



Research Article

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Extraction, Purification and Characterization of Red Shrimp (*Metapenaeus brevicornis*) Body Lipid

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Abstract

Lipid has been extracted and purified by standard methods from the body of red shrimp (*Metapenaeus brevicornis*) to determine some of its physical and chemical characteristics. Antimicrobial activity of the lipid has been studied against selected bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli*, and fungal pathogens *Macrophomina phaseolina*, *Alternaria alternate* and *Curvularia lunata*. According to GLC, the lipid contains caprylic, myristic, palmitic, stearic and arachidic acids as saturated fatty acids, and oleic acid as unsaturated fatty acid. The lipid has been found active against *S. dysenteriae*, *S. typhi* and *A. alternate*, and not active against *S. aureus*, *M. phaseolina* and *C. lunata*.

Keywords: Red shrimp body lipid, Physical and chemical characteristics, Major fatty acids, Antimicrobial activity.

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1. Introduction

Due to the nutritional and health implications of fish, fish lipids (fats/oils) have received intense interest of analysis by the researchers in recent years. Bang and Dyerberg firstly reported the correlation between a largely marine-based diet and a low myocardial infarction rate among Eskimos [1]. Fish lipids, especially marine fish lipids, contain pharmaceutically important and physiologically active unsaturated fatty acids (UFA) and/or polyunsaturated fatty acids (PUFA). There is evidence that UFA and/or PUFA having the protective effect against coronary heart disease and blood pressure [1-7]. They can reduce platelet adherence and enhance endothelium-dependent vasodilatation [3, 4]. There is also evidence from animal studies that UFA and/or PUFA may protect against ventricular arrhythmia, inhibit the development of atherosclerosis in vein graft, in the aorta and in a variety of arteries [6, 8]. UFA and/or PUFA may reduce the incidence of restenosis in patients undergoing percutaneous transluminal coronary angioplasty [9]. Although about 40 years have passed since the first report by Bang & Dyerberg [1], and many works have been devoted later on attempting to analyze and explain the role of fish lipids against various degenerative diseases, still there is no potential explanation of the mechanism. Still there are many fishes to be

investigated, different parts of which may contain different types of useful chemicals depending on variation in seasons, maturation, water temperature, types of processing and storage, and so on [10, 11]. Analysis of the brain lipid of red shrimp (*Metapenaeus brevicornis*) was performed and reported previously [12]. Caprylic, myristic, palmitic, stearic and oleic acids were found as the five major fatty acid components of *M. brevicornis* brain lipid [12]. The lipid was found active against the selected disease causing bacteria and fungal pathogens [12]. By applying on the selected predatory fishes, different extracts of the brain lipid of *M. brevicornis* were found as non-toxic [12]. However, analysis of the *M. brevicornis* body lipid, which may have some chemical characteristics relevant to pharmacological significance, has yet not been performed. In order to get a comparative perspective between the body and brain lipids of *M. brevicornis*, analysis of the body lipid is necessary. In the present study, physical and chemical properties, major fatty acid composition and antimicrobial activity of *M. brevicornis* body lipid are investigated by following the standard methods.

2. Materials and Methods

Materials

The bodies of *M. brevicornis*, already separated from the heads, were purchased from Sea Fishers Bangladesh Ltd., CPA Complex, Sadarghat, Chittagong, Bangladesh. The bodies of *M. brevicornis* were placed in polyethylene bags, rapidly frozen and stored at -18°C in the laboratory. The frozen period was no longer than 2 weeks. Fresh samples were purchased before performing each section of the experiment.

Extraction and purification of the lipid

Extraction and purification of the total lipids from the body of *M. brevicornis* were performed according to the Bligh and Dyer method [13]. The lower chloroform layer of the separating funnel was collected carefully and chloroform was evaporated with a rotary evaporator at 35°C [12]. The lipid was further dried by blowing a slow stream of nitrogen gas [12]. All experiments were performed with freshly extracted and dried lipids.

