Effects of Cooking on Omega 3 Content of *Sardinella longiceps* Emphasizing an Innovative Method to Elevate Omega 3 Intake

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***Abstract*- Most of the small pelagic fish like *Sardinella longiceps* (Indian oil Sardine) are considered as best sources of Omega 3 Polyunsaturated fatty acids (PUFA). Due to their law cost and availability throughout the year, Sardines are popular and consumed often by people, especially in the developing countries. This study was designed to determine the effects of cooking on Omega 3 polyunsaturated fatty acids of *Sardinella longiceps* by comparing GC/MS fatty acid profiles of raw, boiled and fried fish; and then utilizing the fish in an innovative product (dry soup mix) in order to elevate the omega 3 intake that could be obtained from *Sardinella longiceps*. Omega 3 PUFA content of raw fish was 21.54% (of total fatty acids). With boiling it has decreased to 14.23% and when fried in coconut oil, it was 2.83%. Since average per capita fish consumption of Sri Lanka is 30.5 g, frying, which can be identified as the most popular cooking method of Sardines like small fish (due to lots of tiny bones present with lesser flesh), cannot provide the recommended minimum daily "EPA" Omega 3 intake (0.22 g per person) when taken alone without any other Omega 3 sources. As a solution for this issue, an instant soup powder which was incorporated with fish powder derived from, and fish oil extracted from *Sardinella longiceps* was developed containing 9.31% of Omega 3 PUFA, which is significantly higher than that of fried fish. It can provide 0.6 g of EPA and 1.9 g of DHA per serving which is higher than the recommended minimum daily intake.**

***Index terms*- *Sardinella longiceps*, Omega-3 PUFA, EPA, DHA**

I.INTRODUCTION

Sardinella longiceps or the Indian oil sardine (Yak Salaya in Sinhalese) is a commercially important small pelagic resource in the Indo-Pacific region [1]. Fish is the well-known source of all nutrients except carbohydrates and vitamin C. Fish owns an invaluable nutritional quality and serves as a health-food for the affluent world with the fish oils which are rich in polyunsaturated fatty acids (PUFAs), especially ω-3 PUFAs [2]. These fish oils are extracted from fatty or the oily fish (i.e: small pelagic fish like Herring, *Sardinella* sp. and larger pelagic fish like mackerel, salmon, etc) which are carrying oil in their flesh and the belly cavity around the gut. They contain up to 30% oil, although it can vary among the different species and different individuals of the same species [3]. These fish oils are recognized as predominant dietary sources of omega-3 PUFA, which consist mainly of Eicosapentaenoic acid(EPA C20:5) and Docosahexaenoic acid (DHA C22:6) [4]. Alpha linolenic acid (ALA) is an essential fatty acid and EPA and DHA are metabolized from ALA, increasing the chain length and degree of unsaturation by adding extra double bonds to the carboxyl group [5]. But only a minor quantity of the beneficial ω-3 PUFAs (EPA and DHA) are synthesized in humans, since excess dietary Omega-6 fatty acids associated with a high consumption of vegetable oils may compete with omega-3 for metabolization in the body [6]. Regular consumption of omega 3 rich diets with appropriate content of EPA and DHA can prevent hypertension, cardiovascular diseases, type 2 diabetes, rheumatoid arthritis, Crohn's disease and reduce the risk of Dementia, Alzheimer's diseases, obesity, thrombosis, lung disease, cancer including colon, breast and prostate and some other diseases. And also omega 3 PUFAs can improve the development and functioning of the brain, retina and testis [4],[7],[8]. Unlike in meat, fish fat has minimal content of saturated fatty acids.

With the increasing body of knowledge on the health benefits of omega 3 PUFA, the dietary recommendations suggest that the consumption of omega-3 PUFA should be increased. International Society for the Study of Fatty Acids and Lipids (ISSFAL) suggests the intake of omega-3 fatty acids should be 0.65 g of DHA + EPA per day (0.22 g per day of each as a minimum) [9]. According to FDA (2000), the daily intake of EPA and DHA should not exceed 3.0 g per person per day in the form of fish oil, from food and dietary supplements.

