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Study on chemical characterization and composition of liver lipid of Petambori fish (*Rhinobatus granulatus*) of the Bay of Bengal

[1] Md. Sohel Shaikh, [2] Mohammad Helal Uddin, [*3] Md. Ashraful Hoque

[1] Applied Chemistry Research Laboratory, Department of Chemistry, University of Chittagong, Chittagong, Bangladesh.
[2, 3] Department of Applied & Environmental Chemistry, University of Chittagong, Chittagong, Bangladesh.

Abstract
Chemical characteristics of lipid extracted from Petambori fish and composition of that fish was analyzed by standard method. Various chemical properties e.g. saponification value, saponification equivalent value, acid value, percentage of free fatty acid, iodine value, peroxide value, ester value, thiocyanogen value, titre value, unsaponifiable matter, Henher value, Polenske value, Reichert-Meissl value, cholesterol content etc. of the lipid sample were determined and compared with those of different standard oils. Palmitic acid, Stearic acid and Oleic acid were observed at fatty acid composition of the sample by Thin Layer Chromatography (TLC). Percentages of protein and mineral (N, P, K, Ca) in the liver lipid of Petambori fish were studied by modified kjeldahl method.

Keywords: Petambori fish, Thin Layer Chromatography, Composition

1. Introduction

Lipids and fatty acids are playing a significant role in membrane biochemistry and have direct effect on the membrane-mediated process in human such as osmoregulation, nutrient assimilation and transports (Ibrahim *et al.*, 2004) [1]. The nature and quantity of fish lipids are vary according to the species and habitats. It is known that fish lipids are the main sources of polyunsaturated fatty acids (PUFAs) especially eicosapentaenoic acid (EPA; $C_{20:5}$) and docosahexaenoic acid (DHA; $C_{22:6}$) (Osman *et al.*, 2001) [2]. These two fatty acids cannot be synthesized by the human body and must be obtained from the diet (Linko and Hayakawa 1996) [3]. It has been reported that EPA and DHA have biochemical effect in prevention and treatment of several disorder and diseases such as coronary heart disease, rheumatoid arthritis, asthma, cancers, diabetes and others (Pamela, 2001) [4]. At present, the major sources of EPA and DHA are from the cool deep sea fish oil such as menhaden, cod, sardine, anchove and others. Due to their biochemistry important, local fish's oil should be highlighted in order to discover their potential use. Present study is about the chemical characterization of the solvent extracted oil from the liver lipid of Petambori fish local coastal belts and comparing the results with the data available in literature about chemical aspects of liver lipid of Petambori fish. Percentages of protein and mineral (N, P, K, Ca) in the liver lipid of Petambori fish were studied and its potential to be use on incorporation into human food, animal feed or other Food products of higher value.

(Dorsal view)

Fig 1: Petambori fish (*Rhinobatus granulates*)

(Ventral view)

Fig 2: Petambori fish (*Rhinobatus granulates*)

2. Materials and Methods

2.1 Collection of the marine species

The Petambori fish was collected from the local fish market, Sadarghat, Chittagong Bangladesh and preserved in deep freezer for a few days. The liver was separated and preserved until extraction and further chemical investigation.

2.2 Extraction of lipid

Oil extraction from liver was carried out by solvent extraction method using acetone and ethyl acetate as solvent (Uddin and Majid 2000) [5]. Combined extract was recovered with a rotary evaporator at 45 °C to obtain lipid and dried under flushing with a slow stream of nitrogen gas for the removal of residual solvent. Extracted lipid was used for chemical characterization (Vogel 1975) [6]. Thin layer chromatographic (TLC) investigation of the fatty acids present in the fish oil was done in various solvent systems. Cholesterol and proximate composition of the sample were studied with standard methods (Uddin *et al.*, 2004) [7].

2.3 Chemical characterization

Various chemical properties of the lipid sample were determined under the specific condition of the standard methods (Griffin 1972) [8]. saponification value, saponification equivalent value, acid value, free fatty acid, ester value, iodine value, thiocyanogen value, titre value, unsaponifiable matter, Reichert-Meissl value, Polenske value, Henher value of the lipid were determined by standard methods (Morris *et al.*, 1965, Al-Amin *et al.*, 2014 and Willams 1966) [9,10,11].

