Journal of Food and Nutrition Research, 2015, Vol. 3, No. 10, 661-666
Available online at http://pubs.sciepub.com/jfnr/3/10/7

DOI:10.12691/jfnr-3-10-7

Extraction and Characterization of Oil and Lecithin from Boal (*Wallago attu*) Fish

Md. Rasel Molla[1,*], A.K.M. Asaduzzaman[1], Md. Abdur Rashid Mia[1], Md. Aowrongo Zeb[2], Md. Salim Uddin[1]

[1]Department of Biochemistry & Molecular Biology, Rajshahi University-6205, Bangladesh
[2]Department of Microbiology, Prime Asia University, Dhaka-1212, Bangladesh
*Corresponding author: rasel229@gmail.com

Abstract Consumption of fish is very beneficial to the health and development of the human body. They provide essential nutrients to the human. Fish oil contains higher amount of polyunsaturated fatty acids which have significant effect in maintaining a healthy cardiac life. Biochemical composition of boal (*Wallago attu*) was determined. It was found that fishes are rich sources of protein and other nutrients. Boal contain higher amount of moisture 77.51±0.35 (g%). All the other parameters such as, protein, lipid, total sugar and ash were 13.12±1.24 (g%), 1.94±0.06 (g%), 0.05±0.01 (g%), 1.55±0.05 (g%) in boal. Boal fish oil was extracted using n-hexane by soxhlet apparatus. The percentage of oil from boal fish powder was 18.24(g% w/w). Lecithin was also extracted from this fish fleshes before and after oil extraction. Lecithin was 2.02 (g% w/w) and 3.68 (g% w/w) before and after oil extraction. The physicochemical properties of fish oil and lecithin were investigated. The saponification value, iodine value, acid value, peroxide value, percentage of free fatty acids of boal fish oil and lecithin were 199.27±0.78 (mg KOH/g), 129.67±0.63 (mg I/g), 16.78±0.01 (mg KOH/g), 11.65±0.15 (meq O_2/kg), 8.43±0.09 (%). and 124.56±1.23 (mg KOH/g), 89.26±1.44 (mg I/g), 11.31±1.52 (mg KOH/g), 4.26±0.55(meq O_2/kg), 5.68±0.76 (%). The oxidative stability of boal fish lecithin was also measured. Boal fish lecithin showed higher oxidative stability due to the presence of natural antioxidant. Fatty acid composition of boal fish oil and lecithin was measured by gas chromatography (GC). The important polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were found to be 1.35% and 1.98%. But lecithin contains only 9% DHA and other monounsaturated fatty acids. This fish oil and lecithin also contain higher amount of monounsaturated fatty acid and average amount of polyunsaturated fatty acids. Therefore, we can use this fish oil and lecithin in edible purpose, food industry and pharmaceutical industry.

Keywords: *fish oil, lecithin, fatty acid compositions, oxidative stability, boal*

Cite This Article: Md. Rasel Molla, A.K.M. Asaduzzaman, Md. Abdur Rashid Mia, Md. Aowrongo Zeb, and Md. Salim Uddin, "Extraction and Characterization of Oil and Lecithin from Boal (Wallago attu) Fish." *Journal of Food and Nutrition Research,* vol. 3, no. 10 (2015): 661-666. doi: 10.12691/jfnr-3-10-7.

1. Introduction

One of the major sectors of agriculture is represented by the fisheries sector, because fish becomes an integral part of the food culture of populations in many countries. The composition of the fish including protein and lipid, and the contribution of micronutrients, especially vitamin A and mineral from different types of fish species have been focused at the end of last century [1]. In recent years, researchers are interested on the different bioactive compounds including polyunsaturated fatty acids, phospholipids, lecithin, peptides and pigments obtained from various fish species especially from marine fishes [2]. Natural bioactive compounds have diversified structures and functionalities that provide excellent properties to the molecules for the production of nutraceuticals, functional foods and food additives [3]. Polyunsaturated fatty acids and lipid soluble bioactive compounds have been attracted much attention for health benefits. There is commercial interest in obtaining polyunsaturated fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), because these ω-3 fatty acids are potential in the prevention of human diseases, such as in lowering blood cholesterol and thus preventing heart diseases. It is also known that ω-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, arthritis, others inflammatory and autoimmune disorders, and cancer [4]. Fish oil is important not only for their application in food, but also for industrial applications, such as the production of pharmaceuticals, cosmetics, paints and so more. Fish oil is composed of triglycerides containing polyunsaturated fatty acids, most notably EPA and DHA. Lecithin is a sticky fatty substance composed mainly of phospholipid mixtures especially phosphatidylcholine (PC) and phosphatidylethanolamine (PE) with small amount of glycerides, neutral lipids, and other suspended matter [5]. Lecithin from egg yolk, plant tissues and animal are usually utilized for its emulsifying, dispersing and stabilizing properties in the food, pharmaceutical and cosmetic industries. Pharmacologically, the lecithin is

