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Quality and Fatty Acid Composition of Lipids from Head of Indian Mackerel (*Rastreliger kanagurta*) and Tigertooth Croaker (*Otolithes ruber*)

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Abstract

Fish processing discards contain valuable nutrients such as proteins, lipids and minerals. The lipid from mackerel and croaker head extracted by alkali solubilization was recovered and characterized for quality parameters such as peroxide value (PV), free fatty acid (FFA), thiobarbituric acid reactive substances (TBARS) and fatty acid composition. PV, FFA and TBARS values of lipids recovered from head of mackerel was higher than that of croaker. Both mackerel and croaker head lipids had higher amount of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) and lower amount of polyunsatuarated fatty acids (PUFA) compared to fatty acids from muscle of the same fishes. Mackerel head lipids had higher PUFA content (30.81%) than that of croaker (18.71%). The SFA content was dominated by palmitic acid while the MUFA content was dominated by palmitoleic acid. Docosahexaenoic acid (DHA; C22: 6 ω3) and Eicosapentaenoic acid (EPA; C 20: 5 ω3) were the dominant PUFA in both the fish-head lipids. The study revealed the fish head waste as a potential source of ω-3 fatty acids which can be recovered by alkali solubilization process.

Keywords: Head waste, mackerel, tigertooth croaker, fatty acid composition, PUFA, lipid recovery

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Introduction

Fish as a component of healthy diet has been well recognized all over the world and a continuous raise in the global demand is expected (FAO, 2016). Large number of fish meat based consumer convenient products are emerging and thus huge quantity of by-products are generated which accounts for about 65-70% of weight of the raw material (Elavarasan et al., 2017; Kumar et al., 2017). Generally, fish processing waste is either discarded or considered as a low value raw material, which meets the demand of fish meal industry (Ghaly et al., 2013). However, fishery by-products are increasingly being recognized as secondary raw material and being utilized for the production of high value products with functional and bioactive properties such as gelatin, protein hydrolysate and omega-3 fatty acids concentrate. Fishery byproducts could also be used as an important source of nutrition (Ninan et al., 2009; Murthy et al., 2012; Tahergorabi et al., 2014; Renuka et al., 2016).

Heads and fillet cut-offs can be used directly as food or turned into products such as fish sausages, cakes, fish cutlets for human consumption (Reddy & Bhandary, 2015). For this purpose, the meat or meat proteins are to be recovered. Recovering the meat portion from the head waste is often difficult (Ruthu et al., 2014). Alkali solubilization and iso-electric point (pI) precipitation technique is useful to recover proteins and lipids from the head simultaneously. During the alkali solubilization process lipids are liberated which could be recovered and be used as a potential source of unsaturated fatty acids (Khoddami et al., 2012; Tahergorabi et al., 2014).

The estimated Indian marine fish landing for the year 2016 was 3.63 million tonnes which is found to be stagnating. The stagnation or reduction in marine fish landing necessitates improved/effective utilization of available resources (CMFRI, 2017). Indian mackerel and croaker are two important resources being used for domestic as well as for export market. Mackerel, a fatty fish is well known for its poly unsaturated fatty acid content and mainly consumed in the domestic market. The total landings of mackerel during 2016 was 2 49 241 which is 6.67% of total marine fish landings of India (CMFRI, 2017). On the other hand, croaker is an ideal raw material for surimi (wet concentrate of myo-fibrillar proteins) industry because of its better gel forming capacity (Elavarasan et al., 2017). Surimi industry generates significant quantity of by-products. Croakers has account for 4.22% of the total marine landings in India (CMFRI, 2017). With this background, the present study aimed to recover lipids from mackerel and croaker fish head waste and to compare fatty acid composition with the muscle lipids. Quality of recovered fish head waste lipids has also been investigated.

