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Comparative Study of the Characteristics of Seed Oil and Seed Nutrient Content of three Varieties of *Cucumis sativus* L.

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Abstract. The physicochemical characteristics of oils and nutrient contents of the seeds of three varieties of *Cucumis sativus* namely, Shahi-50, Naogaon-5 and Naogaon-Green, have been reported. Profile of fatty acid composition was not wholly similar in all varieties and unsaturated fatty acids were more than 77%, of which linoleic acid was 61.9-62.2%. High degree of unsaturation was indicated with lower peroxide value (3.7-4.2) and FFA (1.1-1.6%). Triacylglycerols and neutral lipids were the most abounded components recorded as 82.1-83.7% and 92.1-94.0%, respectively. The seeds contained potentially useful amounts of lipid (28.0-31.1%) and protein (14.8-15.9%) and other nutrients.

Keywords: seed oil, fatty acid, nutrient contents, *Cucumis sativus*

Introduction

For developing a healthy population the relation between food, nutrition and health has to be reinforced. Developing countries try to achieve this purpose through the exploitation of available local resources. Knowledge of the nutritive value of local dishes and local foodstuff is necessary for increased cultivation and consumption of highly nutritive crops, and supplementing the nutrients of the staple carbohydrate foods of those who cannot afford adequate proteinaceous foods of animal origin.

The Cucurbitaceae is a medium-size plant family, primarily found in the warmer regions of the world, consisting of economically important species, whose fruits are used for nutrition and medicinal purposes. Many Cucurbitaceae seeds are rich in oil and protein, and although none of these oils has been utilized on an industrial scale, many of them are used as cooking oil in some countries of Africa and the Middle East (Mariod *et al*., 2009). *Cucumis sativus*, locally known as Sassa, is one of the species which is cultivated largely in Bangladesh. Its three popular local varieties which vary morphologically from each other, are Shahi-50 (Type-1: Fruit long, dark green, covered with long triangular ovate leaves; seed narrow, straw in colour), Naogaon-5 (Type-2: Fruit medium, light green, covered with medium triangular ovate leaves; seed medium, straw in colour) and Naogaon-Green (Type-3: Fruit small, deep green, covered with small triangular ovate leaves; seed straw in colour). *C. sativus* seeds, besides possessing medicinal qualities, are also a rich source of proteins (28.68%) and lipids (53.76%) (Achu *et al*., 2005).

Extensive researches have been carried out on oils in many countries where vegetable oils are used as house hold and commercial purposes. Peris-Vicente *et al*. (2005) studied drying oils by analysis of fatty acids obtained after acidic hydrolysis of the oils, using HPLC with fluorescence detection for obtaining best resolution of peaks and detector selectivity than with GC-FID methods, and better sensitivity than that achieved with HPLC-UV-VIS detection. Peris-Vicente *et al*. (2007) developed an analytical method for the study of drying oils by analyzing the released fatty acids, using direct infusion mass spectrometry with negative ion electrospray ionization (ESI), avoiding derivatization and separation. Peris-Vicente *et al*. (2006) developed a chromatographic method for characterizing natural waxes by means of their characteristic chemical

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composition. For characterizing the waxes, fatty acids and hydrocarbons were taken as characteristic compounds. Peris-Vicente *et al.* (2004) presented an analytical study of drying oils by analysis of the fatty acids, obtained by acid hydrolysis of the oils, using HPLC by UV-VIS detection for obtaining best resolution of peaks and detector selectivity (FID) than with gas chromatography methods.

The proximate and fatty acids composition of seeds or seed kernels of *C. sativus* from different origins and varieties have been reported widely (Fokou *et al.*, 2009; Mariod *et al.*, 2009; 2004; Achu *et al.*, 2005). Three of the prominent local varieties of *C. sativus*, as mentioned above, have gained large acceptance in the northern part of Bangladesh. The aim of this work was to determine physicochemical characteristics, acylglycerol class, lipid class and fatty acid composition of oils and nutrient contents of the seeds of the three varieties (Type-1, Type-2 and Type-3) of *C. sativus* as a basis of comparison.