used in the treatments for hypercholesterolemia, neurologic disorders, liver ailments, and intramuscular and intravenous injectable and parenteral nutrition formulations [6]. The application of lecithin is also recognized to modify the immune system by activating specific and nonspecific defense systems. Soybeans and egg yolk are the main commercial sources of lecithin [7,10]). In soybean lecithin, unsaturated fatty acids are present in very low percentage as compared to saturated fatty acids. Moreover, lecithin from soybeans does not contain ω-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In nutritional supplements, egg phospholipids cannot also play a significant role because they have a relatively high cholesterol level and unfavorable fatty acid profiles [8,9]. The importance of lecithin analysis is to find the quality index, properties, fatty acid composition, immunologic and neurologic function. As a result, we can use lecithin according to the functional properties of lecithin. Recent studies suggest that a lecithin enriched diet can modify the cholesterol homeostasis and lipoprotein metabolism. Lecithin diet modifies the cholesterol homeostasis in the liver, increasing the activity of 3-hydroxy-3 methylglutaryl coenzyme A reductase (HMG-CoA) reductase and cholesterol 7 alpha-hydroxylase and decreasing the microsomal acyl CoA: cholesterol acyltransferase (ACAT) activity. One of the most spectacular properties of lecithin is its ability to reduce the excess of LDL cholesterol. It also promotes the synthesis in the liver of great amount of HDL, 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 contain choline that is used in treatment of neural disorder. Lecithin is one of the nature elements that have dispersing properties. That is why it can emulsify fat, avoiding its absorption [8,9,10]. Most of the research work has been carried out to extract oil mainly from marine fishes. On the other hand, very few studies have been performed on the extraction of lecithin from fish sources. Bangladesh is a densely populated country and more than ninety percent people depend on the fresh water fishes. In Bangladesh, the main sources of fresh water fishes are ponds, haors, beel and rivers where boal are available as wild fish. Based on the consideration in increasing demand of the functional foods, the aim of this study is to estimate the nutritional status of boal fish and to characterize the oil and lecithin from boal fish. The aim of this study was to estimate the nutritional status of the selected fish species, to extract and characterize the fish oil and lecithin.

2. Materials and Methods

2.1. Materials

The fresh boal fish were collected from local market of Rajshahi, Bangladesh.

2.2. Sample Preparation

The fish fleshes were separated from bone and were sun-dried for about 72 hours. The sun-dried fleshes were then grinded by mechanical grinder and stored at -20°C until further analysis. Then, oil was extracted from both fishes by Soxhlet extraction apparatus using n-hexane and stored at 4°C for further analysis. Lecithins were extracted from fish powder according to the method of Palacios and Wang (2005) [11] modified by Uddin [7].

2.3. Extraction of oil from Boal Fish

The extraction of oil was carried out in a Soxhlet apparatus using n-hexane as solvent. Oil, triglyceride portion of boal fishes was extracted by suitable solvents under the operating condition. Continuous Soxhlet extraction apparatus was used for the extraction of oil (Suter *et al*., 1986) [12]. About 60-70 gm of sun dried powder from boal fishes was placed into the extraction thimble and the extraction was run about 5-6 hours until the colour of the condensed solvent at the top of the apparatus was clear. The solvent was evaporated at low temperature (almost ambient temperature) and the fish oil was stored at 4°C temperature.

2.4. Isolation of Lecithin

Lecithins were extracted from stored fish powder and Soxhlet extracted residues according to the method of Palacios and Wang [11] modified by Uddin [7]. In briefly, 100 ml of ethanol (95%) was added to 30 g of fish powder residues and stirred for almost 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 precipitate of residue was further extracted with 100 ml of ethanol (95%) and followed centrifugation, the supernatant was added to the previous ethanol extract. Twice volume of hexane was added to the ethanol extract for separating the neutral lipids from the polar lipids. The ethanol phase was then collected 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 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.

