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Comparative Study on Characteristics of Seed Oils and Nutritional Composition of Seeds from Different Varieties of Tobacco (*Nicotiana tabacum* L.) Cultivated in Bangladesh

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Abstract: The objectives of this study are to determine characteristics of seed oils and nutritional compositions of seeds from three varieties (Gidri, Virginia and Jati.) of tobacco. Present results revealed specific gravity (0.9235-0.9296), refractive index (1.4763-1.4815), pour point [-17-(-18)°C], flash point (135-142°C), fire point (154-162°C), cloud point [-15-(-18)°C], smoke point (215-230°C), cetane index (38.2-42.8), iodine value (134.37-138.20), saponification value (185.05-189.11), saponification equivalent (296.65-303.17), acid value (3.02-4.23), free fatty acid (1.51-2.12%), ester value (182.03-184.88), unsaponifiable matter (1.39-1.45%), peroxide value (1.77-2.56) and Reichert-Meissl value (0.38-0.45). No significant differences ($p<0.05$) were observed among the varieties for refractive index, pour point, ester value, unsaponifiable matter and Reichert-Meissl value. High values ($p<0.05$) for thermal properties flash point, fire point and smoke point were found in Gidri seed oil while Jati seed oil had highest cetane index. Iodine and peroxide values were low in Gidri seed oil compared to other varieties. Jati had the lowest in saponification and acid values. Glyceride classes were estimated to be monoglycerides (1.01-1.15%), diglycerides (3.75-6.39%) and triglycerides (88.65-91.39%) whereas lipid classes to be neutral lipid (83.10-85.02%), glycolipid (7.16-11.36%) and phospholipid (5.15-6.98%). No significant differences ($p<0.05$) were observed among the varieties for triglyceride and neutral lipid contents. Saturated and unsaturated fatty acids present in the oils were separated and amounted to be (11.01-14.32%) and (82.73-86.75%), respectively, depending upon the varieties. GLC analysis showed the presence of significantly different ($p<0.05$) percentage of fatty acids from series palmitic ($C_{16:0}$) to arachidic ($C_{20:0}$). Linoleic acid was the principal ranging from 67.40-72.10%. Among the varieties Jati seed oil was highest in unsaturated fatty acids. All varieties contained large amounts of lipid (42.29-45.72%), protein (19.21-21.05%), crude fiber (14.58-16.89%) and other essential nutrients. Except for the ash content, there were significant differences ($p<0.05$) in the levels of all parameters among the sample tested. The knowledge of the present studies on different varieties of tobacco seeds could be important to its appropriate industrial use and for improvement in the nutritional value.

Key words: Tobacco, seed oil, fatty acid, nutritional composition

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INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is a commercial crop that is grown in limited areas, mostly Rangpur, Kushtia and Dinajpur districts of Bangladesh such that the economy of the people of these districts is dependent on the cultivation and marketing of tobacco leaf. Tobacco seeds are light brown to black in color, tiny and tough in texture. Tobacco seeds are the waste product of the tobacco leaf industries. Every year huge quantities are being disposed of but these seeds were found to be potentially rich in oil, 32.5% reported elsewhere (Rahman *et al*., 1997) which is higher than that contained in many plant sources (Daramola and Adegoke, 2007; Amoo *et al*., 2006; Applcquist *et al*., 2006; Serap *et al*., 2002). The nutritional value of tobacco seed oil is better than groundnut and cotton seed oils and comparable to safflower oil. Refined tobacco seed oil is used as an edible oil in some European countries (Talaqani *et al*., 1986). Fats and oils are also very important indigenous raw materials for many edible and non-edible purposes. The physico-chemical properties of fats or oils are directly related to their glyceride composition and chemical constitution (Abu Sayeed *et al*., 2004). Hence knowledge of these compositional factors is important in connection with research aimed at finding alternative uses for tobacco seeds and the important of these fat and fat products for specific uses.

