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Physico-chemical characterization and microbial studies of the muscle lipid of Indian mackerel (*Rastrelliger kanagurta*) of the Bay of Bengal

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Abstract
Lipid was extracted from the muscle of Indian mackerel (*Rastrelliger kanagurta*) of the Bay of Bengal by solvent extraction method and various physical and chemical constants were characterized and compared with those of standard oils and fats. Fatty acid profile was evaluated by Gas-Liquid Chromatography (GLC) which indicated the presence of myristic acid, palmitic acid, stearic acid, lignoceric acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. The composition of fatty acids showed that total Polyunsaturated Fatty Acids (PUFA %) were higher than Monounsaturated Fatty Acids (MUFA %) i.e. [30.4141% >28.4568%] and ω-3 Polyunsaturated Fatty Acids (ω-3 PUFA) were higher than ω-6 Polyunsaturated Fatty Acids (ω-6 PUFA) i.e. [24.0288%>6.3853%]. The lipid sample was evaluated in terms of microbial activities (bacterial and fungal activities) by standard methods. Percentages of mineral content (N, P, K and Ca) of lipid containing muscle of the selected specimen were analyzed quantitatively. Thus it is concluded with various important information regarding industrial, pharmaceutical and nutritional aspects.

Keywords: Lipid, Indian mackerel, PUFA, GLC, microbial activities

1. Introduction
Bangladesh is enclosed with a long coastal belt of the Bay of Bengal along its southern boundary and most of the people depend on fish as a source of protein which contributes about 80% of the animal protein [1]. Indian mackerel, locally known as Aila fish, is an important part of the fishery resources of Bangladesh. It is found on a large scale in the Bay of Bengal but public are not conscious about the importance of this fish and enough data of the food value and pharmaceutical aspects are not available also. Currently, the biochemical effects of fish oils in human health and nutrition [2, 3] have placed renewed emphasis on the apparent difference in the compositions of fish oils [4]. It is known that fish lipids are the main sources of polyunsaturated fatty acids (PUFAs) especially eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) [5] and they have biochemical effect in prevention and treatment of several disorder and diseases such as coronary heart disease, rheumatoid arthritis, asthma, cancers, diabetes and others [6]. Lipids and fatty acids also play a significant role in membrane biochemistry and have direct effect on the membrane mediated process in human such as osmoregulation, nutrient assimilation and transport [7]. Recently, researchers are giving more highlight on physico-chemical analysis, microbial study and fatty acid profile of lipid of various marine fishes but results of such types of studies on Indian mackerel are much unknown or less reported. The present investigation is apprehensive with the extraction of the muscle lipid of Indian mackerel with a view to finding out the PUFAs presence in it and studying its physico-chemical and microbial characteristics including quantification of mineral content and comparing the results with the data available in literature about pharmacological aspects.

Fig 1: Indian mackerel (*Rastrelliger kanagurta*)

2. Materials and Methods

2.1 Collection and Identification of the marine species

The export quality title fish Indian mackerel (*Rastrelliger kanagurta*) of the Bay of Bengal was collected from the local fish market, Sadarghat in port and marine city Chittagong (22°20'18.24" N 91°49'54.05" E) which is the financial centre of southeastern Bangladesh. The specimen was identified at the Institute of Marine Sciences & Fisheries of the University of Chittagong.

2.2 Extraction of the lipid

The lipid was extracted from the muscle of Indian mackerel by Bligh and Dyer method using acetone and ethyl acetate as solvent. The extract thus obtained was dried, free of solvent first by rotary evaporation and finally by blowing a slow stream of nitrogen gas. Analytical grade chemicals and reagents were used. Solutions were prepared according to the standard procedures [8, 9, 10].

2.3 Physico-chemical characterization

Refractive index, specific gravity, viscosity and moisture content of the muscle lipid and ash content, crude fat, crude fiber content of the de-oiled muscle of the Indian mackerel were determined by standard methods [11]. Saponification value, saponication equivalent value, acid value and percentage of free fatty acid (as oleic acid), iodine value, acetyl value [12], peroxide value [13], thiocyanogen value, Richert Meissl value, Polenske value [11], Henher value, Elaiden test [14] and quantity of unsaponifiable matter [15] of the muscle lipid of Indian mackerel were determined by standard methods.

