INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 6, p. 92-102, 2015

RESEARCH PAPER OPEN ACCESS

Extraction, biochemical analysis and characterization of oil and lecithin from two selected fish species

Sayad Md. Didarul Alam[1*], Md. Selim Uddin[1], Sohel Hasan[1]

Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh

Key words: PUFA (Poly unsaturated fatty acid); FFA (Free fatty acid); EPA (Eicosapentaenoic acid); DHA (Docosahexaenoic acid).

http://dx.doi.org/10.12692/ijb/7.6.92-105 Article published on December 24, 2015

Abstract

Nutrients from Fishes have beneficiary effects on health. To estimate and compare the nutritional status, biochemical compositions of two fish species, Ayre (*Sperata aor*) and Kani pabda (*Ompok bimaculatus*), were determined. Kani pabda fish contained higher amount of protein (15.295%) than Ayre fish (13.119%) whereas, lipid content of Ayre was higher (1.90%) than that of Kani pabda (1.31%). All other parameters like moisture, ash and total sugar content were investigated and found to be high in Ayre compared to Kani pabda. The percentage of oil from Ayre and Kani pabda fish powder were 18.22 and 12.59, respectively. The acid value, percentage of FFAs, iodine value and peroxide value of Ayre fish oil were found to be 16.59 (mg KOH/g), 8.342%, 129.337 (mg I/g oil) and 11.55 (meq O_2/1000g), respectively, higher than those of Kani pabda fish oil. Similar parameters have also been studied for lecithins extracted from both species having higher oxidative stability due to the presence of natural antioxidants. Consumption of these fish species can prevent malnutrition diseases being a part of proper balanced diet and as a rich source of micronutrients. Furthermore, oil and lecithin isolated from the fish powder provide unsaturated fatty acid molecules that can become useful in food and pharmaceutical industries.

* **Corresponding Author:** Sayad Md. Didarul Alam ✉ didarulalam06@gmail.com

Introduction

Fishes provide about 60% of animal protein in the diet of common Bangladeshi people (Belton *et al*., 2011; Roos *et al*., 2007). A number of regional studies have confirmed the significance of fish items in a Bangladeshi diet (Minkin *et al*., 1997; Hels *et al*., 2002). Fish oil plays vital role in remediation of many diseases and is a protective mean of various types of abnormalities such as heart diseases, diabetes mellitus, atherosclerosis, cancers, inflammation, hypertension, obesity, rheumatoid arthritis, osteoporosis and schizophrenia. In addition, important Omega-3 fatty acids, most notably eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oil can also combat some diseases such as asthma, multiple sclerosis and systemic lupus erythematosus (Iso *et al*., 2001; Nagakura *et al*., 2000). Emerging evidences from epidemiological and experimental studies indicate a relationship between dietary fat and the risk of cancer (Wu *et al*., 2005; Hong *et al*., 2005; Suzuki *et al*., 2004; Maillard *et al*., 2002). Fish oils are enriched with polyunsaturated fatty acids (PUFAs) of the ω3 family and tend to diminish the cholesterol level in blood. Several investigators have demonstrated that diets enriched with fish oil can reduce the growth rates of implanted tumors *in vivo* (Han *et al*., 2009). Supplementation of fish oil may prevent development of heart failure through alterations in cardiac phospholipids that favorably impact inflammation and energy metabolism (Shah *et al*., 2009).

Nowadays, effects of different bioactive compounds (polyunsaturated fatty acids, phospholipids, lecithin and pigments) from various fish species on health are being intensively studied. Bioactive peptides from various fish protein hydrolysates have shown numerous bioactivities like antihypertensive, antithrombotic, immune-modulatory and antioxidative activities. It has been reported that some peptides derived from fish showed antihypertensive activity to inhibit the action of angiotensin I-converting enzyme (ACE) and were found to be even stronger than many natural peptides (Kim *et al*., 2000). Collagen and gelatin from fish are currently used in diverse fields including food, cosmetics, and biomedical industries (Kim *et al*., 2006).

Pharmacological use of lecithin is included in treatments for hypercholesterolemia, neurologic disorders and liver ailments. Lecithin has also been used to modify the immune system by activating specific and nonspecific defense systems (Uddin *et al*., 2011). Recent studies suggest that a lecithin-enriched diet can modify the cholesterol homeostasis and lipoprotein metabolism (Amouni *et al*., 2010). 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 (LeBlanc *et al*., 2003).

Generating evidence on biochemical composition of fish is vital as this will provide information on precise nutrient abundance of particular species and will help the nutritionists and dieticians in issuing 'dietary guidelines' for societal benefit. In the present study, oil and lecithin were extracted and characterized from two fish species, *Sperata aor* and *Ompok bimaculatus,* commonly known as Ayre and Kani pabda, respectively. Several investigations have been done on the effects of consumption of nutrient dense fresh water fishes (Kongsbak *et al.,* 2008; Thilsted *et al.,* 1997) and it is very important to find out the links between human nutrition and fisheries (Roos *et al*., 2007). Proximate composition analyses of *Sperata seenghalaa* (belongs to the same genus with *Sperata aor*) and *Ompok bimaculatus* are already reported (Mohanty *et al*., 2011; Hei and Sarojnalini *et al*., 2012). But due to physiological, geographical and ecological factors, these values vary widely. Therefore, the assessment of different nutritional parameters in the selected fish species was taken into account in our research.