Analysis of physical and chemical characteristics

Refractive index, specific gravity and acetyl value [14], iodine value, acid value, saponification value and Reichert-Meissl value [15], saponification equivalent and percentage of unsaponifiable matter [16], Thiocyanogen value, Henher value and peroxide value [17], Polenske value [18], Kitchener value [19] and Titre value [20] of the body lipid of *M. brevicornis* were determined by standard methods. Each experiment was performed for three times, and the average value is reported. All chemicals were purchased from Sigma Chemicals, BDH, Fisher, and Merck.

Analysis of fatty acids by GLC

The fatty acid composition of *M. brevicornis* body lipids was investigated by GLC after conversion of the acids into the correspondent methyl-esters [21]. 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 (LKB BROMMA 2220 recording integrator). 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 [22]. 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 $4^{\circ}\text{C}/\text{min}$. 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 **Figure 1**, denoted as [1] – [11]. Fatty acid components in *M. brevicornis* body 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 [23]. Quantitative data were corrected for differences in detector responses through analysis of authentic standards of each reported fatty acid [23,24].

Analysis of antimicrobial activity

The lipid was analyzed for its antimicrobial activities against the selected bacteria *Staphylococcus aureus* (gram positive, G^+), *Bacillus subtilis* (G^+), *Salmonella typhi* (gram negative, G^-) and *Escherichia coli* (G^-), and fungal pathogens *Macrophomina phaseolina*, *Alternaria alternate* and *Curvularia lunata*. Disc diffusion method [25,26] and poisoned food technique [25,27] were followed for screening the lipids against the bacteria and fungi, respectively.

3. Results and Discussion

Some physical and chemical constants of *M. brevicornis* body lipid, investigated and determined by following the standard methods in the present study, are presented in **Table 1**. Along with the GLC chromatograph of standard fatty acid methyl-esters, **Figure 1** represents the GLC chromatograph of *M. brevicornis* body lipid. Detailed fatty acid composition of *M. brevicornis* body lipid is presented in **Table 2**. Antimicrobial activities of *M. brevicornis* body lipid against four selected bacterial pathogens and three selected fungal pathogens are presented in **Table 3**.

The refractive index (RI) of lipids is a reflection of its unsaturation. At 30°C, the RI of *M. brevicornis* body lipid (**Table 1**) is found as lower than that of the brain lipid [12], which reflects less unsaturation of the body lipid than the brain lipid of *M. brevicornis*. The RI of cod liver oil is higher but the RI of lard oil is lower than *M. brevicornis* body lipid [28]. The specific gravity (Sp. gr.) of *M. brevicornis* body lipid is found as lower than that of the previously reported brain lipid [12]. The Sp. gr. of *M. brevicornis* body lipid (**Table 1**) is lower than cod liver oil and lard oil, but higher than beef tallow [28]. The result reveals that *M. brevicornis* body lipid contains glyceraldehydes of long chain saturated fatty acid, which is lower than that of the brain lipid of *M. brevicornis* [12].

The iodine value (IV) is a measure of the degree of unsaturation of lipids. The IV of *M. brevicornis* body lipid (**Table 1**) is lower than that of the brain lipid [12], which reflects that the brain lipid is more unsaturated than the body lipid. The IV of *M. brevicornis* body lipid (**Table 1**) is lower than the Gulf and Atlantic menhaden and sardine body lipids [19,29] and cod liver oil [28], but higher than butter fat, lard oil and beef tallow [28]. The IV of *M. brevicornis* body lipid indicates that the lipid is unsaturated, but not highly, and is less unsaturated than *M. brevicornis* brain lipid. The acid value (AV) of *M. brevicornis* body lipid (**Table 1**) is higher than that of the brain lipid [12]. The AV of the body lipid is higher than lard oil and beef tallow but lower than cod liver oil [28].

The saponification value (SV) indicates the average molecular weight of a lipid. The SV of *M. brevicornis* body lipid (**Table 1**) is found as higher than the brain lipid [12]. The SV of *M. brevicornis* body lipid is higher than the Atlantic menhaden and sardine body lipids [19,29], cod liver oil, lard oil and beef tallow [28]. The saponification equivalent (SE) of *M. brevicornis* body lipid (**Table 1**) is higher than *M. brevicornis* brain lipid [12]. The SV and SE of the body lipid of *M. brevicornis* indicate the possibility of the presence of high molecular weight fatty acid contents in the lipid. The percentage of free fatty acid (FFA%, as oleic) of *M. brevicornis* body lipid (**Table 1**) is found to be higher than that of the brain lipid [12]. The FFA% of *M. brevicornis* body lipid is higher than the Atlantic menhaden and sardine body lipids [19, 29].