Materials and Methods

Plant materials and chemicals. Ripe fruits of the three varieties of *C. sativus* were collected in the year 2006 from an experimental plot located in Rajshahi city, Bangladesh. The seeds were separated from the fruits manually and washed several times with water to remove foreign materials. Afterwards, the seeds were dried in sunlight for four consecutive days and then in an electric oven at 40 °C until constant weight was reached. The seeds were ground to a fine powder, packaged and stored at 4 °C prior to analysis. Solvents such as petroleum ether, diethyl ether, benzene, chloroform, acetone, methanol etc. were obtained from Merck (Darmstadt, Germany) and BDH (Poole, England). Silica gel (60-120 mesh) and silica gel (HF_{254}) were products of Merck (Darmstadt, Germany). Esters of fatty acids and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade unless otherwise specified and results were expressed on dry weight basis.

Analysis of *C. sativus* 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 (Buchi Labortechnik AG, Postfach, Switzerland) and the percentage of oil content was computed.

Physical and chemical characteristics. Specific gravity of the oil was determined at 23 °C with the help of a specific gravity bottle. Refractive index of clear oil was determined at 28 °C using Abbe Refractometer (ATAGO T-series, Model-3T, Texas, USA) following IUPAC (1979) method. Iodine value, unsaponifiable matter, Reichert-Meissl value and acetyl value were determined by the methods depicted by Ranganna (1986), while the saponification value, percentage of free fatty acid (FFA) and peroxide value were determined according to the methods described by Williams (1966).

Separation of acylglycerols. The oil was separated into mono-, di- and triacylglycerols by silica gel (60-120 mesh) column chromatography. The solvent systems used to elute the column were similar to those described by Gofur *et al.* (1993a). For quantitative determination of acylglycerol classes, the sample (529.8 mg in 3 mL petroleum ether) was adsorbed on the top of the column; triacylglycerols were eluted with benzene, diacylglycerols with a mixture of diethyl ether and benzene (1:9, v/v) and monoacylglycerols with diethyl ether. Fractions were collected at approx. 1.5-2 mL/min. Elution was monitored by thin layer chromatography (TLC). Purity of the separated fractions was confirmed by TLC, using silica gel (HF_{254}) and hexane-diethyl ether 80/20 (v/v) as solvent system. Spots were visualized with chromic-sulphuric acid at 180 °C.

Fractionation of lipids. A total of 553 mg lipid extracted from the seeds by the method of Bligh and Dyer (1959) was fractionated into three major lipid groups: neutral lipid, glycolipid, and phospholipid by silica gel column chromatography (Gofur *et al.*, 1993b). Neutral lipids were eluted with chloroform, glycolipids with acetone and phospholipids with methanol. Approximately 0.5-1.0 mL fractions were collected per minute and elution was monitored by TLC. Solvents were evaporated in vacuum rotary evaporator and percentages of these fractions were determined by gravimetric method.

Fatty acid composition of oil. Fatty acids of *C. sativus* seed oil were determined as their methyl esters prepared by boron-trifluoride methanol complex method (Morrison and Smith, 1964). A GCD PYE Unicam gas chromatograph (PYE Unicam Ltd., Cambridge, UK) 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. 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 temperature for all GLC analysis was 240 °C. The peaks were identified by comparison with standard fatty acid methyl esters.

Analysis of *C. sativus* seeds. Moisture, ash and crude fibre contents were determined by AOAC (1990) methods. Lipid content was estimated by the method of Bligh and Dyer (1959) using a solvent mixture of chloroform and methanol (2:1 v/v). The micro-Kjeldahl (Buchi Labortechnik AG, Switzerland) method of AOAC (1990) was employed to determine total nitrogen and the protein content was calculated from the total nitrogen, using N × 6.25. Water soluble protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. Determination of starch content was based on analytical method outlined elsewhere (Clegg, 1956). Total sugar content was determined by colorimetric method (Dubois *et al.*, 1951) and total carbohydrates were calculated by the difference (Rahim *et al.*, 1999).

