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Physical and chemical characteristics, major fatty acids, antimicrobial activity and toxicity analysis of red shrimp (*Metapenaeus brevicornis*) brain lipid

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Abstract

Physical and chemical characteristics of red shrimp (*Metapenaeus brevicornis*) brain lipid have been determined by standard methods. Through GLC analysis, caprylic, myristic, palmitic, stearic and oleic acids have been found as the five major fatty acid components of *M. brevicornis* brain lipid. The lipid has found active against disease causing bacteria *Shigella dysenterial*, *Salmonella typhi* and *Staphylococcuss aureus*, and fungal pathogens *Macrophomina phascolma*, *Alternaria alternate* and *Curvularia lunata*. By applying on predatory fishes, *Heteropneustes fossilis* and *Anabas testudineus*, different extracts of *M. brevicornis* brain lipid have showed a minimal toxic effect.

Keywords: Physical and chemical characteristics; *Metapenaeus brevicornis*; Brain lipid; Antimicrobial activity; Toxicity

1. Introduction

A great amount of effort has been put into fish and fish lipids since the first report by Bang and Dyerberg (1972) pointing out a correlation between a largely marine based diet and a low myocardial infarction rate among Eskimos. Due to certain pharmacological activities against various degenerative diseases, fish lipids have received intensive clinical interest (Lee et al., 1985; Rosenberg, 2002). Fish consumption can reduce heart diseases and there is a clear relationship between dietary intake of fish and the likelihood of developing coronary heart disease (Albert et al., 2002; Bucher, Hengstler, Schindler, & Meier, 2002). Although many works have been devoted attempting to analyze and explain the role of fish lipids against various degenerative diseases, still there are many fishes to be investigated. Different parts of fishes can contain different types of useful chemicals depending on variation in seasons and maturation (Henderson & Tocher, 1987; Joseph, 1982). As not all fish lipids are edible, the non-edible fish lipids can be used for commercial purposes. Indian sub-continent, particularly Bangladesh, is blessed with a large water masses, the Bay of Bangle at the southern part. Red shrimp (*Metapenaeus brevicornis* Edwards, 1837) is found in the Bay of Bangle and the Indo-Pacific, and from West Pakistan to Indian, Malaysian, Thai, Indonesian and East Borneo waters (Bhuiyan, 1985; Tirmizi & Bashir, 1979). Profound information on *M. brevicornis* lipid is still not available. However, muscle extract of *M. brevicornis* has been reported to be expressed high azocoll lytic activity compared to extracts of many other prawn varieties, and the activity has also been found to be inhibited to a small extent by dithiothreitol (Karani, Gore, & Nair, 1996, 1999).

M. brevicornis, commercially caught in the coastal area of Chittagong and Khulna districts, Bangladesh, is locally known as "Lal-Chingri". The body of *M. brevicornis* is exported to many Asian and European countries, and the head is generally discarded during the processing. The chemicals, obtained from the head of *M. brevicornis*, can

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be used as raw materials for different commercial purposes. It will also be helpful to reduce environmental pollutions in the locality. Though several industries are interested on this issue, the main problem is the lacking of chemical information on *M. brevicornis* head and/or brain lipid. The aim of the present study is to investigate the approximate physical and chemical properties, major fatty acid components, antimicrobial activity and toxicity of *M. brevicornis* brain lipid for prospective commercial uses.

2. Materials and methods

2.1. Materials

The heads of the matured *M. brevicornis* were collected from Sea Fishers Bangladesh Ltd., CPA Complex, Sadarghat, Chittagong, Bangladesh. The brain of *M. brevicornis* were separated from the heads and preserved at −18 °C in the laboratory. Fresh samples were collected before performing each section of the experiment.