2.4 Microbial analysis

The microbial activity of muscle lipid of Indian mackerel was studied against four bacteria and four fungi. For the detection of antibacterial activities the disc diffusion method [16] was followed. The antifungal activity was assessed by food poison technique [16]. Nutrient agar (NA) and potato dextrose agar (PDA) were used as basal medium for the test of bacteria and fungi respectively. Chloroform was used as a solvent to prepare desired solution (10 % and 5 %) of the lipid sample. Proper control was maintained with chloroform.

2.5 Estimation of minerals

By applying the standard methods, minerals (N, P, K and Ca) of lipid containing muscle were determined [17].

2.6 Chromatographic examinations

The identification of fatty acid components from GLC analysis was carried out on the basis of relative retention time and was quantified by measuring the peak area in comparison with standard value [18].

3. Results and Discussion

3.1 Physical characteristics

The yield of total lipid content extracted from the muscle of Indian mackerel was found to be 32.95 mg/g. This may claim valuable demand for edible purpose due to their higher lipid level. The refractive index of the muscle lipid of Indian mackerel was found to be 1.4735 at 28 °C (Table 1). Refractive power of oils or fats varies somewhat widely and chiefly governed by the proportion and degree of unsaturation present. It is also an intensive property of any substance. The present result indicates that the muscle lipid from the specimen contained moderate amount of unsaturated fatty acids. This was also supported by its iodine value.

The specific gravity of the lipid solution of the muscle lipid of Indian mackerel was determined and found to be 0.946 at 28 °C (Table 1).

The viscosity of the lipid solution of the sample was found to be 313.27 milipoise at 28 °C (Table 1). From the result of viscosity we got an idea about the intermolecular hydrogen bonding in the lipid sample. The present result suggested that there is a few hydroxyl group and few free acid molecules may present in the lipid sample. This observation is supported by low acetyl value and low acid value of the lipid sample.

Table 1: Physical constants of the muscle lipid of Indian mackerel and other lipid sample

Name of the sample	Refractive index	Specific gravity	Viscosity (mp)
Brain lipid of Baghda Chingri	1.4736	0.941	303.26
Liver lipid of Spanish fish	1.4630	0.919	361.00
Muscle lipid of Cuttle fish	1.4731	0.973	320.32
Body lipid of Vetki fish	1.4745	0.923	290.38
Muscle lipid of Indian mackerel	1.4735	0.946	313.27

The moisture content of the muscle lipid of Indian mackerel was determined and found to be 1.82% (Table 2). The moisture content in fixed oils or fats is varying slight and only small amounts are generally present. The ash, crude fat and crude fiber content of the de-oiled muscle was calculated and found to be 0.94%, 2.32% and 2.11% respectively (Table 2).

Table 2: Moisture content of muscle lipid; ash, crude fat and crude fiber content of the de-oiled muscle of Indian mackerel.

Name of the sample	Moisture content (%)	Ash content (%)	Crude fat content (%)	Crude fiber content (%)
Muscle lipid of Indian mackerel	1.82%	0.94	2.32	2.11

3.2 Chemical characteristics

The saponification value and saponification equivalent of the muscle lipid of Indian mackerel was found to be 208.12 and 269.556 respectively (Table 3). Saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fat or oil. On the

hand, saponification equivalent is directly proportional to the average chain length of fatty acid present. The results clearly indicate that the lipid sample contains higher proportion of high molecular weight fatty acids.

The acid value of the muscle lipid of Indian mackerel was found to be 1.18 (Table 3). The percentage of free fatty acid (FFA), as oleic, was also calculated from acid value and was found to be 0.594% (Table 3). Acid value is a measure of proportions of free fatty acids in the oil or fat. Hence, low acid value of the extracted lipid sample is an indication of freshness and low percentage of free fatty acid is an indication of suitability of the lipid for edible purpose. The ester value was calculated and found to be 206.94 (Table 3).This value indicates the amount of ester present in the lipid sample.

The iodine value of 112.36 (Table 3) indicates that the lipid contains moderate proportion of unsaturated fatty acid components and is of semidrying type. This is also approved by the results of the physical properties and confirmed by the Elaiden test.