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

The solvent extracts of three varieties of *C. sativus* seeds yielded on an average, 24.7% oil, which is similar to the value of 25.8% reported by Mariod *et al.* (2009). Information on detailed characteristics of oil and nutritional composition of seeds from *Cucumis* species are too scanty for meaningful comparisons.

The estimated specific gravities (Table 1) of *C. sativus* seed oils (0.9174-0.9192 at 23 °C) are in good agreement with the value 0.9140 at 30 °C for the same oil, but higher than the value 0.8840 at 30 °C for *C. prophetarum* seed oil reported by Mariod *et al.* (2009). Refractive indices of the oils were found to be 1.4645-1.4665 at 28 °C, being higher than 1.434 at 40 °C (Mariod *et al.*, 2009) for the same oil and 1.424 at 28 °C (Mian-Hao and Yansong, 2007) for *Cucumis melo* hybrid 'ChunLi' seed oil. Specific gravity and refractive index which are very stable parameters and used for checking the identity of oils, did not differ significantly ($P<0.05$) between the analyzed samples. Iodine values estimated for *C. sativus* (112.0-114.3) were much lower than the value 89.5 (Mian-Hao and Yansong, 2007) for *Cucumis melo* hybrid 'ChunLi' seed oil, but were consistent with the reported value of 114.0 for the same seed oil (Mariod *et al.*, 2009). Referring to Table 1, therefore, the oil samples of Type-1, in contrast to other samples in the present investigations have lower tendency to become rancid by oxidation. Saponification values of the three samples were in the range 189.8-194.8, being lower than the value 209.0 reported by Sadou *et al.* (2007) for *Cucumis metuliferus* seed oil. The estimated comparatively low saponification values indicate the presence of higher proportion of higher fatty acids. No significant difference ($P<0.05$) in iodine and saponification values were found between the sample means of Type-2 and Type-3. The content FFA (1.1-1.6%) of the *C. sativus* seed oils was similar to the value 1.3% cited in the literature (Mariod *et al.*, 2009), but lower than the value 0.75% (Mian-Hao and Yansong, 2007) reported for *Cucumis melo* hybrid 'ChunLi' seed oil. Results regarding FFA contents indicate more suitability of the oil sample of Type-2 for probably edible purpose as it contained significantly ($P<0.05$)

Table 1. Physical and chemical characteristics of *Cucumis sativa* seed oils

Characteristics	Type-1	Type-2	Type-3
Specific gravity at 23 °C	0.9174 ± 0.0021^{a}	0.9192 ± 0.0008^{a}	0.9185 ± 0.0018^{a}
Refractive index at 28 °C	1.4655 ± 0.0040^{a}	1.4645 ± 0.0042^{a}	1.4665 ± 0.0023^{a}
Iodine value (Hanus)	112.0 ± 0.85^{a}	113.6 ± 0.62^{b}	114.3 ± 1.25^{b}
Saponification value (mg KOH/g)	194.8 ± 0.66^{b}	189.9 ± 1.00^{a}	189.8 ± 1.41^{a}
Free fatty acids (%) as oleic	1.6 ± 0.15b	1.1 ± 0.15^{a}	1.4 ± 0.05b
Unsaponifiable matter (g/100g)	1.3 ± 0.20^{a}	1.2 ± 0.05^{a}	1.4 ± 0.10^{a}
Peroxide value (mEq/kg of oil)	3.7 ± 0.17^{a}	4.2 ± 0.26^{b}	4.0 ± 0.17^{ab}
Reichert-Meissl value	0.61 ± 0.07^{a}	0.81 ± 0.09^{b}	0.71 ± 0.06^{ab}
Acetyl value	2.5 ± 0.10^{a}	2.9 ± 0.34^{ab}	3.0 ± 0.20^{b}

Values are mean ± standard deviation of three experiments; means in the same row with different superscript are significantly ($P<0.05$) different.

lower percentage of FFA than those contained in the rest of the samples.