Frega *et al*. (1991) studied the chemical composition of different varieties (Bright Italia, Kentucky 104 and Bright V) of tobacco seeds. Talaqani *et al*. (1986) investigated the fatty acids composition of the seed oil of certain tobacco varieties cultivated in Northern Iraq. Moreover, researches have been carried out on the characterization of seed oil and nutritional compositions of tobacco seeds (Mukhtar *et al*., 2007; Giannelos *et al*., 2002; Waheed *et al*., 2001; Patel *et al*., 1998; Rahman *et al*., 1997), but studies on different varieties of tobacco cultivated in Bangladesh are few. The present study was conducted to determine physico-chemical characteristics and fatty acid composition of seed oil and compare some important nutrients found in three varieties (Gidri, Virginia and Jati) of tobacco seeds cultivated in an experimental plot located at Nilphamari district, Bangladesh.

MATERIALS AND METHODS

Collection and Processing of Plant Specimens

Tobacco seeds were collected in March, 2005 from ripe fruits of the plant grown in an experimental plot located at Jaldhaka in the district of Nilphamari, Bangladesh. The varieties reported herein, which were all cultivated in homogeneous conditions and were varied morphologically from each other, were Gidri, Virginia and Jati. The seeds were separated from the fruits and washed several times with water to remove the foreign materials. Afterwards the seeds were dried in the sunlight for four consecutive days and then in an electric oven at 40°C until a constant weight was reached. The seeds were ground to a fine powder and stored in a refrigerator at 4°C prior to the analysis. All chemicals and biochemicals used were of analytical grade unless otherwise specified and results were expressed on dry weight basis.

Analysis of Tobacco Seed Oil

The oil from the powdered seeds was extracted with light petroleum ether (40-60°C) in a soxhlet apparatus for about 24 h and the solvent was removed by rotary vacuum evaporator. The crude oil thus obtained was fractionated in a column packed with neutral alumina in petroleum ether using petroleum ether-diethylether (70:30) as the eluting solvent and the percentage of oil content was computed.

Physical and Chemical Characteristics

The specific gravity of the oil was determined at 28°C with the help of a pycnometer. Refractive index of the clear oil was determined at 28°C using Abbe Refractometer following IUPAC method

(IUPAC, 1979). ASTM testing methods (ASTM, 1958) were followed for determining pour point, flash point, fire point, cloud point and cetane index. Smoke point was estimated according to the methods of AOCS (1980). Iodine value was determined according to the method of Wijs while percentage of unsaponifiable matter and Reichert-Meissl value were determined according to the method of Ranganna (1986). Saponification value, saponification equivalent, acid value, percentage of Free Fatty Acid (FFA), ester value and peroxide value were determined according to the methods described by Williams (1966).

Separation of Glycerides

The oil was separated into mono-, di- and triglycerides by silica gel (70-230 mesh) column chromatography. The solvent systems used to elute the column were similar to those described by Gofur *et al.* (1993). For quantitative determination of glyceride classes, the sample (800 mg in 4.0 mL petroleum ether) was adsorbed on the top of the column and triglycerides were eluted with benzene, diglyceride with a mixture of diethyl ether and benzene (1:9, v/v) and monoglyceride with diethyl ether. Approximately 1.5-2 mL min^{-1} fractions were collected. Elution was monitored by TLC. The purity of the separated fractions was confirmed by TLC, using silica gel and hexane-diethyl ether 80/20 (v/v) as solvent system. Spots were visualized with chromic-sulphuric acid at 180°C. The weight percentage of each glyceride class was determined by gravimetric method. Diglycerides were calculated by subtracting the weight of Free Fatty Acid (FFA) as determined by standard method (Williams, 1966) from the weight of diglyceride fraction.

Fractionation of Lipids

A total of 740 mg of lipids extracted from tobacco seeds by the method of Bligh and Dyer (1959) was fractionated into three major lipid groups: neutral lipids, glycolipid and phospholipid by silica gel column chromatography. Neutral lipids were eluted with diethyl ether, glycolipids with acetone and phospholipids with methanol (Rouser *et al*., 1967). Approximately 0.5-1.0 mL fractions were collected per minute and elution was monitored by TLC. Solvents were evaporated by vacuum rotary evaporator. Lipids in different classes were identified by comparing their R_f values with those of standards and percentages of these fractions were determined by gravimetric method.

