http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (6): 810-815, 2005
ISSN 1028-8880

A Comparative Analysis on Physico-chemical Characteristics of Oil Extracted from Six Different Parts of Hilsa fish (*Hilsa ilisha*)

[1]K.A. Salam, [2]A.K.M. Motahar Hossain, [2]A.H.M. Khurshid Alam, [1]F. Pervin and [1]N. Absar
[1]Department of Biochemistry and Molecular Biology,
[2]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: The physico-chemical characteristics of oils extracted from six different parts of hilsa fish (*Hilsa ilisha*) like dorsal, ventral, tail, egg, liver and brain were analyzed. The physical characteristics such as the specific gravity, refractive index, smoke point, flash point, fire point, cloud point, solidification point and pour point of the hilsa fish oils from the different parts presently examined were ranged from 0.920 to 0.932, 1.4700 to 1.4722 at 25°C, 220 to 228°C, 322 to 330°C, 350 to 364°C, 2 to 2.4°C, -10 to -5°C and -7 to 6°C, respectively. The chemical properties such as saponification value, iodine value, peroxide value, acid value, % FFAs and unsaponifiable matters of the hilsa fish oils from different parts were found to be varied from 180.28 to 194.00, 80.70 to 126.40, 7 to 10, 4.16 to 12.00, 2.08 to 6.00 and 1.58 to 7.00%, respectively. The saturated and unsaturated fatty acids present in the oil samples were mainly myristic acid (5.44 to 7.24%), palmitic acid (22.00 to 27.08%), stearic acid (4.00 to 6.32%), palmitolenic acid (12 to 14%), oleic acid (26.08 to 29.78%), linoleic acid (0.92 to 2.20%) and linolenic acid (0.82 to 1.08%). The storage effect on the hilsa fish oils, which were obtained from different parts for the production of fatty acids by the action of lipase have been studied after storing the samples at low temperature (-10 to 0°C) and at room temperature (25 to 28°C). The contents of % FFA were initially low but increased rapidly on storage. It has been shown that lipase enzyme in hilsa fish oil is active even at temperature -10°C. The hydrolytic deterioration of hilsa fish oils were found to be more effective at 0°C than that from -10°C. Further, the qualities of hilsa fish oils were deteriorated slightly further when stored at 25°C.

Key words: Physico-chemical characteristics, fatty acids, storage effects, hydrolytic deterioration

INTRODUCTION

A large number of populations in our country have been suffering from malnutrition. There are many kinds of rivers in our country and all the rivers have a lot of fishes, which are good sources of nutrients specially proteins. For the ignorance of people, they do not know the nutritive value of different kinds of fishes. Hilsa fishes (*Hilsa ilisha*) are one of them.

Fish are highly perishable and spoilage sets in soon after their landing. In the tropical climate condition, deterioration is especially rapid due to the presence of the activities of lipase. The marketing of hilsa fish thus becomes race against time to bring the fish to the consumers before the quality is reduced below on acceptable level. Fatty acids present in the fish undergoes oxidation causing rancidity and brown coloration. Common salt (NaCl) acts as preservative in the preservation of hilsa fish by preventing bacterial growth and destroying or inactivating most enzymes. Very few works have been done on the preservation of hilsa fish at BCSIR, Dhaka and BAU, Mymensingh.

Diets rich in fish oil have been associated with decreased risks of cardiovascular disease. This is thought to be related to the high quantities of omega-3-poly-unsaturated fatty acids found in many types of fish, particularly marine fish. These fatty acids have been reported to reduce platelet aggregation and monocyte adhesion, increased erythrocyte deferability, alter prostaglandin synthesis, lower blood pressure and possibly improve plasma lipids in lowering the levels of LDL-cholesterol. Recent studies in rabbits have been shown that hilsa fish oil reduces blood cholesterol level and arrests the progress of atherosclerosis. Investigation to the mechanism of above action showed that hilsa fish oil significantly depressed the activity of hepatic HMG-COA reductase enzyme which is the rate limiting enzyme in cholesterol biosynthesis.