Materials and Methods

Indian mackerel (*Rastreliger kanagurta*) and tigertooth croaker (*Otolithes ruber*) were procured from local fish markets of Ernakulam, India and brought to the laboratory in iced condition at 1:1 ratio (Fish: Ice; w/w). Fishes were washed with chilled potable water and beheaded manually. Heads were collected, packed in high density polyethylene packs, frozen in an air blast freezer and stored in cold storage at -20 ± 2°C until further use.

Frozen head wastes were thawed in running tap-water. About 4.0 kg of thawed fish head waste was chopped manually with a knife. The chopped fish head waste was ground paste using a household grinder (MX-AC350, Super Mixer Grinder, Panasonic, Panasonic Appliances India Co., Ltd, India). The paste was transferred to a stainless steel vessel equipped with a mechanical stirrer. Potable water was added to the paste (paste: water; 1:3) and stirred for 15 min with the speed of 180 rpm to remove the water soluble components. The lipid mass floating on the surface of water was harvested using a hand-held net with fine mesh (30 micron). The water was removed by filtering the mass in a nylon mesh. The residual mass was transferred back to the stainless steel vessel and into which precooled 0.1 M NaOH solution was added at 1 : 3 ratio (residual mass: alkali; w/w) and stirred at 180 rpm for 30 min. During the entire process, the temperature was maintained below 10°C using chilled alkali solution. The creamish lipid mass accumulated on the wall of vessel and floating on the surface was collected using a fine meshed net. The collected lipid masses was pooled, transferred to an air tight container and stored in refrigerated condition (4 ± 2°C). All the analyses were carried within 48 h of storage.

Head wastes from mackerel and tigertooth croaker were analyzed for proximate composition viz., moisture, crude protein, total lipids and ash according to the methods described in AOAC (2012). In brief, for moisture analysis, the sample was dried in a hot air oven at 105 ± 2°C till constant weight was obtained and the moisture content was quantified gravimetrically. To determine crude protein, the nitrogen content in the sample was determined by Kjeldahl method and the factor of 6.25 was used for the conversion of nitrogen into protein. Total lipid was determined gravimetrically by extracting the lipid using petroleum ether in a soxhlet apparatus. The ash content in the sample was determined gravimetrically by incinerating the sample in a muffle furnace.

Free fatty acid value (FFA) and peroxide value (PV) of lipid recovered by alkali solubilization was determined according to the methods described in AOAC (2000). FFA and PV were expressed as percentage of oleic acid to the total lipids and O_2 kg^{-1} of lipid, respectively. Thiobarbutiric acid (TBA) value was determined as described by Tarladgis et al. (1960) and expressed as mg of malonaldehyde (MDA) kg^{-1} of lipid. In addition, iodine value (g 100 g^{-1}) and saponification value (mg of KOH g^{-1} of lipid) were determined according to the methods explained in AOAC (2000).

Fat was extracted from muscle and head waste lipids (creamy mass recovered during alkali solubilization) of mackerel and tigertooth croaker according to the method described by Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared according to Metcalfe et al. (1966). Accurately 150 mg of oil was mixed with 6 ml methanolic NaOH in a flask and refluxed. After the disappearance of fat globules, 7 ml BF_3 methanol was added to the mixture and boiled for 2 min and then 15 ml of saturated NaCl was added and mixed vigorously for 15 sec. From the reacted mixture, fatty acids were

extracted using 30 ml petroleum ether. Fatty acid extract was washed with water and dried by filtering through anhydrous Na_2SO_4. The excess solvent was evaporated under a stream of nitrogen in a warm water bath (TW20, Julabo, Germany). Fatty acid profile was analyzed as per AOAC (Method 991.39: AOAC, 2000). Methyl esters of the fatty acids obtained after trans-esterification process were separated by GC (Gas Chromatography) Varian CP 3800 USA) equipped with a capillary column. The peak detection was performed by flame ionization detector in the presence of hydrogen (60 psi) and air (40 psi). Nitrogen at 80 psi at a flow rate of 1.2 ml min^{-1} was used as a carrier gas. The initial temperature was set at 120°C and programmed to increase up to 250°C at a rate of 4°C min^{-1}. Identification of fatty acids separated were carried out by comparing the retention time of standard fatty acid methyl esters (Supelco 37 component FAMEmix, Analytical standard, Sigma-Aldrich Co., St.Louis, MO, USA). The results are expressed in percentage as relative peak area of each fatty acid methyl esters to total area of the identified fatty acid methyl ester peaks.