2.4 Estimation of minerals

By applying the standard methods, percentages of minerals (N, P, K, Ca) of lipid containing liver were determined.

2.5 Chromatographic examinations

The liver lipid of Petambori fish was subjected to TLC examination and its fatty acid composition was identified by comparing the R_f values of different spots of chromatograms with those of standard fatty acids reported earlier in different solvent systems (Rahman et al., 2009 and Randerath K) [16,17].

3. Results and Discussion

3.1 Chemical characterization

The saponification value of the liver lipid of Petambori fish was found to be 274.50 (Table 1). The saponification equivalent value of the liver lipid of Petambori fish was found to be 204.70 (Table 1). Saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fat or oil (Das, 1989) [12]. The saponification equivalent value is directly proportional to the average chain length of the fatty acids present in the fat or oil. This result clearly indicate that the lipid sample contain higher proportion of high molecular weight fatty acids. The acid value of the liver lipid of Petambori fish was found to be 2.30 (Table 1). The percentage of free fatty acid (FFA), as oleic, calculated from acid value was found to be 0.50 (Table 1) for the lipid sample. Acid value indicates the proportions of free fatty acid in the oil or fat. Low acid value is an indication of freshness of the oil or fat and suitability of the lipids for edible purpose. So the liver lipid of Petambori fish might be used for edible purpose (Loury, 1966) [13]. The ester value of the liver lipid of Petambori fish was found to be 272.00 (Table 1). This result indicates that ester may present in the lipid sample. The iodine value of the liver lipid of Petambori fish was found to be 106.66 (Table 1). Iodine value gives an estimation of the degree of unsaturated fatty acids in the triglyceride molecules of the fat or oil. This value indicates that the lipid sample contain moderate proportion of unsaturated fatty acids and is of semidrying type which is supported by Elaiden test. The peroxide value is an indication of unsaturation of fats or oils. The most unsaturated fats or oils absorb more oxygen, form greater amounts of unstable hydro peroxides and show higher peroxide value. The peroxide value of the liver lipid of Petambori fish was found to be 33.60 (Table 1). It can be concluded from the result that the liver lipid under investigation contained good amount of unsaturated fatty acids (Hoque *et al.*, 2015) [14]. The thiocyanogen value of the liver lipid of Petambori fish was found to be 63.00 (Table 1).This observation is in conformity with the findings that the lipid sample has moderate iodine value and peroxide value. The acetyl value is a measure of hydroxylated fatty acids in a fixed oil or fat. The acetyl value of the liver lipid of Petambori fish was found to be 12.50 (Table 1). This result indicates low content of free hydroxyl groups present in the lipid sample. The titre value of the liver lipid of Petambori fish was found to be 25.20 (Table 1). This value indicates that the lipid sample is of fat type which support the semisolid condition at room temperature. Unsaponifiable matter is defined as those substances which are not saponified by alkali and which are soluble in petroleum ether or ether. In general, if a fixed fat or oil contain unsaponifiable matter present in excess of about 2 %, there is reason to support adulteration. The unsaponifiable matter in the liver lipid of Petambori fish was found to be 1.48% (Table 1). This result indicate that the lipid sample may contain a small amount of unsaponifiable matter such as sterols, vitamins A & D, hydrocarbons etc (Ahmed *et al.*, 2014) [15]. The Reichert-Meissl value of the liver lipid of Petambori fish was found to be 0.64 (Table 1). Since the Reichert-Meissl value is a measure of the volatile water soluble lower fatty acids present in the fat or oil, so the low Reichert-Meissl value of the lipid sample is an indication of low content of volatile water soluble fatty acids. The Polenske value of the liver lipid of Petambori fish was found to be 0.066 (Table 1). The Polenske value represents a measure of volatile water insoluble but alcohol soluble fatty acids (Ali et al., 2014) [18]. The low Polenske value as obtained is a support of the presence of small amount volatile water insoluble but alcohol soluble fatty acids in the lipid sample. The Henher value of the liver lipid of Petambori fish was found to be 58.11(Table 1). The result indicates the higher percentage of water insoluble nonvolatile fatty acids present in the lipid sample. The liver lipid of Petambori fish was found to form a 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. Semidrying oils absorb oxygen from air slowly and thicken after keeping exposed to air for some time but do not dry up and the iodine value varies between 95 and 140. The amount of cholesterol in the liver lipid of Petambori fish was found to be 2840.20 mg/100g (conc). A comparatively higher amount of cholesterol was observed in the liver lipid of Petambori fish. It can be suggested that the liver of Petambori fish is less useful for edible purpose due to the cholesterol level. The effect of storage time on the lipid sample showed a significant variation in different properties.