No study with fish oil has been carried out on Bangladeshi population yet. Therefore a study will be undertaken to know the physico-chemical characteristics of the hilsa fish oil.

Corresponding Author: Kazi Abdus Salam, Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh
Tel: +88 0721 750041-9/4109 Fax: +88 0721 750064

MATERIALS AND METHODS

Materials: *Hilsa ilisha* were collected from the fish landing center of Rajshahi Shaheb Bazar which were caught mainly in the river of Padma. It was cut into six different parts like dorsal, ventral, tail, egg, liver and brain.

Study on the physical characteristics of the oil

Extraction of oil from hilsa fish: Oil, triglycerides portion of hilsa fish, which was extracted by suitable solvent (n-Hexane) under the operating condition. Continuous Soxlet Extraction Device was used for the extraction of oil[1].

Determination of specific gravity of the oil: The specific gravity of the oil was determined by means of a specific gravity bottle using the following formula[2].

$$\text{Specific gravity} = \frac{\text{(Sp. Gr.) Weight of oil in bottle}}{\text{Weight of distilled water in bottle}}$$

Determination of the refractive index of the oil: Refractive index of a medium is the ratio of the speed of light at a definite wavelength in vacuum to its speed in the media.

Determination of smoke point, flash point and fire point of the oil: The smoke point, flash point and fire point of fish oils of different parts were determined according to the Official Methods of the American Oil Chemist's Society[3].

Determination of pour point, cloud point and solidification point of oil: The pour point, cloud point and solidification points were determined according to the ASTM Standard Methods for lubricating oils[4].

Study on the chemical characteristics of the oil

Determination of iodine value of the oil: Iodine value of the oil was determined by Hanus method using the formula[5].

$$\text{Iodine value (IV)} = \frac{\text{S x (X-Y) x0.127}}{\text{W}} \times 100$$

Where:

S = Strength of the sodium thiosulphate
X = mL of thiosulphate required in the blank experiment
Y = mL of thiosulphate required in the test experiment
W = Weight of oil

A blank experiment was performed exactly in the same manner without the oil.

Determination of saponification value and saponification equivalent: Saponification Value is the number of mg of KOH required to saponify 1 g of oil. The saponification value was determined using the following formula[6].

$$\text{Saponification value (SV)} = \frac{\text{5.61x (A-B) x Strength of acid used}}{\text{W}}$$

Where:

A = Number of mL of acid required for blank experiment
B = Number of mL of acid required for test experiment
W = Weight of oil

Saponification equivalent was calculated from the saponification value using the formula[7].

$$\text{Saponification equivalent (SE)} = \frac{56100}{\text{SV}}$$

Determination of peroxide value of the oil: Peroxide value is expressed in terms of milli equivalent of active oxygen per kg of oil. Peroxide value of the oil was determined using the following formula[8].

$$\text{Proxide value (PV)} = \frac{\text{(S-B) x N}}{\text{W}} \times 1000$$

Where:

S = Titration with sample
B = Blank titration
N = Exact normality of thiosulphate
W = Weight of test portion

A blank test was carried out simultaneously in the same manner as described above.

Determination of % FFA (as oleic) of the oil: The percentage of free fatty acids (% FFA) was measured using the following formula (as oleic)[9].

$$\text{\%FFA (as oleic)} = \frac{\text{V x S x 28.2}}{\text{W}}$$

Where:

V = mL of alkali required to neutralize
S = Strength of alkali
W = Weight of oil

Determination of acid value: It is the number of mg of KOH required to neutralized the free fatty acids present in 1 g of oil. This is used for determining the rancidity due to the presence of free fatty acids.