Materials and methods

Sample collection and preparation

Due to their availability throughout the country in all seasons, Ayre and Kani pabda fishes were collected from the local market in Rajshahi. After cleaning, bones were removed from fishes, and the flesh was packed in polyethylene bags to store in a refrigerator (at 4°C) for experimental purposes.

Biochemical analysis of fish flesh

Moisture content was determined by the conventional procedure (ICOMR, 1971) while ash content was determined by the AOAC method (AOAC, 1980). Anthrone method was used to measure the sugar content (Jayaraman, 1981). Reducing sugar content was quantified by dinitrosalicylic acid method (Miller, 1972; AOAC, 2000). Non-reducing sugar was also determined in accordance to the method given in AOAC (2000). Anthrone method was used to measure the glycogen content of fish flesh (Clegg, 1956). Total protein and water-soluble protein contents of fishes were determined by the micro-kjeldahl method (AOAC, 1995) and Lowry method (Lowry *et al.*, 1951), respectively. Finally, lipid content of Ayre and Kani pabda fishes were determined by the method of Bligh and Dyer (Bligh and Dyer *et al.*, 1959).

Extraction of oil from Ayre and Kani pabda fishes

The stored fish samples were sun-dried for about 72 hours and grinded by mechanical grinder. Oil was extracted from both fishes by Soxhlet extraction apparatus using n-hexane and stored at 4°C for further analysis (Bahl *et al.,* 2001). Parameters like specific gravity, iodine value, acid value, percentage of free fatty acid, peroxide value, saponification value and saponification equivalent of fish oil were determined by using Hanus *et al.*, (1996), IUPAC (1977), AOCS (1998) and conventional procedure (IUPAC, 1977). Saponification equivalent was calculated from the saponification value. The percentage of free fatty acid was calculated from the acid value. The amount of unsaponifiable matters present in the oil was also determined (Jayaraman, 1981).

Extraction of Lecithin

The stored fish samples obtained by mechanical grinding were used for lecithin extraction according to the method of Palacios *et al.* (2005), modified by Uddin *et al.* (2011). 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 containing mainly polar lipids with very small amounts of neutral lipids was collected using a separator funnel. The precipitate was again extracted with 100 ml of ethanol and after centrifugation and the supernatant was added to that ethanol extract. Twice volume of hexane was mixed with the ethanol extract to separate the neutral lipids from the polar lipids. The ethanol phase was evaporated at 40°C. The remaining lipid residue was dissolved in hexane. Volume of this hexane solution was measured and five times the volume of chilled acetone (4°C) was added to it (with slow-stirring) to precipitate the gummy material. The mixture was placed in an ice bath for 15 min and then centrifuged at 5000 rpm for 10 min. After discarding supernatant, the collected precipitate (fish lecithin) was stored at −20° C until further analysis.

The iodine value of Ayre and Kani pabda fish lecithin was measured by the method of Hanus *et al.* (1996). The saponification value and saponification equivalent of Ayre and Kani pabda fish lecithin were determined according to the method of IUPAC (1977). The acid value of Ayre and Kani pabda fish lecithin was also determined using IUPAC (1977) method. AOCS (1998) method was used to determine the peroxide value of Ayre and Kani pabda fish lecithin.

Measurement of oxidative stability of Ayre and Kani pabda fish lecithin

To measure the oxidative stability, emulsions of lecithin in water were oxidized at 37°C. 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. Linoleic acid and standard β-carotene

were used to measure the oxidative stability of fish lecithin. The mixture was properly homogenized using a homogenizer. Oxidative stabilities were checked by the thiocyanate (TC) (Mitsuda *et al.*, 1966) and thiobarbituric acid (TBA) methods (Salih *et al.*, 1987; Pikul *et al., 1989*), which were used to measure the antioxidant activity. In this study, these two methods were conducted to measure the quality of the extracted lecithin in terms of its oxidative stability.

Statistical analysis

All the experiments were carried out in triplicates and the result was presented as mean ± S.E.

Results and discussion

Biochemical analysis

Moisture plays an important role in the metabolism and growth of plants and animals and contributes in most of the physiological reactions in plant and animal tissues. Ayre and Kani pabda fish were observed to contain 77.45% and 74.56% of moisture which was in line with the value obtained for *Sperata seenghalaa* (79.40±0.09%) (Mohanty *et al.*, 2011). Most of the inorganic constituents or minerals were present in ash which was found to be 1.52% and 1.15%, in Ayre and Kani pabda, respectively. Ayre and Kani pabda fish contained very small amount of lipids with a total lipid content of 1.90% and 1.31%, respectively (Table-1). In *Sperata seenghalaa*, the ash content and amount of lipid were recorded as 0.90±0.08% and 1.40±0.79% (Mohanty *et al.*, 2011).

