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DEVELOPMENT OF AN OMEGA 3 ENRICHED INSTANT SOUP POWDER FROM SARDINELLA LONGICEPS

A.H.G.S. Udari*, I. Wickramasinghe, M.V.E. Attygalle
* Department of Food Science and Technology, University ofSri Jayewardenepura, Nugegoda, Sri Lanka.
Department of Zoology, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.

ABSTRACT

"Indian Oil Sardine" or Sardinellalongiceps is an abundantly caught small pelagic fish of commercial importance in the fisheries industry ofSri Lanka.Being considered as an omega-3 rich fish, effects of cooking on its fatty acid profiles(Gas chromatography- mass spectroscopy) were studiedin comparison with that of raw fish.Omega 3 polyunsaturated fatty acid (PUFA) content of raw fish was 21.54% (of total fatty acids). Cooking has decreased the value down to 14.23% and 2.83%, respectively in boiled and fried fish.Development of a value added food product was designed by incorporating fish powder and fish oil derived from Sardinella longiceps along with corn flour (as the basis), tomato powder, milk powder, spices, salt and dehydrated vegetables.The developed Instant soup powder contained9.31% (of total fatty acids) of Omega-3 PUFA, which is significantly higher than that of fried fish. It can provide 0.6 g of Eicosapentaenoic acid (EPA) and 1.9 g of Docosahexaenoic acid (DHA)per serving which are higher than the recommended daily minimum intake. As a whole or a part of a meal, an instant soup enriched with Omega 3 can be more nutritious and a convenient way to elevate the Omega 3 intake.

KEYWORDS: Sardinella longiceps, Omega-3 PUFA, EPA, DHA, soup.

INTRODUCTION

Coastal pelagic fisheries have a higher consideration as the backbone of the fishing industry in Sri Lanka, which contributes about 26% of the total fish production in the country [1]. "Indian Oil Sardine" or *Sardinella longiceps* is a small pelagic fish which is harvested abundantly and commercially available throughout the year. Although post harvest loss in Sri Lankan fishery is considerably high; and this particular fish species is easily affordable with its low cost, still there is no evidence of utilizing it in a possible value added food product efficiently. Processing of low cost fish species into value added food products is one of the most important food processing technologies.

Fatty or oily fish comprising of small pelagic fishsuch as herring, sardines, anchoviesand large pelagic fish such as mackerel, salmon, have oil in their flesh and the belly cavity around the gut. Considering fish fats, most small pelagic fish including Sardines contain around 30% of omega-3 polyunsaturated fatty acids in their total fatty acids profile [2]. The major fatty acids among omega 3 long chain polyunsaturated fatty acids are Eicosapentaenoic acid (EPA; C20:5) and Docosahexaenoic acid (DHA; C22:6) contributing a very high proportion (nearly 85%) to the total n-3 PUFAs. [2][3]. EPA and DHA are synthesised from Alpha Linolenic acid (ALA) and other fatty acids in the omega-3 pathway [4]. 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 oilsmay compete with omega-3 for metabolization in the body [5]. 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 disase, 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 [6]-[9].

Cooking and preservation techniques could probably cause modifications in fatty acids composition due to the possible oxidation. [10]-[11]. However, still there are no clear and adequate records on the effect of cooking on fatty acid profile and quality of fish [6]. Present study compares fatty acid profiles of raw, boiled and fried

Sardinella longiceps in order to reveal the effects of cooking on the fatty acid composition, especially with regard to Omega-3 PUFA (EPA and DHA).

Industrial efforts of incorporating long chain omega-3 polyunsaturated fatty acids (omega-3 PUFA) into foods and dietary supplements has been increasing since the last decade, with the growing body of knowledge on their invaluable health benefits.According to guidelines formulated by the Food and Drug Administration (FDA) and other recognized bodies on Human Nutrition, the minimum average daily intake of Omega-3 PUFA (EPA + DHA) ranged between 0.2 g – 0.65 g per person.

An effort of development of innovative food product enriched with Omega-3 PUFA from Sardinella longiceps was carried out in this study. Without radical changes in eating habits and without much effort on preparation this product could elevate the omega-3 PUFA intake.

Using dry soup mixes is a very fast form of cookery and also popular among communities the world over.The frantic rhythm of modern life has changed the food preparation methods and obviously instant foods are preferred by consumers. And people have become more aware of healthy food and therefore, "Omega-3 enriched instant soup mix" would be a good and convenient nutritional choice. And also it comes as a dry product assuring longer period of shelf life than broth.