Acid value was determined using the formula[10].

$$\text{Acid value (Av)} = \frac{V \times S \times 56.1}{W}$$

Where:
V = mL of alkali required to neutralize
S = Strength of alkali
W = Weight of oil

Determination of the quantity of unsaponifiable matter of the oil: The unsaponifiable matter is a fraction of fat or oil that remain insoluble after saponification of the fat sample by alkali. The unsaponifiable matter includes the sterols, higher alcohol's, pigment and hydrocarbons.

The amount of unsaponifiable matter present in the oil was determined using the method as described[11].

The quantity of unsaponifiable matter present in the 100 g of oil was calculated from the following formula

$$\text{Unsaponifiable matter (USM)} = \frac{\text{Weight of unsaponifiable matter}}{\text{Weight of oil taken}} \times 100$$

Determination of fatty acids composition of the oil: The fatty acids composition of hilsa fish oils were determined as their methyl esters which were prepared by the boron trifluoride methanol complex method[12].

The amounts of fatty acids were calculated from the peak areas computed by LKB electronic integrator.

Storage effect of hilsa fish oil at different temperatures: The samples of different parts of fresh hilsa fish oil were placed in cellophane bags and stored in a temperature controlled refrigerator (Lec Refrigerator Ltd. UK). After attaining the desired temperature i.e. -10 to 0°C, the %FFAs of the oils contained in the fish samples were measured after each five days interval upto 120 days. For the storage at room temperature (25 to 28°C), the fish samples were kept in the laboratory room (humidity, 60-65%) and the % FFAs were measured after each five days interval up to 120 days.

RESULTS AND DISCUSSION

Physical characteristics of hilsa fish oil

Specific gravity: The specific gravity of fats or oils does not vary as general rule to an extent, which makes this properly useful in discriminating between one fat and another. The specific gravity of particularly all fats or oils lies between 0.9 to 0.9544. As shown in the Table 1, the specific gravity of the hilsa fish oils presently examined from the different parts were varied from 0.920 to 0.932 at 25°C. The specific gravity of the oil from brain gave the maximum value (0.932) while that from the dorsal and ventral gave the minimum value (0.92). The values obtained in the present studies are quite similar to that reported for rice bran oil (0.916-0.92) by Mattil[13].

Refractive Index (RI): The refractive power of oils or fats varies somewhat widely and is chiefly governed by the proportion and degree of unsaturated matter present. It was found that the RI of the oils from the different parts of hilsa fish varied from 1.4700 to 1.4722 at 25°C (Table 1). The hilsa fish oil from dorsal and tail gave the maximum value (1.4722) while that from egg and liver gave the minimum value (1.4700). For comparison, it may be mentioned that the refractive index of Brassica, Linseed oil, Sesame oil, Sunflower oil and Olive oil are 1.470, 1.478, 1.475, 1.466 and 1.466, respectively[14,15]. The present value of this investigation is quite similar to that of the reported values.

Smoke point (°C): Smoke point of the different parts of hilsa fish oils were varied from 220 to 228°C (Table 1). The smoke point of the oil from brain gave the maximum value (228°C), while that from dorsal, ventral and tail gave the minimum value (220°C) in each case.

Flash point (°C): According to the Table 1, the flash point of the different parts of hilsa fish oils were ranged between 322 to 330°C. It was found that the flash point of oil from brain gave the maximum value (330°C) while that from liver oil gave the minimum value (322°C).

Fire point (°C): As described in Table 1, the fire point of the different parts of hilsa fish oils were between 350 to 364°C. It was found that the brain oil gave the highest fire point followed by oil from ventral and so on.

Cloud point (°C): As shown in the Table 1, the cloud point of the different parts of hilsa fish oils were found to be ranged from 2 to 2.4°C. It was also found that the oil from dorsal, ventral and tail has the same cloud points.

