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ISOLATION, CHARACTERIZATION AND STUDY OF MICROBIAL ACTIVITIES OF THE BRAIN LIPID AND CHEMICAL ANALYSIS OF THE BRAIN OF BAGHDA CHINGRI (*Penaeus monodon*) OF THE BAY OF BENGAL

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The brain lipid of Baghda chingri (*Penaeus monodon*) was extracted and characterized with respect to various physical and chemical constants and compared with those of standard oils and fats. Thin layer chromatographic (TLC) and gas liquid chromatographic (GLC) investigation of the lipid showed the presence of myristic, palmitic, stearic, oleic, linoleic, arachidic and some other unidentified fatty acids. The microbial activities of the lipid were investigated. Nitrogen, phosphorus, potassium and calcium contents of the total brain containing the lipid were determined.

Key words: Lipid, Chingri, Polyunsaturatted fatty acid (PUFA), TLC, GLC.

Introduction

Bangladesh earns substantial amount of foreign exchange by exporting shrimps prawns and lobster to many countries of the world including USA, UK and Japan. Processing of these is associated withe expulsion of head and brain. Literature survey (Endinkeau and Kiew 1993; Gutierrez and Da Silva 1993; Heyden 1994; van Schacky *et al* 1999, Harper and Jacobson 2001; Bucher *et al* 2002; Holub 2002) shows that fish lipids including brain lipids of shrimps and prawns contain pharmaceutically important and physiologically active ω-3 and ω-6 unsaturated fatty acids, which play an effective role in reducing cardiovascular problems. This phenomenon has attracted investigators to analyze fish lipids of both marine and fresh water origins for polyunsaturated fatty acids (PUFA). Lovern (1953) has found that PUFA are present in high ratios in lipids of marine fish of which acids of ω-3 configuration are predominant Bang and Dyerberg (1975) studied the dietary habits of the Eskimos, based on fish oils containing higher proportion of PUF As dominated by ω-3 fatty acids. Both ω-3 and ω-6 fatty PUFAs have been found to inhibit the biosynthesis of cholesterol in liver (Murray *et al* 1990). These essential fatty acids constitute integral part of nervous tissues in the brain as complex lipid. The exportable marine species from Bangladesh, Baghda chingri (*Penaeus monodon*) is remarkable from demand point of view. The present investigation is concerned with the isolation of the brain lipid of Baghda chingri with a view to find out the PUFAs presence in it and studying its physico-chemical and microbial characteristics including the chemical analysis of brain.

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Experimental

The lipid was extracted from the brain of Baghda chingri by Bligh and Dyer method (Gurr and James 1977) using chloroform: methanol (2:1, v/v). The extract thus obtained was dried, free of solvent first by rotary evaporation and finally by blowing a slow stream of nitrogen gas. The yield was 25%. The refractive index, moisture, crude fat, crude fibre and ash contents of the lipid were determined by standard methods (Ranganna 1991). Saponification value, saponification equivlent value, acid value and percentage of free fatty acid (as oleic), iodine value, acetyl value (Griffin 1972), peroxide value (Morris 1965), thiocyanogen value, Reichert - Meissl value and Polenske value (Ranganna 1991), Henher value, Elaiden test result (Das 1989) and the quantity of unsaponifiable matter (Williams 1966) of the lipid were determined by standard methods.

The fatty acid mixture was prepared from the lipid sample, which was then converted into corresponding methyl esters by proper treatment with methanolic solution of sulphuric acid and purified (Loury 1966 and 1967; Mangold and Kammereck 1961). Thin layer chromatographic separation was made using thin layer of silica gel as the stationary phase and petroleum ether: ether (80:20, v/v) as the mobile phase. The separation of methyl esters depending on R_f values was visualized by spraying with a 0.2% ethanolic solution of 2,7-dichlorofluo-

rescein air drying and inspection under UV - light. Mixture of standard methyl esters of fatty acids was used for comparison and the fatty acid composition of the lipid was identified from R_f values. The methyl esters prepared as before were also analysed by GLC. (Mangold and Kammereck 1961; Loury 1966 and 1967). A portion of the sample was injected into one end of the column of the GLC equipment (PYE-UNICAM PU 4500, Phillips) using a flame ionization detector and a chart recorder. A column (internal diameter 2mm, length 1.5 meter) was filled with 10% diethyl glycol succinate (DEGS) on 100 - 200 (British Std. Sieve) mesh. The injector temperature was 230°C and the detector temperature was 250°C. The temperature of the column was programmed initially at 100°C for 1 min, then 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 Chemical Company, USA) were used for identification of the peaks. The fatty acids present in the lipid under investigation were thus identified by comparison of relative retention time and peak position. The percentage of the acids was computer estimated from the GLC peaks.

