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Research article

Characterization of oil and lecithin from pioly (*Aspidoparia morar*) fish

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Key words: Fish oil, Lecithin, Oxidative stability, Saponification.

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Abstract

Pioly fish oil was extracted from fish powder using n-haxane as a solvent. Lecithin containing mainly phospholipids was also isolated from pioly fish powder. The amount of extracted oil was approximately 0.11 g/g fish powder. The percentage of lecithin from pioly fish powder was 2.13, whereas it was 3.67% for the residues after the extraction of oil. Oil and lecithin from pioly fish powder were characterized by the determination of iodine value, acid value, percentage of FFA content, peroxide value and saponification value to know the quality for comparison to commercially available oil and lecithin from other sources. The iodine values of oil and lecithin for fish flesh powder were 81.28 and 55.95, respectively. Saponification value of pioly fish oil and lecithin were 191.37 and 119.11 mg KOH/g. Acid value and peroxide value of pioly fish oil and lecithin were in acceptable ranges. Lecithin from pioly fish showed high oxidative stability indicating potential for commercial purposes.

Introduction

Fish is consumed all over the world as rich sources of protein and lipid. Fish is also an integral part of the food culture of populations in many countries. Therefore, fisheries sector represents one of the major sectors of agriculture. Fish and fish oil are an important source of essential dietary components such as micronutrients, especially vitamin A and mineral, polyunsaturated long chain fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1,2]. Currently, much attention have been paid by the researcher on the bioactive compounds including polyunsaturated fatty acids, phospholipids, lecithin, peptides and pigments obtained from various fish species [3]. It is well known that polyunsaturated fatty acids and lipid soluble bioactive compounds have strong role in favor of human health. Polyunsaturated fatty acids especially ω-3 fatty acids have an important role in the prevention of human diseases, such as in lowering blood cholesterol, coronary artery disease, hypertension, arthritis, others inflammatory and autoimmune disorders and cancer [4].

Lecithin is a sticky fatty substance which includes mainly phospholipids (especially phosphatidylcholine [PC] and phosphatidylethanolamine [PE]), small amount of glycerides, neutral lipids, and other suspended matter. It is widely used for its emulsifying properties in the food, pharmaceutical and cosmetic industries. Lecithin has also been used to modify the immune system by activating specific and nonspecific defense systems and pharmacologically in the treatment for hypercholesterolemia, neurologic disorders and liver ailments [5-7]. The important property of lecithin is its ability to reduce the excess of LDL cholesterol. It also promotes the synthesis of a great amount of HDL in the liver, the beneficial cholesterol. Bile acid secretion with high levels of cholesterol and phospholipids is encouraged by lecithin-rich diets when compared with diets without lecithin. Lecithin contains choline that is used in treatment of neural disorder. Lecithin is one of the natural elements that have dispersing properties. That is why it can emulsify fat, avoiding its absorption [8-10]. The principal commercial sources of lecithin are egg yolk and soybeans [5,8]. Lecithin obtained from these two sources is rich in saturated fatty acids as compared to unsaturated fatty acids. Therefore, lecithin from egg yolk and soybeans does not play vital role as nutritional supplements. Lecithin from fish sources has many nutritional health benefits due to the presence of unsaturated fatty acids including suspended bioactive compounds.

Many research works have been performed to extract oil from fishes especially from marine fishes. There is very few studies have been carried out on the extraction of

lecithin from fish sources. Bangladesh is highly dense populated country. Majority of the people depend on the fresh water fishes due to presence of lot of ponds, haors, beel and rivers. Pioly (*Aspidoparia morar*) is a small genus of cyprinid fishes and also known as Monah, Boreala, Chelluah etc. in different places [11,12]. It is found in natural sources such as rivers, lakes and farming ponds. However, considering the increasing demand of the functional foods, the aim of this study was to extract and to characterize the oil and lecithin from pioly fish for commercial utilization in health benefit purpose.

Experimental

Preparation of sample

Pioly fish was collected from the local market in Rajshahi. After collection, the samples were cleaned by fresh cold water and viscera were removed. The fish fleshes were separated from bone and were sun-dried for about 72 hours and grinded by mechanical grinder. Then, the sun-dried fish powder was stored at 4^0C in polyethylene bag for further analysis.