The amount of total protein in Ayre and Kani pabda was 13.119% and 15.295%, respectively**.** These values can be considered low comparing to the previous results (20.06±1.13% and 30.51±2.19%, respectively) (Mohanty *et al.*, 2011; Hei *et al.*, 2012). The results also displayed 1.178% and 1.525%, of total water soluble protein in both species (Table-1).

Table 1. Nutrient content of Ayre and Kani pabda fish.

Parameters (%)	Species	
	Ayre	Kani pabda
Moisture	77.45±0.352	74.56±0.648
Ash	1.52±0.047	1.15±0.050
Total soluble sugar	0.043±0.001	0.033±0.001
Reducing sugar	0.0018±0.001	0.0015±0.001
Non reducing sugar	0.045±0.001	0.036±0.01
Glycogen	0.910±0.014	1.104±0.01
Total protein	13.119±1.229	15.295±0.609
Water soluble protein	1.178±0.083	1.525±0.056
Total lipid	1.90± 0.047	1.31±0.029

All experiments were carried out in triplicates and the result was presented as mean ± S.E.

The sugar content in Ayre and Kani pabda was found to be 0.043% and 0.033% respectively. Additionally, 0.0018% and 0.0015% of reducing sugar were present in Ayre and Kani pabda, respectively. Glycogen content was determined to be 0.910% and 1.104% in Ayre and Kani pabda. Table-1 showed that the quantity of non-reducing sugar of Ayre and Kani pabda as 0.045% and 0.036%, respectively.

Extraction of oil from Ayre and Kani pabda fishes

The oil obtained by soxhlet extraction from Ayre and Kani pabda was found to be 18.22% and 12.59%, respectively (Fig.1A). Specific gravity of fats and oils does not vary as a general rule to an extent, which makes this property useful to discriminate one from the other. Specific gravity of oils were found to be 0.902 and 0.877 for Ayre and Kani pabda, respectively (Table-2). Iodine values give an estimation of the amount of unsaturated fatty acids in the triglyceride molecules of fat and oil and it was observed that the Iodine value of Ayre fish oil (129.337 mg I/g oil) was higher than that of Kani pabda fish oil (110.002 mg I/g oil) (Table-2). Therefore, the Ayre fish oil had higher tendency to

become rancid by oxidation than the Kani pabda fish oil. On the other hand, Ayre fish oil may have tremendous health benefit due to the presence of higher unsaturated fatty acids. Saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fat or oil. The comparatively high saponification values indicate the presence of low proportion of lower fatty acids. Saponification values were found to be 198.995 (mg KOH/g) and 145.943 (mg KOH/g) for Ayre and Kani pabda fish oil respectively, whereas the saponification equivalents were found to be high in Kani pabda compared to Ayre (Table-2). These results also indicated that Ayre and Kani pabda fish oil contained high proportion of higher chain fatty acids. Acid value is the measurement of free fatty acids present in the oils or fats. Acid value of Ayre fish oil was found to be 16.59 (mg KOH/g). The percentage of free fatty acid of Ayre fish oil calculated from acid value was found to be 8.342. On the other hand, the acid value of Kani pabda oil was found to be 13.85 (mg KOH/g). The percentage of free fatty acid was found to be 7.00 for Kani pabda fish oil (Table-2).

Table 2. Chemical characteristics of oils extracted from Ayre and Kani pabda fish.

Parameters	Species	
	Ayre	Kani pabda
Specific gravity	0.902±0.003	0.877±0.004
Iodine value (mg I/g oil)	129.337±0.615	110.002±0.179
Acid value (mg KOH/g)	16.59±0.01	13.85±0.04
Percent of free fatty acid (%)	8.342±0.093	7.00±0.526
Peroxide value (meq O_2/1000g)	11.55±0.15	7.87±0.01
Saponification value (mg KOH/g)	198.995±0.780	145.943±0.880
Saponification equivalent	280.518±1.100	380.719±2.179
Unsaponifiable matter (%)	9.452±0.483	6.101±0.230

All experiments were carried out in triplicates and the result was presented as mean ± S.E.

Acid value and percentage of free fatty acid of Ayre fish oil were higher than that of Kani pabda fish oil. Peroxide value of oil is used as a measurement of rancidity which occurs by auto-oxidation. It was interesting that the peroxide value of Ayre fish oil was significantly higher than that of Kani pabda fish oil (Table-2). This result was in line with the higher iodine value of Ayre fish oil, because high unsaturated fatty acids have a greater liability for the rancidity of oil. The unsaponifiable matter in Ayre fish oil was 9.452%; whereas in Kani pabda fish oil it was 6.101%. The oil from Ayre fish contained little higher amounts of unsaponifiable matter (Table-2).