The ester value (EV) is a relative measure of the amount of ester present in a lipid. The EV of *M. brevicornis* body lipid (**Table 1**) is higher than the brain lipid [12]. The acetyl value of *M. brevicornis* body lipid (**Table 1**) is found to be lower than the brain lipid [12], but higher than cod liver oil, lard oil and beef tallow [28], which reveals that *M. brevicornis* body lipid contains lower content of free hydroxyl groups than *M. brevicornis* brain lipid, but higher than cod liver oil, butter fat, lard oil and beef tallow. This assumption is also supported by the value of RI (**Table 1**). The Thiocyanogen value (TV) of *M. brevicornis* body lipid (**Table 1**) is lower than the brain lipid [12]. The TV of the body lipid of *M. brevicornis* is an indication of the presence of high molecular weight unsaturated fatty acid contents.

The Henher value (HV%) is a measure of water insoluble fatty acids in a lipid. The HV% of *M. brevicornis* body lipid (**Table 1**) is lower than the brain lipid [12], which is an indication of the presence of lower percentage of water insoluble fatty acids in the body lipid than the brain lipid of *M. brevicornis*. The Reichert-Meissl value (RMV) and Polenske value are measures of volatile fatty acids in lipids. The RMV of *M. brevicornis* body lipid (**Table 1**) is found as higher than the brain lipid [12], which is an indication for the presence of higher percentage of volatile water-soluble fatty acids in the body lipid than the brain lipid. The RMV of *M. brevicornis* body lipid is higher than cod liver oil, lard oil and beef tallow, but lower than that of butter fat [28]. The Polenske value of *M. brevicornis* body lipid (**Table 1**) is higher than the brain lipid [12], which indicates the presence of higher percentage of volatile alcohol-soluble but water insoluble fatty acids in the body lipid than the brain lipid.

The Kirschner value (KV) is a number dependent on the amount of soluble silver fatty acids in the Reichert-Meissl distillate. The KV of *M. brevicornis* body lipid (**Table 1**) is higher than that of the brain lipid [12]. It reflects that the body lipid contains higher amount of soluble silver fatty acids in the Reichert-Meissl distillate than that of the brain lipid. The percentage of unsaponifiable matter (USM%) of *M. brevicornis* body lipid (**Table 1**) is found to be lower than the brain lipid [12]. The USM% of the body lipid is not exceeded the limit of 2%, and therefore, it is assumed that the lipid does not contain foreign matters [30]. The peroxide value (PV) of *M. brevicornis* body lipid (**Table 1**) is lower than that of the brain lipid [12], which supports the observation indicated by the IV and TV of the lipid. The Titre value is the solidifying point of mixed fatty acids in a lipid. The Titre value of *M. brevicornis* body lipid (**Table 1**) is lower than the brain lipid [12].

GLC analysis of *M. brevicornis* body lipid shows that about 91% and 7.2% of the weight of the fatty acids consists of saturated and unsaturated acids, respectively (**Table 2**). The lipid may also contain some other fatty acids in small amounts (**Figure 1**), which could not be identified due to non-availability of standards in the laboratory. However, fatty acid contents in different parts of fishes can vary depending on variation in seasons and maturation [11,31]. The percentage of caprylic acid (8:0) in *M. brevicornis* body lipid (**Table 2**) is found as lower than that of the brain lipid [12]. The percentage of myristic (14:0), palmitic (16:0) and stearic (18:0) acids in *M. brevicornis* body lipid

(Table 2) are higher than the brain lipid of *M. brevicornis* [12] and cod liver oil [32]. Absence of arachidic acid (20:0) in *M. brevicornis* brain lipid was reported previously [12]. However, *M. brevicornis* body lipid contains 1.4% arachidic acid (Table 2). This may play an important role in the structural and constitutional difference between the body and brain. However, it is not obvious, and therefore, widespread experimental studies are still in demand. Myristic, palmitic and stearic acids are important ingredients of soaps; the mixture of myristic and lauric acids improves the quality of soaps; Zn, Ca, Mg and Al-stearates are used in face, bath and talcum powders; and the mixture of palmitic and stearic acid or stearine is used in manufacturing of candles, shaving soaps, emulsifying agents, etc. [28]. Hence, *M. brevicornis* body lipid can be considered to produce industrial grade saturated fatty acids, i.e. myristic, palmitic and stearic acids.