The peroxide value is an indication of unsaturation present in fats and oils. The more unsaturated fats or oils absorb more oxygen, form greater amount unstable hydro peroxide and show higher peroxide value. The peroxide value of the muscle lipid of Indian mackerel was found to be 122.72 (Table 3). It can be concluded from the result that the muscle lipid under investigation contained good amount of unsaturated fatty acids. Thiocyanogen value of the sample was found to be 58.82 (Table 3). This observation is in conformity with the findings that the lipid sample has moderate iodine value and peroxide value.

Acetyl number is a measure of the number of hydroxyl groups present in a fat or oil. The acetyl value of 10.75 (Table 3) indicates low content of free hydroxyl group in the lipid sample. The titre value of the muscle lipid of Indian mackerel was found to be 26.2 °C (Table 3). This value indicates that the lipid sample is of fat type which supports its semisolid condition at room temperature.

The unsaponfiable matter in the muscle lipid of Indian mackerel was found to be 0.622% (Table 3). Unsaponifiable matter is defined as those substances which are not saponified by alkali and which are soluble in ether or petroleum ether. In general, if a fixed oil or fat contains unsaponifiable matter in excess of about 2%, there is reason to support adulteration. The result indicates that the lipid sample may contain a small amount of unsaponifiable matter such as sterols, vitamins A and D, hydrocarbons etc.

The Reichert-Meissl value of the muscle lipid of Indian mackerel was found to be 1.06 (Table 3). Since the Reichert-Meissl value is a measure of the volatile water soluble lower fatty acids present in the fat or oil, so the lower Reichert-Meissl value of the lipid sample is an indication of low content of volatile water soluble fatty acids.

The Polenske value of the muscle lipid of Indian mackerel was found to be 0.86 (Table 3). The Polenske value represents a measure of volatile water insoluble but alcohol soluble fatty acids. The Polenske value as obtained is a support of the small amount volatile water insoluble but alcohol soluble fatty acids in the lipid sample.

The Henher value of the muscle lipid of Indian mackerel was found to be 92.63% (Table 3). This result indicates the higher percentage of water insoluble, non-volatile fatty acids present in the lipid sample

The Kirschner value of the muscle lipid of Indian mackerel was found to be 0.341. This result indicates the presence of trace amount of fatty acid in the Reichert-Meissl distillate which forms soluble silver salt. The muscle lipid of Indian mackerel was found to form cloudy solutions with bromine and a precipitate appeared due to the insoluble bromide during the experiment. Hence, the lipid is marine oil (fish oil).

The muscle lipid of Indian mackerel was found to form treacle-like consistency with mercuric nitrate, $Hg(NO_3)_2$ solution after 24 hours during the experiment. Hence, the lipid is of semi-drying type. The amount of cholesterol in the muscle lipid of Indian mackerel was found to be 20.66 mg/g. A lower amount of cholesterol is observed in the muscle lipid of Indian mackerel. It can be suggested that the lipid of India mackerel fish is more suitable for edible purpose with respect to its cholesterol level.

The effect of storage time on the muscle lipid of Indian mackerel showed a significant variation in different values. Acid value, peroxide value increased with increasing time of storage and R-M value, thiocyanogen value, titre value and iodine value decreased with increasing time of storage. That means, the quality of the lipids deteriorated with increasing time of storage.

Table 3: Chemical constants of the muscle lipid of Indian mackerel and some related fats and oils.