Cooking fish could cause modifications in fatty acids; especially due to the possible oxidation, the quantity of PUFA remaining in the food when it is eaten, that is, after storage and/or cooking, could be lower than the initial content in the raw product [10]. Yet there are no clear and adequate reports on the effect of cooking on fatty acid profile and quality of fish [11].

This study compares the fatty acid profiles of raw fish, fried fish, boiled fish of *Sardinella longiceps* and also the developed soup powder which has been incorporated with a fish powder and fish oil derived from the same fish species aiming the enhancement of the Omega 3 intake that could be obtained from *Sardinella longiceps*. This could be used to study the effect of cooking on the fatty acid composition of the *Sardinella longiceps* with relevance to the raw fish. And also it could suggest the extent and the effectiveness of the Omega 3 enrichment of the value added product which was developed to enhance the Omega intake by means of *Sardinella longiceps*, comparatively to the form of cooked fish.

II. RESEARCH METHODOLOGY AND MATERIAL USED

2.1 Sample preparation for determination of the effect of cooking on the Omega 3 PUFA content of Sardinella longiceps:

Samples of *Sardinella longiceps* were purchased from the fish landing site in Pitipana, Negambo, Sri Lanka. Purchased fish were kept frozen in ice box and taken to the aquatic laboratory at Department of Zoology-University of Sri Jayewardenepura where they were washed well with running tap water. A part of the cleaned fish was descaled, deheaded, and were cut to remove gut and back bone and crushed with motor and pestle. Another part of the above cleaned fish was deep fried using coconut oil and then they were crushed with motor and pestle. The remaining part of the fish was boiled in water and then crushed with motor and pestle.

2.2 GC/MS Analysis of fatty acid profiles of raw and cooked fish:

Fat of raw and cooked fish and also fish soup powder were extracted by using the Chloroform-methanol extraction method [12]. Six drops of test portion was taken into a screw capped tube. It was then dissolved in 3ml of Benzene, 1.5ml of Sodium methoxide and 4.5ml of Methanol. Then it was heated in a water bath for about 45 minutes. Next it was taken from the water bath and cooled to the room temperature. Then 10ml of distilled water was added into it and mixed well. (A white emulsion formed, when the Fatty acid methyl esters were completely formed). Then 9ml of Hexane was added into it, and shaken a little and kept until the Hexane layer separated. The separated Hexane layer was concentrated by evaporating it. The methyl esters of fatty acids were analysed by GC/MS (Model Agilent, 7890 and Agilent,5975; Cinert XLEI/CIMSD with Triple-Axis Detector; 30mm x 0.25mm column), according to GC/MS method [13]. The temperature of the injector and detector were 250 ^{0}C and 270 ^{0}C respectively. The initial temperature of the oven was set at 100^{0}C. The temperature was brought up to 170 ^{0}C at the rate of 20^{0}C/min during ramp 1. Afterwards, during ramp 2, the temperature was gradually increased to 230^{0}C at the rate of 2^{0}C/min followed by ramp 3 during which the temperature was risen up to 280^{0}C at the rate of 5^{0}C/min. Fatty acids in the samples were identified by retention time index available at Department of Chemistry, University of Sri Jayewardenepura.

2.3 Fish powder preparation: Fish were washed and cleaned well and head, scales, fins, bones and the guts were removed. And they were then cut in to small pieces. Thereafter they were dipped in concentrated lime juice (which was prepared with the juice of six matured medium sized lime fruits mixed with 150ml of clean water) for 5-10 minutes. Then fish were steamed in an electric steamer with boiling water for 15-20 minutes, and the steamed fish were dried in a drying cabinet maintained at 35$\pm$5^{0}C for 24 hours. Finally dried fish were powdered separately and fish powder was sieved to be mixed with corn flour.