Solidification point (°C): The solidification points of the different parts of hilsa fish oils were found to be in the

Table 1: Physical characteristics of hilsa fish oil extracted from different parts

	Name of the parts					
Constants	Dorsal	Ventral	Tail	Egg	Liver	Brain
Specific gravity (at 25°C)	0.920	0.920	0.922	0.926	0.924	0.932
Refractive index (at 25°C)	1.472	1.472	1.472	1.470	1.470	1.471
Smoke point (°C)	220	220	220	224	224	228
Flash point °C	325	325	325	328	322	330
Fire point (°C)	352	350	352	360	356	364
Cloud point (°C)	2.0	2.0	2.0	2.2	2.4	2.1
Solidification point (°C)	-9.4	-10	-9.6	-8.4	-9.2	-5.0
Pour point (°C)	-6.8	-7.0	-6.4	4.0	6.0	5.0

ranges of -10 to -5°C (Table 1). From the experimental data it was found that the solidification point of the oil from brain gave the maximum value (-5°C) while that from ventral gave the minimum value (-10°C).

Pour point (°C): The pour point of hilsa fish oils were found to be ranged between -7 to 6°C (Table 1). It is clear that the pour point of the oil from liver has maximum value whereas that from ventral has minimum value.

Chemical characteristics of hilsa fish oil

Saponification value and Saponification Equivalent: The Saponification value of the hilsa fish oils from different parts were found to be ranged from 180.28 to 194.00 (Table 2). It was found that the oil from dorsal and ventral gave the maximum value and that from egg gave the minimum value. This result is found to be very similar to that reported for rice bran oil by Murti *et al.*[16].

Of the oils examined, the oil from egg gave the maximum saponification equivalent (311.18) followed by that from liver (310.36) and so on in decreasing order.

Iodine value: Iodine value is defined as grams of iodine absorbed by 100 g of fat. The iodine values of hilsa fish oils from different parts were ranged between 80.70 to 126.40 (Table 2). As found the oil from liver gave the maximum value (126.40) while that from brain gave the minimum value (80.70). Some vegetables oil namely olive oil, linseed oil, sunflower oil and cottonseed oil have the iodine values of 80-85, 175-200, 125-141 and 102-114, respectively[17]. The present values of this investigation are quite similar to that of the reported values.

Peroxide value: The peroxide value is the milli equivalent of peroxide oxygen combined in a kilogram of oil. The peroxide values of hilsa fish oils from different parts were ranged between 7 to 10 and highest value is found in the liver and lowest is in the brain (Table 2).

Acid value and % of FFA: Acid value is a measure of the hydrolysis that has occurred in a fat and is defined as the number of milligrams of potassium hydroxide required to neutralized the free fatty acids in 1 g of oil or fat.

The acid values of hilsa fish oils from different parts were found to be varied from 4.16 to 12.00 and % FFA from 2.08 to 6.00. The oils from the liver have higher acid value and % FFA than those of oils from any other parts (Table 2).

Unsaponifiable matter: The unsaponifiable matter of oil represents the presence of a mixture of several alcohols. All fish oils contained cholesterol; other alcohols in the unsaponifiable matter include pigments, vitamin A and D, glycerol ethers (Salachyl, chimyl and butyl alcohols) and fatty alcohols. Vitamin E is also present in small amounts.

The unsaponifiable matters present in the presently examined hilsa fish oils from different parts were varied from 1.58 to 7.00%. Highest amount of unsaponifiable matter is present in the oil from brain and lowest amount is present in that from ventral.

Fatty acid composition of the different parts of hilsa fish oil: The fatty acids composition of the oils from six different parts of hilsa fish (Table 3) showed that the saturated fatty acids present in the oil samples were mainly myristic acid (5.44 to 7.24%), palmitic acid (22.00 to 27.08%) and stearic acid (4.00 to 6.32%). The unsaturated fatty acids present in the oil samples were mainly palmitolenic acid (12 to 14%), oleic acid (26.08 to 29.78%), linoleic acid (0.92 to 2.20%) and linolenic acid (0.82 to 1.08%). Highest amount of total unsaturated fatty acids was present in oil from egg and lowest amount in oil from brain. It was found that hilsa fish contained the highest amount of oleic acid. The oil from egg contained the highest amount of oleic acid (29.78%) followed by that from liver (29.44%), tail (28.40%), dorsal (28.32%), ventral (28.00%) and brain (26.08%).