Separation of Saturated and Unsaturated Fatty Acids

Separation of saturated and unsaturated fatty acids from about 48 g of oil was carried out by lead-salt ether method (Abu Sayeed *et al*., 2004; Williams, 1966). Briefly, the oil was saponified with alcoholic caustic soda to obtain soap solution. A slight excess of lead acetate solution was added to the soap solution to form lead salts of fatty acids, which were then separated. Ether was added to the mixture of lead salts and the whole mixture was warmed and then cooled at 0°C for 24 h. The precipitated lead salts of saturated fatty acids so formed were separated from the solution of lead salts of unsaturated fatty acids by filtration. The lead salts of the unsaturated fatty acids were obtained by removing the ether from the ethereal solution. Each group of lead salts was suspended in water and treated with sufficient hydrochloric acid to form fatty acids and lead chlorides. The mixture was then extracted with ether which was subsequently evaporated to obtain the different fatty acids.

Fatty acid Composition of Oil

Fatty acid composition of tobacco seed oil was determined as their methyl esters prepared by boron-trifluoride methanol complex method (Morrison and Smith, 1964). A GCD PYE Unicam gas chromatograph equipped with a flame ionization detector was used to determine the fatty acid methyl esters. Nitrogen carrier gas was used at a flow rate of 30 mL min^{-1}. Fatty acids were separated on a 1.8 m×2 mm i.d. glass column packed with 6% BDS (butanediol succinate polyesters) on solid support

Anakorm ABS (100/120) mesh. Analysis was carried out at isothermal column temperature 190°C, injector and detector temperatures for all GLC analysis were 230°C. The peaks were identified by comparison with standard fatty acid methyl esters.

Analysis of Tobacco Seed

Moisture, ash and crude fiber contents were determined by AOAC methods (AOAC, 1990). Lipid content was estimated by the method of Bligh and Dyer (1959) using a solvent mixture of chloroform and methanol (2:1 v/v). Total protein content determined by the micro-Kjeldahl method (AOAC, 1990) and calculated from total nitrogen using the formula: N×6.25. Water soluble protein was determined by the method of Lowry (1951) using bovine serum albumin as the standard. Starch content (Clegg, 1956) and total carbohydrate (Rahim, 1999) were also measured.

Statistical Analysis

All data were expressed as the mean and Standard Deviation (SD) of three experiments and were subjected to one way analysis of variance (ANOVA). Mean values were compared at $p<0.05$ significant level by Duncan's multiple range test using SPSS 11.5 software package.

RESULTS AND DISCUSSION

Solvent extraction of three different varieties (Gidri, Virginia and Jati) of tobacco seeds yielded an average of about 29.82% oil, which is slightly lower than the value of 32.5% reported by Rahman *et al.* (1997). Information on detailed characteristics of seed oil and nutritional compositions of seeds from other plant sources are too scanty for meaningful comparisons.

As can be seen from Table 1, specific gravities of the tobacco seed oils (0.9235-0.9296 at 28°C) are very close to the value obtained for *Mesua ferrea* seed oil which is 0.9287-0.9312 at 31°C (Abu Sayeed *et al*., 2004). Refractive indices of the oils in the present investigations were found to be 1.4763-1.4815 at 28°C which were higher compared to that obtained for *Pentaclethra marcrophylla* (1.4650 at 26°C) (Odoemelam, 2005) and *Mesua ferrea* (1.4690-1.4739 at 30°C) (Abu Sayeed *et al*., 2004) seed oils. The experimental values are in good agreement with the values 1.4740-1.480 at 28°C reported by Gofur *et al*. (1993) and 1.4732-1.4737 at 25°C reported by Rahman *et al*. (1997) for the same oil. Specific gravity and refractive index are very stable parameters and should be used for checking the identity of oils. Highest values are obtained with increasing degree of unsaturation, as well as with larger molecular weights (Deuel, 1951; Peach and Tracey, 1955). No significant difference ($p<0.05$) in refractive index and pour point was found for the different seed oil samples. Flash, fire and smoke points of Gidri seed oil appeared to be significantly higher ($p<0.05$) than those of the rest samples. Cloud point and cetane index were found to be significantly lower and higher ($p<0.05$), respectively in the sample of Jati seed oil. Smoke, fire and flash points of a fatty material are measures of its thermal stability when heated in contact with the air. Fatty acids are much less stable than glycerides; hence the smoke, fire and flash points of ordinary oils depend principally upon their content of free fatty acids (Mattil Karl *et al*., 1964).