Proximate composition and lipid quality parameters were analyzed in triplicates using independent samples. The results are expressed as mean with standard deviation. Fatty acid composition was analyzed using pooled fat extract from two independent samples. Homogeneity of variance was tested using ANOVA with the help of software $(SPSS)^R$.

Results and Discussion

In the present study, lipids from head of two fish species (mackerel and croaker) were recovered using alkali solubilization process and their quality parameters were determined. A comparison was made between the fatty acid compositions of lipids recovered from head waste and muscle of the respective fishes.

Proximate composition of mackerel and croaker head waste is presented in Fig. 1. The data on head waste of croaker has been retrieved from Elavarasan et al. (2017). Lipid content of croaker head waste was higher than mackerel head waste. Among other major bio-chemical constituents, protein content was higher in mackerel head waste. On dry basis (db), the lipid content of croaker and mackerel head waste was 23 and 18%, respectively. The lipid content in the head wastes from tuna, sardine and tilapia were reported to be 13.5, 5.67 and 5.7% respectively (Khoddami et al., 2009; Swapna et al., 2010 Nguyen et al., 2011).

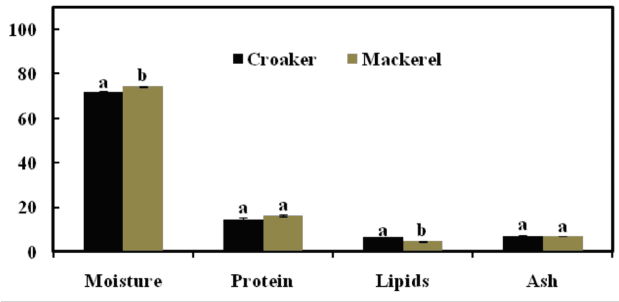

Fig. 1. Proximate compositions of mackerel and croaker fish head wastes. The different letters on the error bars indicate the significant difference within the parameter between the samples

The quality of lipids recovered from mackerel and croaker fish head waste is presented in Table 1. Free fatty acid value (FFA), peroxide value (PV) and Thiobarbituric acid reactive substances (TBARS) value of mackerel head lipids were 0.27% of total lipids as oleic acid, 5.64 meq O_2 kg^{-1} and 7.05 mg malonaldehyde kg^{-1} of lipids, respectively and found to be higher than croaker head waste lipids. Free fatty acids (FFA) are formed by the hydrolysis of oils and fats. The lower FFA value indicates better quality and may have better storage stability (Khoddami et al., 2012). The level of FFA formed

Table 1. Quality of lipids recovered from head of mackerel and croaker

Quality parameters	Mackerel Head Lipids	Croaker Head Lipids
FFA (% of lipids as oleic acid)	0.27 ± 0.02[a]	0.13 ± 0.03[b]
PV (meq O_2 kg^{-1} of lipid)	5.64 ± 0.05[a]	1.51 ± 0.02[b]
TBARS (mg of malonaldehyde kg^{-1} of lipids)	7.05 ± 0.01[a]	3.46 ± 0.01[b]
Iodine value (g 100 g^{-1})	40.00 ± 2.00[a]	52.00 ± 3.00[b]
Saponification value (mg of KOH g^{-1})	186.50 ± 12.50[a]	229.50 ± 14.50[b]

would vary with process time, temperature and moisture content (Mahesar et al., 2014). Peroxide value and TBARS value correspond to formation of primary and secondary lipid oxidation products due to oxidation of lipids. In general, the crude fish oil has been reported to have the PV in the range of 3-20 meq O_2 kg^{-1}. PV value of 8 meq O_2 kg^{-1} is considered to be acceptable limit for human consumption (Boran et al., 2006). In the present study, the PV of mackerel and croaker head waste lipids was well below 8 meq O_2 kg^{-1}, indicating the suitability for human consumption. Similarly, head waste lipids from *Euthynnus affinis* were reported to have a PV of 7.31 meq O_2 kg^{-1} (Khoddami et al., 2012). TBARS value of fish head waste lipids obtained in the present study is comparable with the available report on fresh anchovy fish oil and crude sardine fish oil (Chakraborty & Joseph, 2015).