The body lipid of *M. brevicornis* contains about 7.2% unsaturated fatty acid, i.e. oleic acid (18:1) [Table 2], which is lower than that of *M. brevicornis* brain lipid [12]. It may also have an important role in the structural and constitutional difference between the body and brain, which is obscure, and further studies are, therefore, necessary to establish the phenomena. UFA can inhibit the development of various degenerative diseases, and information on the mechanism for the role of UFA against degenerative diseases is still obscure. Therefore, further studies on this aspect are required. *M. brevicornis* body lipid can be considered to produce industrial and pharmaceutical grade UFA. Oleic acid can easily be removed by scourine, and therefore, it is extensively used as a lubricant for wool prior to spinning, and is used in preparation of emulsifying agents, in compounding of rubber, in manufacture of polishes and in producing its esters, such as butyl oleate [28].

Antibacterial and antifungal activities of *M. brevicornis* body 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. According to Table 3, the bacterial pathogens *S. dysenterial* and *S. typhi* are sensitive towards the body lipid of *M. brevicornis*, whereas the bacterial pathogen *S. aureus* showed no inhibitory activity (Table 3). *S. dysenterial*, *S. typhi* and *S. aureus* were found sensitive towards *M. brevicornis* brain lipid [12]. Both *S. dysenterial* and *S. typhi* showed the minimum zone of inhibition for 10 μ l and maximum for 20 μ l *M. brevicornis* body lipid activity (Table 3). The inhibition zone by *S. dysenterial* and *S. typhi* for 10 and 20 μ l *M. brevicornis* body lipid is seemed to be lower than that of the brain lipid [12]. The body lipid of *M. brevicornis* is, therefore, assumed less active against *S. dysenterial* and *S. typhi* than the brain lipid.

It is evident from the data that the fungal pathogen *A. alternate* is sensitive towards *M. brevicornis* body lipid, and *M. phascolma* and *C. lunata* showed no inhibitory activity (Table 3). *M. brevicornis* brain lipid [12] is seemed to be less active against *A. alternate* than the body lipid (Table 3). Moreover, *M. brevicornis* brain lipid was reported as active against *M. phascolma*, *A. alternate* and *C. lunata* [12]. Therefore, it is obvious that *M. brevicornis* body lipid is less active than *M. brevicornis* brain lipid against *M. phascolma*, *A. alternate* and *C. lunata*. However, it is difficult to explain. The presence of less UFA acid in *M. brevicornis* body lipid than the brain lipid may have an important role in variation of the antimicrobial activity of the lipids.

Table 1. Some physical and chemical properties of *M. brevicornis* body lipid.

