

Assessment of EPA, DHA and DPA Level in Fish Food Cooked Using South Asian Method

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DOI: <https://doi.org/10.51244/IJRSI.2026.13010070>

Received: 30 December 2025; Accepted: 05 January 2026; Published: 31 January 2026

ABSTRACT

Fish play an important role in human nutrition as they contain omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA; C₂₀:5n-3) and docosahexaenoic acid (DHA; C₂₂:6n-3) and docosapentaenoic acid (DPA, C₂₀:5n-3). These particularly, omega-3 fatty acids play a role in preventing heart disease and have anti-inflammatory and anti-thrombolytic effects. The commonly consumed marine fishes (n=8) used to assess the amount of EPA, DHA and DPA in raw fish as well as the cooked fish with coconut milk and fried with coconut oil. The results show that fatty acid compositions changed significantly during fish preparation by either cooking as curry or frying in coconut oil, and resulted in lower nutritive values than raw fish. Particularly, the healthy lipids EPA and DHA have been lost during fish preparation. These results reveal that traditional Asian fish cooking and frying method are not good method of fish processing for consumption. The adding to the evidence that consuming fried fish is less beneficial to human health than eating fish cooked in coconut cream as curry.

Key words: Fish, EPA, DHA, DPA, cooking, frying

INTRODUCTION

Consumption of fish provides a vital contribution to the survival and health of a significant portion (nearly 135 billion of people) of the world's population. Lipids in fish mainly consist of long-chain polyunsaturated fatty acids (LCPUFAs) such as eicosapentaenoic acid (EPA, C₂₀:5n-3), docosahexaenoic acid (DHA C₂₂:6n-3) and docosapentaenoic acid (DPA, C₂₀:5n-3) which belong to the physiologically important group of omega-3 fatty acids. The increase in consumption of unsaturated fat, along with a reduction in saturated fats, lowers serum cholesterol in humans and causes a positive impact on human health. PUFAs particularly, omega-3 fatty acids play a role in preventing heart disease and have anti-inflammatory and anti-thrombolytic effects (Bowen *et al.*, 2016). These fatty acids cannot be synthesized and can only be obtained through the diet. The nutritional benefit of fish consumption is mainly attributed to the effect of omega-3 polyunsaturated fatty acids, which are thought to have several potential cardio protective benefits (Swanson *et al.*, 2012). PUFAs are produced only by plants and phytoplanktons, and are essential for the functioning of all higher organisms. Omega-3 and Omega-6 fatty acids are chain elongated and desaturated in the body and retro conversion of DHA to EPA often takes place and can thereby be an important precursor for eicosanoids like prostaglandins, thromboxanes, leucotrienes and hydroxyl-fatty acids (Gutierrez *et al.*, (2025). . In addition, two new families of lipid mediators such as resolvins and protectins that are derived from omega-3 FAs, have potent anti-inflammatory, neuroprotective and pro-resolving properties (Sehan, 2006). When large amounts of very long-chain omega-3 fatty acids are ingested, there is a high incorporation of EPA and DHA into membrane phospholipids which might alter the physical characteristics of cell membranes. One of the major concerns with the intake of omega-3 fatty acids has been the high degree of unsaturation and thereby the possibility of promoting peroxidation. The consumption of marine fish that have high omega-3 fatty acid is reported to promote the reduction of death by cardiovascular diseases (CVD) in Japan and Greenland (Yano *et al.*, 1988; Bang, 1976). Nearly 50 % of population of Sri Lanka consumes inland fish and the lipid, omega-3- fatty acids and tocopherol contents in muscles of these fish are not known. Modification in the fatty acid profiles in fish by culinary technologies for cooking fish alters the nutritive value of the final product (Garcia-Arias *et al.*, 2003). The oxidation and changes in lipid profile of fish lipid due to cooking can lead to certain medical disorders such as higher risk of atherosclerosis (Modugo *et al.*, 2011),

The traditional fish eating habits in Sri Lanka is eating fish cooked in curry with coconut cream or fried in coconut oil, unlike raw fish consumed by the far eastern population. In modern societies, quantitatively, the most important source of omega-3 fatty acids are α -linolenic acid (ALA, C18:3) found mostly in vegetable oils, but these are without any eicosapentaenoic acid (EPA, C20:5n-3) or docosahexaenoic acid (DHA, C22:6n:3) (Ponnampalam., *et al.* (2021). Significant amounts of very long chain omega-3 fatty acids are obtained from fatty fish (herring, mackerel, salmon, trout, eel, anchovies and sardines). The ratio of these two (EPA& DHA) and DPA fatty acids however, is differed between the species of fish. The omega-3 fatty acids in fatty fish are not synthesized in the fish itself but plants and phytoplanktons synthesize it before the marine fatty acids are transferred through the food chain to the fish.