2.2. Extraction and purification of lipid

Extraction and purification of the total lipid from the brain of *M. brevicornis* were performed according to the Bligh and Dyer method (Bligh & Dyer, 1959). The brain (≈2 g wet weight) was firstly grounded with purified sand and 10 ml distilled water. A 30 ml of chloroform-methanol mixture (2:1 v/v) was added and mixed well with vigorous shaking. The mixture was kept for 12 h in dark. At the end of this period, 20 ml chloroform and 20 ml distilled water were added further. The mixture was shaken thoroughly in a separating funnel and allowed to settle. The lower layer of chloroform was collected carefully. Chloroform was evaporated with a rotary evaporator at 35 °C. The lipid was further dried by blowing a slow stream of nitrogen gas. All experiments were performed with freshly extracted and dried lipid.

2.3. Physical and chemical characteristics analysis

Refractive index, specific gravity and acetyl value (Griffin, 1966), iodine value, acid value, saponification value and Reichert-Meissl value (Sharma, 1995), saponification equivalent and percentage of unsaponifiable matter (Williams, 1966), Thiocyanogen value, Henher value and peroxide value (Jacobs, 1945; Morris, 1965), Polenske value (Ranganna, 1991), Kitchener value (Das, 1988) and titre value (Findlay, 1973) of the lipid were determined by standard methods. Each experiment was performed for three times, and the average is reported. All chemicals were purchased from Sigma Chemicals, BDH, Fisher, and Merck.

2.4. GLC analysis of fatty acids

The fatty acid composition of *M. brevicornis* brain lipid was investigated by GLC after conversion of the acids into the correspondent methyl-esters (Loury, 1967; Metcalfe, Schmitz, & Pelka, 1966). To study the sample by GLC, a portion of the converted sample was injected into one end of the column of the GLC equipment (PYE-UNICAM PU 4500, Column rov – 1, Phillips) having a flame ionization detector and a chart recorder. The column (internal diameter 2 mm, length 1.5 m) was filled with 10% diethyl glycol succinate polyesters (DEGS) supported on 100–120 (British Std. Sieve) mesh Diatomic C (Misra et al., 1983). The injector and detector temperatures were 230 °C and 250 °C, respectively. The initial column temperature was programmed at 100 °C for 1 min, and then, was allowed to rise to 225 °C at a rate of 4 °C/min. Nitrogen gas was used as the carrier gas at a flow rate of 11.3 ml/min. Standard methyl esters of caprylic, nonanoic, capric, undecanoic, lauric, myristic, palmitic, stearic, oleic, arachidic and behenic acids (Sigma Chemicals) were used for identification of the peaks. Peak position and relative retention time of the standard fatty acid methyl-esters (FAME) in GLC chromatograph are shown in Fig. 1 and denoted as [**1**]–[**11**]. Fatty acid components in *M. brevicornis* brain lipid were identified by comparing retention times with those obtained from the standard FAME. Quantitative composition was measured based on the percentage of a specific peak area to the total peak area (Garcia, Aguilar, Cordova, Suarez, & Bolanos, 2000). Quantitative data were corrected for differences in detector responses through analysis of authentic standards of each reported fatty acid (Garcia et al., 2000; Leslie, 1966; Srivastava & Srivastava, 1987).

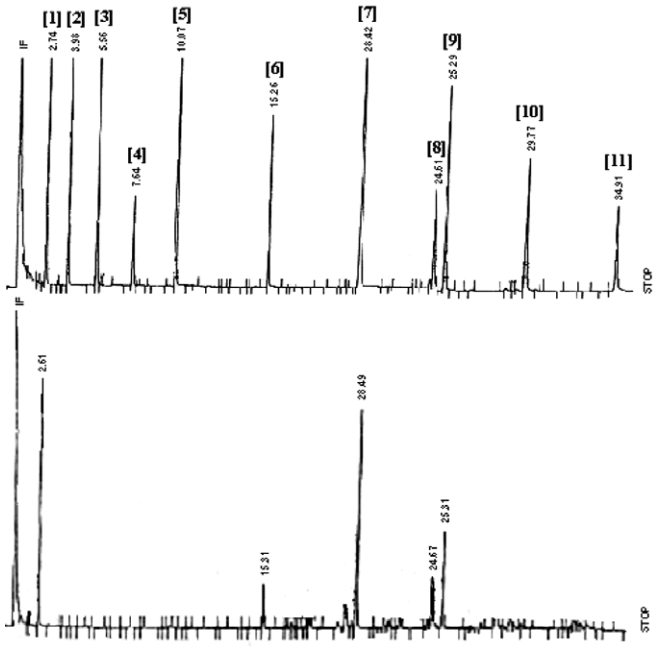

Fig. 1. GLC chromatogram of the standard fatty acid methyl ester mixture and of *M. brevicornis* brain lipid.