Extraction of lecithin

The percentage of lecithin from Ayre and Kani pabda are shown in Fig.1B. Before oil extraction it was found that the amount of lecithin from Ayre and Kani pabda were 2.015% and 2.07%, respectively. On the other hand, after oil extraction, amounts of lecithin were 3.675% and 3.1%, respectively. These results showed that, the percentage of lecithin was increased after extracting the oil from both fish species. Iodine value gives an estimation of the amount of unsaturated fatty acids in the triglyceride molecules. The iodine value of Ayre fish lecithin was higher compared to Kani pabda fish lecithin (Table-3).

Table 3. Characterization of fish lecithins from Ayre and Kani pabda.

Parameters	Species	
	Ayre	Kani pabda
Iodine value (mg I/g oil)	90.021±1.450	76.894±1.466
Acid value (mg KOH/g)	11.311±1.519	7.901±0.907
Peroxide value (meq O_2/1000 g)	4.263±0.5515	2.563±0.621
Saponification value(mg KOH/g)	124.564±1.226	108.043±1.964
Saponification equivalent	450.390±4.435	519.131±9.712
Percent of free fatty acid (%)	5.684±0.763	4.292±0.456

All experiments were carried out in triplicates and the result was presented as mean ± S.E.

So, the Ayre fish lecithin had higher tendency to become rancid by oxidation than the Kani pabda fish lecithin.

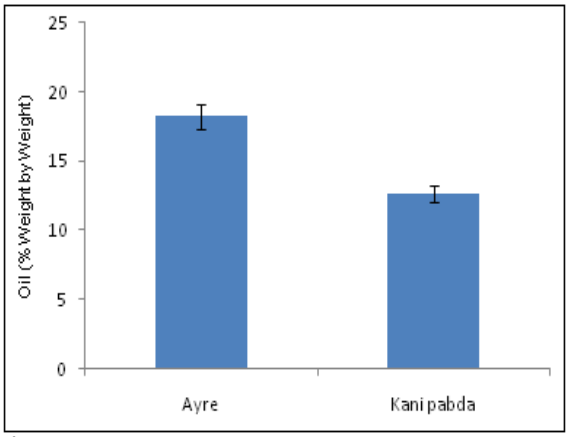

Fig. 1A. Percentages of oil extracted from Ayre and Kani pabda.

The saponification value of Ayre and Kani pabda fish lecithin were 124.564 (mg KOH/g) and 108.043 (mg KOH/g), respectively (Table-3). Whereas the saponification equivalents were calculated from saponification value and found to be 450.390 for Ayre and 519.131 for Kani pabda fish lecithin, respectively. The saponification value of Ayre fish lecithin was higher than that of Kani pabda fish lecithin.

When lecithin rancidify, triglycerides are converted into fatty acids and glycerol, causing an increase in acid number. 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. Acid value of Ayre and Kani pabda fish lecithin are presented in Table-3. The percentage of free fatty acid of Ayre fish lecithin calculated from acid value was found to be 5.684. On the other hand, the percentage of free fatty acid was found to be 4.292 for Kani pabda fish lecithin. Acid value was used to measure the quality index of lecithin. The lower acid value of Ayre and Kani pabda fish lecithin indicated the higher quality of product.

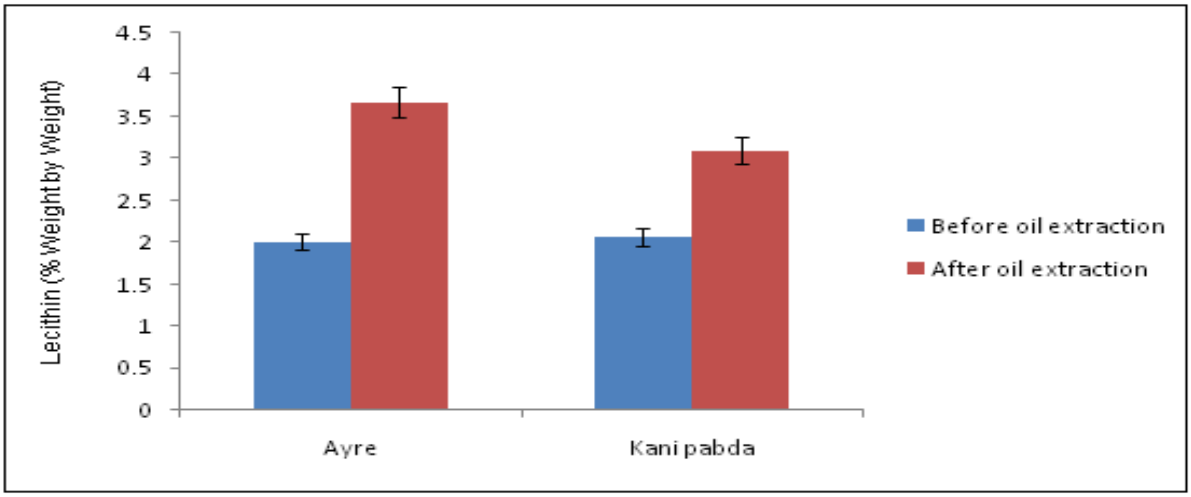

Fig. 1B. Percentages of lecithin extracted from Ayre and Kani pabda.