2.5. Characterization of Fish Oil and Lecithin

The iodine value of oil and lecithin was measured by the method of Hanus [13]. 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 grams of fat or oil. Saponification value was measured by IUPAC [14]. 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 is related to the molecular weight of fat or oil and therefore provides information on the mean molecular weight of the combined fatty acids. The acid value was measured according to the official method of IUPAC [14]. 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 [15]. The peroxide value is defined as the milliequivalent of peroxide oxygen combined in a kilogram of oil.

2.6. Determination of Fatty Acid Composition by Gas Chromatography (GC)

GC analysis was performed to determine the fatty acid compositions of oil and lecithin from boal fish. A Hewlett Packard gas chromatograph (6890 Series II GC system) with a fused silica capillary column (100 m length x 0.25 mm internal diameter, 0.2 µm of film, Supelco, Bellefonte, Pennsylvania, USA) was used. The fatty acid methyl esters were firstly prepared according to the AOCS official method of Ce 2-66 (AOCS) [15]. Nitrogen was used as the carrier gas (1 mL/min) of the fatty acid methyl esters. The oven temperature was programmed according to Uddin [7]. The initial temperature, 130°C was constant for 3 min and then increased to 240°C at a rate of 4°C/min followed by a hold at 240°C for 10 min. Temperature both for injector and detector were 250°C. Fatty acid methyl esters were identified by comparison of retention time and standard fatty acid methyl esters mixtures (Supelco, Pennsylvania, USA).

2.7. Oxidative Stability of Fish Lecithin

To measure the oxidative stability, emulsions of lecithin in water were oxidized at 37°C. 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. Deionized and degassed water were used for emulsion preparation. The mixture was properly homogenized by a homogenizer. 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) and thiobarbituric acid (TBA) 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.

2.7.1. Thiocyanate Method (TC Method)

The oxidative stability of fish lecithins were measured by using the method of Mitsuda [16]. The peroxide formed by lipid peroxidation reacted with ferrous chloride and formed ferric ions. Ferric ions then combined with ammonium thiocyanate and produced ferric thiocyanate. In briefly, 0.1 ml of emulsion solution was added to 4.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Then 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl was added to the reaction mixture. Exactly, 3 min after addition, the absorbance of a red color was measured at 500 nm. The absorbance was recorded at 120 hours' intervals during the incubation.

2.7.2. Thiobarbituric Acid Method (TBA Method)

The oxidative stability of fish lecithins were measured by using the method of Ottolenghi [17]. The extent of lipid peroxidation was evaluated by TBA method. Malonadehyde, the product of lipid breakdown caused by oxidative stress, binds with TBA to form a red complex of thiobarbituric acid reactive substance (TBARS). In 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.

3. Results and Discussion

3.1. Biochemical Composition

The result of the mean percentage of moisture, protein, fat, carbohydrate contents and ash of boal fish are showed in Table 1. Biochemical composition of boal (*Wallago attu*) was determined. It was found that fishes are rich sources of protein and other nutrients. The percentage of moisture were 77.51±0.354 for boal. On the other hand, protein and lipid content of boal was 13.125±1.237 and 1.94±0.049. All the other parameters such as, total sugar and ash were 0.05±0.01 and 1.55±0.05 in boal.

Table 1. The nutrient content of boal fish

Parameters	Boal
Moisture (g %)	77.51± 0.35
Total protein (g %)	13.12±1.24
Total lipid (g %)	1.94± 0.06
Water soluble protein (g %)	1.18±0.08
Glycogen (g %)	0.91±0.01
Total soluble sugar (g %)	0.05±0.01
Non reducing sugar (g %)	0.04±0.01
Reducing sugar (g %)	0.01±0.1
Ash (g %)	1.55±0.05

All data are presented by mean value of 2 replicate ±SD (SD was mentioned at least 0.01)