CVD accounted for 17.3 million deaths in 2008, representing 30% of all global deaths. Of these deaths, an estimated 7.3 million were due to CHD and 6.2 million due to stroke (WHO, 2011). By 2030, it is predicted that around 24 million people will die each year from CVD, mainly from CHD and stroke. These are projected to remain as the leading causes of death (WHO, 2011). Geographically, the Indian subcontinent, which makes up one quarter of the world's population, is the region critically affected by CVD (Ramaraj & Chellappa, 2008). Those who have their origin in the Indian sub-continent are collectively known as South Asians and this includes people from India, Pakistan, Bangladesh, Nepal, Sri Lanka and Buttan (Joshi *et al* 2007). Studies have identified a three to five-fold increase in the risk of myocardial infarction and cardiovascular death in South Asians as compared to other ethnic groups (McKeigue *et al.*, 1989; Eapen *et al.*, 2009) since their diet, culture, religion and life styles are different from other ethnic groups. The global prevalence of CHD in South Asians is significantly higher than any other ethnic group during the past forty years (Purnani and Merchant (2020). This study is mainly focused on identifying essential fatty acids such as , EPA, DHA and DPA in highly consumed varieties of fish found in the Batticaloa district, and determining the fate of these fatty acids in cooking by the local community and CVD patients. The omega-3- polyunsaturated fatty acid (ω -3PUFA) in the fresh fish, cooked fish in curry and fried fish of the highly-consumed food fishes were also assessed and the effects on PUFA by the cooking method was evaluated.

METHODOLOGY

The most commonly consumed marine fish in Sri Lanka were collected from the urban and local markets of the Batticaloa district of Eastern Sri Lanka and immediately brought to the cafeteria wrapped in polythene bags. Fish were cleaned and muscle tissue was removed from the mid abdomen of the body. This tissue was washed with cold water and used for cooking and frying. Traditional fish cooking method was followed with the coconut milk and oil and the variety of spices. The fish was cleaned thoroughly, and the muscle (trunk and abdomen) was cut into pieces (65 ± 3 g) and weighed (1.5 kg). The fish pieces were transferred into a bowl and two teaspoons of salt was sprinkled with one teaspoon of turmeric. The dish set aside for 10 minutes. Next, a medium size aluminum pan was kept on medium heat, and coconut oil (50 ml) was added, followed by addition of mustard seeds, fenugreek, fennel seeds, curry leaves, and cinnamon sticks. The mixture was left for 30 seconds. Then, garlic, green chillies, and chopped onion were added for 4–5 minutes until the onion became translucent. Sliced tomatoes were then added and left for 2 minutes, followed by addition of chilli powder, coriander powder, cumin powder, and salt. After stirring well, 2 liters of diluted coconut cream and tamarind pulp were poured into the mixture and cooked for 4 minutes. When the gravy boiled for 5 minutes, the pieces of fish gently were slid into the gravy and left for another 5-10 minutes to cook under low heat with the pan covered. Finally, two cups of concentrated coconut cream (50ml) were added into the boiling fish curry and cooked for another 5 minutes at low heat. Fresh coriander leaves were sprinkled on the curry, and the fish curry was kept away from the flame. (Plate .1). Fried fish also were made. The fish trunk was weighed, and the fish was transferred into the bowl, two teaspoons of salt and one teaspoon of turmeric were sprinkled over it and set aside to marinate for 10 minutes. Coconut oil (Turkey, Sri Lanka) was used for frying. One liter of coconut oil was added into the frying pan and boiled for 5 minutes. At about 92 ± 02 °C, marinated fish were slid into the oil for frying. The fish were stirred for 20 minutes until they were golden brown.