2.4 Fish oil extraction : Fish were washed and cleaned well and head, scales, fins, bones and the guts were removed. Fish were then cut in to pieces

and were cooked in water until considerable amount of oil droplets appeared on the surface of the water. The oil layer was then decanted along with water and allowed to cool down to room temperature. Then the decanted liquid mixture was centrifuged at 1000rpm for about 20 minutes. Then the top oil layer was decanted into a separating funnel and allowed to stand for about 10 minutes. The separated oil layer was then filtered through anhydrous sodium sulphate and it was collected into a 100 ml small beaker.

2.5 Selection of best fish soup powder formula: Four different formulations of soup powder were made using different fish powder: corn flour ratios. Soup was prepared by re-constitution of instant soup powder with boiled water and were served to 30 untrained panellists and five point hedonic scale test was performed with the panel.

2.6 Selection of best soup powder formula enriched with Fish oil: The selected formula from first sensory evaluation (according to 2.5) was developed in to four different formulas with different soup powder: fish oil ratio and the soup were served to a sensory panel of 30 untrained panelists for ranking test.

2.7 Determination of caloric value of the developed soup powder: Analysis of Moisture, Ash, Crude protein, crude fiber and Total fat of the developed instant fish soup powder were determined as described by AOAC (2000**)** [14].

2.8 Microbiological analysis: Microbiological analysis of Total plate count, Enumeration of Yeast and Moulds, Enumeration of *Staphylococcus aureus* , and Detection and Enumeration of Coliform, Faecal Coliform and *Escherichia coli* were carried out according to SLS 516: 1991[15].

III. RESULTS AND DISCUSSION

3.1 Comparison of Omega 3 Polyunsaturated Fatty acid composition of raw and cooked Sardinella longiceps:

Table 1: Fatty Acid Composition of raw,boiled and fried *Sardinella longiceps* (%w/w) of totalfattyacids

Fatty Acids		**% (of Total Fatty Acids)**		
C18:3 (n-3)	Linolenic acid (ALA)	0.88 ± 0.205^{b}	0.33 ± 0.174^{c}	1.24 ± 0.102^{a}
C20:5 (n-3)	Eicosapentaenoic acid (EPA)	10.73 ± 0.181^{a}	7.87 ± 0.225^{b}	0.57 ± 0.115^{c}
C22:6 (n-3)	Docosahexaenoic acid (DHA)	9.93 ± 0.187^{a}	6.03 ± 0.130^{b}	1.02 ± 0.171^{c}
% total Omega 3 PUFA		21.54 ± 0.145^{a}	14.23 ± 0.207^{b}	2.83 ± 0.045^{c}
% total Omega 6 PUFA		18.16 ± 0.233	15.71 ± 0.152	15.25 ± 0.301
% total Polyunsaturated fatty acids		39.70 ± 0.212	29.94 ± 0.139	18.08 ± 0.130
% total Saturated fatty acids		37.81 ± 0.105	39.58 ± 0.308	54.80 ± 0.037

Different superscription in each raw is significantly different.