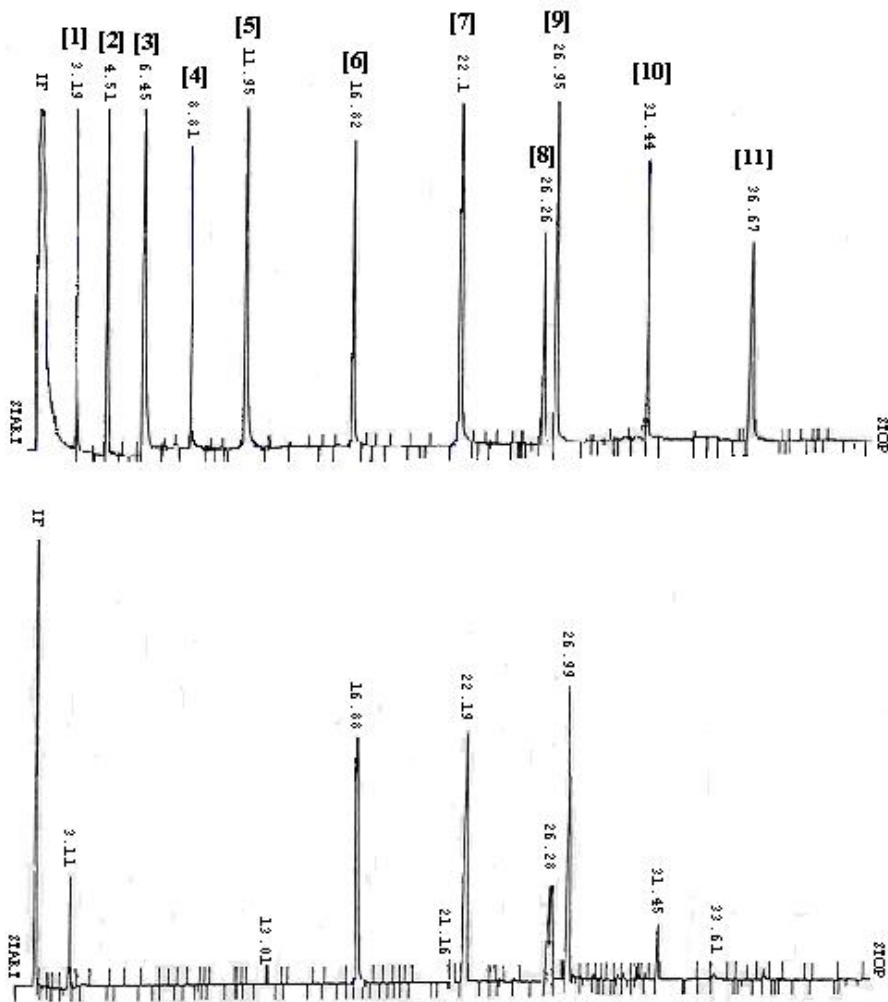
Properties	<i>M. brevicornis</i> body lipid
Refractive index (R.I.) [at 30°C]	1.468
Specific gravity (Sp. gr.) [at 30°C]	0.9114
Iodine value (I.V.)	97.46
Acid value (A.V.)	1.88
Saponification value (S.V.)	268.62
Saponification equivalent (S.E.)	229.3
Percentage of free fatty acid (F.F.A.%)	4.6
Ester value (E.V.)	271.2
Acetyl value	9.97
Thiocyanogen value (T.V.)	19.11
Henher value (H.V.%)	53.52
Reichert-Meissl value (R.M.V.)	1.06
Polenske value	0.73
Kitchener value (K.V.)	0.684
Percentage of unsaponifiable matter (U.S.M.%)	1.986
Peroxide value (P.V.) in milliequivalents per 1000 g.	160.88
Titre value (°C)	23.2

Table 2. Relative percentage of the major fatty acids of *M. brevicornis* body lipid.

Fatty acid	<i>M. brevicornis</i> body lipid
Caprylic (8:0)	3.6
Myristic (14:0)	20.9
Palmitic (16:0)	28.3
Stearic (18:0)	36.8
Oleic (18:1)	7.2
Arachidic (20:0)	1.4

Table 3. Antimicrobial activities of *M. brevicornis* body lipid

Name of the bacteria \bar{E}	Diameter of inhibition zone (mm)	
	10 μ l lipid	20 μ l lipid
<i>Shigella dysenterial</i>	5	6.1
<i>Salmonella typhi</i>	1.7	1.9
<i>Staphylococuss aureus</i>	–	–
Name of the fungus \downarrow	Diameter of inhibition zone (mm)	
	10 μ l lipid	20 μ l lipid
<i>Macrophomina phascolma</i>	–	–
<i>Alternaria alternate</i>	0.22	0.25
<i>Curvularia lunata</i>	–	–

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

4. Conclusion

It is obvious from the present study that *M. brevicornis* body lipid contains both of the saturated and unsaturated fatty acids, i.e. caprylic, myristic, palmitic, stearic and oleic acids. It also possesses antimicrobial activity against bacterial and fungal pathogens, i.e. *S. dysenteriae*, *S. typhi* and *A. alternata*. This particular characteristic may be helpful in various aspects, especially in expanding the use of the lipid for industrial, nutritional and pharmaceutical purposes.

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