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Research Paper

FATTY ACID COMPOSITION, PHYSICO-CHEMICAL AND ANTI-OXIDANT PROPERTIES OF ALMOND SEED (*Terminalia catappia* L) OIL AND ITS THERAPEUTIC USES

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Abstract

Almond (*Terminalia catappa* L.) is an underutilized crop which belongs to a group of nuts with hard shelled seeds enclosing a single edible kernel. Almond was generally believed to have originated in Malaysia and is distributed throughout the old world tropic and tropical America. Almond trees are a source of beauty, inspiration, food and medicine. In the northern part of Bangladesh, lots of Almond trees have been planted in the road side that are producing lots of Almond seeds which are being wasted every day in the street or are being dumped this important plant materials in the garbage due to lack of knowledge of its nutritional, commercial and medicinal values. In this study, at the first time in our country, Bangladesh Almond oil was extracted from Almond seed kernel by solvent extraction method and purified by column chromatography and found to be about 31% of oil. The fatty acid composition of the Almond oil was determined by GLC. The percentage composition of individual fatty acids was found to be Palmitic Acid (52.40 %), Linoleic Acid (18.53%), Oleic Acid (16.58%), Stearic Acid (5.37%) and Behenic Acid (7.10%). The amount of saturated and unsaturated fatty acids was found to be 64.87% and 35.11%, respectively. The physicochemical properties of almond seed oil were - colour (yellow), refractive index

(1.454), specific gravity (0.971), acid value (0.52), saponification value (190 mgKOH/g), iodine value (103 mg/g), peroxide value (0.61) and free fatty acids (0.26 mg/g). These results indicate that Almond oil could be a good source of edible oil for human consumption like other vegetable oils and its high content of polyunsaturated and monounsaturated fatty acids is of potential interest especially fighting against artheclerosis, high blood pressure, cardiovascular diseases. Antioxidents such as Vitamin A, Vitamin E and Vitamin K1 were detected in Almond seed oil by conventional method except Vitamin D. Almond seed oil might also be used as hair oil since palmitic acid and oleic acid are the key ingredients in hair oil and it contains also Vitamin A and Vitamin E.
Key words: Almond seed oil, Fatty acids, and GC.

INTRODUCTION

Fats and oils are a significant food source and are supplying essential fatty acids like linoleic acids fo human health. Fats and oils are also used for producing drug dispersants in therapeutics and play an important functional and sensory roles in food product [1,2]. Vegetable oils are broadly used in the purpose of cooking and food processing, pharmaceutical, cosmetics and chemical industries [3,4].

Almond nut is one of the best popular nuts in the world. Its kernels are usually used as snack foods also are used as components in a variety of processed foods, especially bakery and confectionary products and seeds are also rich in unsaturated fatty acids (USFA), protein and oil as nutritional values.

Almond (*Terminalia catappa* L.) is an underutilized crop which belongs to a group of nuts with hard shelled seeds enclosing a single edible kernel. Almond was generally believed to have originated in Malaysia and is distributed throughout the old world tropic and tropical America. It is also reported that Almonds is a species of tree of the Western and Central Asia including China, Turkestan, Kurdistan, Afghanistan and Iran [5]. According to report of FAO the top five almond producers in calendar year of 2003 were the USA at 758,000 metric tons (45% of the global production), followed by Spain (210,000 tons, 12%), Syria(139,000 tons, 8%), Iran (105,000 tons, 8% and Italy 991,000 tons, 5%).

In Nigeria, it is usually found in the tropical rain forest zones of the southern and western states. The flesh (pulp) of the ripe fruit and kernels which are usually extracted by cracking the nuts are widely eaten across all age groups in Nigeria and particularly in Ekiti State. The Kernels of Almond are often of small size and difficult to extract from inside the seeds and these factors may have contributed to its lack of use in many areas of all over the country.

Almond contains protein, lipid, carbohydrates, minerals and vitamins that are very important for human diet and health . Almond oil is also one of the most important vegetable oils and used as an edible oil in different countries.