As shown in Table 2, iodine values of the tobacco seed oils were determined to be 134.37-138.20, which were similar to the values 137.10-138.00 (Rahman *et al*., 1997) and 136.90-137.90 (Gofur *et al*., 1993) for the same seed oil and lower than the values 161.95 for *Pentaclethra marcrophylla* (Odoemelam, 2005) and 144.57 for *Psophocarpus tetragonolobus* (Amoo *et al*., 2006) seed oils. Specific gravity and iodine value were all characteristic of highly unsaturated oils and significantly lower ($p<0.05$) values obtained for Gidri seed oil indicated lower content of unsaturated fatty acids in this oil compared to those contained in the other samples. In general, the greater the degree of unsaturation i.e., the higher the iodine value, the greater is the risk of the oil or fat to become rancid by

Table 1: Physical characteristics of three varieties (Gidri, Virginia and Jati) of tobacco seed oil

	Varieties		
Characteristics	Gidri	Virginia	Jati
Specific gravity at 28°C	0.9235±0.001158[a]	0.9279±0.000779[b]	0.9296±0.000963[b]
Refractive index at 28°C	1.4763±0.004802[a]	1.4781±0.001651[a]	1.4815±0.002794[a]
Pour point (°C)	-18±0.0.41[a]	-17±0.72[a]	-17±0.37[a]
Flash point (°C)	142±0.81[b]	138±1.41[a]	135±1.41[a]
Fire point (°C)	162±1.41[b]	154±1.41[a]	155±0.81[a]
Cloud point (°C)	-15±0.08[b]	-16±0.67[b]	-18±0.88[a]
Smoke point (°C)	230±0.81[b]	215±1.41[a]	218±1.41[a]
Cetane index	40.5±1.08[b]	38.2±0.90[a]	42.8±0.59[c]

Values are mean±standard deviation of three experiments. Means in the same row with different superscripts are significantly ($p<0.05$) different

Table 2: Chemical characteristics of three varieties (Gidri, Virginia and Jati) of tobacco seed oil

	Varieties		
Characteristics	Gidri	Virginia	Jati
Iodine value (Wijs)	134.37±1.23[a]	138.00±1.41[b]	138.20±0.63[b]
Saponification value (mg KOH g^{-1})	188.21±1.50[b]	189.11±0.68[b]	185.05±1.25[a]
Saponification equivalent	298.09±2.37[a]	296.65±1.07[a]	303.17±2.04[b]
Acid value ((mg KOH g^{-1})	3.65±0.17[b]	4.23±0.12[c]	3.02±0.11[a]
Free fatty acids (%) as oleic	1.83±0.09[b]	2.12±0.06[c]	1.51±0.05[a]
Ester value	184.56±1.40[a]	184.88±0.58[a]	182.03±1.34[a]
Unsaponifiable matter (g/100 g)	1.41±0.03[a]	1.45±0.03[a]	1.39±0.03[a]
Peroxide value (mEq kg^{-1})	1.77±0.02[a]	2.01±0.06[b]	2.56±0.0.05[c]
Reichert-Meissl value	0.38±0.03[a]	0.40±0.02[a]	0.45±0.03[a]