2.5. Analysis of antimicrobial activity

The lipid was analyzed for its antimicrobial activity against the selected bacteria *Shigella dysenterial*, *Salmonella typhi* and *Staphylococcuss aureus*, and fungal pathogens *Macrophomina phascolma*, *Alternaria alternate* and *Curvularia lunata*. Disc diffusion method (Ahmed, Rahman, Chowdhury, Begum, & Anwar, 1998; Bauer, Kirby, Sherris, & Truck, 1966) and poisoned food technique (Ahmed et al., 1998; Grover & Moore, 1962) were followed for screening the lipid against the bacteria and fungi, respectively.

2.6. Toxicity analysis

Toxicity of different extracts of *M. brevicornis* brain lipid was bio-assayed upon two predatory fishes *Heteropneustes fossilis* and *Anabas testudineus* under laboratory condition (Nasiruddin, Azadi, Chowdhury, & Mazumder, 1997). The actual concentrations of doses, used in the bioassays, were calculated in terms of ppm. The bioassays were run in a series of glass aquarium, netted on top, each containing 5 l of tap water and the calculated amount of toxicant. In each experiment, a control was maintained. Five concentrations of each extract, i.e., 50, 100, 250, 500 and 750 ppm doses were used. In each test, a set of five test fish were released at random in each concentration and each dose of the toxicant was replicated two times. The test exposure period was 24 h. All the bioassays were conducted under laboratory condition. The average water temperature was 26 ± 0.5 °C and average system pH during the experiments was 6.2 ± 0.05. Behavior of the fish was recorded in terms of their movements and abnormalities. The rate of fish mortality was counted only with those fishes, which were killed within 24 h of treatment.

3. Results and discussion

M. brevicornis brain lipid is investigated for some physical and chemical characteristics, major fatty acid components as well as for antimicrobial capability and toxicity to determine its nature and find out its suitability for a given purpose.

3.1. Physical characteristics

Physical characteristics of *M. brevicornis* brain lipid along with some other reported lipids are presented in Table 1. At 30 °C, the refractive index (RI) and specific gravity (Sp. gr.) of the lipid are found as 1.476 and 0.936, respectively, which indicate that the lipid consists of glyceraldehydes of long chain saturated fatty acid.

3.2. Chemical characteristics

Chemical characteristics of *M. brevicornis* brain lipid and some other reported lipids are presented in Table 1. The iodine value (IV, which is a measure of the degree of unsaturation) of *M. brevicornis* brain lipid is found to be lower than menhaden and sardine body lipids and cod liver oil. The value of IV supports that the lipid is unsaturated but not highly. The fact is also supported by the relatively low thiocyanogen value (TV, Table 1) as well as peroxide value (PV, Table 1).

The acid value (AV) of the lipid is calculated as 1.75. This relatively low AV makes the lipid more nutritive. The saponification value (SV) of the lipid is found to be higher than menhaden and sardine body lipids and cod liver oil (Table 1). The saponification equivalent (SE) of the lipid is found to be 215.42. These relatively low SV and SE indicate the probability of the presence of high molecular weight fatty acid contents in the lipid.