It is used for cooking in some parts of South America and the nuts may be consumed fresh after extraction from the shell or preserved by drying or smoking and consumed up to a year later.

It is light yellow in color. Indian almond is rich in vitamin E and high in unsaturated fat [S Ziller,1994]. Almond contains high percentage of oil having edible properties like soybean oil as reported in the literature. The Almond oil may be used for edible purposes after refining and the crude oil for soap making.

Many studies have been conducted to assess the physic-chemical properties of the almond seed oil such as Ozean et al, have studied the physic-chemical composition of Turkey almond kernels and oils [6].Yada et al, also have studied the physical properties of almond pit and kernel [7].Moayedi et al, have also studied the chemical compositions of oils from several wild almond species [8]. Food and drug administration also claim that eating 42g of almond every day as part of a diet low in saturated fat and cholesterol which may reduce the risk of coronary heart disease [9].Almond kernels contain unsaturated fatty acids such as oleic, linoleic and linolenic acid. These three fatty acids are very important for human nutrition and are helpful in the diet [8,10] and almost twenty species of wild almond have been reported in Iran[11].

Several studies have been done on the Nutrient composition of Almond and Physicochemical properties of Almond oil in India, Nigeria, Australia, USA, Thailand, Iran, Japan and other countries, but no detail information regarding the fatty acid composition and physicochemical properties of Almond seed oil as well as Therapeutic uses are available in our country even though we are cultivating Almond Tree and producing lots of Almond seeds that are wasting every day in every time in the street as well as dumping this important plant materials in the dustbin without knowing its nutritional, commercial and medicinal values.

Considering the nutritional value of Almond and the industrial and edible importance of Almond seed oil, detail fatty acid composition, Physico-chemical and Anti-oxidant properties and Therapeutic uses of Almond seed oil was investigated at the first time in Bangladesh by this study. Additionally , the physic-chemical properties of Almond seed oil were compared with other two edible oils namely Sunflower oil and a fresh brand Soybean oil.

MATERIALS AND METHODS

Collection and Preparation of Seeds Sample

Matured and Ripened Almond Seeds were collected from the Campus of Khwaja Yunus Ali Medical College and Hospital, Enayetpur, Sirajgonj and sun dried for sufficient time. Almond seeds were crashed by mechanically and kernels of Almond Seeds were separated from the flesh and sun dried for six hours and stored in an air tight plastic container in refrigerator of our laboratory of the Department of Biochemistry and

Molecular Biology, Noakhali Science and Technology University, Noakhali, Bangladesh for experimental analysis. Two different brands, one Sunflower oil and one fresh brand of Soybean oil were collected from local markets of Noakhali city, Bangladesh for qualitative analysis.

Extraction and Purification of Almond Oil

The Almond Seed Kernel oil was extracted from Almond Seeds by Solvent Extraction method of Ogunsua et al., Badifu, 1989 [12] using petroleum ether as extracting solvent. The solvent was removed under reduced pressure in a rotary evaporator. The oil obtained from Almond seeds was purified over a neutral Alumina column using ether-diethyl ether (70:30, V/V) as eluting solvent. The purity of the oil was checked by normal TLC (Thin Layer Chromatography).

Fatty Acid Composition of Almond Seed Kernel Oil

The fatty acid composition of Almond oil was determined by Gas Liquid Chromatography (GLC).The fatty acid methyl esters of the Almond oil and those of the standard fatty acids were prepared by direct esterification [13].

GLC Examination

A Pye-Unicam gas chromatograph equipped with a flame ionization detector was used.Fatty acids were separated on a glass column (length 1.5m, i.d. 2 mm), which was packed with Gas Chrom P (100-120 mesh) and coated with 10% diethylene glycol succinate polyesters. Nitrogen was used as a carried gas at a flow rate of 20-30 ml/min. The temperatures of the injector, detector and column were maintained at 220^0C, 160^0C and 180^0C, respectively. Gas chromatographic peaks were identified by comparison with those of methyl esters of standard fatty acids in respect of their retention time. Peaks were measured by a Pye-Unicam electronic integrator. The percentage of each peak was calculated as % of total area of all the peaks.

