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EXTRACTION AND CHARACTERIZATION OF COWPEA (*VIGNA UNGUICULATA* L. WALP) SEED OIL

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Abstract

The physical-chemical characteristics of oils extracted from three varieties of *Vigna unguiculata* L. Walp seed (LBS-1, LBS-2 and LBS-3) were analyzed. The physical characteristics such as specific gravity, refractive index, smoke point, flash point, fire point, cloud point solidification point and Pour point of the seed oil from the different varieties ranged from 0.91 to 0.932, 1.4719 to 1.4922, 220 to 221°C, 85.12 to 85.2°C, 91.99 to 92.10°C, 2.0 to 2.1°C, -8.4 to -8.43°C, -6.0 to -6.02°C, respectively. The chemical characteristics such as saponification value, saponification equivalent, iodine value, peroxide value, acid value, % of free fatty acid and unsaponifible matter ranged from 173.00 to 179.00, 277.12 to 280.62, 110 to112, 10.00 to 10.99, 1.3 to 1.6, 2.66 to 3.31 and 2.76 to 3.22 respectively. The oil was found to contain high levels of unsaturated fatty acids, especially linoleic acid (33.5-37.8) and linolenic acid (20.1-22.0). The dominant saturated acid was palmitic (19.0-20.1). In the light, the iodine value was dropped from 110 to 99, 112 to 101 and 111 to 100 for LBS-1, LBS-2 and LBS-3 respectively at the end of one month while the peroxide value was increased from 10.99 to 50.32, 10.21 to 47.35 and 10 to 48 for LBS-1, LBS-2 and LBS-3 respectively. The oil exhibited good physicochemical properties and could be useful as edible oils and in cosmetics industry.

Key words: Characterization, *Vigna unguiculata* L. Walp, Seed oil, Unsaturated fatty acid

Introduction

Seed oils are used in making soap, hair shampoo, alkyd resin, lubricants, paints, cosmetics, pharmaceuticals, drying agents, and other industrial purposes. They also play important roles in health benefits as antioxidant, antiseptic, antiviral, detoxifier, carcinogenic, depurative, diuretic, emenagogue, stimulant, cytophylactic, tonic etc.

Vigna unguiculata L. Walp (Bengali name: Barbati, English name: Cowpea), is an annual, warm season herbaceous legume (Davis et al 1991). It was reported to have originated in Africa, Asia and South America (Ng and Marechal 1985; Summerfield *et al.* 1985). It is one of the most important crops in terms of food value and wide adaptability. The seed contain 18.70-21.22% protein and 2.48%-3.03% oil (Ashraduzzaman *et al.* 2009; Mahadevappa & Piyara 1978, 1981; Onwuliri & Obu 2002). The seed is diuretic and used to strengthen the stomach. When boiled and eaten as a food it is considered to destroy worms in the stomach (Chopra *et al.* 1982). An infusion of seed can be taken orally to treat amenorrhea whilst powdered roots eaten with porridge are believed to treat

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painful menstruation, epilepsy and chest pain (Van Wyk & Gericke 2000). The seeds are cooked with the roots of other herbs to treat blood in urine and bilharzias (Nyazema, 1987; Kritzinger *et al.* 2004). Zia-ul-Haq *et al.* (2010) reported that *Vigna unguiculata* L. oil contains tocopherols which protect polyunsaturated fatty acids in cell membranes and lipoproteins from oxidation and are believed to play a preventive role in diseases associated with oxidative stress like cancer, cardiovascular diseases, cataracts, age-related mascular degeneration, central neurodegenerative diseases and diabetes mellitus (Brigelius-Flohe *et al.* 2002).