Peroxide value is also used as a quality index of lecithin. Peroxide value of Ayre and Kani pabda fish lecithin were found to be 4.263 (meq O_2/1000 g) and 2.563 (meq O_2/1000 g), respectively (Table-3). The peroxide values of food grade lecithin recommended by FAO/WHO are found to be less than 10 meq O_2/1000 g (Nieuwenhuyzen *et al.*, 2008). Ayre fish lecithin showed higher peroxide value as compared to Kani pabda fish lecithin. This result agreed with higher iodine value of Ayre fish lecithin because high saturated fatty acids have a higher tendency to become rancid.

Oxidative stability of Ayre and Kani pabda fish lecithin

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 Ayre and Kani pabda fish lecithin are shown in Fig.

2A-2B and Fig. 3A-3B. 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 the 15th day.

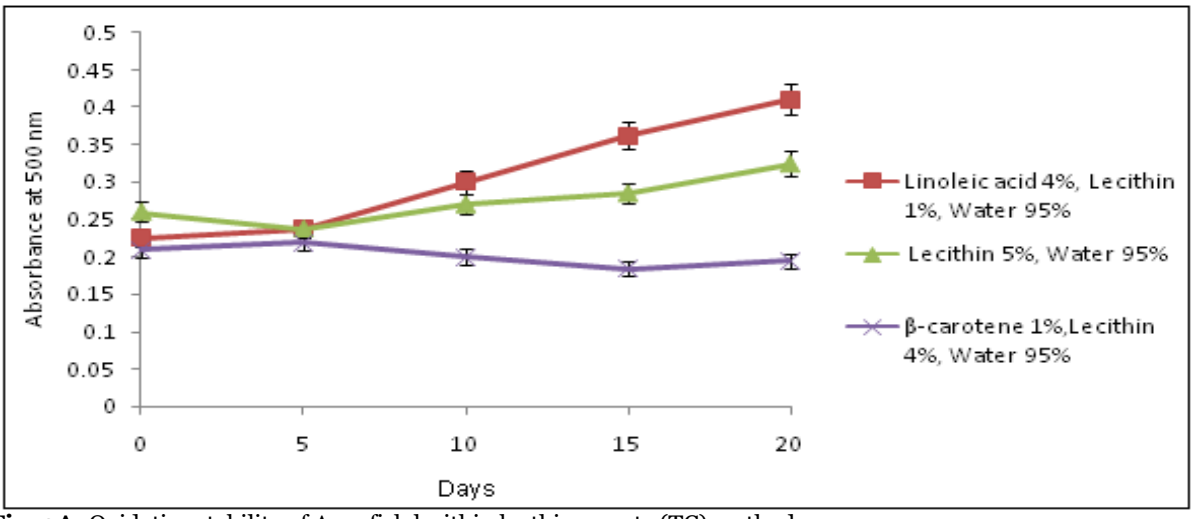

Fig. 2A. Oxidative stability of Ayre fish lecithin by thiocyanate (TC) method.

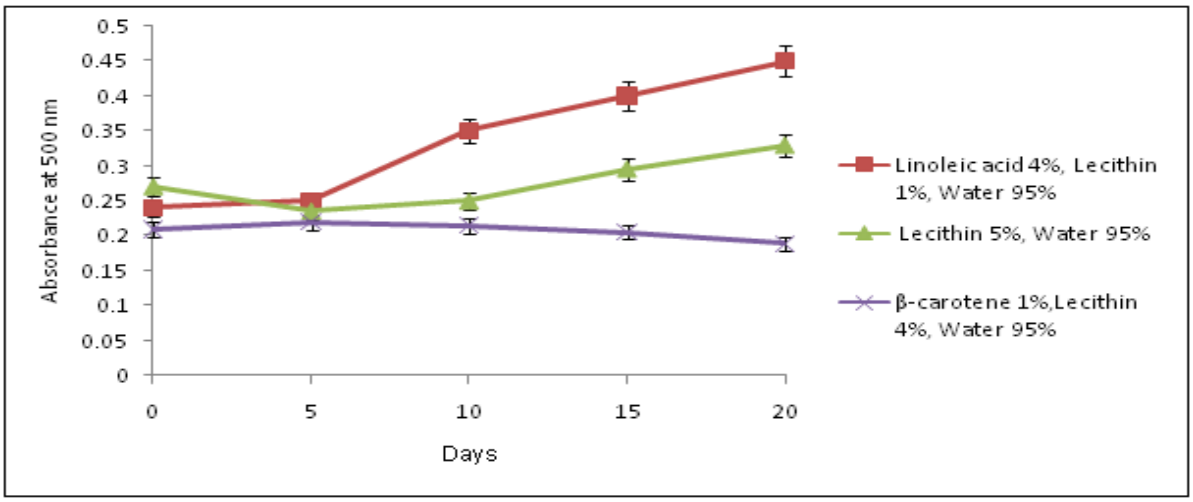

Fig. 2B. Oxidative stability of Kani pabda fish lecithin by thiocyanate (TC) method.