Storage effect of hilsa fish oil at different temperature: The conversion of fatty oils (hilsa fish oil) isolated from different parts of hilsa fish into fatty acids by the action of lipase have been studied in the present investigation

Table 2: Chemical characteristics of hilsa fish oil extracted from different parts

	Name of the parts					
Constants	Dorsal	Ventral	Tail	Egg	Liver	Brain
Saponification value	194.00	194.00	192.00	180.28	180.76	182.24
Saponification equivalent	289.17	289.17	292.19	311.18	310.36	307.84
Iodine value (Hanus method)	101.30	102.00	101.40	100.22	126.40	80.70
Peroxide value m. eq. O_2/Kg oil	8.00	8.60	8.00	9.28	10.00	7.00
Acid value	4.28	4.16	4.30	7.16	12.00	8.72
% FFA (as oleic)	2.14	2.08	2.15	3.58	6.00	4.36
Unsaponifiable matter (%)	1.66	1.58	1.82	4.60	3.72	7.00

Table 3: Fatty acid compositions of hilsa fish oil extracted from different body parts

	Name of the parts					
Fatty acids	Dorsal	Ventral	Tail	Egg	Liver	Brain
$C_{14:0}$	5.44	5.70	5.50	6.60	5.90	7.24
$C_{16:0}$	24.70	25.30	24.86	26.40	27.08	22.00
$C_{18:0}$	6.20	6.32	6.30	5.70	5.00	4.00
$C_{16:1}$	13.00	12.30	13.11	14.00	12.00	12.18
$C_{18:1}$	28.32	28.00	28.40	29.78	29.44	26.08
$C_{18:2}$	1.16	1.10	1.02	2.18	2.20	0.92
$C_{18:3}$	0.92	0.96	0.92	0.90	1.08	0.82

after storing the samples at low temperature (-10 to 0°C) and at room temperature (25 to 28°C).

Figure 1 shows the hydrolytic deterioration of hilsa fish oils at low temperature (-10°C). It was found that the contents of % FFA in the oils obtained from different parts of the fish samples were initially low but increased rapidly on storage. After storage of 120 days at -10°C, the oils from liver, brain, egg, ventral, dorsal and tail contained 45.82, 44.16, 42.61, 39.87, 35.88 and 33.30 % FFA, respectively. From this finding, it was concluded that the lipase enzyme in hilsa fish oil is active even at temperature -10°C.

Figure 2 shows the hydrolytic deterioration of hilsa fish oils from different parts at low temperature (0°C). It was found that the hydrolytic deterioration of hilsa fish oils were more effective at 0°C than that from -10°C, which might be due to higher activity of lipase at 0°C. After storage of 120 days at 0°C, the hilsa fish oils from liver,