Oil have been extracted and characterized from many plant sources: *Cicer arietinum* seed (Zia-ul-Haq *et al.* 2007a), *Vigna radiate* seed (Zia-ul-Haq *et al.* 2008a), rubber seed (Abdullah and Salimon 2009), *Khaya senegalensis* (Desr.) *A. juss* seed (Ayo *et al.* 2007), grape seed (Pardo *et al.* 2009), *Terminalia catappa* seed (Nzikou *et al.* 2010), breadfruit seed (Ajiwe *et al.* 1995). Some research work also reported on the *Vigna unguiculata* L. Walp seed oil (Zia-ul-Haq *et al.* 2010) commonly grown in Pakistan, but data on extraction and characterization of Barbati (*Vigna unguiculata* L. Walp) seed oil locally grown in Bangladesh is not available. Therefore the aim of the present study was undertaken to reveal the extraction and characterization of oil from Barbati (*Vigna unguiculata* L. Walp) seed locally available in Bangladesh.

Materials and Methods

Ripe pods of barbati (*Vigna unguiculata* L. Walp) used for the work were collected in the year 2007 from the experimental plot located at Rajshahi city, Rajshahi, Bangladesh. The varieties reported herein, which were all cultivated in homogeneous conditions and differed morphologically from each other, were: LBS-1 (pods are short, straight and red; seeds are somewhat round, small, smooth and red), LBS-2 (pods are long, cylindrical and deep green; seeds are somewhat elongated, kidney shaped, and deep red) and LBS-3 (pods are short, somewhat curved and light green; seeds are small, kidney shaped, and red) commonly found in Bangladesh (Ashraduzzaman *et al.* 2009) (Fig 1).

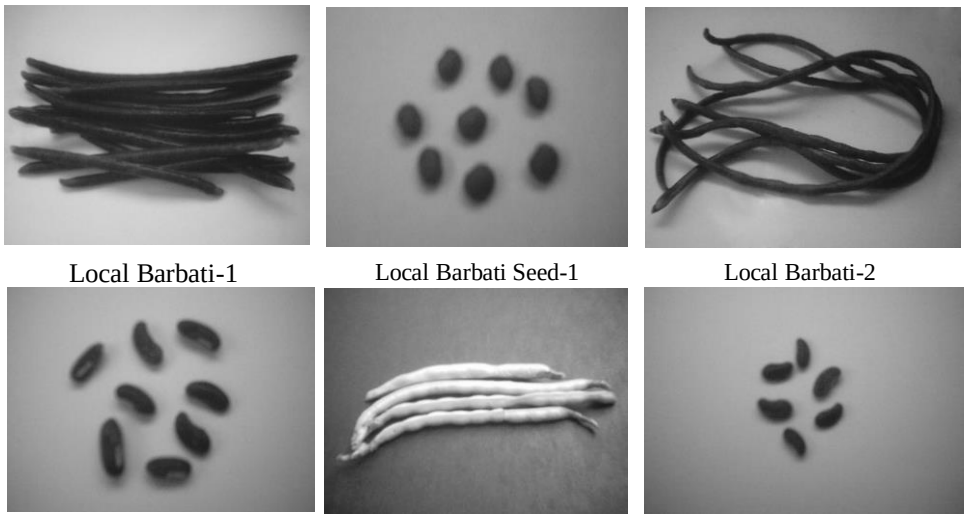

Fig.1. Photographs of three verities of Barbati pods (*Vigna unguiculata* L.) and their seeds.

The seeds were separated from the flesh of the fruits manually and washed several times with water to remove the foreign materials. Afterward, the seeds were dried in the sunlight for four consecutive days and again in an electric oven at 40°C until a constant weight were reached. The seeds were ground to a fine powder, packed and stored in a refrigerator at 4°C prior to analysis.

Oil Extraction

For oil extraction (Soxlhet method), 500g of ground seeds were placed into a cellulose paper cone and extracted using n Hexane in a 5-l Soxhlet extractor for 8 h according to Pena *et al.* (1992). The oil was then recovered by evaporating of the solvent using rotary evaporator (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60 °C for 1 h. All experiments were done in triplicates**,** and the mean and standard deviations were calculated.