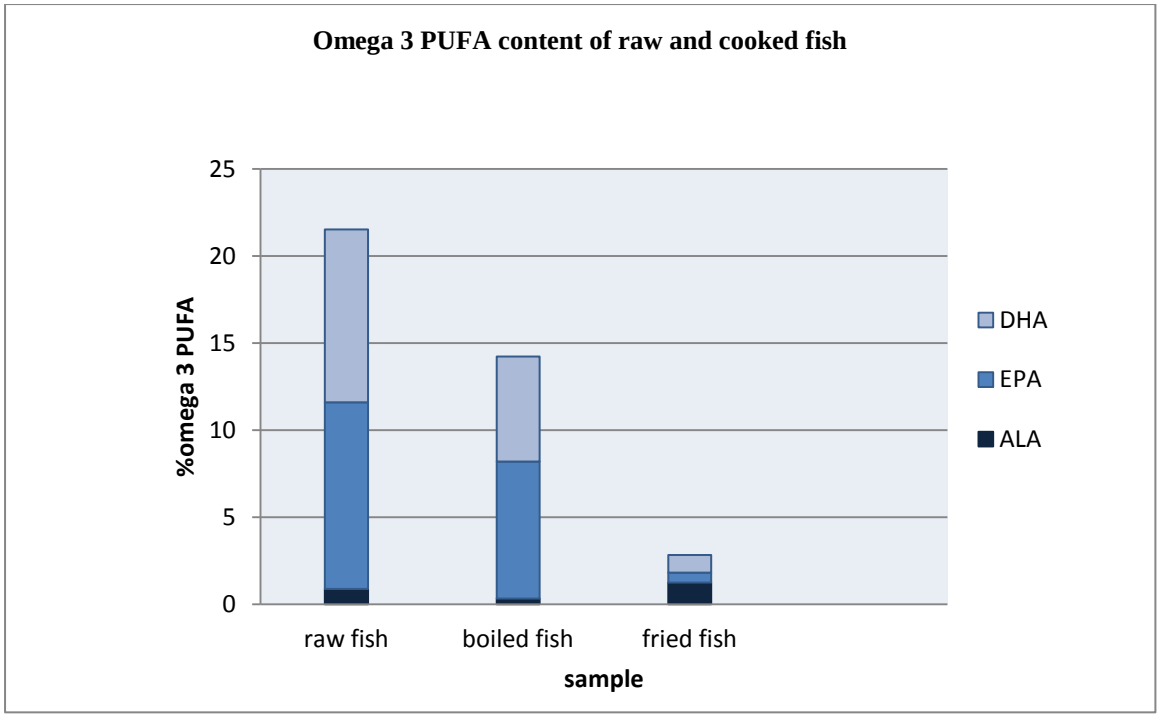

Figure 1. Bar chart of omega-3 percentages raw, boiled and fried fish

All three samples were containing omega 3 fatty acids. ALA content of the raw Sardinella longiceps was (0.88 ± 0.105) % and it has significantly increased when the fish was fried but was significantly decreased when the fish was boiled. Percentage of EPA was (10.73± 0.081) % for the raw fish and there was a huge decline in that value when the fish was fried. It has also decreased to a considerable extent when the fish was boiled. DHA content of the raw fish was (9.93 ± 0.087)% and as same as in EPA content, the least DHA was recorded in fried fish and lower levels in boiled fish (Figure 1). Saturated fatty acid levels of fried fish were drastically higher than those of raw and boiled fish.

According to the suggestions of International Society for the Study of Fatty Acids and Lipids (ISSFAL), intake of omega-3 fatty acids should be 0.65 g of DHA + EPA per day (0.22 g per day of each as a minimum). Therefore when compared to the average per capita fish intake of Sri Lanka, which is about 30.5 grams per day , according to the department of fisheries and Aquatic both raw and boiled fish the values were below 5 but for fried fish it was above 5. Therefore the quantity of omega 3 fatty acids present in the fried fish was inadequate. But in the case of boiled fish resources[16], fried fish can provide DHA in recommended amounts but EPA content that can be provided by fried fish is only around 0.17 g, which is lower than recommended minimum value, 0.22g.

Table 2: lipid quality indices for raw and cooked samples

Sample type	PUFA/SFA	n6/n3
Raw fish	1.05	0.84
Boiled fish	0.76	1.10
Fried fish	0.33	5.39

3.2 Lipid quality of raw and cooked Sardinella longiceps

The minimum value for PUFA/ SFA ratio recommended is 0.45 [17]. According to the calculated results, both boiled and raw fish gave values above 0.45 but fried fish only gave 0.33 revealing that the lipid quality of fried fish cannot satisfy the desired standard.