The fish lecithin showed significantly increased oxidation after 20 days. 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 and 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 (Uddin *et al.*, 2011). Gogolewski *et al.* (2000) also reported that long chain polyunsaturated fatty acids (esterified with polar lipids) had synergistic effects with antioxidants.

In overall, considerable variations were found in the biochemical compositions of Ayre and Kani pabda fish. Protein and lipid content of both fishes were high. The iodine value of Ayre was higher than that of Kani pabda, which indicated that the Ayre fish oil contained more unsaturated fatty acid than Kani pabda fish oil.

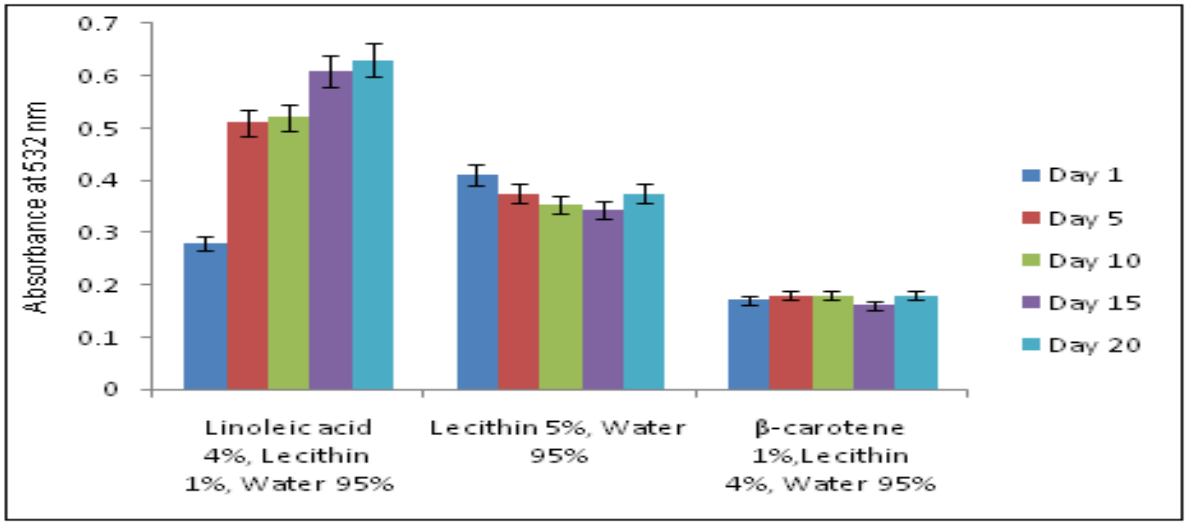

Fig. 3A. Oxidative stability of Ayre fish lecithin by thiobarbituric acid (TBA) method.

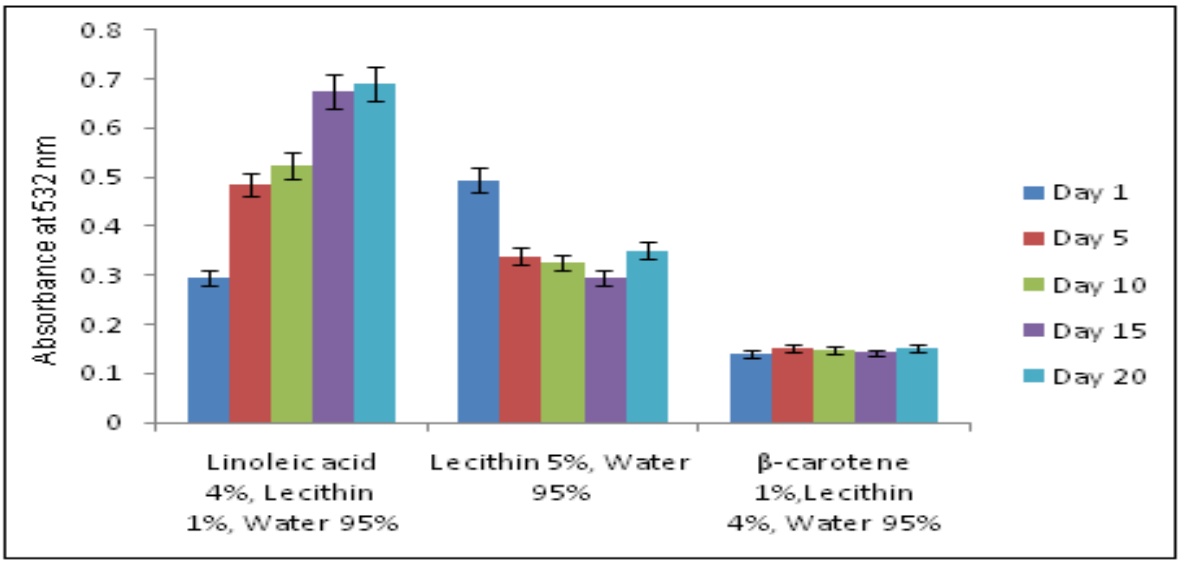

Fig. 3B. Oxidative stability of Kani pabda fish lecithin by thiobarbituric acid (TBA) method.