MATERIALS AND METHODS

Determination of the effect of cooking on the Omega 3 PUFA content of Sardinella longiceps

Sample preparation

Samples of *Sardinella longiceps* were purchased from the fish landing site in Pitipana, Negambo. Purchased fish were kept frozen in ice box and taken to the aquatic laboratory at Department of Zoology-USJP 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.

Comparison 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;CinertXLEI/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 at100^{0}C. The temperature was brought up to 170 ^{0}C at the rate of 20^{0}C/min duringramp 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}Cattherateof5^{0}C/min. Fatty acids in the samples were identified by retention time index available at Department of Chemistry, University of SriJayewardenepura.

Fish powder and fish oil 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.

Fish powder preparation

A part of the fish pieces was 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+50C for 24 hours. Finally dried fish were powdered separately and fish powder was sieved to be mixed with corn flour.

Fish oil extraction

The remaining part of the fish pieces was 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.

Sensory evaluation

Sensory evaluation was carried out in two steps and the results were analysed using Kruskal-Wallis nonparametric method in MINITAB statistical software.

Selection of best fish soup powder formula

Four different formulations of soup powder were made using different fish powder: corn flour ratios as given in Table 1.Soup was prepared by re-constitution of instant soup powder with boiled water and were served to30 panellists and five point hedonic scale test was performed with the panel.

Table 1: Different formula with different Corn flour: Fish powder ratio tested for the best fish soup powder formula by sensory analysis

Ingredient	% of total sample weight			
	125	**137**	**193**	**157**
Corn flour	50	40	30	20
Fish powder	10	20	30	40
Milk powder	10	10	10	10
Tomato powder	6	6	6	6
Dehydrated carrots	6	6	6	6
Dehydrated leaves	4	4	4	4
Spice mix	7	7	7	7
Salt	6	6	6	6
Citric acid	1	1	1	1

Selection of best soup powder formula enriched with Fish oil

The selected formula from first sensory evaluation was developed in to four different formulas with different soup powder: fish oil ratio and the soups were served for a sensory panel of 30 panellists for ranking test.

Table 2: Fish soup powder to fish oil ratio

Sample No	Fish soup powder %	Fish oil %
125	97	3
131	95	5
193	93	7
157	91	9

Determination of fatty acid profile of developed instant soup powder

The method followed was as same as in determination of the effect of cooking on the fatty acid composition of *Sardinella longiceps.*

Proximate analysis

Parameters of the proximate analysis (Moisture, Ash, Crude protein, crude fiber and Total fat) of the developed instant fish soup powder were determined as described by AOAC (2000)[14].

Determination of Shelf life

The selected best Instant fish soup powder was packed (30g per one pack- 3 servings) and well-sealed in packets made with laminated packaging material of 30 BOPP 25 MCPP. The following tests were performed in two week intervals for 6 weeks. Total plate count and Yeast and mould count according to SLS 516: 1991; Moisture content (Oven drying method, AOAC) and Peroxide value [15].

RESULTS AND DISCUSSION

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

Table 3: Fatty Acid Composition of raw andcooked Sardinella longiceps (%w/w of total fatty acids)

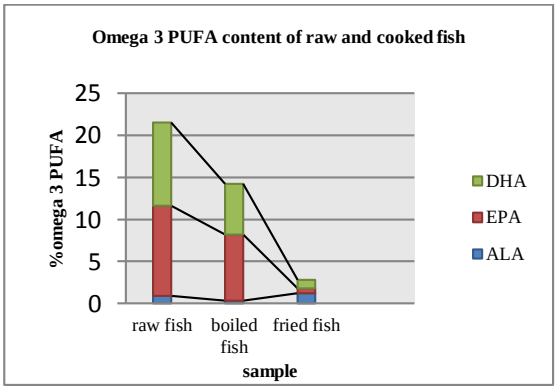
Fatty Acids		% (of Total Fatty Acids)		
Nomenclature	Systematic name	Raw fish	Boiled fish	Fried fish
C18:3 (n-3)	Linolenic acid (ALA)	0.88 ± 0.205^{b}	0.33 ± 0.174^{c}	1.24 ± 0.102^{a}
C20:5 (n-3)	Eicosapentaenoic acid (EPA)	10.73 ± 0.181^{a}	7.87 ± 0.225^{b}	0.57 ± 0.115^{c}
C22:6 (n-3)	Docosahexaenoic 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
% total Monounsaturated fatty acids		22.49 ± 0.215	30.48 ± 0.125	27.12 ± 0.125

The means with the different superscripts within a raw are significantly different at $p<0.05$

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.

