic acid (DHA) in steamed and boiled cooked samples were slightly lower when compared to those of raw muscle in *Leiognathus dussumieri*.

Marichamy *et al.* (2009) [14] reported that arachidonic acid is the principle omega-6 fatty acid in the brain and together with Docosahexaenoic acid (DHA) which is important for the brain development of infants. The present study showed that the concentration of docosapentaenoic (DPA) acid decreased in all cooked fish samples. Concentrations of all n-3 and n-6 fatty acids in deep fried cooked samples of *Gazza minuta* and *Leiognathus dussumieri* were very low. Similar results were reported by Candela *et al.* (1998) [4] for fried sardines and mackerel. In a study of fish by Sikorski and Kolakowska (2003) [16], it was reported that heating for 20 minutes at 160 ^{0}C could reduce the DHA contents to 45% and EPA levels to 20%. They also found that temperature is more effective than the duration of heating in the cooking process (Xiao and Joanne, 2007) [17]. Zakipour Rahimabadi and Dad (2012) [18], also reported that oil absorption in the frying process in turn dilutes the concentration of other fatty acids.

In view of the above results, the fried fish cannot be a good source of the omega-3 and omega-6 fatty acids and also Mozaffarian *et al.* (2003) reported that the consumption of fried fish did not reduce the risk of fatal ischemic heart disease due to changes in fatty acid composition by frying oil. However, if there is a need to increase the energy density of a diet, then fried fish could make an important impact. The ratio of n-6/n-3 is known to be of dietetic importance because it is the key factor for balanced synthesis of eicosanoids in organism (Xiao and Joanne, 2007) [17]. The ratios of PUFA/SFA for different cooking methods of two pony fish species studied here are given in table 6. A minimum value for PUFA/SFA ratio recommended by nutritionists is 0.45. Present study indicated that the PUFA/SFA ratios for steamed and boiled cooked fish were well above the recommended value but deep fried fish muscles were well below the recommended value.

Lipid content and fatty acid composition in fish vary from species to species, and with age, sex and diet. In addition to these biological factors, water temperature, time of capture, water salinity, cooking process, and cooking temperature also have an effect (Hoffman *et al.* 1994, Marichmy *et al.* 2009). Gall *et al.* (1983) [6, 10, 14] reported that deep fried fillets absorb cooking oil during cooking which resulted in increased levels of the major cooking oil fatty acids in the deep fried fillet with a corresponding decrease in the relative amounts of other major fillet fatty acids on a percent composition basis.

Further absorption of cooking oil fatty acids appears to be inhibited if the original level in the fillet is high.

Due to effects of frying oil, generally frying method has a greater negative effect in comparison with other common methods of fish cooking with regards to health benefits (Garcia-Arias *et al.* 2003; Larsen *et al.* 2010) [7, 13]. However consumption of fried fish in comparison to other cooked fish has shown absence of lower risk on cardiac benefits. The n3/n6 fatty acid ratios for different kind of cooking methods were given in table 4. Nutritionists believe that the desirable n6/n3 fatty acid ratio should be 5 at a maximum. According to the present results the n6/n3 fatty acid ratios were well below the recommended value. Fried fish intake was also associated with structural abnormalities indicative of systolic dysfunction and potential coronary atherosclerosis (Maozaffarian *et al.* 2006) [15].

Finally it can be suggested that from a health point of view, frying the fish in oil reduce its benefits. The deep fat frying method is accepted by consumers because of the unique flavor- texture combination and also flavor characteristics. One should consider reducing the negative effects of frying by choosing a better frying oil, better size and thickness of muscle, pre-frying preparation (whole fish, muscle with skin, coating) and better time and temperature of frying.

Table 4: Total n-3/n-6 fatty acid ratio of cooked fish muscles of *Leiognathus dussumieri and Gazza minuta.*

	Leiognathus Dussumieri			***Gazza minuta***		
	Steaming	**Boiling**	**deep frying**	**Steaming**	**Boiling**	**Deep frying**
n-3	13.8	13.8	2.5	25.1	29.9	1.1
n-6	9.4	9.3	2.3	4.1	4.3	1.9
n-3/n-6	1.46	1.48	1.08	6.12	6.95	0.57

Table 5: Total PUFA/SFA fatty acid ratio of cooked fish muscles of *Leiognathus dussumieri and Gazza minuta.*

	Leiognathus Dussumieri			***Gazza minuta***		
	Steaming	**Boiling**	**deep frying**	**Steaming**	**Boiling**	**Deep frying**
Sfa	27.9	28.8	73	41.6	36.5	88.6
Mufa	31.3	30.8	8.1	16	14.5	6.8
Pufa	25.4	20.6	5.2	32.4	38.5	3.2
Pufa/Sfa	2.03	1.78	0.18	1.16	1.45	0.11

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