A portion of the lipid sample was screened for its anti-bacterial activity by disc diffusion method (Bauer *et al* 1966) against four human pathogenic bacteria, viz. *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi and Escherichia coli.* Another portion was screened for its anti - fungal activity by poisoned food technique (Grover and Moore 1962) against three phyto pathogenic fungi, viz. *Macrophomina phaseolina, Alternaria alternata* and *Curvularia lunata*. Nutrient Agar and Potato Dextrose Agar were used as basal medium for anti-bacterial and anti-fungal activity tests respectively. Dimethyl formamide (DMF) was used as a solvent for preparation of lipid solution of desired concentration (1%).

The brain was first sun dried with occasional stirring, and then vacuum oven dried at 40°C. The dried materials were ground in an electric grinder into 60 mesh powder and digested according to the Modified Kjeldahl Method. Percentage of N, P, K and Ca were estimated by standard procedure (Ranganna 1991).

Results and Discussion

The physical and chemical characteristics of the brain lipid investigation may help to evaluate its suitability for a given purpose. Some of the physical and chemical constants are shown in Table 1, for comparison with of standard commercial oils. The refractive index of the lipid was found to be 1. 4736, a quite high value that is an indication of moderate amount of unsaturation in the fatty acid components. The moisture, ash, crude fat and crude fibre contents of the lipid were found to

Table 1

Physical and chemical constants of the brain lipid of Baghda chingri and some related fats and oils (Williams 1966; Lange 1987; Das 1989)

Name of the sample	S.V.	S.E.V	A.V.	F.F.A (%)	I.V.	T .V.	Acetyl Value	U.S.M. (%)	R.M.V.	P.V.	H.V.	R.I.
Olive oil	190-195	287-295	0.6-1.5	0.25-0.60	80-88	75-83	10.04	0.5-1.2	0.6-1.5	0.5	0.6	1.4657 - 1.4667
Sunflower oil	190-194	287-295	0.6-2.4	0.15-0.45	125-140	78.4-81.3	---	0.3-0.9	0.5	---	---	1.4659 - 1.4721
Cotton seed oil	192-198	283-292	1.0-5.0	0.4-0.9	103-111	61-69	0.7-12.2	0.8-1.8	0.95	---	94.2	1.4743 - 1.48
Linseed oil	189-195	287-296	4.0	0.5-0.75	175-200	---	---	1.0-1.5	---	---	94.8	1.479 - 1.480
Soybean oil	190-195	287-295	1.27-1.54	0.35-0.85	129-137	77-85	---	0.7-1.6	0.5-2.5	0.2-1.0	---	1.4723 - 1.4756
Coconut oil	255-260	210-250	2.5-10.0	---	8.2-9.6	6.1-70	---	0.15-0.7	7.0-8.0	15-17	82	1.4530
Palm-kerneloil	248	220-250	----	---	15-18	---	---	---	28	---	94.2	---
Sardine oil	189.8-193.8	---	2.2-21.7	---	138.8-	---	---	---	---	---	---	---
Whale oil	184-200	---	0.3-51.4	---	126.9	---	---	---	---	---	---	---
Brain lipid of Baghda chingri	229.25	244.71	1.11	0.56	95.83	43.63	10.58	0.566	1.04	0.796	95.32	1.4736 at 28°C

S.V, Saponification value; S.E.V, Saponification equivalent value; A.V, Acid value; F.F.A, Free fatty acid; I.V, Iodine value; T.V, Thiocyanogen value; U.S.M, Unsaponifiable matter; R.M.V, Reichert - Meissl value; P.V, Polenske value; H.V, Henher value, R. I, Refractive Index.

be 2.102%, 0.93%, 1.85% and 1.34%, respectively. The comparatively high saponification value and saponification equivalent value, 229.25 and 244.71, respectively, indicate the presence of higher proportion of high molecular weight fatty acid components. The acid value and percentage of free fatty acid (as oleic) were found to be 1.11 and 0.56, respectively. The low values of these characteristics are an indication of the suitability of the lipid for edible purpose. The iodine value of 95.83 indicates that the lipid contains moderate proportion of unsaturated fatty acid components and is of semidrying type, also confirmed by the Elaiden test. The peroxide value of 194.95 and thiocyanogen value of 43.63 also indicates moderate content of unsaturated fatty acid components. The acetyl value of 10.58 is an indication of low content of free hydroxyl groups in the lipid. The low value of unsaponifiable matter 0.566% (w/w) indicates that the lipid contains a small amount of unsaponifiable sterols, tocopherols, vitamins A and D, hydrocarbons and so on. The Reissert-Meissl and Polenske value, 1.04 and 0.796, respectively are an indication of low content of both volatile water soluble and volatile water insoluble but alcohol soluble fatty acid components in the lipid sample. The higher Henher value of 95.32% is an indication of high percentage of water insoluble nonvolatile fatty acid components present in the lipid.