Physicochemical properties of Almond oil

Determination of Colour

Colours of Almond seed oil, Sunflower oil and Soybean oil (Fresh Brand) were observed by visual parallelism.

Determination of Refractive index

Refractive index of all three clear oils were determined by using Abbe Refractometer following standard IUPAC method [14].

Determination of Specific gravity

The specific gravityof Almond seed oil, Sunflower oil and Soyabean oil (Fresh Brand) were determined at 30^0C by a Pycnometer.

Determination of Acid Value

For the determination of Acid value, 0.5 gm of each oil was taken in each 250 ml conical flask and dissolved it with 25 ml of solvent mixture that prepared freshly by diethyl ether and absolute alcohol (1:1). Then the contents were heated to boiling and the flask was shaken thoroughly, in order to dissolve the free fatty acids as completely as possible. The solution was cooled and then titrated with 0.1M alcoholic KOH solution with constant shaking using phenolphthalein as indicator until the pink color persisted after vigorous shaking. End point was reached when the pink colour persisted for 30 seconds in the hot solution after vigorously shaking. A blank titration was carried out simultaneously. Triplicate analysis of each sample was conducted.

Determination of Saponification value

Exactly 1.0 g of the oil sample was dissolved in 3 ml chloroform in a 50 ml conical flask. Then 25 ml of 1% phenolphthalein was added and solution was titrated to a colourless end point with 0.5MHCL.A blank titration was carried out in all respect [15]. Triplicate analysis of each sample was conducted.

Determination of Iodine Value

At first Hanus Iodine Reagent was prepared by dissolving 13.2g of iodine with glacial acetic acid in a 500 ml volumetric flask by warming over water bath. When the iodine was completely dissolved, the solution was cooled and 1.5 ml pure Br_2 (sulfur free) was added in it and diluted up to 500 ml with glacial acetic acid.

Then exactly 0.3 gm of oil sample was transferred into a dry 250 ml glass stoppered reagent bottle and add 10 ml of chloroform to the bottle and also to the other dry 250 ml glass stoppered reagent bottle containing no oil sample that was serve as a control. In the next step, 30 ml of Hanus Iodine Reagent was added to each bottle. Stoppered, mixed and placed in the dark for exactly for 30 minutes. After 30 minutes 10 ml of 15% KI solution was added to each bottles and mixed well. This step assured that any free iodine remaining in the chloroform solution is extracted in the KI solution. In the next step 75 ml of distilled water was added to each bottle and it was then titrated with 0.1 N $Na_2S_2O_3$ solution until the solution becomes light yellow. Then added a few drops of 1% starch solution and completed the titration until the blue colour was just disappeared. The control was treated in a similar way. Triplicate analysis of each sample was conducted.

Determination of Peroxide Value

Exactly 1.0 g of KI and 20 ml of solvent mixture (glacial acetic acid: chloroform, 2:1 v/v) were added to 1.0 g of the oil sample and the mixture was boiled for one minute. The hot solution was poured into a flask containing 20 ml of 5% KI solution. Few drops of starch solution were added to the mixture and the letter was titrated with 0.025M $Na_2S_2O_3$ solution .A blank titration was also carried out without oil.

Determination of Free Fatty Acids of the Oil Samples

20 ml of ethanol: diethyl ether (1:1, v/v) mixture was transferred to 250 ml conical flask and 2 ml of 1% phenolphthalein solution was added to it and the mixture was neutralized using 0.10 M NaOH solution.Then 5 g of each oil sample was added to the neutralized mixture and titrated against 0.10 M NaOH solution with constant shaking until a pink colour developed and persisted for 15 minutes.The titre values were used to obtain the free fatty acid value [16].