Soxhlet extraction of oil from pioly fish

The extraction of oil was performed in a Soxhlet apparatus using n-hexane as solvent. About 60-70 g of sun-dried powder from fish fleshes was placed into the extraction thimble and the extraction was run about 5-6 hours until the color of the condensed solvent at the top of the apparatus was clear. The solvent was then evaporated at low temperature (ambient temperature) and the fish oil was stored at 4°C temperature.

Isolation of lecithin from pioly fish

The extraction of lecithin from pioly fish powder and Soxhlet extracted residues were carried out according to the method of Palacios and Wang [13] modified by Uddin *et al.* [14]. Figure 1 shows the schematic representation of lecithin isolation from pioly fish powder. Briefly, 30 g of fish powder and 100 ml of ethanol (95%) were taken in a beaker and stirred for about 12 hours by a magnetic stirrer. The mixture was then centrifuged at 6000 rpm for 10 min. The supernatant that contained mainly polar lipids with very low amounts of neutral lipids was collected in a separatory funnel. The residue was further extracted with 100 ml of ethanol (95%) and followed centrifugation, the supernatant was added to the previous ethanol extract. To separate the neutral lipids from the polar lipids, twice volume of n-hexane was added to the ethanol extract. The ethanol phase was then collected from separatory funnel and evaporated at 40°C. The remaining lipid residue was dissolved in hexane. A fifth volume of chilled acetone (4°C) to hexane was added to the n-hexane mixture with slow stirring for precipitation of the gummy material. The mixture was placed in an ice bath for 15 min and then centrifuged at 5000 rpm for 10 min. The collected precipitate called fish lecithin was stored at - 20°C until further analysis.

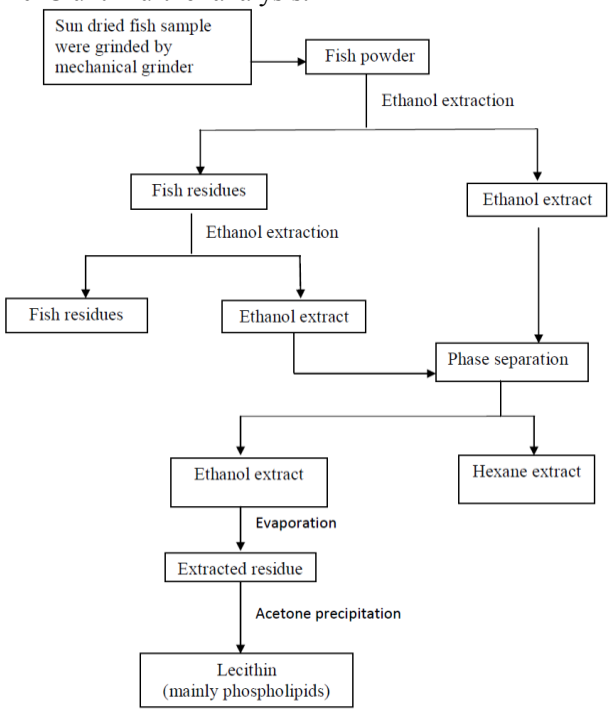


Figure 1. Schematic representation of isolation of lecithin from pioly fish powder

Characterization of fish oil and lecithin

Specific gravity of oils was measured by the method of Hsieh *et al.* [15]. Specific gravity of a substance represents how much heavier the substance to the same volume of water at a particular temperature and it may be defined as the ratio of the weight of the definite volume of substance to the weight of the definite volume of water at a particular temperature. The iodine value of oil and lecithin was measured by the method of Hanus *et al.* [16]. The iodine value of fat or oil is the amount of halogen absorbed under specific conditions and is expressed as the number of grams of iodine per 100 g of fat or oil. Saponification value was determined by IUPAC [17]**.** The saponification value of the fat or oil is the number of milligrams of potassium hydroxide required to saponify completely 1 g of fat or oil. The saponification value provides information on the mean molecular weight of the combined fatty acids. The acid value was measured according to the official method of IUPAC [17]. The acid value was the amount of milligrams of KOH required to neutralize the acids present in 1 g of sample. Peroxide value was determined by the method of AOCS [18]. The peroxide value is defined as the milli-equivalents peroxide per 1000 g (meq/1000 g) of sample.