A study of the effect of storage time showed an increase of acid value and peroxide value and a decrease of R-M value, thiocyanogen value and iodine value with time. That means, the quality of the lipid deteriorates with storage time.

Thin layer chromatographic (TLC) analysis showed the presence of myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$), arachidic acid ($C_{20:0}$), arachidonic acid ($C_{20:1}$) and erucic acid ($C_{22:1}$), well-separated in petroleum ether: ether (80:20) solvent system. The chromatogram showed several other spots, which could not be identified due to non - availability of suitable standards in the laboratory. These unidentified acids may be some PUFAs such as eicosapentaenoic acid ($C_{20:5}$), docosahexaenoic acid ($C_{22:6}$) etc. which are available in marine plants, phytoplankton, zooplankton, algae etc. (Beare 1962; Dyerberg 1986) on which the Baghda chingri lives on.

Qualitative and quantitative information about myristic acid, palmitic acid, stearic acid, oleic acid and arachidic acid present in the lipid has been obtained from GLC (Table 2). These acids comprised of about 33% of the total acids present in the lipid as calculated from the area of the peaks in the chromatograph. The chromatograph showed several other peaks, which could not be identified due to non - availability of suitable standards in the laboratory. However, TLC analysis in a different laboratory (conducted by the same authors) showed the presence of linoleic, linolenic, arachidonic, erucic acids in addition to the acids identified and quantified by GLC.

It is evident from Table 3 that the lipid sample has positive activity against all the test pathogenic bacteria. Maximum inhibition was found in the case of *Staphylococus aureus* (28 mm) and minimum in *Bacillus subtilis* (12 mm), while oil soaked paper discs were used. *Staphylococcus aureus* and *Salmonella typhi* were found sensitive towards the lipid sample at 0.05 ml / disc and 0.1 ml / disc while *Bacillus subtilis* and *Escherichia coli* showed very low or no inhibitory activity. It is evident from Table 4 that the mycelial growth of all test fungi was stimulated by the lipid sample. It is hoped that this work employing the lipid sample as chemical test will help the development of pesticides and medicines for human diseases.

The brain of Baghda chingri was found to contain a good amount of nitrogen (3.54) as well as protein (Proteineous nitrogen), which is well-balanced in respect of essential amino acids. The percentage of phosphorus, 0.5506 indicates that phospholipid may be presented in the lipid, which was extracted from the brain. The percentage of potassium was found to be 1.123. Calcium content of brain, 0.914% may help in the formation of rigid bone structure of the community children in their growing age who eat these Baghda chingri (Table 5).

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Table 2

Fatty acids as obtained by GLC analysis of methylated brain lipid of Baghda chingri

Peak number of acids identified by GLC	Name of the fatty acids	Inference	Retention time, RT	Area	Relative area, %
1	Myristic acid	$C_{14:0}$	16.52	8722	3.748
2	Palmitic acid	$C_{16:0}$	21.83	31280	13.443
3	Stearic acid	$C_{18:0}$	26.07	18483	7.943
4	Oleic acid	$C_{18:1}$	26.70	16291	7.001
5	Arachidic acid	$C_{20:0}$	31.21	1572	0.676

Table 3
Anti-bacteria screening data of the brain lipid of Baghda chingri

Name of the bacteria	Diameter of inibition zone in mm		
	Oil soaked disc	0.05 ml / disc	0.1 ml / disc
Staphylococcus aureus	28	23	25
Bacillus subtilis	12	-	-
Salmonella typhi	26	20	22
Escherichia coli	13	-	-

'--' means no inhibition

Table 4
Anti-fungal screening data of the brain lipid of Baghda chingri

Name of the fungi	% Inhibition after 5 days
Macrophomina phaseolina	- 2.09
Curvularia huna ta	- 20.229
Alternaria alternata	- 1.1730

-ve sign indicates the stimulation of test fungi

Table 5
Percentage of nutrient elements in the brain of Baghda Chingri

Name of the sample	N	P	K	Ca
Brain of Baghda Chingri	3.54	0.5506	1.123	0.914

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