Antioxidant Properties of Almond Oil

Antioxidants such as Vitamin A, Vitamin E and Vitamin K1 of Almond oil except Vitamin D were detected by conventional method and the presence of these antioxidants were confirmed by TLC.

RESULTS

In order to Extract and purify Almond oil, Almond seeds as presented in Figure-1 were crashed by mechanically and their Kernels as presented in Figure-2 were separated and the separated kernels were sundried for sufficient time and used for the extraction and purification of oil and the contents of the oil was about 31% (Figure-3).

In order to characterize the Almond oil, fatty acid composition, Physicochemical properties and Antioxidant properties were determined as the methods described in the Methods section and the results are presented in Table-1, Table-2 and Figure-4, Figure-5, Figure-6, Figure-7, Figure-8, Figure-9, Figure 10 and Figure 11, respectively and compared with Sunflower oil and a market bland soybean oil (Fresh). The major fatty acids present in Almond seed oil as presented in Table-1 was identified to be palmitic acid (52.40%), Linoleic acid (18.53%), Oleic acid (16.58%), Stearic acid (5.37%) and Behenic acid (7.10%) by GLC.

Table-1: Fatty acid composition (% of methyl fatty acids) of Almond oil by GLC (by wt%):

Fatty Acid	Composition (%)
Palmitic Acid	52.40%
Linoleic Acid	18.53%
Oleic Acid	16.58%
Stearic Acid	5.37%
Behenic Acid	7.10%
Saturated Fatty Acids	64.87%
Unsaturated Fatty Acids	35.11%

Figure-1: Almond Seeds

Figure-2: Almond Seed Kernel

Table-2: Physicochemical Properties of Almond seed oil, PSK oil, sunflower oil and fresh soyabean oil.

Characteristics	**Almond Seed oil**	**Sunflower oil**	**Soybean oil (Fresh Brand)**
Color	Yellow	Yellow	Yellow
Refractive index (25^0C)	1.454	1.4735	1.4722
Specific gravity	0.971	0.924	0.921
Acid Value	0.52	0.6	0.374
Saponification value	190	188	210
Iodine value	103	120	105.26
Peroxide value	0.61	0.669	2.68
Free Fatty Acids (as oleic acid)	0.26	0.262	0.18

Figure-3: Extracted and Purified Almond Oil (about 31%)

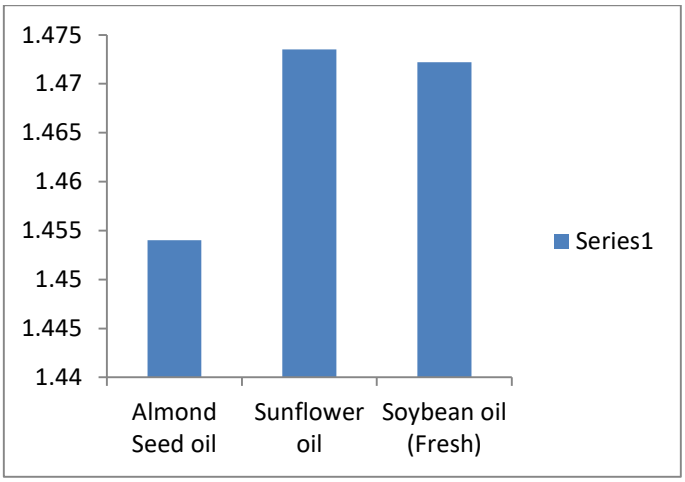

Figure 4: Refractive index of Almond seed oil, Sunflower oil and Soybean oil (Fresh)