Oxidative stability of fish lecithin

Oxidative stability of lecithin is associated with the degradation by oxygen in the air and the double bonds of an unsaturated fatty acid can undergo cleavage, releasing volatile aldehydes and ketones**.** Emulsions of lecithin in water were prepared and oxidized at 37°C for measuring the

oxidative stability. Three emulsions of lecithin in water (w/w) (linoleic acid 4%, lecithin 1%, water 95%; lecithin 5%, water 95%; β-carotene 1%, lecithin 4%, water 95%) were prepared. The oxidative stability of fish lecithin was measured by using linoleic acid and β-carotene as standard. Oxidative stabilities were checked by the thiocyanate (TC) [19] and thiobarbituric acid (TBA) [20] methods, which were used to measure the antioxidant activity. In this study, these two methods were performed to measure the quality of the extracted lecithin in terms of its oxidative stability.

TC method

The oxidative stability of fish lecithin was measured according to the method of Mitsuda *et al.* [19]. The peroxide formed by lipid peroxidation reacted with ferrous chloride and formed ferric ions which combined with ammonium thiocyanate and produced ferric thiocyanate. Briefly, 0.1 ml of emulsion solution was added to 4.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of a red color was measured at 500 nm. The absorbance was recorded at 120 hours intervals during the incubation.

TBA method

The TBA method was used to evaluate the extent of lipid peroxidation according to the method of Ottolenghi [20]. The product of lipid breakdown caused by oxidative stress is malonadehyde which binds with TBA to form a red complex of thiobarbituric acid reactive substance (TBARS). Briefly, 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid were added to 1 ml of emulsion solution. The mixture was heated at 100°C for 10 min in a boiling water bath. After cooling, the mixture was centrifuged at 3000 rpm for 20 min. Absorbance of the supernatant containing TBARS was measured at 532 nm.

Results and Discussion

Extraction of oil and lecithin from pioly fish

The amount of oil extracted from pioly fish flesh powder by Soxhlet extraction using n-hexane was approximately 0.11 g/g fish powder. It was found that oil content of marine fishes was much higher than that of fresh water fishes. The oil obtained in *Scomber scrombrus* fish species was 28.24% [21]. The percentages of lecithin from fish powder and extracted residues are shown in Figure 2. It was found that the percentage of lecithin increased from 2.13 to 3.67 after the extraction of oil. It could be explained in two ways- firstly, after oil extraction, the sample load for the isolation of lecithin was increased and secondly, the presence of non-polar lipid in the sample may reduce the isolation of lecithin. Uddin *et al.* [12] reported that the percentage of lecithin from squid viscera was 4.25, which was almost similar to this result. On the other hand, 25% lecithin was found in deoiled residues of anchovy obtained by supercritical carbon dioxide extraction [22]. The percentage of lecithin may vary on the amount of phospholipid presented in sample.

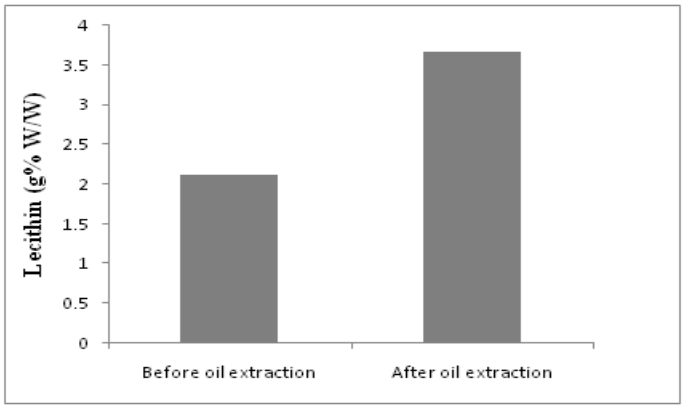


Figure 2. The percentage (g%) of lecithin from pioly fish powder

Characterization of fish oil and lecithin

The specific gravity of pioly fish oil was found to be 0.91 (Table 1). Specific gravity of fats and oils does not vary as a general rule to an extent, which makes this property useful in discriminating between one to another. The specific gravity of practically all fats or oils lies between 0.90 and 0.95. The specific gravity of fish oil was closely related to the value of 0.927 for *Sarddneila bongiceps* fish species [23]. Table 1 shows the iodine value, acid value, percentage of FFA content, peroxide value and saponification value of pioly fish oil and lecithin. These parameters provide the quality index of the fish oil.