Figure 1



Bar chart comparison of omega-3 percentages raw, boiled and fried 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 (the department of fisheries and Aquatic resources), 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.

Sensory Evaluation

Selection of best fish soup powder formula

According to the results obtained from Kruskal-Wallis Test (MINITAB 15) for five point hedonic scale sensory evaluation for the four different soup powder formulae , sample 125, which was having the least fish powder percentage, has shown a significant difference in colour, appearance, odour, texture and taste when compared with highest fish powder containing sample 157, whereas there was no significant difference when it was compared with the sample 131, that was having the second least fish powder percentage. But sample 125, has shown significant difference in Overall acceptability of the soup when compared with all the other three samples those were having comparatively higher fish powder percentages than in 125 (Figure 2).

Figure 2

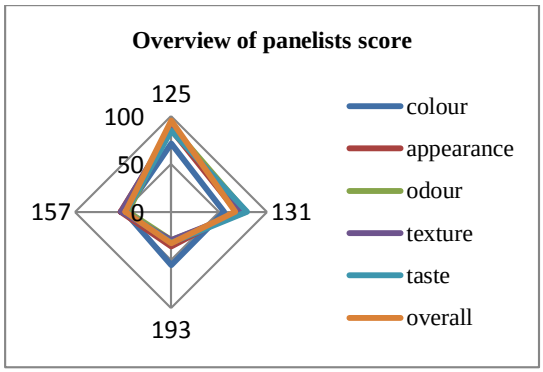
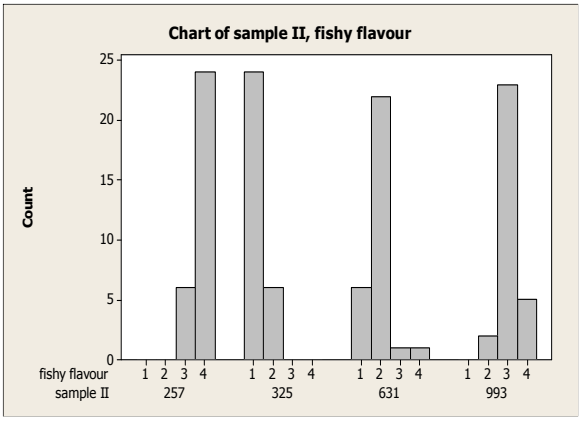


Chart of panellists' score for sensory parameters versus sample Selection of best soup powder formula enriched with Fish oilSelection of best soup powder formula enriched with Fish oil

The soup powder formula which was selected from the first sensory evaluation was further developed by incorporating fish oil extracted from *Sardinella longiceps*. Results of the ranking test performed with 30 panellists for selection of the best Fish oil: soup powder ratio which gives the most acceptable fishy flavour were analysed according to the Kruskal-Wallis Test. According to the Figure 3, sample 325, that was having the least fish oil percentage has ranked as the soup sample with the most acceptable fishy flavour.