Name of the Sample	S.V.	S.E.V.	A.V.	F.F.A. (%) (as oleic)	E.V.	I.V.	P.O.V.	Acetyl Value (%)	T. V.	Titre value (°C)	H.V.	U.S.M. (%)	P.V.	R.M.V.
Olive oil	190-195	287-295	0.6-1.5	0.25-0.60	---	80-88	---	10.4	75-83	17.26	0.6	0.5-1.2	0.5	0.6-1.5
Sunflower oil	190-194	287-295	0.6-2.4	0.15-0.45	---	125-140	---	---	78.4-81.3	17	---	0.3-0.9	---	0.5
Cotton seed oil	192-198	283-292	1.0-5.0	0.4-0.9	---	103-111	---	0.7-12.2	61-69	30.37	94.2	0.8-1.8	---	0.95
Linseed oil	189-195	287-296	4.0	0.5-0.75	---	175-200	---	---	---	---	94.8	1.0-1.5	---	---
Soyabean oil	190-195	287-295	1.2-1.5	0.35-0.85	---	129-137	---	---	77-85	22-27	---	0.7-1.6	0.2-1	0.5-2.55
Muscle lipid of Hilsa	203.25	276.01	3.108	1.56	---	92.55	55.05	10.255	52.54	---	93.27	0.74	0.764	0.965
Brain lipid of Baghda Chingri	229.255	244.71	1.11	.56	28.14	95.83	194.95	10.58	43.63	27.2	95.32	0.566	0.796	1.04
Brain lipid of Kerani Chingri	214.11	262.06	1.04	0.52	13.07	100.38	192.26	10.82	45.29	26.7	92.19	0.641	0.694	0.95
Muscle lipid of Cuttle fish	260.87	215.05	1.78	0.89	258.77	106.82	109.45	12.95	54.82	27.5	77.98	1.10	0.72	0.91
Muscle lipid of Indian mackerel	208.12	269.556	1.18	0.594	206.94	112.36	122.72	10.75	58.82	26.2	92.63	0.622	0.86	1.06

Abbreviations: S.V.= Saponification Value; S.E.V.= Saponification Equivalent Value; A.V.= Acid Value; F.F.A.= Free fatty acid; E.V.= Ester Value I.V.= Iodine Value; P.O.V.= Peroxide Value; T.V.= Thiocyanogen Value; H.V.= Henher Value; U.S.M.= Unsaponifiable Matter; P.V.= Polenske Value; R.M.V.= Reichert-Meissl Value

3.3 Microbial activities of the lipid sample

In the present study, the muscle lipid of Indian mackerel fish was selected and screened for antibacterial activities against four human pathogenic bacteria and antifungal activities against four phytopathogenic fungi.

3.3.1 Bacterial activity test

The antibacterial activities of the lipid sample were studied against two gram positive and two gram negative bacteria. Paper discs soaked in lipid solutions (10% and 5%) were used. It is evident that the lipid sample solution was found to be active against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*, but not active against *Bacillus cereus* (Table 4). Indian mackerel showed higher zone of inhibition against *Salmonella typhi* (21 mm) than others test bacteria. The muscle lipid of Indian mackerel has no inhibitory activity against *Bacillus cereus*.

Table 4: Antibacterial activity of the muscle lipid of Indian mackerel

Name of bacteria	Type of sample	Zone of inhibition (diameter in mm) after 48 hours		
		Treatment	Control	Differences
Salmonella typhi	10%	21	0	21
	5%	10	0	10
Staphylococcus aureus	10%	18	0	18
	5%	9.5	0	9.5
Escherichia coli	10%	16	0	16
	5%	7.5	0	7.5
Bacillus cereus	10%	0	0	0
	5%	0	0	0

3.3.2 Fungal activity test

The antifungal activities of the lipid sample were studied against four phyto-pathogenic fungi. It is evident that the muscle lipid of Indian mackerel did not show any inhibition on mycelial growth of *Curvularia lunata* but highly stimulated (Table 5). Except these the mycelial growth of almost all test fungi was inhibited by the lipid sample. The muscle lipid of Indian mackerel showed higher zone of inhibition against *Alternaria alternata* (12.654mm) than others.

Table 5: Percent growth inhibition of test fungi by the muscle lipid of Indian mackerel

Name of the fungi	Type of sample	% inhibition after 5 days
		Muscle lipid of Indian mackerel
Fusarium equiseti	10%	11.124
Aspergillus fumigateus	10%	8.328
Alternaria alternata	10%	12.654
Curvularia lunata	10%	-12.734

(-) means no inhibition

3.4 Estimation of minerals (N, P, K and Ca)

Most of the people of our country have been suffering to a great extent from protein malnutrition. Indian mackerel contain a good amount of nitrogen (5.125%) as well as protein (proteineous nitrogen) which is well balanced in respect of essential amino acids (Table 6). The percentage of phosphorus (1.736) indicates that phospholipid may present in the lipid sample. The percentage of potassium (1.153) in the muscle of Indian mackerel may be helpful to increase blood pressure for those people having low blood pressure. The percentage of calcium in the muscle of Indian mackerel fish was found to be 0.653 (Table 6). It may help in the formation of rigid bone structure of the community children in their growing age who eat this marine species.