Higher oxidative stability was found in lecithin from both Ayre and Kani pabda fishes. Therefore, it can be concluded that fish oil and lecithin isolated from these fish species provide unsaturated fatty acids that can be useful in food industries as well as in pharmaceutical industries.

Acknowledgement

The authors are very thankful to National Institute of Science and Technology, Ministry of Science and Technology, Government of the People's Republic of Bangladesh for financial assistance.

References

Amouni MM, Eder de CP, Priscila GM, Maricene S, Patricia M. 2010. Influence of Soy Lecithin Administration on Hypercholesterolemia. Hindawi Publishing Corporation, Cholesterol **2010**, Article ID 824813, 4 pages. http://dx.doi.org/10.1155/2010/824813

AOAC. 1980. Official methods of analysis, 13th Edition. Association of official analytical chemists, Washington DC.

AOAC. 1995. Official methods of analysis, 16th Edition. Association of official analytical chemists, Washington DC.

AOAC. 2000. Official methods of analysis, 17th Edition. Association of official analytical chemists, Washington DC.

AOCS. 1998. Official Methods and Recommended Practices of the American Oil Chemists' Society. American Oil Chemists' Society, Champaign, III.

Bahl BS, Bahl A. 2001. In: Advanced Organic Chemistry, S. Chand and. Co. Ltd. India.

Belton B, Karim M, Thilsted S, Jahan KM, Collis W, Phillips M. 2011. Review of Aquaculture and Fish Consumption in Bangladesh. Studies and Reviews **76,** 2011-53 p, The World Fish Center, Penang,

Bligh EG, Dyer W. 1989. Total Lipid Extraction and Purification, can. Jour. Biochem. Physiol **37,** 911-7.

Clegg KM. 1956. The application of the anthrone reagent to the estimation of starch in cereals. J. Sci. Food Agric. **7,** 40-44.

Gogolewski M, Nogala-Kalucka M, Szeliga M. 2000. Changes of the tocopherol and fatty acid contents in rape seed oil during refining. Eur J Lipid Sci Technol **102,** 618–23. http://dx.doi.org/10.1002/1438-9312(200010)102:10<618::AID-EJLT618>3.0.CO;2-6

Han J, Ma I, Hendzel M, Allalunis-Turner J. 2009. The cytotoxicity of gamma-secretase inhibitor I to breast cancer cells is mediated by proteasome inhibition, not by gamma-secretase inhibition. Breast Cancer Res **11(4),** R57. http://dx.doi.org/10.1186/bcr2347

Hanus Method. 1996. AOAC 920. 158 (ISO 3961:1996 Animal and vegetable fats and oils--Determination of iodine value.

Hei A, Sarojnalini C. 2012. Proximate Composition, Macro and Micro Mineral Elements of Some Smoke-dried Hill Stream Fishes from Manipur, India. Nat. Sci. **10(1),** 59-65.

Hels O, Hassan N, Tetens H, Thilsted SH. 2002. Food consumption, energy and nutrient intake and nutritional status in rural Bangladesh: changes from 1981–82 to 1995–96. Eur. J. Clin. Nutr. **57,** 586–594.

Hong MY, Bancroft LK, Turner ND, Davidson LA, Murphy ME, Carroll RJ, Chapkin RS, Lupton JR. 2005. Fish oil decreases oxidative DNA damage by enhancing apoptosis in rat colon. Nutn Cancer **52(2),** 166-175.

Hsieh TCY, Williams SS, Vejaphan W, Mayers SP. 1988. Characterization of volatile components of menhaden fish oil. Journal of American Oil Chemistry Society **66,** 114-117.

ICOMR. 1971. A Manual of Laboratory Techniques. Indian Council for Medical Research. National Institute of Nutrition, India, 2-6 p.

Iso H, Rexrode KM, Stampfer MJ. 2001. Intake of fish and omega-3 fatty acids and risk of stroke in women. JAMA **285,** 304-312.

IUPAC. 1977. Standard methods for the analysis of oils, Fats and Derivatives. 5th Edition. Pergamon Press, Method-11.D.3., 56-60 p.& Method-2. D.7, **69** (1976/1977).

Jayaraman J. 1981. Laboratory manual in Biochemistry (1st Edition). Wiley Eastern Ltd. New Delhi, India.

Keyur BS, Monika K, Duda KM, O'Shea GC, Sparagna DJ, Chess RJ, Khairallah, Isabelle

RF, Xu W, Robert C, Murphy, Rosiers CD, William CS. 2009. The cardio protective effects of fish oil during pressure overload are blocked by high fat intake. Hypertension. **54(3),** 605–611. http://dx.doi.org/10.1161/HYPERTENSIONAHA.109.135806

Kim TK, Choi BI, Han JK, Hong HS, Park SH, Moon SG. 2000. Hepatic tumours: contrast agent-enhancement patterns with pulse inversion harmonic US. Radiology **216(2),** 411-417.