Physical characteristics

The specific gravity of the sample oil was measured using specific gravity bottle following the procedures as described by Pearson, (1976). The refractive index was determined with calibrated Abbey refractometer using the method described by Dawodu and Omole (2000). The smoke point, flash point and fire point of different seed oils were determined according to the Official Methods of the American Oil Chemist's Society, (AOCS 1984). The cloud point and solidification points were determined according to ASTM (1952) standard Methods for lubricating oils.

Chemical characteristics

Saponification value, saponification equivalent and Iodine value were determined by using the standard method of AOAC (1984). The peroxide value, acid value and free fatty acid were determined by using the standard method of AOAC (1990).

The amount of unsaponifiable matter present in the oil was determined using the method as described by Device and Williams (1961). The quantity of unsaponifiable matter present in the 100 g of oil was calculated from the formula as described (Williams 1966).

Determinations of fatty acids

The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 µl of n-hexane to 50 mg of oil followed by 50 µl of sodium methoxide using the method of Cocks *et al.* (1966). The mixtures were vortex for 5 s and allowed to settle for 5 min. The top layer (1 µl) was injected into a gas chromatograph (Model GC- 14A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionization detector and a polar capillary column (BPX70 0.25), 0.32 mm internal diameter, 60 m length and 0.25 µm film thickness (SGE Incorporated, USA) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 240 °C and column temperature was 110 °C held for one minute and increased at the rate of 8 °C/min to 220 °C and held for one minute. The run time was 32 min. The fatty acid methyl esters peaks were identified by comparing their retention time with those of the standards. Percent relative fatty acid was calculated based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

Storage effect of V. unguiculata seed oil at different temperature

The oil samples of different varieties of *V. unguiculata* seed were stored in a temperature controlled refrigerator at 0°C, room temperature (25°C) and temperature controlled oven (at 50°C). The iodine value and peroxide value were measured after each one week intervals up to 4 weeks.

Results and Discussion

The oil obtained from *V. unguiculata* seeds had a light yellow color. The physical and chemical properties and fatty acid compositions are shown in Table 1-3.

The oils were showed to have specific gravity of 0.915 (LBS-1), 0.912 (LBS-2) and 0.91 (LBS-3) g/cm^3, which is comparable to the values reported in seeds of groundnut oil (0.918). The refractive index of the oils was ranged from 1.4719 to 1.4922 (Table-1). The value of this investigation is quite similar to that of the refractive index of sufflower seed oil (1.4735) and *mesua ferra* seed oil (1.469-1.4739) (Rafiquzzaman, *et al.* 2006; Abu Sayeed et al., 2004). Thermal properties, i.e. smoke point, flash point, fire point, cloud point, etc. of the oil samples of *V. unguiculata* seed do not appear to be significantly different from each other.

Table 1. Physical characteristics of different varieties of barbati seed oil.

Physical characteristics	Varieties		
	LBS–1	LBS–2	LBS–3
Specific gravity (at25^{0}C)	0.915±16^c	0.912±11^b	0.91±12^a
Refractive index (at 25^{0}C)	1.4922±22^c	1.4719±23^a	1.480±24^b
Smoke point (^{0}C)	220±22^a	221±21^a	220±02^a
Flash point (^{0}C)	85.12±12^a	85.2±11^a	85.14±32^a
Fire point (^{0}C)	92±51^a	92.10±16^a	91.99±19^a
Cloud point (^{0}C)	2.0±04^a	2.0±08^a	2.1±09^a
Solidification point (^{0}C)	-8.43±11^a	-8.42±10^a	-8.4±10^a
Pour point (^{0}C)	-6.0±19^a	-6.02±18^a	-6.012±18^a

LBS =Local Barbati Seed. Values are mean ± standard deviation of three experiments. Mean in the same row with different superscripts are significantly (P <0.05) different.

As shown in Table 2 the saponification numbers of all varieties of *V. unguiculata* seed oil were lower than 200 with low acid values (1.3-1.6) and high iodine numbers (102-112). These results suggest that the experimental seed oils have higher oxidative stability and protection during storage and processing. The high iodine values are due to its high contents of unsaturated fatty acids. These results are similar with those of Zia-ul-Haq *et al*., (2007b, 2008b, and 2010) who found approximately similar profile in seed oil of different leguminous crops such as, *Cicer arietinum*, *Vigna radiate* and *V. unguiculata*. The free fatty acid value of the oils lowers than 4, as % oleic acid indicate that these oils are very much stable.