Iodine values give an estimation of the amount of unsaturated fatty acids in the triglyceride molecules of fat and oil. The iodine values of oil and lecithin for fish flesh powder were 81.28 and 55.95, respectively. These values were lower as compared to marine fishes but almost similar to fresh water fishes [23]. Paul *et al.* [24] reported the iodine value of 83.23 for *Channa marulius* fish oil. The low iodine value of lecithin might be obtained due to oxidation of polyunsaturated fatty acid during long isolation process.

Acid value was used to determine the acidity of oil and lecithin. Acid value of pioly fish oil and lecithin were found to be 13.44 and 9.24 mg KOH/g, respectively. In literature, acid value of food grade fish oil should not be more than 3 mg KOH/g of oil, but the acceptable limit for acid value was reported to be 7-8 mg KOH/g [25]. On the other hand, the lower acid value of pioly fish lecithin indicated the high quality of product. The acid value of this fish lecithin was much lower than the value of 33.1 for squid viscera [14] and 28.12 for anchovy lecithin [22]. The acid values of food grade lecithin recommended by FAO/WHO are found to be up to 36 mg KOH/g of lecithin [26]. The percentage of free fatty acid of pioly fish oil and lecithin calculated from acid value were 6.75 and 4.64, respectively. Due to the presence of moisture in the sample, free fatty acid may be liberated by its hydrolytic rancidity.

Saponification value is directly related to the chain length of fatty acids. Higher saponification value indicates the presence of low molecular weight fatty acid in triglycerides. The long chain fatty acids have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fats or oil and therefore, high molecular weight. The saponification value of pioly fish oil and lecithin were 191.37 and 119.11 mg KOH/g, respectively. Several researchers reported the saponification value of fish oil from various species such as 259.5 mg KOH/g for menhaden, 180.9 mg KOH/g for *Seriola nigrofasciata*, 205.7 mg KOH/g for *Channa striatus* and 199.66 mg KOH/g for *Channa marulius* [24,27]. According to AOCS, standard saponification value for fish oil is 180-200 mg KOH/g [28]. Therefore, it was found that saponification value of pioly fish oil was similar to other good quality fish oil. On the other hand, saponification value of lecithin was very low because lecithin mainly contained high molecular weight fatty acids.

Peroxide value is used for the measurement of rancidity, which occurs by auto oxidation. Peroxide value of pioly fish oil and lecithin were found to be 9.24 and 3.97 meq/1000 g, respectively. Bimbo [29] has reported that peroxide value of crude fish oil was between 3 and 20 meq/1000 g. This indicated that the lipid oxidation rate in extracted fish oil was low. In contrast, the peroxide value of food grade lecithin recommended by FAO/WHO was found to be up to 10 meq/1000 g lecithin [26], which was also much higher than the lecithin obtained in this study. Lee *et al.* [22] reported that the peroxide value of lecithin from anchovy was 4.66-6.79 meq/1000 g, which are very close to the peroxide value of pioly fish lecithin.

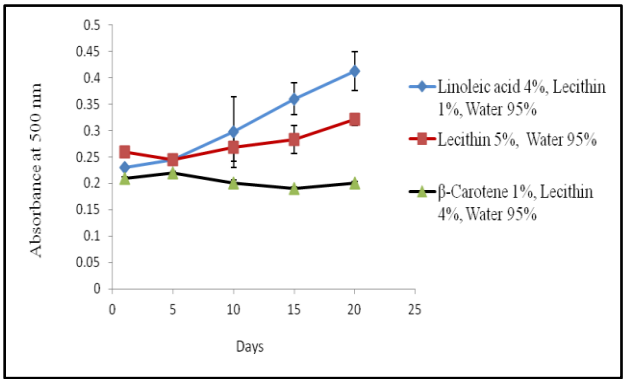
Table 1. Characteristics of the oil and lecithin obtained from pioly fish as a quality index