Comparison of fatty acid composition of lipids recovered from head waste and muscle from the same fishes is presented in Table 2. In both mackerel and croaker, total saturated fatty acids (SFA) were higher in head waste lipids than the muscle lipids. Saturated fatty acid content was dominated by palmitic acid. Total monounsaturated fatty acids (MUFA) were higher in head waste lipids compared to muscle lipids of mackerel, whereas croaker had almost similar content of MUFA both in head waste and muscle lipids. Among the monounsaturated

Table 2. Comparison between fatty acid composition of mackerel and croaker head waste lipids with respective fish muscle lipids

Fatty acids	Fatty acid composition (%)			
	Mackerel Head	Mackerel muscle	Croaker Head	Croaker muscle
C14:0	10.55	2.04	4.07	3.08
C15:0	1.31	0.56	0.79	0.52
C16:0	29.15	19.6	39.15	35.06
C17:0	1.44	0.99	1.19	0.96
C18:0	8.73	13.31	8.52	8.5
Σ SFA	**51.18**	**36.5**	**53.72**	**48.12**
C16:1	11.89	3.29	14.39	12.21
C18:1	5.63	5.25	12.61	13.35
C20:1	0.25	0.15	0.39	0.5
Σ MUFA	**17.77**	**8.69**	**27.39**	**26.06**
C18:2ω-6	1.97	2.02	0.87	0.88
C18:3ω-6	0.38	0.15	0.12	0.15
C18:3ω-3	1.57	0.88	0.64	0.59
C20:2ω-6	0.23	0.23	0.25	0.27
C20:3ω-6	0.21	0.32	0.22	0.21
C20:3ω-3	0.2	0	1.21	0.22
C20:4ω-6	2.23	6.22	2.88	3.58
C20:5ω-3	10.77	8.35	6.03	7.62
C22:6ω-3	13.25	36.53	6.49	12.06
Σ PUFA	**30.81**	**54.7**	**18.71**	**25.58**
ω-3	25.79	45.76	14.37	20.49
ω-6	5.02	8.94	4.34	5.09
ω-3/ω-6	**5.14**	**5.12**	**3.31**	**4.03**

SFA – Saturated fatty acids; MUFA- Mono unsaturated fatty acids; PUFA- Poly unsaturated fatty acids

fatty acids, palmitoleic acid content was more in both fish head waste lipids. PUFA (polyunsaturated fatty acids) content was higher in muscle lipids than the head waste lipids in both mackerel and croaker. A higher ω-3, ω-6 and ω-3/ω-6 ratio was recorded for mackerel head waste lipids compared to croaker head waste lipids. Both mackerel and croaker muscle lipids contained higher levels of ω-3 and ω-6 fatty acids. The docosahexaenoic acid (DHA; C_{22}: 6 ω3) and Eicosapentaenoic acid (EPA; C_{20}: 5 ω3) were the dominant PUFA in lipids extracted from mackerel and croaker fish.

Utilization of fishery by-products for the production of high value compounds is being researched worldwide. Alkali solubilization process is gaining much attention as a useful technique to recover proteins and lipids simultaneously from fishery waste. Currently, there is an increasing demand for fish oil for direct human consumption. Present investigation has demonstrated the use of alkali solubilization process for recovering lipids for the utilization of head waste of mackerel and croaker. The lipids from fish head wastes contained rich amount of polyunsaturated fatty acids (EPA, DHA) and monounsaturated fatty acids (Palmitoleic acid) that have significant nutritive value.

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