Figure 3



Bar chart of panellist score for different samples

1-Most preferred 2-Prefer secondly
3- Prefer thirdly 4-Least preferred

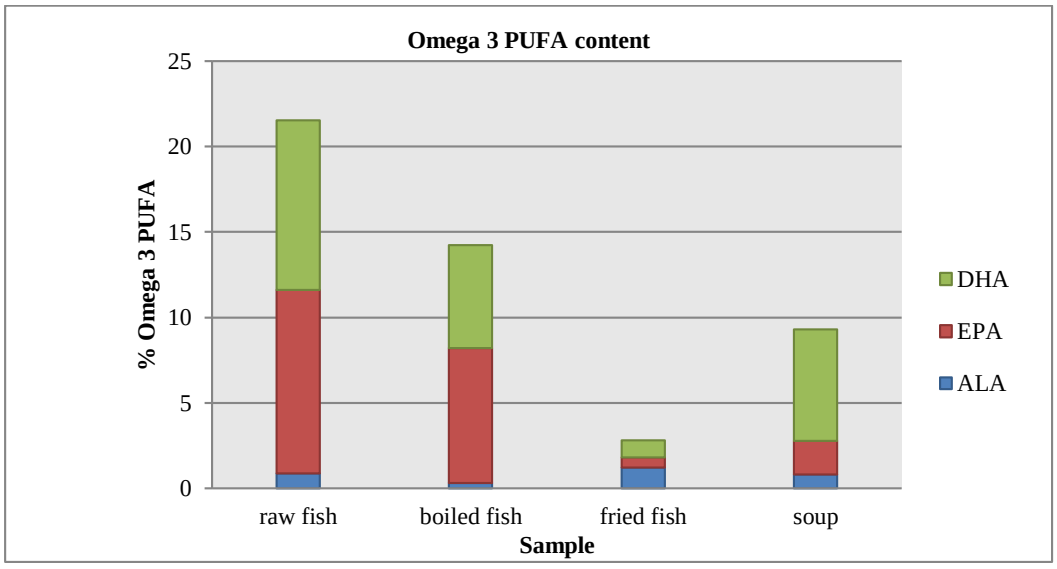
Fatty acid composition of Instant fish soup powder

The statistical analysis on the % Omega -3 PUFA of the four samples (Raw fish, boiled fish, fried fish and soup powder) exhibited a significant difference. 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 instant soup powder was lower than that of boiled fish and higher than fried fish. Figure 4 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 (ISSFAL).

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

Fatty Acids		% (of Total Fatty Acids)
Nomenclature	Systematic name	
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
% total Monounsaturated fatty acids		34.48 ± 0.036

Figure 4



Comparison of Omega 3 PUFA content of raw, cooked fish and soup

Lipid quality indices of Omega 3 enriched instant fish soup powder

PUFA/SFA ratio

Total PUFA/ Total SFA of the soup powder= 25.48/40.04 = 0.64. The minimum value recommended for PUFA/ SFA is 0.45 [16]. 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.

n6/n3 ratio

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 [17]. 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.

Proximate Analysis

Since this fish soup powder has been incorporated with dehydrated vegetables, leaves and spices like ingredients total ash content and the crude fiber were quite higher than those of the commercially available instant soup powders. 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) [18].

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

Parameter	% (w/w)
Moisture	10.61
Ash	3.18
Crude protein	7.12
Total fat	7.95
Crude fiber	4.64
carbohydrate	66.5

Determination of the shelf life

According to the results shown in table 6, moisture content of the soup powder had increased over the considered time period of six weeks and the value recorded in the 6th week was lower than the maximum standard limit according to SLS 643 (dry fish products) [19]. Peroxide value had also increased over time and at the end of the 6th week peroxide value was 5.3 meq/kg, which was lower than the general SLS standard value for cereal based dry products (10meq).

Both the Total plate count and the count of yeast and moulds have increased over time and it has recorded a rapid acceleration of the growth of microbial populations from 4th week to 6th week. Yet the values for both total plate count and yeast and moulds at the end of 6th week complied the specification for dried fish products (SLS 643, Food Act 26, 1986) And also AIIBP : Microbiological Specifications for Dry Soups and Bouillons [20].

Evaluation of the values obtained for the tested parameters of the shelf life determination study, it can be concluded that the product is safe to be consumed after 6 weeks of storage and the shelf life of the product would be more than 6 weeks (1 ½ months).

Table 6: Changes in tested parameters during storage

Weeks	% Moisture	Peroxide value (Meq/kg)	Total plate count (CFU/g)	Yeast and moulds (CFU/g)
0	7.61	0.6	4.0×10^2	1.0×10^2
2	7.76	1.7	7.0×10^2	1.2×10^2
4	7.95	3.2	1.3×10^3	1.5×10^2
6	8.52	5.3	4.1×10^3	1.7×10^2

CONCLUSION

Raw *Sardinella longiceps* contains more than 20% of Omega 3 in its fatty acid profile and this is nearly 55% of the total PUFA content of the fish. The results conclude that cooking, especially frying, which is the most popular method of cooking of small fish with less flesh and larger numbers of tiny bones, may significantly reduce Omega 3 poly unsaturated fatty acids content. This can be due to the heat treatments that used to cook fish and/or the leaching of EPAand DHA to the cooking medium. Hence, frying *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. Omega 3 enriched instant 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 PUFAs, each in higher amount than 0.22g, the recommended. Therefore the developed Omega 3 enriched fish soup powder can be suggested as a well-balanced omega 3 supplement.

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