Table 1
Some physical and chemical properties of *M. brevicornis* brain lipid and other marine lipids (Agarwal, 1994; Das, 1987, 1988; Williams, 1966)

Properties[a]	Menhaden		Sardine			Cod liver oil	*M. brevicornis* brain lipid
	Gulf	Atlantic	Herring	Pilchard	Anchovy		
Refractive index (RI) (at 30 °C)	–	–	–	–	–	–	1.476
Specific gravity (Sp. gr.) (at 30 °C)	–	–	–	–	–	–	0.936
Iodine value (IV)	145–150	150–185	120–150	166–194	180 (max)	137–166	109.15
Acid value (AV)	–	–	–	–	–	–	1.75
Saponification value (SV)	–	185–190	185–195	185–195	191–193	171–179	260.42
Saponification equivalent (SE)	–	–	–	–	–	–	215.42
Percentage of free fatty acid (FFA%)	–	3 (max)	0.2–0.5	0.1–1.3	1 (max)	–	4.09
Ester value (EV)	–	–	–	–	–	–	258.67
Acetyl value	–	–	–	–	–	–	11.20
Thiocyanogen value (TV)	–	–	–	–	–	–	24.36
Henher value (HV%)	–	–	–	–	–	–	68.98
Reichert-Meissl value (RMV)	–	–	–	–	–	–	0.97
Polenske value	–	–	–	–	–	–	0.69
Kitchener value (KV)	–	–	–	–	–	–	0.61
Percentage of unsaponifiable matter (USM%)	–	0.6–1.6	2–3	0.5–2	3 (max)	0.54–2.68	3.0
Peroxide value (PV)	–	–	–	–	–	–	192.70 milliequivalents per 1000 g
	–	–	–	–	–	–	96.35 millimoles per 1000 g
Titre value (°C)	–	30.34	23–27	28–32	3.9	–	24.8

[a] " – " Not reported/unknown.

The percentage of free fatty acid (FFA%, as oleic) of *M. brevicornis* brain lipid is found to be higher than menhaden and sardine body lipids and cod liver oil (Table 1), which indicates that the lipid can be used as an industrial ingredient, such as in soap manufacture. The ester value (EV) of the lipid is found to be 258.69. The acetyl value of the lipid (Table 1) indicates the presence of low content of free hydroxyl groups in the lipid. The fact is also supported by the relatively low RI.

The Henher value (HV%) of the lipid (Table 1) indicates the presence of high percentage of water insoluble fatty acids with high molecular weight. The relatively low Reichert-Meissl value (RMV) of the lipid (Table 1) is an indication of low content volatile water-soluble fatty acids. The relatively low Polenske value (Table 1) indicates low content of the volatile alcohol-soluble but water-insoluble fatty acids in the lipid. The low RMV and Polenske value indicate presence of comparatively higher fatty acids in the lipid as indicated by the low SV. The Kirschner value (KV) of the lipid suggests the presence of trace amount of soluble silver fatty acids in the Reichert-Meissl distillate.

The percentage of unsaponifiable matter (USM%) exceeds the limit of 2% (Table 1), which indicates that the lipid contains some foreign matters, such as unsaponifiable sterols, tocopherols, vitamins A and D, and unsaturated hydrocarbons, etc. (Chatten, 1966).

The PV of the lipid supports the observation indicated by the low IV and TV (Table 1).

The titre value of the lipid is found to be 24.8 °C.

3.3. Major fatty acid composition

GLC analysis of *M. brevicornis* brain lipid shows that about 80% of the weight of the fatty acids consists of saturated acids, i.e., caprylic (8:0), palmitic (16:0), myristic (14:0) and stearic (18:0) acids. Palmitic, myristic and stearic acids are important ingredients of soaps (Agarwal, 1994). Mixtures of myristic and lauric acids improve the quality of soaps (Agarwal, 1994). Zn, Ca, Mg and Al salts of stearic acid are used in face, bath and talcum powders (Agarwal, 1994). Stearine, the mixture of palmitic and stearic acid is used in manufacturing of candles, shaving soaps, emulsifying agents, etc. (Agarwal, 1994).