Values are mean±standard deviation of three experiments. Means in the same row with different superscripts are significantly ($p<0.05$) different

oxidation (Egan *et al*., 1981). Therefore, seed oil from Gidri tobacco, in contrast to other varieties in the present investigations has lower tendency to become rancid by oxidation. Saponification values of the oils in the three samples were in the range 185.05-189.11 whereas saponification equivalents were calculated from saponification values to be 296.65-303.17. The present saponification values are in good agreement with 190.34 (Amoo *et al*., 2006) for *Psophocarpus tetragonolobus* seed oil and with the reported value 189.20-190.50 (Rahman *et al*., 1997) for the same oil. Significantly lower ($p<0.05$) saponification value was found in the sample of Jati seed oil. The esters of the low molecular weight fatty acids require the most alkali for saponification, so that the saponification value is inversely proportional to the mean of the molecular weights of the fatty acids in the glycerides present. Since many oils have somewhat similar values, the saponification value is not, in general, as useful for identification purposes as the iodine value(Egan *et al*., 1981). Moreover, saponification value outside the range of 190-200 indicates that the fatty acid has a mass higher or lower than the average size of the more common fats (Williams, 1966). These comparatively low saponification values as reckoned, indicate the presence of higher proportion of higher molecular weight fatty acids. The percentage of free fatty acids (1.51-2.12) from the three varieties of tobacco seed oil was similar to the value 1.90-2.30 cited in the literature (Rahman *et al*., 1997) for the same oil. Ester values of oils in the three samples were calculated as 182.03-184.88 from acid value and saponification value, which were not significantly different ($p<0.05$) in all reported samples.

Tobacco seed oils in the samples of different varieties contained unsaponifiable matters 1.39-1.45%, which were close to that obtained for *Mesua ferrea* seed oil that is 1.44-1.50% (Abu Sayeed *et al*., 2004), but lower than those obtained for *Psophocarpus tetragonolobus* (1.63%) and *Orchid fruit myristica* (1.79%) seed oils (Amoo *et al*., 2006). Amounts of unsaponifiable matters under the present investigations were lower than the value 1.57-1.60% for the same seed oil reported by Gofur *et al*. (1993). Unsaponifiable matter includes hydrocarbons, higher alcohols and sterols

(e.g., cholesterol, phytosterol). Most oils and fats of normal purity contain less than 2% of unsaponifiable matter (Egan *et al*., 1981). No significant inter-variety differences ($p<0.05$) in the level of unsaponifiable matters were observed. The oils from the three varieties of tobacco seeds gave peroxide values of 1.77-2.56 mEq kg^{-1} which were determined in normal laboratory conditions. Fresh oils usually have peroxide values well below to 10 mEq kg^{-1}. A rancid taste often begins to be noticeable when the peroxide value is in between 20 and 40 mEq kg^{-1}. In interpreting these data, however, it is necessary to take into account the particular oil or fat involved (Egan *et al*., 1981). Lower peroxide value as obtained indicating that tobacco seed oils are quality oil. Reichert-Meissl values of the samples were assayed as 0.38-0.45, which were much lower than the values 5.85-6.03 obtained for *Mesua ferrea* (Abu Sayeed *et al*., 2004) seed oil. At $p<0.05$, no significant difference in the Reichert-Meissl values could be detected for all samples tested.

The total amount of oil was separated into mono-, di- and triglyceride fractions by means of column chromatography and the results are shown in Table 3. The triglycerides content varied from 88.65 to 91.39%, while diglycerides from 3.75 to 6.39% and monoglycerides from 1.01 to 1.15%. Significantly higher ($p<0.05$) amounts of diglycerides (6.39%) were detected in the Jati tobacco seed oil. Moreover, the total recovery of glyceride was about 96.12% (average) indicating that tobacco seed oils contained lower amount of nonglyceride than that contained in *Mesua ferrea* seed oil (Abu Sayeed *et al*., 2004). No significant difference ($p<0.05$) in triglyceride compositions to account for about 90.15% (average) of the total weight of oil, among the three samples were observed; moreover it was almost similar to the values reported by other authors (Gofur *et al*., 1993). Mono and particularly diglycerides occur naturally in oils and fats, where their presence is initially due to partial hydrolysis of the oil by enzyme action in the fruit or seed. Monoglyceride are surface-active materials, that is to say they have both polar, water-soluble and non-polar, fat-soluble groups. It is for this reason that the higher monoglycerides are of great importance as emulsifiers in the food industry. They are particularly valuable for producing stable oil-in-water emulsions and they are also crystal promoters. Thus, a fat containing a small amount of monoglyceride will set quickly to a micro-crystalline matrix. This property makes the monoglycerides useful in preventing oil exudation from fatty materials (Devine and Williams, 1961). Gofur *et al*. (1993) reported that tobacco seed oil contained an average of 2.29% monoglyceride which were higher than that found out in the present work. This variation may be attributed to the seed sources including location, variety, cultural practice during production, soil type or a combination of two or more of these factors.