3.2. Characterization of Oil

The oil yield obtained from boal flesh powder by Soxhlet extraction using n-hexane was approximately 0.18 g g^{-1} fish powder. The lipid content of boal flesh powder was lower than that of marine fishes. Table 2 shows the iodine value, percentage of FFA content, acid value, peroxide value and saponification value of fish oil and lecithin. These parameters provide the quality index of the fish oil and lecithin. The saponification value of boal fish oil was 199.27. The saponification value of Aji-aji fish oil is higher than Menhaden oil which is 259.5±2.0 and 180.9±2.0, respectively. This value indicates that Aji-aji fish oil contains shorter fatty acid chain length with lower molecular weight compared to Manhaden oil [18]. The iodine values of boal fish oil and lecithin were found to be 129.66 and 89.26, respectively. These values were lower as compared to marine fishes but higher to fresh water fishes [19]. Iodine value has been determined to estimate the degree of unsaturation and the relative amounts of unsaturated fatty acids in the triglyceride molecules. The high iodine values of boal fish oil and lecithin indicate the presence of higher amounts of unsaturated fatty acids in the samples. Iodine value gives an estimation of the degree of unsaturation and the relative amounts of unsaturated fatty acids in the triglyceride molecules of the fat. It may be suggested that the oil under investigation contains higher amounts of unsaturated fatty acids as its iodine value was calculated to be 96.17. A higher percentage of free fatty acid (above 1.5%) is a determination or indication of unsuitability of the oil for edible purpose. So the fish oil of *M. vittatus* might be suitable for edible purposes as it contains free fatty acid less than 1.5%.

Table 2. Chemical characteristics of the oils obtained from boal fish

Parameters	Boal fish oil	Boal fish lecithin
Saponification value (mg KOH/g)	199.27±0.78	124.56±1.23
Iodine value (mg I/g oil)	129.67±0.63	89.26±1.44
Acid value (mg KOH/g)	16.78±0.01	11.311±1.519
Peroxide value (meq O_2/Kg)	11.65±0.15	4.263±0.5515
Percent of free fatty acid (%)	8.43±0.09	5.68±0.76
Specific gravity	0.90±0.01	

All data are presented by mean value of 2 replicate ±SD (SD was mentioned at least 0.01)

In this study, 18.24% (w/w) oil was isolated from boal fish powder. The percentage of oil from boal are shown in Figure 1. Iodine value, percentage of FFA content, acid value, peroxide value and saponification value of fish oil are given in Table 2. These parameters provide the quality index of the fish oil and lecithin. The iodine values were found to be 129.67. These values were higher than the values of 83.23 for *Channa marulius* fish oil [20]. The higher degree of unsaturation i.e. the higher iodine value, the greater is the liability of the oil or fat to become rancid by oxidation [21]. The comparatively high saponification values indicate the presence of low proportion of lower fatty acids. The saponification value was found to be 199.27 for boal fish oil. Acid value is the measurement of free fatty acids present in the oils or fats. From investigation, the acid value of boal fish oil was found to be 16.78. The percentage of free fatty acid of boal fish oil calculated from acid value was 8.43. The acid value of 6.72 and percentage of free fatty acid of 3.38 for Tilapia fish oil were reported by Kadir [22]. Peroxide value is the milligram equivalents of peroxide oxygen combined in a kilogram of oil and peroxide value of oil is used as a measurement of rancidity which occurs by autoxidation. The peroxide value of boal fish oil was 11.6. This value was higher than the value of 2.5 for Aji-aji fish oil [19].

3.3. Characterization of Lecithin

The percentage of lecithin from boal are shown in Figure 1. Before and after oil extraction it was found to be 2.02% and 3.68%, respectively. These result showed that, the percentage of lecithin was increased after extracting oil from boal. The amount of lecithin from squid viscera was 4.25% [7]. Iodine value, saponification value, percentage of FFA content, acid value and peroxide value are presented in Table 2. The saponification value of boal fish lecithin was 124.56. 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 triglyceride. Iodine values give an estimation of the amount of unsaturated fatty acids in the triglyceride molecules. Iodine value determines the degree of unsaturation of fat or oil. The iodine values were measured by the Hanus method and were found to be 89.26 for boal. The acid value was used to determine the acidity of the lecithin. Due to the presence of moisture in lecithin, FFA may be liberated by its hydrolytic rancidity. Determination of FFA content therefore provided an index of the quality of the fish lecithin. From investigation, the acid value of the boal fish lecithin was found to be 11.31. The percentage of free fatty acid of boal fish calculated from acid value and was found to be 5.68. Acid value was used to measure the quality index of lecithin. The lower acid value of boal fish lecithin indicated the higher quality of product. The acid value of this fish was much lower than the value of 33.10 for squid viscera [7]. The acid values of food grade lecithin recommended by FAO/WHO are found to be up to 36 mg KOH/g of lecithin [23]. Peroxide value is used for the measurement of rancidity which occurs by auto oxidation. Peroxide value is also used as quality index of lecithin. Peroxide value of boal fish lecithin are shown in Table 2. Peroxide value of boal fish lecithin was found to be 4.26. The peroxide values of food grade lecithin recommended by FAO/WHO are found to be up to 10 milliequivalent/1000 g [23].