C. sativus seed oils contained unsaponifiable matter (Table 1) ranging from 1.2% in Type-2 to 1.4% in Type-3, being similar to 1.1% for the same oil reported by Mariod *et al.* (2009). No significant inter-variety differences ($P<0.05$) in the level of unsaponifiable matter were observed. The peroxide values in the samples of *C. sativus* ranging from 3.7 to 4.2 mEq/kg were slightly higher than 3.5 mEq/kg revealed by Mariod *et al.* (2009), but much lower than 8.0 mEq/kg reported by Fokou *et al.* (2009) for the same oil. Fresh oils usually have peroxide values well below 10 mEq/kg. The present experimental results indicate that *C. sativus* seed oils are quality oil. The low Reichert-Meissl values (0.61-0.81) as estimated for *C. sativus* indicate the low content of lower volatile soluble fatty acids, and this value is also in agreement with the low saponification value as obtained. Acetyl values of *C. sativus* seed oils were determined to be (2.5-3.0). Significant differences ($P<0.05$) in mean peroxide, Reichert-Meissl and acetyl values of the sample were noticed.

As shown in Table 2, the triacylglycerol contents varied from 82.1 to 83.7%, while diacylglycerols, from 10.9 to 11.5% and monoacylglycerols, from 1.1 to 1.9%. No significant differences ($P<0.05$) were observed in triacylglycerol and diacylglycerol composition to account for about 82.8% (average) and 11.2% (average), respectively, of the total weight of oil, in the three varieties. Total recovery of acylglycerols was more than 95% indicating that *C. sativus* seed oils contained lower amount of non-acylglycerols than that contained in *Mesua ferrea* seed oil (Sayeed *et al.*, 2004). Of the three samples, Type-3 contained significantly ($P<0.05$) higher amount (1.9%) of monoacylglycerols that can be separated easily by column chromatography and used as emulsifier. Mono- and particularly diacylglycerols occur naturally in oils and fats, where their presence is initially due to partial hydrolysis of the oil by enzyme action in the fruits or the seeds. Monoacylglycerols are surface-active materials, having both polar, water soluble, and non-polar, fat-soluble groups. It is for this reason that the higher monoacyl-glycerol are of great importance as emulsifier in the food industry. They are particularly valuable for producing stable oil-in-water emulsions and are also crystal promoters. Thus, fat containing small amount of monoacylglycerol will set quickly to a micro-crystalline matrix (Devine and Williams, 1961).

Table 2. Acylglycerol composition of *Cucumis sativa* seed oils (wt %)

Varieties	Monoacylglycerol	Diacylglycerol	Triacylglycerol
Type-1	1.5 ± 0.10^{b}	11.5 ± 0.60^{a}	82.1 ± 1.73^{a}
Type-2	1.1 ± 0.17^{a}	11.4 ± 0.40^{a}	83.7 ± 0.29^{a}
Type-3	1.9 ± 0.26^{c}	10.9 ± 0.45^{a}	82.7 ± 1.41^{a}

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

Fractionation of *C. sativus* seed lipids by silica gel column chromatography into neutral lipids, glycolipids and phospholipids was quantified as 92.1-94.0%, 2.2-3.7% and 2.6-3.1%, respectively (Table 3). There were no significant differences ($P<0.05$) in the level of phospholipid contents of different sources. Results also indicated neutral lipids to be the most abundant component of seed lipids, recorded over 92% of the total weight of the lipid. However, the amounts of glycolipids and phospholipids found in *C. sativus* were lower than those of *Nicotiana tabacum*, whereas neutral lipids were higher (Ali *et al.*, 2008).

Table 3. Lipid composition of *Cucumis sativa* seed lipids (wt %)

Varieties	Neutral lipid	Glycolipid	Phospholipid
Type-1	93.3 ± 0.60^{ab}	3.4 ± 0.20^{b}	2.6 ± 0.26^{a}
Type-2	92.1 ± 0.45^{a}	3.7 ± 0.20^{b}	2.7 ± 0.34^{a}
Type-3	94.0 ± 0.79^{b}	2.2 ± 0.26^{a}	3.1 ± 0.45^{a}

