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Research Note

Comparative Analysis of Fatty Acid Profile of Fish oils Extracted from *Diaphus watasei* and *Sardinella longiceps*

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Fish oils are a rich natural source of long-chain polyunsaturated fatty acids (PUFA), especially those of the n-3 series, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Beneficial health effects of n-3 PUFA are well demonstrated and include the prevention of a number of diseases such as coronary heart disease, inflammation, hypertriglyceridemia, allergies, hypertension, arthritis, autoimmune disorders and cancer (Von Schacky, 2003). Studies in newborns indicate that DHA is essential for the normal functional development of retina and brain, particularly in premature infants (Conner, 2000). Currently, the predominant dietary sources of very long-chain n-3 PUFA are oily fish and fish oil supplements (Sahena et al., 2010). In India, fish oil is mainly extracted from oil sardine (*Sardinella longiceps*) and is reported as a rich source of n-3 PUFA such as EPA and DHA (Andrade et al., 1995; Ambasankar & Balakrishnan, 2006). Biochemical composition of various species of myctophid fishes report that they are good sources of protein and minerals and are similar to other common marine fishes except for the fact that they have very high fat content *viz.*, from 4.9 to 28.5% (Gopakumar et al., 1983; Noguchi, 2004). Rajamoorthy et al. (2013) reported the proximate composition of *Diaphus watasei*, *viz.*, moisture, fat, protein and ash content as 63.19, 15.13, 21.40 and 1.33% respectively which clearly indicate the nutritional potential of this species for human consumption and formulation of novel foods.

In the present study, an attempt was made to compare the fatty acid profile of oil extracted from *D. watasei*, a myctophid fish commonly seen in the bycatches of deep sea shrimp trawlers operating off Kerala, with oil extracted from *S. longiceps.*

Myctophid fish, *D. watasei* samples were collected from the bycatch of deep sea shrimp trawlers operating off Kollam and Cochin coasts of Kerala, India and oil sardine, *S. longiceps* samples were bought from a nearby fish market in Cochin. Fish samples were obtained under iced condition in styrofoam boxes and transported to the laboratory in insulated container with ice.

Fish oil was extracted from the whole fish of both species by cooking method (FAO, 1986). Fishes were thawed, washed and then cooked in boiling water for 21 min and allowed to cool down for 15- 20 min. The top layer was collected and passed into separating funnel; remaining stickwater was filtered and solids were separated by pressing. The press liquor obtained was also transferred to separating funnel. Oil was separated from the press liquor and clarified with salt and hot water washes and stored in glass bottles at room temperature.

About 100 mg of oil was saponified by refluxing for about 5 min with 5 ml of 0.5 N methanolic NaOH and esterification was done by refluxing with BF_3-methanol. To the cooled mixture, saturated NaCl was added. Finally the fatty acid solution was extracted with petroleum ether and washed with water. The solvent was evaporated and made up to 1 ml (Metcalfe et al., 1966). Fatty acid methyl esters were analysed by using Perkin Elmer Autosystem XL Gas Chromatograph and Perkin Elmer Turbo

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Mass Spectrophotometer (Norwalk, CTO 6859, and USA). The separation was performed in an Elite 225 capillary column measuring 30 m length and 0.25 mm diameter with a film thickness of 0.25 μm. Helium was used as a carrier gas at a flow rate of 0.5 ml min^{-1}. The initial temperature was maintained at 265°C. The oven temperature was initially held at 110°C for 4 min and was programmed to increase to 240°C at a rate of 2.7°C min^{-1}, held at 240°C for 3 min and then programmed to increase to 280°C and held for 5 min. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. The total run time was about 62.15 min.

Analysis were performed in triplicate and averaged. Results are expressed as mean ± SD and Student's t-test was used to assess statistical significance.

Table 1 illustrates the fatty acid components *viz.*, saturated fatty acid, (SFA) mono unsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of oil extracted from *D. watasei* and *S. longiceps*. The total oil content was higher in *S. longiceps* (17.29% on wet weight basis) than *D. watasei* (15.2%). SFA and PUFA were found in comparatively higher amount in sardine oil than in oil extracted from *D. watasei* but MUFA content was higher in the latter (Fig. 1). In the present study, in *Diaphus* oil, significantly higher level was found in total MUFA when compared with the oil of *S. longiceps* ($p<0.001$). The fatty acid composition of *D. watasei* oil consists of SFA-37.49, MUFA- 42.49 and PUFA-14.07% whereas in oil of *S. longiceps*; the SFA, MUFA and PUFA were found to be 42.17, 23.61 and 30% respectively.