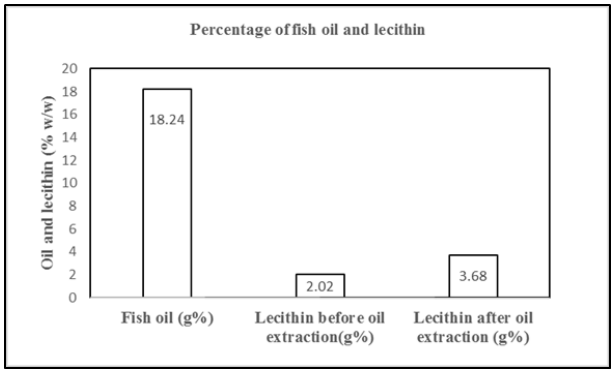

Figure 1. Soxhlet extraction of oil and Isolation of Lecithin before and after oil extraction

Table 3. Fatty acid compositions (percent) of Boal fish oil and lecithin

Fatty acid compositions	Boal fish oil	Boal fish lecithin
	Fatty acid (%)	Fatty acid (%)
Myristic acid (C14:0)	1.96	**N.D**
Myristoleic acid (C14:1)	0.96	13.6
Palmitic acid (C16:0)	23.87	25.9
Palmitoleic acid (C16:1)	6.14	**N.D**
Stearic acid (C18:0)	9.45	14.6
Oleic acid (C18:1)	22.87	13.6
Elaidic acid (18:1)	3.8	10.5
Linoleic acid (C18:2)	10.25	**N.D**
r-Linolenic acid (C18:3)	1.05	**N.D**
Arachidic acid (C20:0)	1.82	**N.D**
cis-11,14-Eicosadienoic acid + Behenic acid (C20:2 + C 22:0)	3.85	**N.D**
Linolenic acid (C18:3)	1.29	**N.D**
Heneicosanoic acid + cis-11,14,17-Eicosatrienoic acid (C21:0 + C20:3)	1.9	**N.D**
Tricosanoic acid (C23:0)	0.72	**N.D**
Arachidonic acid (C20:4)	0.61	**N.D**
cis-8,11,14-Eicosatrienoic acid (C20:3)	1.14	**N.D**
Erucic acid (C22:1)	2.64	12.9
cis-13,16-Docosadienoic acid (C22:2)	1.79	**N.D**
EPA (C20:5)	1.35	**N.D**
Lignoceric acid + Nervonic acid (C24:0 + C24:1)	0.56	**N.D**
DHA (C22:6)	1.98	9

N.D: Not Detected

3.4. Fatty Acid Compositions of Boal Fish Oil and Lecithin

The fatty acid compositions of boal fish oil and lecithin obtained by GC are shown in Table 3. The percentages of the total polyunsaturated fatty acid in oil and lecithin were 19.46% and 9.0%, respectively. In oil the important polyunsaturated fatty acids EPA and DHA were found to be 1.35% and 1.98%, respectively. These values were lower than the value of 7.7% EPA and 14.5% DHA for Salmon fish oil [24]. But lecithin contains only 9% DHA which is lower than 14.5% of squid viscera lecithin [5].

Among the monounsaturated fatty acids, C18:1 was present in higher amounts in oil and lecithin. The most significant saturated fatty acids were C16:0 (23.87%) and (25.90%) in oil and lecithin, respectively.