Table 4 shows the percentages of the neutral lipids, glycolipids and phospholipids. Neutral lipids account for 83.10-85.02% of total lipids while only 7.16-11.36% glycolipids were detected. Phospholipids make up 5.15-6.98% of total lipids. No significant difference in the neutral lipid content of the tobacco seed oils studied was observed. However, the contents of glycolipids and phospholipids from these seeds were found to be significantly different ($p<0.05$). Neutral lipids were found to be the most abundant component of seed lipid. Significantly ($p<0.05$) greater amounts of glycolipids and lower amount of phospholipids were recorded in the sample of Gidri tobacco than those contained in other varieties. In this study, tobacco seed lipid were found to contain lower content of neutral lipids and higher contents of glycolipids and phospholipids compared with the results obtained for *Mesua ferrea* seed lipid (Abu Sayeed *et al*., 2004).

The saturated and unsaturated fatty acids present in the tobacco seed oils were separated by lead salt ether method. The result showed that saturated and unsaturated fatty acid content varied from 11.01-14.32% and 82.73-86.75%, respectively, depending upon the varieties analyzed herein (Table 5). Significantly higher ($p<0.05$) amounts of saturated fatty acids (14.32%) in Gidri seed oil and of unsaturated fatty acids (86.75%) in the Jati seed oil were detected.

Table 6 shows that tobacco seed oils contain both saturated and unsaturated fatty acid ranging from $C_{16:0}$ to $C_{20:0}$. The predominant fatty acids were linoleic, oleic and palmitic. The minor fatty acids

Table 3: Glyceride composition (weight %) of three varieties (Gidri, Virginia and Jati) of tobacco seed oil

Varieties	Monoglyceride	Diglyceride	Triglycerides
Gidri	1.01±0.03^{a}	4.50±0.21^{b}	90.41±1.27^{a}
Virginia	1.15±0.06^{b}	3.75±0.08^{a}	91.39±0.91^{a}
Jati	1.12±0.03^{ab}	6.39±0.19^{c}	88.65±1.23^{a}

Values are mean±standard deviation of three experiments. Means in the same column with different superscripts are significantly ($p<0.05$) different

Table 4: Lipid composition (weight %) of three varieties (Gidri, Virginia and Jati) of tobacco seed lipid

Varieties	Neutral lipids	Glycolipids	Phospholipids
Gidri	83.10±0.54^{a}	11.36±0.90^{c}	5.15±0.20^{a}
Virginia	83.70±1.12^{a}	9.78±0.26^{b}	5.53±0.05^{b}
Jati	85.02±1.31^{a}	7.16±0.26^{a}	6.98±0.08^{c}

Values are mean±standard deviation of three experiments. Means in the same column with different superscripts are significantly ($p<0.05$) different

Table 5: Fatty acids (saturated and unsaturated) of three varieties (Gidri, Virginia and Jati) of tobacco seed oil

Varieties	Saturated fatty acids (%)	Unsaturated fatty acids (%)
Gidri	14.32±0.58^{b}	82.73±0.61^{a}
Virginia	12.20±0.43^{a}	84.98±1.15^{ab}
Jati	11.01±0.81^{a}	86.75±1.24^{b}

Values are mean±standard deviation of three experiments. Means in the same column with different superscripts are significantly ($p<0.05$) different

Table 6: Fatty acid composition (%) of three varieties (Gidri, Virginia and Jati) of tobacco seed oil