An appreciable amount of unsaturated fatty acid, i.e., oleic acid (18:1) is also found to be present in the lipid. Epidemiological evidence suggests that ingestion of a diet rich in fish and fish lipids may be related to reduce incidence of atherosclerosis and other cardiovascular diseases (Albert et al., 2002; Bucher et al., 2002; Kromhaut, Bosschietger, & Coulander, 1985). The possibility is that unsaturated fatty acids (UFA), especially polyunsaturated fatty acids (PUFA), present in fish lipids, might have anti-atherogenic properties (Leaf & Weber, 1988; Lee et al., 1985; Ware, 1999). Treatment by fish oils has produced an interesting contribution to the reduction of plasma triglycerides in hyperlipidemia associated with increased VLDL (very low-density lipoproteins, Jacotot, 1991). There is evidence that dietary fish lipids can inhibit the development of atherosclerosis in vein graft in dogs, in the aorta of some rabbit models, in swine coronary arteries and in a variety of arteries in non-human primates (Greenberg, 1991). Dietary supplementation with cod-liver oil improves endothelium-dependent relaxations in hypercholesterolemic and atherosclerotic blood vessels, and this effect could explain, in part, the beneficial effect of UFA, i.e., Ω-3 fatty acids, on the occurrence of cardiovascular diseases (Boulanger, Schini, Shimokawa, Lüscher, & Vanhoutte, 1991). Fox and DiCorleto (1991) have shown that emulsions of Ω-3 fatty acid, containing 30% of Ω-3 fatty acid, inhibit the production of platelet-derived growth factor by confluent cultures of bovine aortic endothelial cells. Safflower oil, containing preliminary Ω-6 PUFA, has less than one-tenth of the inhibitory activity of Ω-3 fatty acid, and peanut oil, containing mostly saturated and monounsaturated lipids, is inactive (Fox & DiCorleto, 1991). Bruckner (1991) has suggested that Ω-3 and Ω-6 fatty acids, with adequate antioxidants, most likely increase peripheral capillary blood flow by altering vascular tone and blood viscosity, and Ω-3 fatty acids, without adequate antioxidant present, may be detrimental with regard to peripheral capillary flow. However, data on these points are still insufficient, and further studies are, therefore, required. GLC chromatograph of *M. brevicornis* brain lipid is shown in Fig. 1 and detailed fatty acid composition, along with some other reported lipids, is presented in Table 2. The lipid may also contain some other fatty acids in small amounts, which are not identified.

3.4. Antimicrobial activity

Antibacterial and antifungal activities of *M. brevicornis* brain lipid were investigated by standard methods against selected bacteria and fungus. The selection was made based on the availability of the bacteria and fungus in the laboratory.

Antibacterial activity of *M. brevicornis* brain lipid against three bacterial pathogens – *S. dysenterial*, *S. typhi* and *S. aureus* is presented in Table 3. The result indicates that for 10 μl of the lipid, *M. brevicornis* brain lipid showed the maximum zone of inhibition against *S. typhi* and the minimum against *S. aureus*. On the other hand, for 20 μl of the lipid, *M. brevicornis* brain lipid showed the maximum zone of inhibition against *Shigella dysenterial* and the minimum against *S. aureus*.

Table 4 represents the antifungal activity of *M. brevicornis* brain lipid against three fungal pathogens – *M. phascolma*, *A. alternate* and *C. lunata*. The result proves that the lipid showed the maximum zone of inhibition against *C. lunata* and the minimum against *A. alternate*.

3.5. Toxicity

The toxicity of different extracts of *M. brevicornis* brain lipid was studied by applying them on two predatory fish species – *H. fossilis* and *A. testudineus*. Upon exposure to

Table 2
Relative percentage of the major fatty acids of *M. brevicornis* brain lipid and some other marine lipids

Fatty acid[f]	*Merluccius hubbsi*[a]	*Monodonata turbinata*[b]	Cod liver oil[c]	*Euthynnus pelamis*[d]		*Corallina officinals*[e]	*Dictyota dichomota*[e]	*Cladophora fascicularis*[e]	*Metapenaeus brevicornis* brain lipid
				Muscle	Liver				
Caprylic (8:0)	–	–	–	–	–	–	–	–	39.3
Myristic (14:0)	2.8	2.3	4.7	2.74–4.64	1.17–2.16	2.48	10.32	9.76	10.5
Palmitic (16:0)	18.0	17.85	13.7	17.01–23.54	14.24–20.57	29.46	23.05	21.94	23.8
Stearic (18:0)	3.2	12.22	2.5	3.91–6.72	5.8–7.52	0.58	2.4	0.23	6.4
Oleic (18:1)	14.9	16.46	–	10.62–13.39	7.06–13.19	–	–	–	18.7

[a] Méndez and González (1997).
[b] Miletic et al. (1991).
[c] Indarti et al. (2005).
[d] Watanabe et al. (2005).
[e] Johns et al. (1979).
[f] " – " Not reported/unknown.