Quality index	Oil	Lecithin
Specific gravity	0.91	-
Iodine value (mg I/g oil)	81.28±0.52	55.95±1.38
Acid value (mg KOH/g)	13.44±0.03	9.24±1.78
Free fatty acid (%)	6.75±0.07	4.64±0.76
Saponification value (mg KOH/g)	191.37±0.89	119.11±1.28
Peroxide value (meq/1000 g)	9.24±0.14	3.97±0.82

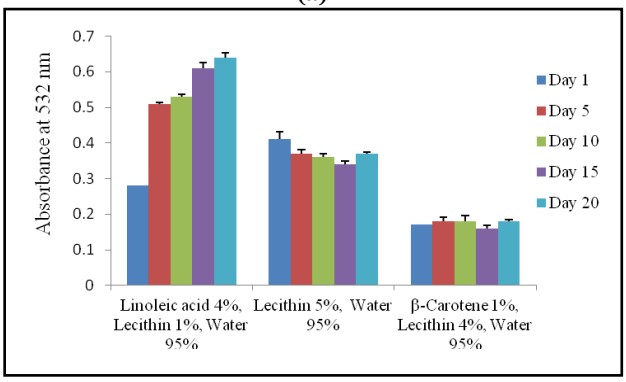
Mean value of 2 replicates ± SD (SD was mentioned at least 0.01).

Oxidative stability of lecithin

The oxidative stability represents the susceptibility of a food or edible oil to lipid oxidation, which causes rancid odors and flavors. Oxidative stability may be used to provide information regarding the efficacy of antioxidants, the effect of impurities and evaluation of refining processes of fats and oils. The oxidative stabilities of pioly fish lecithin are shown in Figure 3 (a) and (b). In this study, the oxidation trend was evaluated instead of determining the absolute state of oxidation of lecithin. It was found that the absorbance value of lecithin with linoleic acid emulsions increased from earlier. Auto-oxidation was indicated by the increase in absorbance value due to formation of peroxides during incubation. Lecithin emulsion with β-carotene showed lower absorbance values indicating low levels of lipid peroxidation. β-carotene, a strong antioxidant inhibited the peroxide formation of the lipids by peroxidation over a certain period. On the other hand, only lecithin emulsion showed low levels of lipid peroxidation until 15 days and after that increased significantly from the initial level of lipid peroxidation. Initially, fish lecithin emulsion showed slightly higher absorbance as compared to lecithin within the linoleic acid emulsion. It might be happened due to the presence of peroxide from the oxidation of neutral lipids of fish lecithin during processing. In TBA method, high absorbance values were also observed in lecithin with linoleic acid emulsion as compared to only lecithin emulsion and lecithin emulsion with β-carotene. The results from TBA method were agreement with the results of TC method. However, pioly fish lecithin has higher oxidative stability. The presence of small amounts of natural antioxidants might be one of the causes of its higher oxidative stability and it was highlighted by other researchers [14, 22]. Gogolewski *et al.* [30] also reported that long chain polyunsaturated fatty acids esterified with polar lipids had synergistic effects with antioxidants. High oxidative stabilities of lecithin from animal and plant sources were also reported by using different methods [31, 32].



(a)



(b)

Figure 3 (a) and (b). Oxidative stability of pioly fish lecithin: (a) TC method and (b) TBA method. Results are the mean value of 3 replicates ± SD

Conclusion

Pioly fish oil and lecithin were isolated from fish powder in this study. Both fish oil and lecithin were characterized by the determination of iodine value, saponification value, FFA content, acid value and peroxide value. These parameters referred that the quality index of oil and lecithin from pioly fish were satisfactory as compared to that of other fresh water fishes. The oxidative stability of lecithin was also higher due to presence of natural oxidants. However, it can be concluded that pioly fish oil and lecithin may have potentiality to be used as food additives and emulsifier in food, pharmaceutical and cosmetic industry.

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