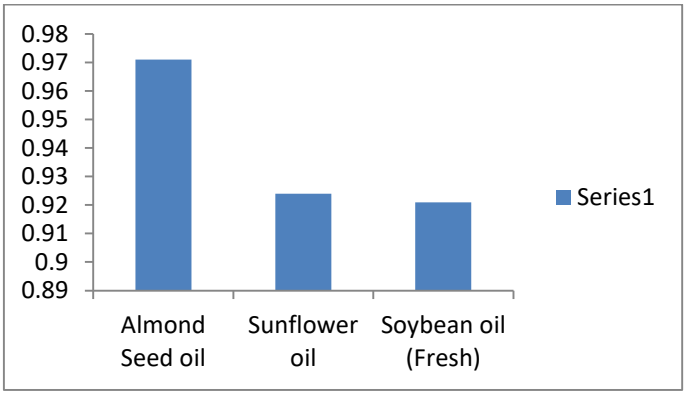

Figure 5: Specific gravity of Almond seed oil, Sunflower oil and Soybean oil (Fresh)

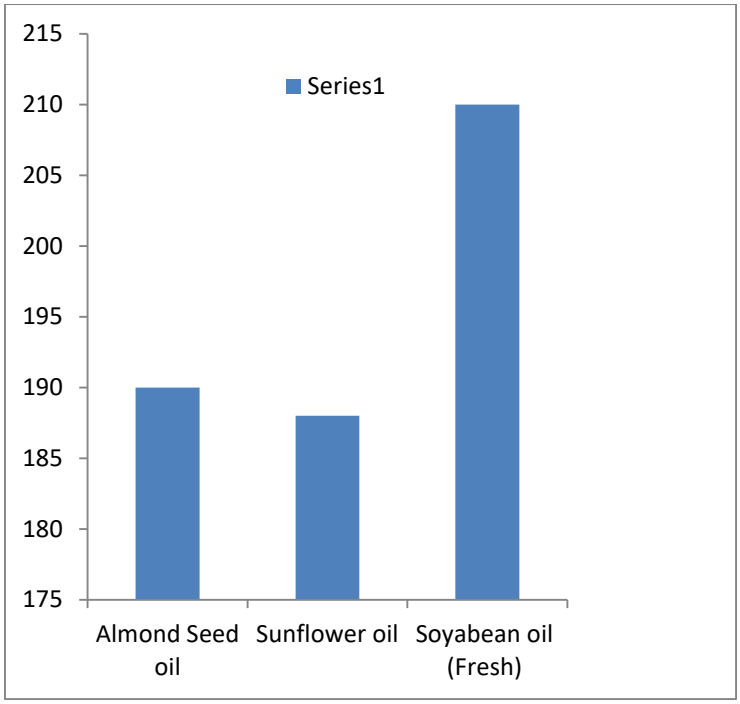

Figure 6: Saponification value of Almond seed oil, Sunflower oil and Soybean oil (Fresh)

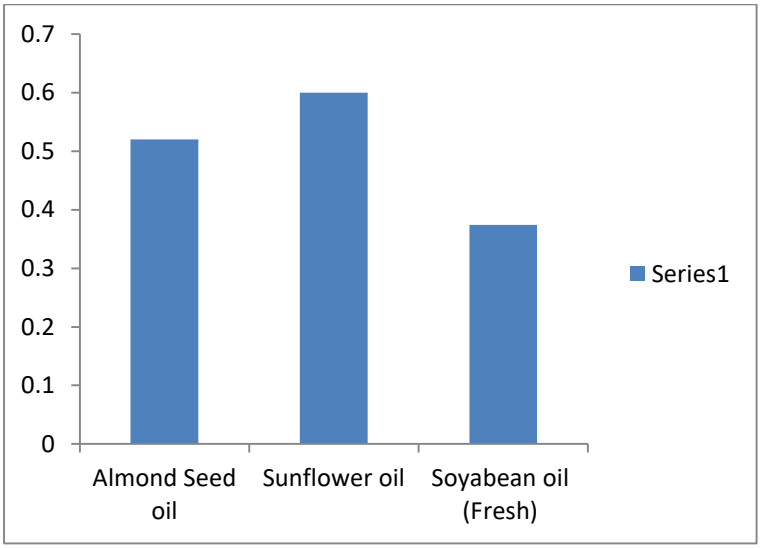

Figure 7: Acid value of Almond seed oil, Sunflower oil and Soybean oil (Fresh)