3.5. Oxidative Stability of Boal Fish Lecithin

The term oxidative stability refers to 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 stability of boal fish lecithin are shown in Figure 2A-B. In this study, the oxidation trend was evaluated instead of determining the absolute state of oxidation of the incubated sample. Lecithin with linoleic acid emulsions showed the increase in absorbance value from the first day. The increase in absorbance value was an indicator of auto-oxidation by formation of peroxides during incubation. Only the fish lecithin emulsion showed low absorbance values indicating low levels of lipid peroxidation until 15 days. The fish lecithin showed significantly increased oxidation after 20 days. In contrast, fish lecithin emulsions with β-carotene showed high oxidative stability. β-carotene, a strong antioxidant inhibited the peroxide formation of the lipids by peroxidation over a certain period. Initially, fish lecithin emulsion showed slightly higher absorbance as compared to lecithin within the linoleic acid emulsion. This might be due to the presence of peroxide from the oxidation of neutral lipids of fish lecithin. In thiobarbituric acid method, the absorbance measured on the 0, 5, 10, 15th day was also similar to the lecithin and lecithin with β-carotene emulsions. However, this value was also high in the lecithin with linoleic acid emulsion indicating a low oxidative stability. On the other hand, a significant increase in absorbance was found on the 20th day of the lecithin emulsion sample. However, fish lecithin showed high oxidative stability. Lecithin from fish may contain small amounts of natural antioxidants that might be one of the causes of its higher oxidative stability [5]. [25] Gogolewski also reported that long chain polyunsaturated fatty acids which 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 [11,26,27].

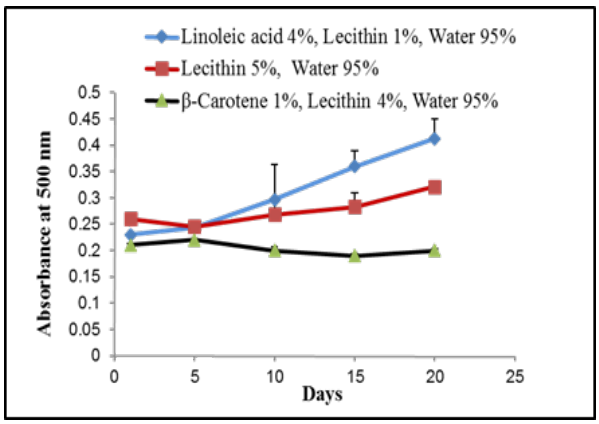

Figure 2-A. Measurement of oxidative stability of boal fish lecithin by thiocyanate (TC) method and All data are presented by mean value of 3 replicate ±SD (SD was mentioned at least 0.01)

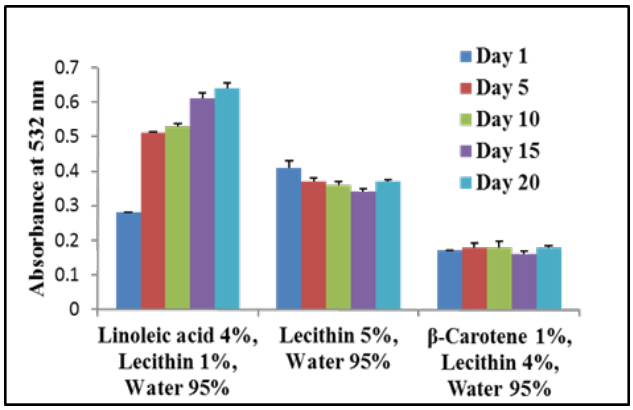

Figure 2-B. Measurement of oxidative stability of boal fish lecithin by thiobarbituric acid (TBA) method and All data are presented by mean value of 3 replicate ±SD (SD was mentioned at least 0.01).

4. Conclusions

In this study, oil and lecithin was isolated from boal fish powder and characterized by measuring the iodine value, saponification value, FFA content, acid value, and peroxide value. The importance of lecithin analysis is to find the functional properties and fatty acid composition due proper utilization of lecithin. Fatty acid composition of oil and lecithin was also measured by gas chromatography. Boal fish oil contains relatively high in monounsaturated fatty acids and an average in polyunsaturated fatty acid especially EPA and DHA. But EPA was not found in lecithin. The oxidative stability of oil and lecithin was also high due to the presence of natural antioxidant. Therefore, it can be concluded that boal fish oil has the potential to be used as a local source of PUFA due to its comparatively high content of EPA and DHA.

Acknowledgements

The authors would like to thanks to the Laboratory of Advanced Processing Technology, Pukyong National University, Busan, South Korea.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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