The desirable ratio of n6 /n3 fatty acids should be 5 at a maximum [18]. For

this ratio is somewhat higher than that of raw fish. Therefore it can be suggested that for obtaining omega 3 polyunsaturated fatty acids in desirable quantities, the consumption boiled fish would be

healthier. Yet the larger amount of tiny bones which cannot be removed when the fish is prepared makes this small fish less preferable to be boiled.

3.3 Selection of best soup powder formula

According to the responds obtained in the sensory evaluation, the selected fish soup powder was containing 9.7g of fish powder and 3.0g of fish oil derived from *Sardinella longiceps.*

Table 3: The selected Fish Soup Powder formula

Soup powder	100g
Corn flour	48.5g
Fish powder	9.7g
Milk powder	9.7g
Tomato powder	5.82g
Dehydrated carrots	5.82g
Dehydrated leaves	3.88g
Spice mix	6.79g
salt	5.82g
Citric acid	0.97g
Fish oil	3.0g

3.4 Fatty acid composition of Instant fish soup powder

The statistical analysis of the % Omega -3 PUFA of the four samples (Raw fish, boiled fish, fried fish and soup powder) exhibited a significant difference. (Figure 2). According to the results raw fish of Sardinella longiceps recorded the highest value of % Omega-3 PUFA and the least value was owned by fried fish. The percentage Omega -3 PUFA content of the Omega 3 enriched soup powder was lower than that of boiled fish and higher than fried fish. Figure 2 shows that EPA content of the soup has been declined whereas DHA content was increased when compared to boiled fish. And both EPA and DHA contents of soup were higher than that of fried fish and lower than raw fish of Sardinella longiceps. Soup powder can provide both EPA and DHA above the minimum daily intake suggested by the International Society for the study of fatty acids and lipids.

Table 4: Fatty Acid Composition soup powder (%w/w of total fatty acids)

Fatty Acids		% (of Total Fatty Acids)
C18:3 (n-3)	Linolenic acid (ALA)	0.81 ± 0.004
C20:5 (n-3)	Eicosapentaenoic acid (EPA)	1.98 ± 0.042
C22:6 (n-3)	Docosahexaenoic acid (DHA)	6.52 ± 0.004
% total Omega 3 PUFA		9.31 ± 0.040
% total Omega 6 PUFA		16.17 ± 0.032
% total Polyunsaturated fatty acids		25.48 ± 0.009
% total Saturated fatty acids		40.04 ± 0.083