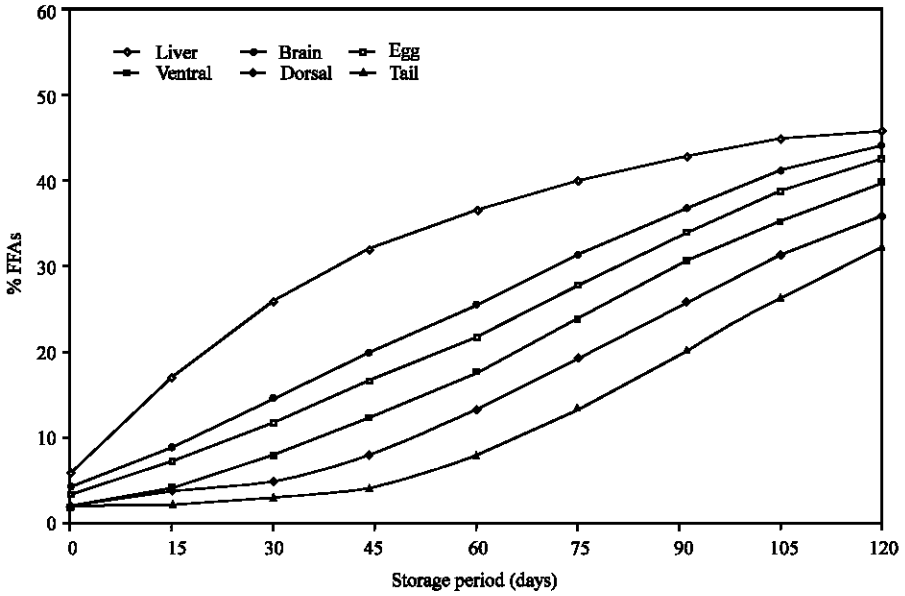

Fig. 1: Effects of storage at low temperature (-10°C) on the formation of FFAs in the different parts of hilsa fish oils

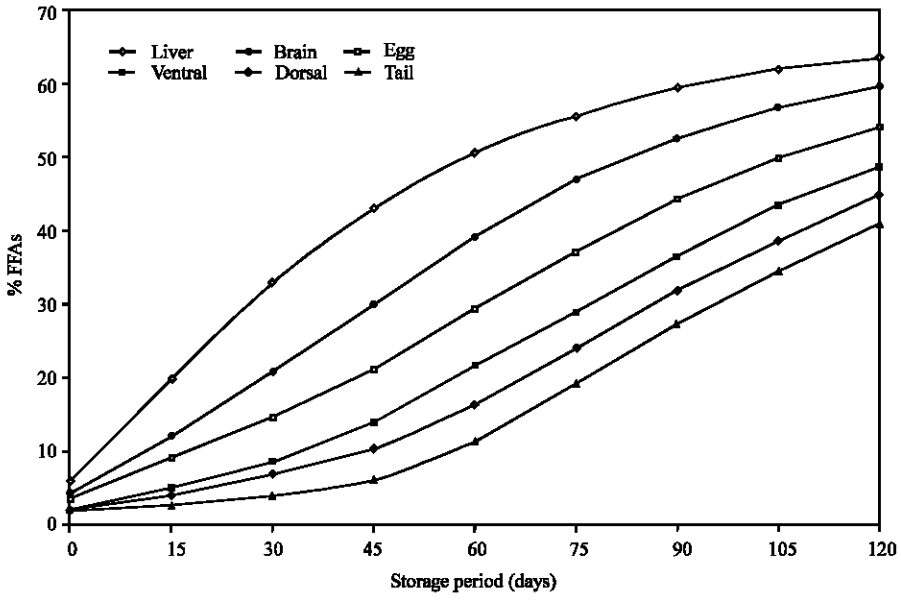

Fig. 2: Effects of storage at low temperature (0°C) on the formation of FFAs in the different parts of hilsa fish oils