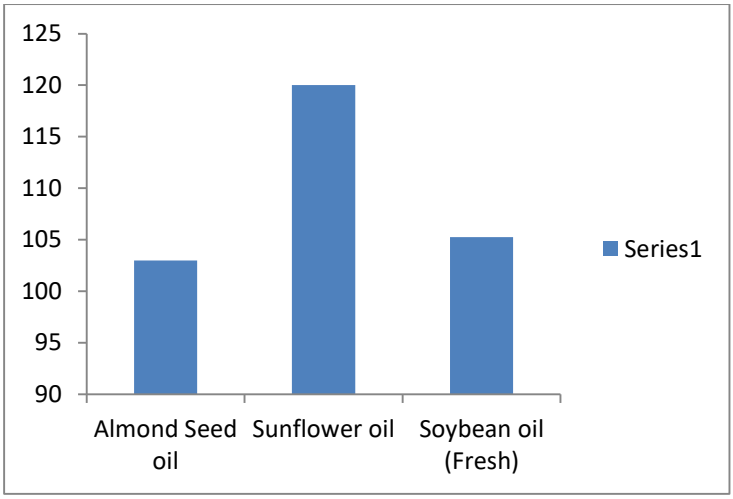

Figure 8: Iodine value of Almond seed oil, Sunflower oil and Soybean oil (Fresh)

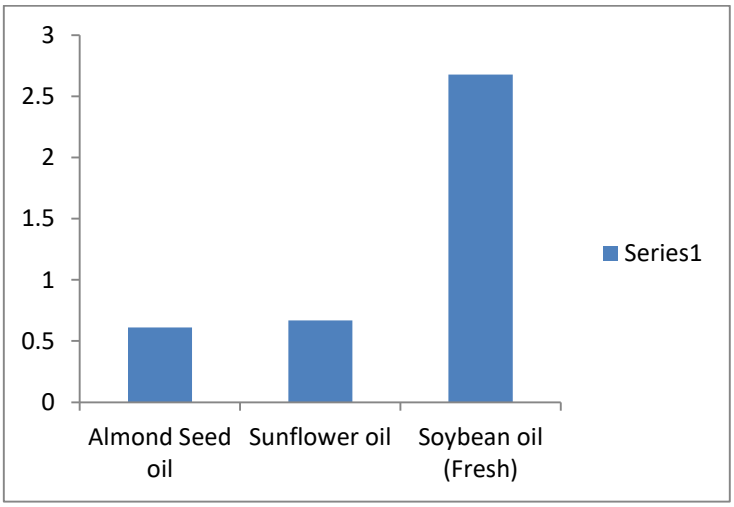

Figure 9: Peroxide value of Almond seed oil, Sunflower oil and Soybean oil (Fresh)

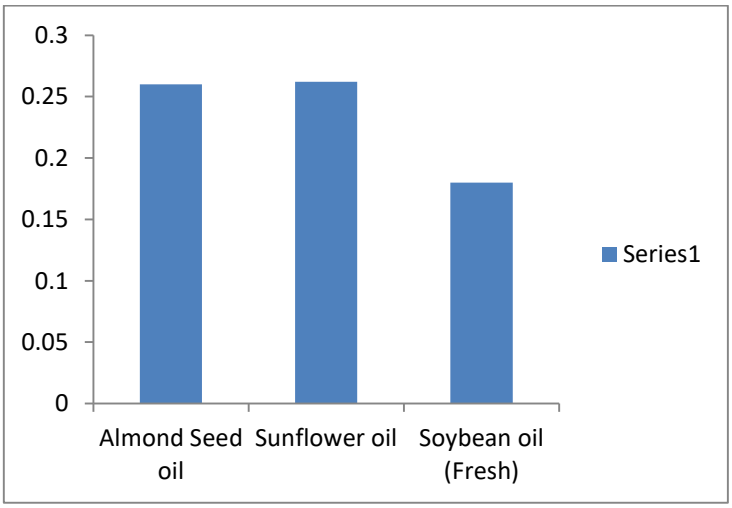

Figure 10: Free Fatty Acids of Almond seed oil, Sunflower oil and Soybean oil (Fresh)