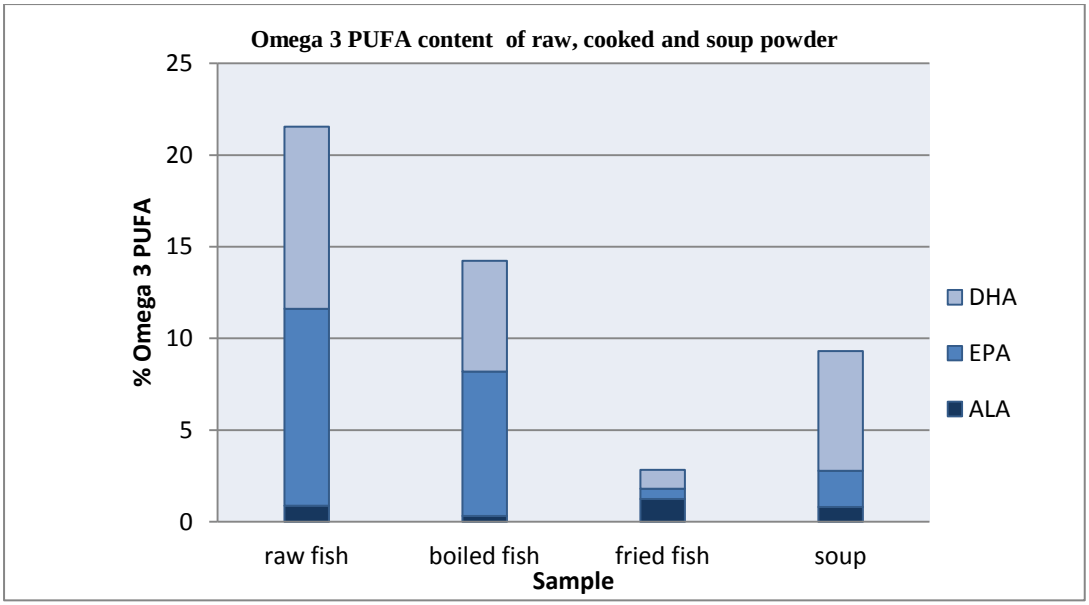

Figure 2: bar chart of Omega 3 PUFA content of raw, cooked fish and soup powder

3.5 Lipid quality indices of Omega 3 enriched instant fish soup powder

Total PUFA/ Total SFA of the soup powder= 25.48/40.04 = 0.64.

The minimum value recommended for PUFA/ SFA is 0.45 [17]. Since value of the omega 3 enriched soup is higher than the recommended minimum value it can be suggested that the soup contains health friendly PUFA more than SFA.

Total n6 PUFA/ Total n3 PUFA = 16.17/9.31 = 1.74.

For being said that it is having an ideal combination of PUFA, the preferable ratio of n6/n3 is considered to be 5 at a maximum [18]. As the value observed for the soup was lower than 5, it can be suggested that the developed soup is having an acceptable PUFA combination.

3.6 Caloric value of the soup powder

According to the observed values for Carbohydrate, protein and fat, the calculated total caloric value of the instant soup powder was 366.03 Kcal/100g. This value was below than the maximum reference value given by USDA National Nutrient Database for Standard Reference for Soup, cream of vegetable, dry, powder (2015) [19].

Table 5: Results of the proximate analysis of the soup powder

Parameter	% (w/w)
Moisture	10.61 $\pm$ 0.233
Ash	3.18 $\pm$ 0.412
Crude protein	7.12 $\pm$ 0.102
Total fat	7.95 $\pm$ 0.204
Crude fiber	4.64 $\pm$ 0.115
carbohydrate	100- (10.61+ 3.18+ 7.12+7.95+ 4.64) = 66.5

3.7 Microbiological Analysis

Both the Total plate count and the count of yeast and moulds complied the specification for dried fish products according to SLS standards [15] and also AIIBP : Microbiological Specifications for Dry Soups and Bouillons [20].

Table 6:Results of Microbiological analysis of soup powder

Total plate count	Yeast and mould	*Staphylococcus aureus*	*E.coil*
4 x 10^2 CFU/g	1 x 10^2 CFU/g	Not detected	Not detected

IV. CONCLUSION AND FURTHER WORK

According to the results obtained for the Fatty acid profiles of raw and cooked (boiled, fried) fish *Sardinella longiceps*, it can be clearly concluded that cooking may result a significant loss of Omega 3 poly unsaturated fatty acids. Frying , the most popular method of cooking *Sardinella longiceps* cannot provide the recommended minimum "EPA" Omega 3 intake (0.22 g per person) when taken

alone without any other Omega 3 sources, as average per capita fish consumption of Sri Lanka is 30.5 g. This can be due to the heat treatments that used to cook fish and/or leaching of fatty acids in to the cooking medium. The results of the study further says that raw *Sardinella longiceps* contains more than 20% of Omega 3 PUFA in its fatty acid profile and this is nearly 55% of the total PUFA content of the fish. Omega 3 enriched soup powder 30g pack can provide 2.5g of EPA + DHA and for one serving it is 0.85g. This value is larger than 0.65, and lower than 3.0g, which are the recommended minimum and maximum daily intakes respectively. And also it can provide EPA and DHA, the biologically important two omega 3 PUFA each in higher amounts than the recommended minimum daily values.

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