Determination of EPA, DHA and DPA of Fresh and Cooked and fried fish

Fat Extraction by Acid Hydrolysis

Muscle tissue from each fish was homogenized using a grinder (Sumeet, Japan). Homogenized muscle samples (3 g) from each fish were weighed in triplicate using an analytical balance (AG204, Mettler, Toledo) and placed

in dried conical flasks. Muscle tissue samples were hydrolyzed by adding 8 ml of distilled water and 10 ml of concentrated hydrochloric acid and incubated at 95 °C in a boiling bath for 45 minutes. The samples were cooled and transferred to Mojonnier flasks. Fat was serially extracted three times with 25 ml volumes of petroleum ether: diethyl ether (1:1 v/v). The upper phase, which contained the lipids, was evaporated to dryness and weighed for further analysis.

Preparation of Fatty acid methyl esters (FAMES)

FAMES were prepared from muscle samples from lipids extracted from each species. Samples were extracted with petroleum ether/diethyl ether solvent mixture according to modified method of AOAC (948.16). The FAs in the total lipids were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% BF₃ (v/v) in methanol (Paquot, 1979).

Fatty acid analysis using gas liquid chromatography

The FAMES were analyzed on a Shimadzu-14A model gas chromatograph (GC) (Shimadzu, Japan), equipped with a flame ionization detector (FID) and fitted with a capillary column (Superlcowax-10 polythene glycol; length, 100 m; I. D, 0.25 µm) (Sigma-Aldrich Co LLC, St. Louis, MO). Injector and detector temperatures were 200 °C and 220 °C. The oven program was initially held at 60 °C for 10 min, then increased at a rate of 1 °C/min to 200 °C over 10 minutes, and then held at 200 °C for 55 min (total run time). The flow rate of the N₂ carrier gas was 1 °C min⁻¹. GC analysis of FAMES was repeated three times for each sample. FAMES were identified by comparison of peak retention times to those of standards (NU prep check- SD 461, USA). Samples were run in split mode (50:1). Results were expressed as FID response area, as relative percentages of peak area obtained from GC-FID chromatogram. The results are given as mean ± SD in Table 1. Data were analysed statistically using one-way analysis of variance (ANOVA), p < .05, using SPSS 10.0. Means were compared using Tukey's multiple comparison test or Student's *t*-test. SAS 9.1.3. was also used to compare the data whether it was significantly different one treatment to other.