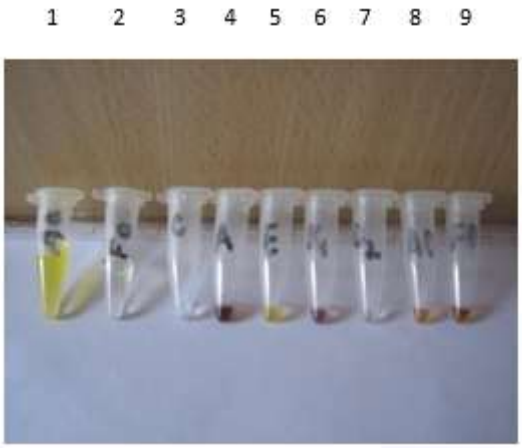

Figure-11: Anti-oxidant Properties of Almond Oil

Lane-1=, AO, Almond Oil, Lane-2=FO, Fresh Soybean oil, Lane-3=C, 40 µl 50% H_2SO_4 in Ethanol as a Control,

Lane-4=A, 5 µl Vit A + 35µl 50% H_2SO_4 in Ethanol—Deep Pink color,

Lane-5= E, 5µl Vit E + 35µl 50% H_2SO_4 in Ethanol—Yellowish-Pink color,

Lane-6=K1, 5 µl Vit K1 + 35µl 50% H_2SO_4 in Ethanol-Brownish-Pink color,

Lane-7=D2, 5 µl Vit D2 + 35µl 50% H_2SO_4 in Ethanol—Whitish color,

Lane-8=AO,5 µl Almond oil + 35µl 50% H_2SO_4 in Ethanol-Yellowish-Pink-Brown (Vit.A, Vit.E(++), and Vit.K1 present),

Lane-9=FO, 5 µl Fresh Soybean oil + 35µl 50% H_2SO_4 in Ethanol—Brownish-Yellow-Pink Color (Vit.A, Vit.E, and Vit.K1(++) present).

DISCUSSION

It is reported in literatures that the most common fatty acids in edible oils and fats are those containing 18 carbons and these are Stearic acid (a saturated fatty acid), Oleic acid (a monounsaturated fatty acid), and Linoleic and linolenic acids (polyunsaturated fatty acids containing two and three double bonds, respectively.) Since our experimental Almond oil contained stearic acid, oleic and linoleic acids and the amount of saturated and unsaturated fatty acids were found to be 64.87% and 35.11%, respectively. So, Almond oil could be a good source of edible oil for human consumption like other vegetable oils and its high content of polyunsaturated and monounsaturated fatty acids is of potential interest especially fighting against arthrosclerosis, high blood pressure, cardiovascular diseases.

From the results it is concluded that the color of the Almond oil is same as the Sunflower oil and a market bland Soybean oil (Fresh) as presented in the Table-2. The Refractive index of Almond seed oil was found to be 1.454 which is less than the value of Sunflower oil (1.4735) and Soybean oil (Fresh) (1.4722) (Figure 4) , but it is in agreement with the value of 1.465 for almond nut oil [17] which indicating that this oil sample contained fairly large amount of unsaturated fatty acids.

The specific gravity of Almond seed oil was found to be 0.971 which is slightly higher that the value of Sunflower oil (0.924) and Soybean oil (0.921) (Figure 5), but this value indicating that it is less dense than water which is in agreement with the value of 0.964 for almond nut oil [17].