	Fatty acid composition (%)		
Fatty acid	Gidri	Virginia	Jati
Palmitic acid (C_{16}: 0)	10.90±0.28^{b}	9.85±0.35^{ab}	8.81±0.40^{a}
Stearic acid (C_{18}: 0)	3.50±0.06^{c}	3.24±0.03^{b}	2.78±0.05^{a}
Oleic acid (C_{18}: 1)	16.60±0.70^{b}	16.40±0.74^{b}	14.52±0.40^{a}
Linoleic acid (C_{18}: 2)	67.40±0.95^{a}	69.21±1.30^{a}	72.10±0.86^{b}
Linolenic acid (C_{18}: 3)	1.30±0.04^{b}	1.10±0.06^{a}	1.50±0.03^{c}
Arachidic acid (C_{20}: 0)	0.20±0.01^{a}	0.20±0.02^{a}	0.29±0.01^{b}

Values are mean±standard deviation of three experiments. Means in the same row with different superscripts are significantly ($p<0.05$) different

Table 7: Nutritive compositions of three varieties (Gidri, Virginia and Jati) of tobacco seed

	Varieties		
Parameters (%)	Gidri	Virginia	Jati
Moisture	5.08±0.13^{a}	5.28±0.12^{a}	5.71±0.04^{b}
Lipid	43.36±0.82^{a}	45.72±1.25^{b}	42.29±0.66^{a}
Ash	3.46±0.04^{a}	3.31±0.02^{a}	3.39±0.10^{a}
Total protein	20.50±0.37^{ab}	19.21±0.68^{a}	21.05±0.82^{b}
Water soluble protein	7.98±0.06^{b}	7.01±0.09^{a}	8.01±0.32^{b}
Starch	4.08±0.12^{a}	4.48±0.13^{b}	4.59±0.10^{b}
Crude fiber	15.12±0.71^{a}	16.89±0.36^{b}	14.58±0.45^{a}
Total carbohydrate	12.48±1.87^{b}	9.59±2.1932^{a}	12.98±0.74^{b}

Values are mean±standard deviation of three experiments. Means in the same row with different superscripts are significantly ($p<0.05$) different

were stearic, linolenic and arachidic. No significant differences ($p<0.05$) were detected in the oleic, linoleic and arachidic acid content of two varieties Gidri and Virginia. The saturated fatty acids, palmitic and stearic acids were found to be highest ($p<0.05$) in Gidri seed oil. This result does not completely agree with some reported works (Gofur *et al*., 1993; Frega *et al*., 1991; Talaqani *et al*., 1986). The difference in our results could be explained by variations in soil and climatic conditions (Egan *et al*., 1981) that could alter fatty acid content in these oils. We have found some differences between the samples studied, regarding the content in fatty acids.

Polyunsaturated fatty acids are very important for human nutrition. Linoleic acid was found to be the predominant polyunsaturated fatty acid in tobacco seed oil ranging from 67.40 to 72.10%; it

was also detected as the highest polyunsaturated fatty acid in Jati variety, while linolenic acid ranged from 1.10 to 1.50. The content of linoleic acid detected in tobacco seed oil was much higher compared to many seed oils such as *Mesua ferrea* (13.68%) (Abu Sayeed *et al*., 2004), Chetoui olives (14.3-20.40%) (Temime *et al*., 2006) and *Dracunculus vulgaris* (23.21%) (Serap *et al*., 2002). Linoleic acid is an essential fatty acid for humans. GLC data also indicated that tobacco seed oils contained mainly unsaturated fatty acids at 85.50-88.41%, while saturated fatty acids were found to be present at 11.59-14.40%. The saturated/unsaturated fatty acids ratio of the oils ranged from 0.0.1310 to 0.1684 in all samples; however, Jati tobacco seed oil displayed higher unsaturation comparing to the other oils with a saturated/unsaturated fatty acids ratio of only 0.1310. These ratios indicate that the samples have a high content in unsaturated fatty acids, which could increase to autoxidation and polymerization, resulting in cross-linked and tough films upon exposure to air. Hence the oil could have industrial applications.