Table 2. Chemical characteristics of different varieties of barbati seed oil.

Chemical characteristics	Varieties		
	LBS–1	LBS–2	LBS–3
Saponification value	177.00±3^b	179.00±21^c	173.00±12^a
Saponification equivalent	280.62±01^c	280.62±13^b	277.12±11^a
Iodine value (Hanus method)	110±22^b	112±23^c	111±22^a
Peroxide value m.eq.o$_2$/Kg oil	10.99±10^c	10.21±11^b	10.00±14^a
Acid value	1.4±44^b	1.6±23^c	1.3±34^a
%FFA(as oleic)	2.66±12^b	3.31±14^c	2.87±13^a
Unsaponifible matter (%)	2.76±10^a	3.22±11^c	2.90±15^b

LBS =Local Barbati Seed. Values are mean ± standard deviation of three experiments. Mean in the same row with different superscripts are significantly (P <0.05) different.

The fatty acids composition of the oils from the three different varieties of Barbati seed (Table 3) as analyzed by GC, showed that the saturated fatty acids present in the oil samples were mainly palmitic acid (19.00% to 20.1%) and stearic acid (5.00% to 6.00%). The unsaturated fatty acids present in the oil sample were in the proportion of linoleic acid (33.50% to 38.80%) followed by linolenic acid (20.1 % to 22.00%) and there after oleic acid (13. to 16%). The present data indicated that the barbati seed oil might also be used as a edible oil as it contains higher amount of unsaturated fatty acids and their contents were found to be varied between 66.82-77.00% and their acid values are significantly low These findings are also similar with Zia-ul-Haq *et al.* (2010).

Table 3. Fatty acid composition of different varieties of barbati seed oil.

Fatty acids	Varieties		
	LBS–1	LBS–2	LBS–3
Palmitic acid ($C_{16:0}$)	20.1 ± 11^{c}	19.7 ± 22^{b}	19.0 ± 0.22^{a}
Stearic acid ($C_{18:0}$)	5.0 ± 0.3^{c}	6.0 ± 0.2^{b}	5.5 ± 0.6^{a}
Oleic acid ($C_{18:1}$)	15.1 ± 0.11^{b}	16.2 ± 1.3^{c}	13.2 ± 0.4^{a}
Linoleic acid ($C_{18:2}$)	34.0 ± 0.2^{b}	37.8 ± 0.9^{c}	33.5 ± 0.1^{a}
Linolenic acid ($C_{18:3}$)	20.1 ± 0.11^{b}	22.0 ± 0.12^{c}	20.12 ± 0.14^{b}

LBS =Local Barbati Seed. Values are mean ± standard deviation of three experiments. Mean in the same row with different superscripts are significantly (P <0.05) different.

The effect of temperature at different storage periods of LBS-1, LBS-2 and LBS-3 on the iodine and peroxide value were shown in table 4-6. The data clearly indicated that the storage of the oils at refrigerator and at 25°C in the dark had very much little effect on the iodine value and peroxide value after 1-week and this trend is continued even after storage for four weeks. On the other hand, if the oils were storage at 50°C these values are changed significantly after 4-weeks. This result indicates the considerable loss of unsaturation in oil by photo-catalyzed reactions, suggesting that the oil is better stored at 25°C in the dark (Table 4).

Table 4. Effect of storage under different conditions on the Iodine value and peroxide values of barbati seed oil (LBS-1).