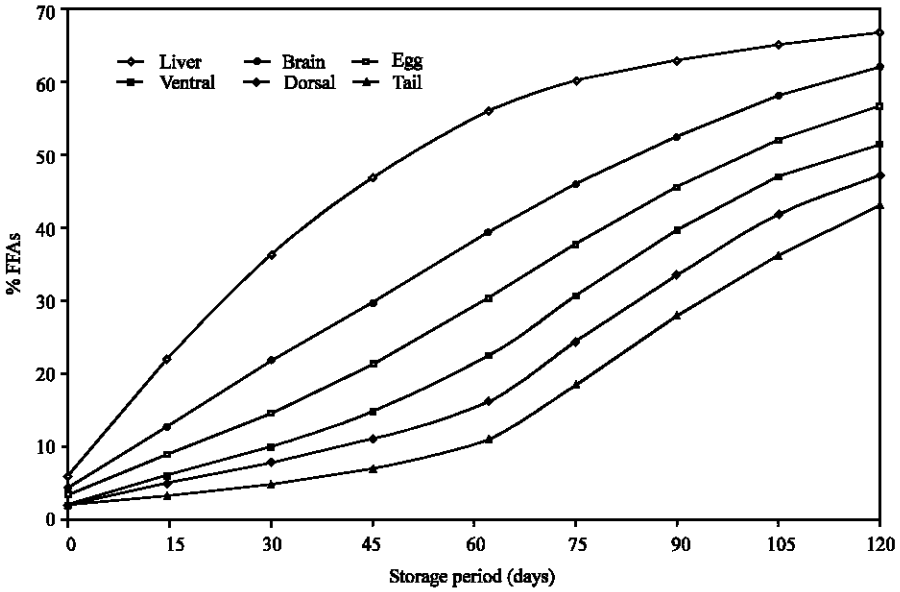

Fig. 3: Effects of storage at room temperature (25 to 28°C) on the formation of FFAs in the different parts of hilsa fish oils

brain, egg, ventral, dorsal and tail were found to be 63.46, 59.51, 54.00, 48.60, 44.91 and 41.00% FFA, respectively.

As shown in Fig. 3, the quality of hilsa fish oils were deteriorated slightly further when stored at 25°C and after 120 days of storage at 25°C, the oils from liver, brain, egg, ventral, dorsal and tail were found to be 66.60, 62.00, 56.72, 51.40, 47.20 and 43.15% FFA, respectively.

REFERENCES

1. South Combe, J.E., 1926. Chemistry of the Oil Industries. 2nd Edn., Constable Company Ltd., London, pp: 144.
2. Hilditch, T.P., 1949. The Industrial Chemistry of Fats and Waxes. 3rd Edn., Balliere Tindall and Cox, London, pp: 83.
3. Official and Tentative Methods and Materials of the American Oil Chemist's Society, 1980. 3rd Edn., Vol. I, USA.
4. American Society for Testing and Materials, 1952. Method No. D., pp: 128-147.
5. Williums, K.A., 1966. Oils, Fats and Fatty Foods. 4th Edn., 'J' and 'A' Churchill Ltd., London, pp: 124.
6. IUPAC, 1967. Standard Methods for the Analysis of Oils, Fats and Derivatives. 6th Edn., Pergamon Press, Oxford, pp: 56.
7. Paech, K. and M.V. Tracey, 1955. Modern Method of Plant Analysis, Berlin, pp: 332.
8. Jacobs, M.B., 1958. The Chemical Analysis of Foods and Foods Products. 3rd Edn., pp: 393.
9. Augustus West, P. and O. Aurelio Cruz, 1933. The Phil. J. Sci., 54: 52.
10. Device, J. and P.N. Williams, 1961. The Chemistry and Technology of Edible Oils and Fats. Pergamon Press Oxford, pp: 127.
11. Williams, K.A., 1966. Oils, Fats and Fatty Foods. 4th Edn., 'J' and 'A' Churchill Ltd. London, pp: 125.
12. Morrison, W.R. and L.M. Smith, 1964. J. Lipid Res., 5: 600.
13. Mattil, 1958. Industries Oil and Fat Products. 3rd Edn., pp: 216.
14. Karim, R., 1992. A comparative study of oil seeds. M.Sc. Thesis. University of Rajshahi, pp: 80.
15. Islam, A., 1994. Chemical studies on the seed of *Polyalthia longifolia*. M.Sc Thesis, University of Rajshahi, pp: 124.
16. Murti, K.S., 1948. J. Am. Oil Chem. Soc., 25: 211.
17. Lal Agarwal, K., 1990. Purification and characterization of toxic and non-toxic proteins from the kernels of semecarpus Anacardiul Linn. M.Sc. Thesis. University of Rajshahi, pp: 107.