It was found that tobacco seeds contained 5.08-5.71% moisture, which were similar to *Pentaclethra marcrophylla* seed (5.30%) (Odoemelam, 2005), but slightly lower than *Garcinia kola* seed (6.30%) (Daramola and Adegoke, 2007). To obtain a product that can be stored for a long time and diminish the probability of the bacterial and fungal agents that could alter the quality through decomposition, the moisture content is important in the preservation of these oils (Aguilera Morales *et al*., 2005). These values (5.08-5.71%) are considered satisfactory for the preservation of this tobacco seed. Among the samples significantly higher ($p<0.05$) amount of moisture content was found in Jati tobacco seed. Tobacco seeds contained total lipids of 42.29-45.72%, which were similar to the reported value 41.30% for the same seed and were lower than the value 53.90% of *Sesamum indicum* seed (Javed Akhtar *et al*., 2000). Ash contents ranged from 3.31-3.46% and these were lower than *Psophocarpus tetragonolobus* seed (4.91%) (Amoo *et al*., 2006) and *Phaseolus coccineus* seed (4.60%) (Aremu *et al*., 2006). Of the three varieties, significantly higher amounts ($p<0.05$) of lipid was detected in Virginia tobacco seed. Ash content can be regarded as a general measure of quality and often is a useful criterion in identifying the authenticity of a food. A high ash content suggests the presence of an inorganic adulterant (Egan *et al*., 1981). Ash content did not differ significantly ($p<0.05$) among the sample means. Total protein (N×6.25) of tobacco seeds was found to be 19.21-21.05% in which 7.01-8.01% of it was water soluble. This value for total protein was higher than the values 3.49% for *Garcinia kola* seed (Daramola and Adegoke, 2007), 9.58% for *Xylopia aethiopica* seed (Barminas *et al*., 1999) and 12.90% for *Kerstingiella geocarpa* seed (Aremu *et al*., 2006). Protein content estimated by micro-Kjeldahl method showed considerably higher value than that given by Lowry method. The Kjeldahl method determines both water-soluble and insoluble proteins while the Lowry method determines concentrations of water soluble proteins only. Starch contents of 4.08-4.59% were reported herein for tobacco seeds and these results were lower than 5.52% for loofah seed (Devine and Williams, 1961). Crude fiber contents ranged from 14.58-16.89% for tobacco seeds, which were higher than the value 2.55% reported for *Garcinia kola* seed (Daramola and Adegoke, 2007), 2.13% for *Pentaclethra macrophylla* seeds (Enujiugha and Akanbi, 2005) and 12.23% for *Psophocarpus tetragonolobus* seed (Amoo *et al*., 2006). Carbohydrate contents (9.59-12.98%) of tobacco seeds were considerably lower than those of *Garcinia kola* seed (83.96%) (Daramola and Adegoke, 2007), *Psophocarpus tetragonolobus* seed (22.30%) (Amoo *et al*., 2006) and *Mesua ferrea* seeds (15.88-18.68%) (Abu Sayeed *et al*., 2004). Except for the ash content, there were significant differences ($p<0.05$) in the levels of all parameters among the sample tested.

CONCLUSION

Taken as a whole, in this study, the physicochemical characteristics can be helpful to identify the quality of oil and oil products for possible industrial or commercial uses. Tobacco seeds of all varieties

reported herein contain higher percentage of unsaturated fatty acids as compared to saturated fatty acids which is the characteristics of vegetable oils. From the quality point of view, tobacco seed oil classified as linoleic oil, is comparable to other oils and can be utilized in paint industries as potential raw material. The findings also imply that tobacco seeds may, therefore, be used as a potentially attractive source of lipid, protein and fiber. Protein content also commends tobacco seeds as a nutritive complement. Our results show considerable variability in most analyzed data which may be attributed to genetic variability and these analytical data will be helpful for the selection of variety. Further study is needed to understand how stage of maturity, growing region, harvesting conditions and storage period influence characteristics of oil and nutritive values of the seeds of tobacco.

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