Kim SE, Mendis E. 2006. Bioactive compounds from marine processing byproducts- A review. Food Research International **39,** 383-393. http://dx.doi.org/10.1016/j.foodres.2005.10.010

Kongsbak K, Thilsted SH, Wahed MA. 2008. Effect of consumption of the nutrient-dense, freshwater small fish *Amblypharyngodon mola* on biochemical indicators of vitamin A status in Bangladeshi children: a randomized, controlled study of efficacy. Br J Nutr **99(3),** 581–597 http://dx.doi.org/10.1017/S000711450781912X

Lowry OH, Rose brough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin-phenol reagent. J. Biol.chem. **193,** 265-275.

Maillard V, Bougnoux P, Ferrari P, Jourdan ML, Pinault M, Lavillonniere F, Body G, Floch OL, Chajes V. 2002. N-3 and N-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. Int. J Cancer, **98,** 78-83.

Miller GL. 1972. Use of dinitrosalicylic acid reagent for the determination of glucose. Amal. Chem **31,** 426-428.

Minkin SF, Rahman MM, Halder S. 1997. Fish biodiversity, human nutrition and environmental restoration in Bangladesh. In: Open water Fisheries of Bangladesh (Tsai, C. & Ali, M. Y., eds.), 75–88 p. The University Press Limited, Dhaka, Bangladesh.

Mitsuda H, Yasumoto K, Iwami K. 1966. Antioxidative action of indole compounds during the autoxidation of linoleic acid. Eiyo to Shokuryo**19,** 210–4. http://dx.doi.org/10.4327/jsnfs1949.19.210

Mohanty BP, Behera BK, Sharma AP. 2011. Nutritional significance of small indigenous fishes in human health. Central Inland Fisheries Research Institute, Kolkata, India. Bulletin No. **162.** ISSN 0970-616X.

LeBlanc MJ, Brunet S, Bouchard G, Lamireau T, Yousef IM, Gavino V, Levy E, Tuchweber B. 2003. "Effects of dietary soybean lecithin on plasma lipid transport and hepatic cholesterol metabolism in rats," Journal of Nutritional Biochemistry **14(1),** 40–48 p. http://dx.doi.org/10.1016/S0955-2863(02)00253-X

Nagakura T, Matsuda S, Shichijyo H, Hata K. 2000. Dietary supplementation with fish oil rich in omega-3 polyunsaturated fatty acids in children with bronchial asthma. Eur Respir J **16,** 861-865.

Nieuwenhuyzen WV, Tomas MC. 2008. Update on vegetable lecithin and phospholipid technologies. Eur J Lipid Science &Technology **110,** 472–86. http://dx.doi.org/10.1002/ejlt.200800041

Pikul J, Leszczynski DE, Kummerow FA. 1989. Evaluation of 3 modified tba methods for measuring lipid oxidation in chicken meat. Journal of Agricultural and Food Chemistry **37(5),** 1309-1313.

Salih AM, Smith DM, Price JF, Dawson LE. 1987. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poultry Science **66(9),** 1483-1488.

Palacios LE, Wang T. 2005. Egg-yolk lipid fractionation and lecithin characterization. J Am Oil Chem Soc **82,** 571–8. http://dx.doi.org/10.1007/s11746-005-1111-4

Roos N, Wahab MA, Hossain MAR, Thilsted SH. 2007. Linking human nutrition and fisheries: incorporating micronutrient dense, small indigenous fish species in carp poly culture production in Bangladesh. Food and Nutrition Bulletin **28(2),** p. S280–S293 (Suppl.)
http://dx.doi.org/10.1177/15648265070282S207

Suzuki S, Akechi T, Kobayashi M, Taniguchi K, Goto K, Sasaki S, Tsugane S, Nishiwaki Y, Miyaoka H, Uchitomi Y. 2004. Daily omega-3 fatty acids intake and depression in Japanese patients with newly diagnosed lung cancer. Brit J Cancer **90,** 787-793.

Thilsted SH, Roos N, Hassan N. 1997. The role of small indigenous fish species in food and nutrition security in Bangladesh. Naga, the ICLARM Quarterly, **20(3-4),** 82-84, 102.

Uddin MS, Kishimura H, Chun BS. 2011. Isolation and Characterization of Lecithin from Squid (*Todarodes pacificus*) Viscera Deoiled by Supercritical Carbon Dioxide Extraction.Journal of Food Science **76(2),** C350-354.
http://dx.doi.org/10.1111/j.1750-3841.2010.02039.x

Wu M, Harvey KA, Ruzmetov N, Welch ZR, Sech L, Jackson K, Stillwell W, Zaloga GP, Siddiqui RA. 2005. Omega-3 polyunsaturated fatty acids attenuate breast cancer growth through activation of a neutral sphingomylinase-mediated pathway. International Journal of Cancer, **117,** 340-348.