Observation	Method of storage	Iodine value	Peroxide value
Control	Refrigerated	110±0.22	10.99±0.10
	Storage at 25°C in dark	110±0.22	10.99±0.10
	Storage at 50°C in light	110±0.22	10.99±0.10
After one week	Refrigerated	109±0.11	11.50±0.22
	Storage at 25°C in dark	109±0.24	11.30±0.21
	Storage at 50°C in light	107±0.19	20.00±0.34
After two weeks	Refrigerated	108±0.25	12.00±0.39
	Storage at 25°C in dark	108±0.33	12.35±0.42
	Storage at 50°C in light	105±0.34	25.12±0.40
After three weeks	Refrigerated	107±0.22	12.30±0.31
	Storage at 25°C in dark	106±0.13	12.44±0.30
	Storage at 50°C in light	103±0.30	30.00±0.24
After four weeks	Refrigerated	105±0.42	12.38±0.43
	Storage at 25°C in dark	106±0.32	12.44±0.40
	Storage at 50°C in light	99±0.22	50.32±0.42

Values are mean ± standard deviation of three experiments.

The increase in the corresponding peroxide values is much greater for the oil when stored in the light and also suggests a high level of photo-catalyzed oxidation of the oil. Both the iodine and peroxide value of oils stored at 25ºC in the dark and refrigerator do not change significantly. Similar results were also reported (Kyari, 2008).

Table 5. Effect of storage under different conditions on the Iodine value and peroxide values of barbati seed oil (LBS-2).

Observation	Method of storage	Iodine value	Peroxide value
Control	Refrigerated	112±0.23	10.21±0.11
	Storage at 25ºC in dark	112±0.23	10.21±0.11
	Storage at 50ºC in light	112±0.23	10.21±0.11
After one week	Refrigerated	111±0.11	11.00±0.21
	Storage at 25ºC in dark	111±0.34	11.10±0.20
	Storage at 50ºC in light	109±0.18	19.30±0.33
After two weeks	Refrigerated	110±0.25	12.00±0.34
	Storage at 25ºC in dark	110±0.33	12.04±0.41
	Storage at 50ºC in light	108±0.44	24.22±0.39
After three weeks	Refrigerated	109±0.32	12.27±0.31
	Storage at 25ºC in dark	108±0.13	12.37±0.30
	Storage at 50ºC in light	105±0.30	29.20±0.24
After four weeks	Refrigerated	106±0.52	12.22±0.43
	Storage at 25ºC in dark	106±0.32	12.34±0.50
	Storage at 50ºC in light	101±0.22	47.35±0.62

Values are mean ± standard deviation of three experiments.

Table 6. Effect of storage under different conditions on the Iodine value and peroxide values of barbati seed oil (LBS-3).

Observation	Method of storage	Iodine value	Peroxide value
Control	Refrigerated	111±0.22	10.00±0.14
	Storage at 25ºC in dark	111±0.22	10.00±0.14
	Storage at 50ºC in light	111±0.22	10.00±0.14
After one week	Refrigerated	110±0.11	11.20±0.21
	Storage at 25ºC in dark	110±0.24	11.00±0.25
	Storage at 50ºC in light	107±0.19	20.20±0.30
After two weeks	Refrigerated	109±0.21	12.00±0.37
	Storage at 25ºC in dark	109±0.32	12.35±0.32
	Storage at 50ºC in light	106±0.32	26.12±0.41
After three weeks	Refrigerated	107±0.20	12.35±0.30
	Storage at 25ºC in dark	106±0.23	12.74±0.60
	Storage at 50ºC in light	103±0.31	32.00±0.22
After four weeks	Refrigerated	104±0.42	12.18±0.33
	Storage at 25ºC in dark	105±0.31	12.14±0.43
	Storage at 50ºC in light	100±0.12	48.00±0.41

Values are mean ± standard deviation of three experiments.

Conclusions

The result of characterization of *Vigna unguiculata* L. Walp seed oil showed that the oil could be utilized successfully as a source of edible oil. It contains high amount of unsaturated fatty acids. High unsaponifiable matters content (2.76-3.22%) guarantees the

use of the oils in cosmetics industry. The production of this oil could be economic benefit to Bangladesh or the other countries where the *Vigna unguiculata* L. Walp is cultivated widely.

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