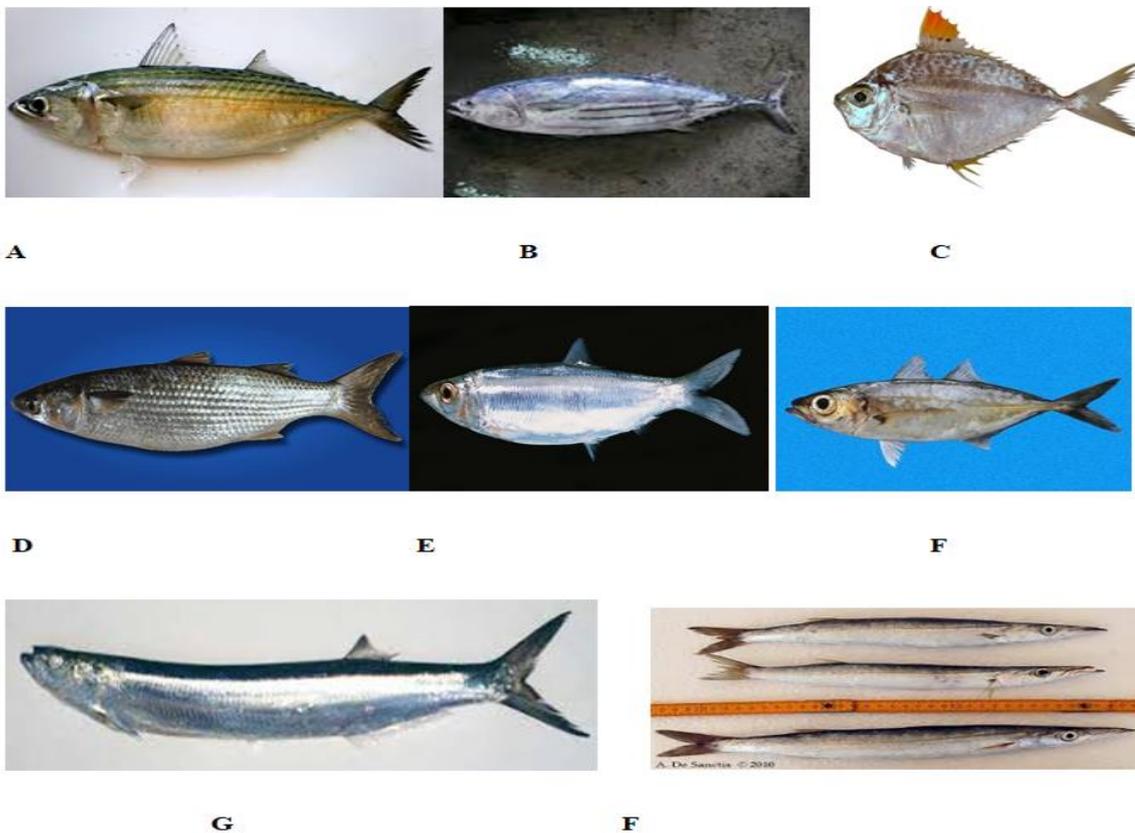


Plate 1. A- Indian mackerel (*Rastrelliger kanagurata*), B- Skipjack tuna (*Katsuwonus pelamis*), C- Pony fish (*Leiognathus bindus*), D- Mullet (*Mugil cephalus*), E- Sardine (*Dussumieria acuta*), F- Mackerel shad (*Selar crumenophthalmus*), G- Wolf herring (*Chirocentrus dorab*), H- Barracuda (*Sphyraena jello*)



Plate 2: Raw , Fried and Cooked fish

RESULTS AND DISCUSSION

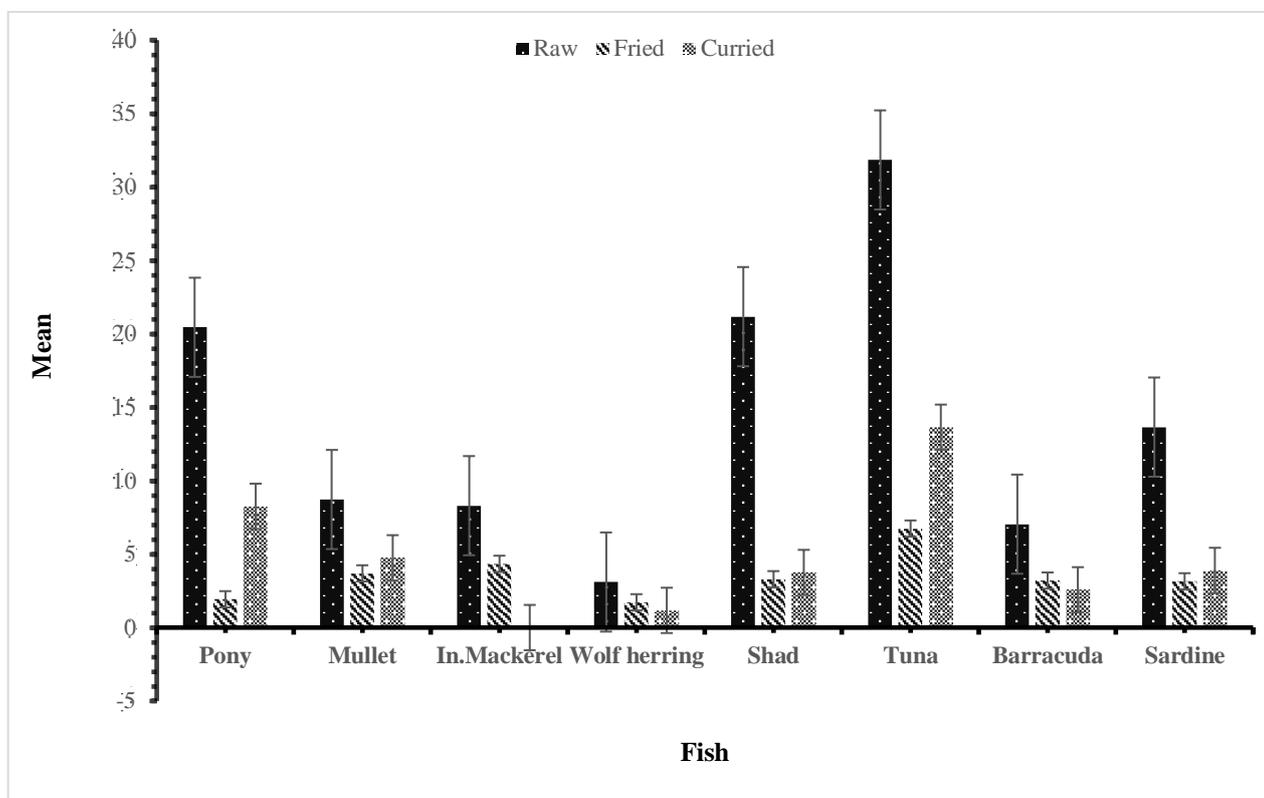


Figure 1. Total EPA and DHA content in raw, fried and curried fish. Data presented as mean±SD EPA & DHA of processed fish