Table 3
Antibacterial activity of *M. brevicornis* brain lipid

Name of the bacteria	Diameter of inhibition zone (mm)	
	10 μl lipid	20 μl lipid
Shigella dysenterial	14	17.1
Salmonella typhi	15.8	16
Staphylococcuss aureus	4	2

Table 4
Antifungal activity of *M. brevicornis* brain lipid

Name of the fungus	Diameter of inhibition zone (mm)	
	10 μl lipid	20 μl lipid
Macrophomina phascolma	11.4	12.1
Alternaria alternate	2.2	3.8
Curvularia lunata	27.5	21.3

the extract, *H. fossilis* showed vigorous movement and repeatedly rose towards the surface to take air. With erratic movement and having no balance, the exposed species became paralyzed and straightened, then, slowly settled to the bottom of the aquarium-water, and ultimately died after different intervals. The effect of distilled water extract on *H. fossilis* showed the lowest mortality (0%) for all doses (Table 5). On the other hand, 50% ethyl alcohol extract gave the lowest mortality (0%) with 50, 100 and 250 ppm doses, and the highest (20%) with 750 ppm dose (Table 5).

In case of *A. testudineus*, the affected fishes jumped upwards, and then, they started moving up and down rapidly. Subsequently, their movement became slow and stopped. Gradually, they allayed to the bottom of the aquarium-water, and ultimately died after different intervals. With *A. testudineus*, the distilled water extract

Table 5
Mortality (%) of *Heteropneustes fossilis* and *Anabas testudineus* at different concentrations of various solvent extracts of *M. brevicornis* brain lipid for 24 h exposure

Name of fish	Name of the solvent extract	Dose (ppm)	Observation-1		Observation-2		Total no. of fish killed	Mortality (%)
			No. of fish taken	No. of fish killed	No. of fish taken	No. of fish killed		
Heteropneustes fossilis	Distilled water	50	5	0	5	0	0	0
		100	5	0	5	0	0	0
		250	5	0	5	0	0	0
		500	5	0	5	0	0	0
		750	5	0	5	0	0	0
	Ethyl alcohol (50%)	50	5	0	5	0	0	0
		100	5	0	5	0	0	0
		250	5	0	5	0	0	0
		500	5	0	5	1	1	10
		750	5	1	5	1	2	20
Anabas testudineus	Distilled water	50	5	0	5	0	0	0
		100	5	0	5	0	0	0
		250	5	0	5	0	0	0
		500	5	0	5	0	0	0
		750	5	0	5	0	0	0
	Ethyl alcohol (50%)	50	5	0	5	0	0	0
		100	5	0	5	0	0	0
		250	5	0	5	1	1	10
		500	5	1	5	0	1	10
		750	5	1	5	0	1	10

showed 0% mortality for all doses (Table 5). With 50% ethyl alcohol extract, the lowest and highest mortalities were 0% and 10% with 50, 100, and 250, 500, 750 ppm doses, respectively (Table 5). From these findings, it can be concluded that the relative toxicity of the extracts on both *H. fossilis* and *A. testudineus* is minimal, and that the toxic ingredients present in the brain lipid of *M. brevicornis* are highly soluble in ethyl alcohol but least in distilled water. It is difficult to assume which types of compounds are responsible for the toxicity. Some cholesterol-based compounds may be involved in this case. Further studies are, therefore, required to ascertain the ingredients responsible for the toxicity, and to gather information regarding to the spectrum of the toxic effects. Finally, *M. brevicornis* brain lipid is assumed almost non-toxic, and can be used for edible purpose after treating with a suitable procedure. It can also be used to produce industrial and pharmaceutical grade fatty acids.

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