Acid value is used as an indicator for edibility of oil and suitability for use in the paint industry. Acid value of almond seed oil was found to be 0.52 which is nearly same to the acid value of Sunflower oil(0.6) and much higher than Soybean oil (Fresh) (0.374) (Figure 6).BSTI standard for acid value is 0.6mg/g max [18]

Saponification value is an indicator that can be used to compare relative molecular masses and indicate the usefulness of oil in industry. The saponification value of almond seed oil was found to be 190 which is almost same to the Saponification value of Sunflower oil (188) and lower than Soybean oil (Fresh) (210) (Figure 7). The Saponification Value of Almond seed oil is within the range of BSTI standard 189-195 [18]The high saponification value recorded in this study for Almond seed oil suggest that this oil contain high molecular weight fatty acids and low levels of impurities. This is evidence that this oil could be used in the soap making industry also [19].

The Iodine value is a measure of the degree of unsaturation in oil and it is an identity characteristics nature of oil. It indicates the degree of unsaturation in the fatty acid of triacyglycerol. This value could be used to quantify the amount of double bond present in the oil which reflects the susceptibility of oil to oxidation. The Iodine value of Almond seed oil was found to be 103 which is almost same to the Fresh band of Soybean oil (105.26) (Figure 8), and it is suitable for edible purposes and also in the manufacture of pastry and cosmetic [20].

The peroxide value is used as an indicator of deterioration of oil. Fresh oils have peroxide values less than 10 mEq/kg.The peroxide value of Almond seed oil was found to be 0.61 which is almost same as the Sunflower oil (0.669) and much less than Soybean oil (**2.68)** (Figure 9),.The peroxide value of Almond oil (0.61) is lower than that expected of rancid oil which ranges from 20.00 to 40.00 mg/g oil [21]. This result shows that this Almond seed oil is not rancid and considered as stable [22].

The free fatty acid value of Almond seed oil was found to be 0.26 which is same as the value of Sunflower oil (0.262), but higher than Soybean oil (Fresh)(**0.18)** (Figure 10). Eckey, E.W., 1954, reported [23] that the allowable limit of free fatty acid for edible oil is 0-3. Therefore this almond oil could be used as an edible oil as the value is within the limit for edible oil. Antioxidants such as Vitamin A, Vitamin E and Vitamin K1 of Almond oil except Vitamin D were detected by conventional method and the results were compared with soybean oil by using Standard Vitamin A, Vitamin E and Vitamin K1.The Yellowish color of Almond oil diluted with Ethanol and reacted with 50% Sulphuric Acid indicating the presence of Vitamin A, Vitamin E and Vitamin K1 as we can see in the Figure-11 (Lane-8) compared with standard Vitamin A(Lane-4), Vitamin E (Lane-5), Vitamin K1(Lane-6), but absence of Vitamin D2 as it is the whitish in color like same as negative control which is 50% of sulphuric acid in Ethanol (Whitish Color). From this

result it is concluded that Almond oil may help to reduce Vitamin A, Vitamin E and Vitamin K deficiency symptoms and diseases and might be useful as a supplementary source of fat soluble vitamins. It is also found in the literature that Vitamin E is one of the most powerful natural antioxidants helping to combat the emergence of free radicals and other damaging elements in the skin. So, our experimental Almond oil may also help to fight against cancer and may help to keep our skin healthy due to presence of Vitamin E.

CONCLUTION

Based on the results of this research findings, it can be concluded that Almond sees have high oil yields (31%) which is comparable to the oil yield of some commercial seed oils. The physico-chemical properties of Almond oil indicated that it could be a good source of edible oil for human consumption like other vegetable oils. The content of polyunsaturated and monounsaturated fatty acids in Almond oil is of potential interest especially fighting against arthrosclerosis, high blood pressure, cardiovascular diseases. The presence of Anti-oxidants such as Vitamin A and Vitamin E in Almond oil will be useful for our skin health and will prevent the signs of aging and may also help to fight against cancer. In conclusion, Almond oil might also be used as hair oil since palmitic acid and oleic acid are the key ingredients in hair oil and it contains also Vitamin A and Vitamin E. The findings of this research also be useful to promote the sustainable cultivation of the almond tree in mountain region and road side of Bangladesh for large-scale oil production.

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