The Sri Lankan style of fish consumption led to a decrease in overall EPA and DHA content (Figure 1). These changes could be interpreted according to the findings of Sioen *et al.*, (2006), who suggested that two mechanisms may occur during frying: absorption of culinary fat by the fish and leaching of soluble fat molecules out of the food. Therefore, it must be noted that when an exchange of fat between the fish and culinary fat takes place, loss of specific fish fatty acids such as EPA and DHA increase considerably (Garcia-Arias *et al.*, 2003). This would imply that the diffusion of EPA and DHA from trout fillets into culinary fat would result in decreased levels of these fatty acids in the cooked samples (Kitson *et al.*, 2009). In addition, Sioen *et al.*, (2006) showed that, during frying, a decrease in total fatty acid content in oily fish changed the fatty acid profile of culinary fat. In contrast, the fatty acid profile in lean fish is similar to that of the culinary fat used. Officially, the recommended intake of EPA plus DHA for humans by the WHO is 1 g/day (Gladyshev *et al.*, 2007). Recently WHO informed that healthy adults has to be consumed 250–500 mg of combined EPA and DHA per day. Owing to the popularity of fried and cooked fish, the actual consumption of EPA and DHA.

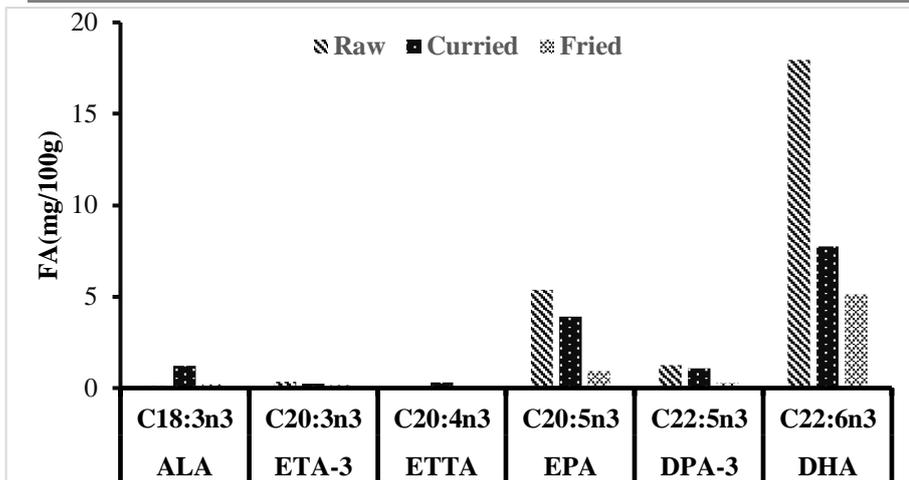


Figure 2. Wolf herring Omega-3 –FA, here, EPA, DPA and DHA amount in Raw, Curry and fried fish

Table 1. The amount of PUFA, omega-3 PUFA and omega-6- PUFA in five fish species of raw, curried and fried fish, Here, R-Raw, C-Curry and F-Fried . mean±SD

Fish Name	Type	PUFA	ω3-PUFA	ω6-PUFA
<i>Leiognathus bindus</i>	R	31.22±20.6	23.65±12.08	6.77±8.08
	F	12.55±0.81	2.44±0.36	9.81±0.32
	C	19.11±0.97	11.27±0.57	7.03±0.57
<i>Mugil cephalus</i>	R	15.03±0.69	7.29±0.39	5.28±0.19
	F	15.46±0.97	5.38±0.45	9.38±0.33
	C	29.15±1.23	13.20±0.41	15.68±0.78
<i>Rasterlliger kanagurata</i>	R	51.20±0.22	25.20±0.10	26.10±0.12
	F	13.54±0.57	5.40±0.50	7.85±0.50
	C	42.29±1.70	23.48±1.70	18.31±1.24
<i>Chirocentrus dorab</i>	R	25.25±1.93	10.10±0.49	15.30±0.41
	F	16.96±0.38	4.35±0.31	12.61±0.07
	C	7.35±0.41	2.35±0.26	4.98±0.15
<i>Selar crumenophthalmus</i>	R	42.82±3.25	23.68±3.12	7.28±1.28
	F	16.82±2.44	4.09±1.26	11.95±1.06
	C	14.50±0.45	4.35±0.34	9.83±0.09
<i>Katsuwonus pelamis</i>	R	46.05±3.04	33.91±0.70	11.22±0.70
	F	17.54±0.81	7.61±0.15	9.61±0.15