Acid value, Peroxide value increased with increasing time of storage and Richert-Meissl value, Thiocyanogen, titre value, iodine value decreased with increasing time of storage. That means, the quality of the lipid deteriorated with increasing time of storage.

Table 1. Chemical constants of the liver lipid of Petambori fish and some related fats and oils (Das, 1989; Williams, 1966, Helal *et al.*, 2000, 2004) [5, 7, 11, 12]

Name of the sample	S.V.	S.E.V.	A.V.	F.F.A. (%) as oleic	E. V	I.V.	P. O. V.	T. V.	Acetyl Value	Titre Value	U.S.M. (%)	R.M.V.	P.V.	H.V.
Linseed oil	189-195	287-296	4.0	0.5-0.75		175-200	---	---	---	---	1.0-1.5	---	---	94.8
Soybean oil	190-195	287-295	1.27-1.54	0.35-0.85		129-137	---	77-85	---	22-27	0.7-1.6	0.5-2.5	0.2-1.0	---
Coconut oil	255-260	210-250	2.5-10.0	---		8.2-9.6	---	6.1-7.0	---	20-24	0.15-0.7	7.0-8.0	15-17	82
Palm Kernel oil	248	220-250	---	---		15-18	---	---	---	---	---	28	---	94.2
Sardine oil	189.8-193.8	---	2.2-21.7	---		138.8-177	--	---	---	---	---	---	---	---
Whale oil	184-200	--	0.3-51.4	---		126	---	---	---	---	---	---	---	---
Liver lipid of Petambori fish	274.50	204.70	2.30	0.50	272.00	106.66	33.60	63.00	12.50	25.20	1.48	0.64	0.066	58.11

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; U.S.M. = Unsaponifiable matter; R.M.V. = Reichert-Meissl value; P.V. =Polenske value; H.V. = Henher value.

3.2 Estimation of N, P, K and Ca in liver lipid of Petambori fish (Ahmed *et al.*, 2014) [15]

Table 2: Percent of nitrogen (N), phosphorus (p), potassium (K) and calcium (Ca) of the liver lipid of Petambori fish.

Name of the sample	Nitrogen (%)	Phosphorus (%)	Calcium (%)	Potassium (%)
Liver lipid of Petambori fish	3.080	1.255	0.650	1.150

3.3 Chromatographic examination

The fatty acid methyl esters mixtures obtained from the liver lipid of Petambori fish was subjected to TLC examination and their fatty acid composition was identified by comparing the R_f values of methyl esters of standard fatty acids in different solvent systems.

Table 3: The R_f values (most related) of thin layer chromatographic examination of the liver lipid of Petambori fish.

Solvent System	R_f values of standard fatty acids			R_f value obtained from the spots of lipid sample			
	PA	SA	OA				
P:E (60:40)	0.921	0.947	0.283	0.284	0.946	0.466	0.641
P:E:A (70:30:1)	0.942	0.961	0.417	0.936	0.941	0.416	0.314
P:H (80:20)	0.813	0.832	0.316	0.300	0.833	0.852	0.814
H:E (80:20)	0.815	0.820	0.201	0.816	0.833	0.200	0.900

Note: P: Petroleum ether, E: Ether, A: Acetic acid, H: Hexane PA-Palmitic Acid, SA-Stearic Acid, OA-Oleic Acid

5. Conclusion

Chemical characterization and proximate composition studies of lipid of Petambori fish were done in this investigation. Presence of good amount of sterols, vitamins A & D, is considered with respect to U.S.M. Percentage of F.F.A. validated suitability of the oil for edible purpose. Semi drying nature of the fish lipid was pointed out by I.V. Chromatographic examinations substantiated the presence of Palmitic acid, Stearic acid and Oleic acid in the liver lipid. Protein and other important minerals (Ca, P and K) were also found with significant values in the lipid of Petambori fish.

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