Table 6: Percent of N, P, K and Ca in muscle of Indian mackerel

Name of the sample	N (%)	P (%)	K (%)	% of Ca (%)
Brain lipid of Kerani Chingri	3.090	0.550	1.061	0.798
Brain lipid of Baghda Chingri	3.540	0.726	1.123	0.914
Liver lipid of Blue Spotted Fantail ray	4.099	2.750	1.180	0.641
Muscle of Cuttle fish	6.533	1.237	1.118	0.450
Muscle of Indian mackerel	5.125	1.736	1.153	0.653

3.5 Chromatographic analysis

Qualitative and quantitative information about myristic acid, palmitic acid, stearic acid, behenic acid, lignoceric acid, myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid present in the muscle lipid of Indian mackerel has been obtained from GLC analysis (Table 7). It is found that the muscle lipid of Indian mackerel contained 41.1289% saturated fatty acid in which palamitic acid (28.0152%) is higher than other saturated fatty acids i.e. myristic acid (6.7394%), stearic acid (4.1146%), lignoceric acid (2.2597%). It is obvious that, it also contains monounstaturated palmitoleic acid (12.0527%) and oleic acid (15.8310%). From the analysis of fatty acid composition of lipid by GLC test, it is found that the experimental lipid contains high proportion of PUFA, polyunsaturated fatty acid (□-3 & □-6).The muscle lipid of Indian mackerel contains 24.0288% □-3 fatty acid in which docosahexaenoic acid (13.8883%) is higher than eicosapentaenoic acid (9.06%) and linolenic acid (1.0805%). On the other hand the lipid contained 6.3853% □-6 fatty acid in which arachidonic acid (5.0333%) is higher than linoleic acid (1.3520 %).

Table 7: Fatty acid composition of the extracted muscle lipid of Indian mackerel

Types of fatty acid	Name of fatty acid		Relative percentage (%)	Total (%)	
Saturated fatty acid	Myristic acid (C14:0)		6.7394	41.1289	
	Palmitic acid (C16:0)		28.0152		
	Stearic acid (C18:0)		4.1146		
	Behenic acid (C22:0)		-----		
	Lignoceric acid (C24:0)		2.2597		
Monounsaturated fatty acid (MUFA)	Myristoleic acid (C14:1)		0.5731	28.4568	
	Palmitoleic acid (C16:1)		12.0527		
	Oleic acid (C18:1)		15.8310		
Polyunsaturated fatty acid (PUFA)	ω-3 PUFA	Linolenic acid (C18:3)	1.0805	24.0288	30.4141
		Eicosapentaenoic Acid (C20:5)	9.0600		
		Docosahexaenoic Acid (C22:6)	13.8883		
	ω-6 PUFA	Linoleic acid (C18:2)	1.3520	6.3853	
		Arachidonic acid (C20:4)	5.0333		

4. Conclusions

The current study can be considered as an attempt to evaluate the local marine resources for total lipid and lipid types, especially for PUFAs (ω-3 and ω-6 fatty acids) which play an important role in reducing cardiovascular problems and overweight. For this regard, extracting total lipid from the muscle of Indian mackerel physico-chemical constants and microbial activities have been tested. Here the results indicate the presence of moderate amount of unsaturated fatty acids in the extracted lipid which was confirmed by R.I., I.V., and T.V. Percentage of F.F.A. validated suitability of the fish oil for edible purpose. Semidrying nature of the muscle lipid of Indian mackerel was pointed by I.V. and confirmed by Elaiden test. Gas-Liquid Chromatographic (GLC) examination substantiated the presence of some important ω-3 PUFA's like eicosapentaenoic acid and docosahexaenoic acid which have medicinal role to reduce blood triglycerides. Due to inhibitory activities of the extracted lipid against few bacteria and fungi, it may also possible to produce topical medicaments like antifungal ointments, antibacterial creams, germicides etc. from the extracted lipid.

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