	C	33.24±2.01	15.65±1.59	17.26±1.59
<i>Sphyraena jello</i>	R	25.35±0.64	10.00±0.13	15.34±0.51
	F	17.19±0.23	5.46±0.12	11.73±0.11
	C	11.62±0.36	4.54±0.19	7.08±0.17
<i>Dussumieria acuta</i>	R	32.48±1.97	17.24±0.91	6.13±0.67
	F	17.92±0.89	3.97±0.65	13.50±0.24
	C	14.21±2.25	8.03±0.45	5.55±1.66

The EPA+DHA:C16:0 ratios are considered a good index to assess lipid oxidation (Osman *et al.*, 2001). Our data showed that fish made into curry (particularly, mackerel and barracuda) had higher lipid oxidation activities than fried samples. Overall, fish processed into curry had higher hypocholesterolaemic: hypercholesterolaemic (HH) ratios (Table 2). The HH ratio is useful in relating PUFA, considered hypocholesterolaemia (decrease in cholesterol levels), to the sum of two saturated fatty acids, namely, myristic acid (C14:0) and palmitic acid (C16:0), considered hypercholesterolaemic (increase in cholesterol levels) (Testi *et al.*, 2006). Stearic acid (C18:0) should be excluded from the saturated fraction in this ratio because it does not behave like a typical saturated fatty acid in regard to affecting cholesterol levels (Unusan, 2007). Asghari *et al.*, (2013) showed that the HH value of raw trout is 2.65, which increases in microwaved and fried samples but decreases significantly (P<0.05) in boiled samples.

Table 2. Hypocholesterolaemic: hypercholesterolaemic fatty acid (HH) ratio in raw, fried, and curried fish

Fish name	Raw fish (HH)	Fried fish (HH)	Curried fish(HH)
Pony fish	0.68	1.3	0.7
Mullet	0.51	1.22	1.41
In. Mackerel	4.17	1.12	2.05
Wolf Herring	0.55	1.25	1.72
Mackerel scad	0.86	1.55	1.31
Tuna	1.32	1.29	1.84
Barracuda	0.96	1.56	1.33
Herrings	0.24	1.41	0.5
R value	-0.5	-0.45	0.64
P value	0.21	0.26	0.09

Therefore, contrary to expectations, frying enhanced the nutritional value of trout from the point of view of increasing the HH ratio. The HH index was higher in all processed fish (fry and curry) compared to raw fish in these findings. Testi *et al.* (2006) found higher values of HH in fish, varying from 2.03 to 2.46. The present study showed that the HH ratio in fried and curried fish ranged from 1.12 to 1.84 (Table 2), and revealed a decrease in cholesterol levels. The content of health beneficial FAs (EPA, DHA) decreased significantly by the traditional cooking methods of Asian tuna fish. By using coconut oil for frying and coconut cream for processing, the

nutritional quality decreased compared to raw fish, whereas the content of health beneficial fatty acids was significantly lower in processed fish. A significant increase in energy content was observed in all cooked samples, whereas the cholesterol levels decreased. But, microwaving is the best cooking method for a healthy consumption of rainbow trout (Asghari *et al.*, 2013).

CONCLUSION

The lipids in fish fillets play an important role in providing taste, flavour, smell, and texture to the fish. Our results show that fatty acid compositions changed significantly during fish preparation by either cooking as curry or frying in coconut oil, and resulted in lower nutritive values than raw fish. Particularly, the healthy lipids EPA and DHA have been lost during fish preparation. These results reveal that traditional cooking and frying method are not good method of fish consumption. The adding to the evidence that consuming fried fish is less beneficial to human health than eating fish cooked in coconut cream as curry.

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