Saturated fatty acids like palmitic acid and stearic acid were found in higher quantity in total saturated fatty acid level of *D. watasei*. In Diaphus oil, among the PUFA, DHA which plays a major role in counteracting various disorders and diseases related

Table 1. Fatty acid profile (%) of oil from *Diaphus watasei* and *Sardinella longiceps*

	Fatty acids	*Diaphus watasei*	*Sardinella longiceps*
Saturated fatty acids (SFA)			
C14	Myristic acid	4.00±0.02	12.02 ±0.03***
C16	Palmitic acid	21.02±0.06	25.22 ±0.92***
C18	Stearic acid	9.07±0.02	4.08 ±0.02***
C20	Arachidic acid	3.40±0.07	0.86 ±0.01***
	Total SFA	37.49±0.17	42.17±1.01***
Mono-unsaturated fatty acids (MUFA)			
C16:1	Palmitoleic acid	2.86±0.03	14.47 ±0.97***
C18:1	Oleic acid	39.51±0.04	8.81 ±0.08***
C20:1	Gadoleic acid	0.12±0.01	0.33 ±0.01***
	Total MUFA	42.49±1.05	23.61±0.08***
Poly-unsaturated fatty acids (PUFA)			
C18:2	Linoleic acid	0.85±0.03	1.24 ±0.01***
C18:3n3	α-Linolenic acid	0.28±0.00	0.73 ±0.02***
C18:3n6	λ-Linolenic acid	0.04±0.00	0.61±0.01***
C20:2	Eicosadienoic acid	0.29±0.02	0.15±0.00***
C20:3	Eicosatrienoic acid	0.04±0.05	0.18±0.01**
C20:4	Arachidonic acid	0.73±0.03	1.56±0.06***
C20:5	Eicosapentaenoic acid	2.83±0.05	16.85±0.90***
C22:6	Docosahexaenoic acid	9.01±0.09	8.67±0.82 NS
	Total PUFA	14.07±1.81	30.00±0.27***

Significance level expressed as *** = $p<0.001$, ** = $p<0.01$ and * = $p<0.05$

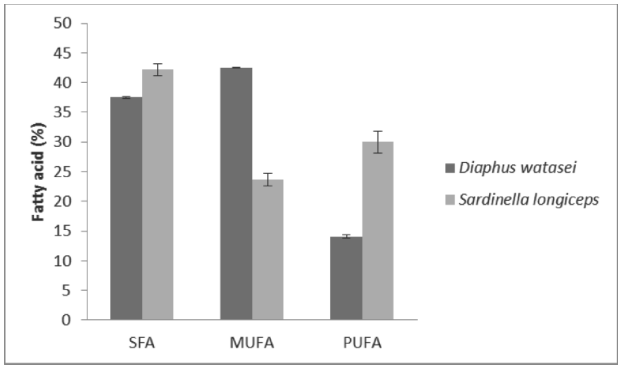

Fig. 1. Comparison of SFA, MUFA and PUFA contents in *Diaphus watasei* and *Sardinella longiceps* oil

to aging, was found in levels comparable to that of sardine oil. Oleic acid is the predominant fatty acid found in *D. watasei* (39.51%) than *S. longiceps* (8.81%) giving a higher MUFA value (42.49%) to *D. watasei oil*. Lea et al. (2002) studied the lipid content, fatty acid composition and calorific value of seven species of the family myctophidae from Southern Ocean and reported relatively high levels of MUFA (33.8–53.5% of total FA) in all species. Oleic acid (39.51%), palmitic acid (21.02%), stearic acid (9.07%) and DHA (9.01%) were found as the predominant fatty acids in the *Diaphus* oil. Oleic acid has been reported to slow down the development of heart diseases and promote the production of antioxidants (Waterman & Lockwood, 2007).

Though myctophid fishes are abundant in the seas around India, they are the least studied and underutilized. In view of their potential and nutritive value it is important to explore the avenues for utilizing myctophid species at par with other commercially important species. Fatty acid profile has revealed the nutritional significance of *D. watasei,* in comparison to other finfish species such as oil sardine. Due to presence of higher level of DHA when compared to sardine oil, *D. watasei* may be considered as a viable source for extraction and purification of DHA, which is essentially required for the development of foetal brain and retina during pregnancy. Since myctophids are not utilized for direct human consumption so far, it may be used as a potential nutrient resource in the formulation of pharmaceutical products and animal feeds, in future.

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