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

The fatty acid patterns (Table 4) of *C. sativus* seed oils were qualitatively similar to those of other plants; linoleic acid (61.9-62.2%) being the major fatty acids followed by oleic acid (15.6-16.5%). Linoleinic acid was detected in trace amount in all the samples. It was also noted that *C. sativus* oils contained mainly unsaturated fatty acids (77.8-79.1%), while saturated fatty acids were 20.9-22.2%. Saturated fatty acids, accounting more than 20%, were palmitic (10.1-10.7%) and stearic (10.2-12.1%). No significant ($P<0.05$) differences were detected in palmitic and linoleic acid contents of the seed oils. The most prominent feature of the fatty acid composition of *C. sativus* seed oils was the high amount of linoleic acid, being slightly lower than that reported by Mariod *et al.* (2009) for the same

seed oil. But the amount of linoleic acid detected herein, was higher as compared to many other seed oils such as that of *Cucumis melo* var. *agrestis* (57.6%) (Mariod *et al.*, 2009), *Cucumis metuliferus* (56.21%) (Sadou *et al.*, 2007), *Cucurbita maxima* (43.0-50.3%), and *Cucurbita argyrosperma* (56.0%) (Applequist *et al.*, 2006), which is likely to satisfy the essential fatty acid requirement of humans. The nutritional value of linoleic acid is due to its metabolism at tissue level, which produces long chain polyunsaturated fatty acids and prostaglandins (Sayanova *et al.*, 2003). The saturated/unsaturated fatty acid ratio of the oils was found to be in the range of 0.2642 to 0.2853 in all varieties; however, Type-3 seed oil displayed higher unsaturation as compared to the others with a saturated/unsaturated fatty acid ratio of only 0.2642. These ratios indicate that the samples have a high content of unsaturated fatty acids, which may make them more attractive for the consumers who wish to ingest this type of acid.

Table 4. Fatty acid composition of *Cucumis sativa* seed oils (%)

Fatty acids	Type-1	Type-2	Type-3
Palmitic acid (C16 : 0)	10.1 ± 0.36^{a}	10.2 ± 0.51^{a}	10.7 ± 0.20^{a}
Stearic acid (C18 : 0)	12.1 ± 0.40^{c}	10.9 ± 0.10^{b}	10.2 ± 0.43^{a}
Oleic acid (C18 : 1)	15.6 ± 0.05^{a}	16.5 ± 0.43^{b}	16.3 ± 0.51^{ab}
Linoleic acid (C18 : 2)	61.9 ± 0.49^{a}	61.9 ± 0.45^{a}	62.2 ± 0.32^{a}
Linoleinic acid (C18 : 3)	0.3 ± 0.10^{a}	0.5 ± 0.17^{ab}	0.6 ± 0.20^{b}

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

The nutrient contents of *C. sativus* seeds are reported in Table 5. The seeds contained moisture (6.7-7.3%), higher than the value of 4.41% reported by Mariod *et al.*, (2009) and 5.65 % reported by Achu *et al.* (2005) for the same seeds. The three samples did not show significant ($P<0.05$) differences in moisture content. For the preservation of a product for a long time and to diminish the probability of bacterial and fungal growth, that could alter the quality through decomposition, the content of moisture is important (Aguilera-Morales *et al.*, 2005). *C. sativus* seeds contained total lipids, 28.0-31.1%, higher than the value of 3.3-4.1% reported for *Castanea sativa* (Neri *et al.*, 2010). Ash contents was in the range 3.4-4.2%, similar to the reported values 4.0 (Mariod *et al.*, 2009) and 3.5 (Achu *et al.*, 2005), but lower than the values 8.3 (Mariod *et al.*, 2009) for *Cucumis prophetarum* and 5.7 (Mariod *et al.*, 2009) for *Cucumis melo* var. *flexuosus* seeds.

Table 5. Nutrient contents of *Cucumis sativa* seeds

Parameters (g/100 g)	Type-1	Type-2	Type-3
Moisture	7.2 ± 0.26^{a}	7.3 ± 0.36^{a}	6.7 ± 0.30^{a}
Lipid	28.0 ± 0.45^{a}	28.8 ± 0.26^{b}	$31.1\ 0.34^{c}$
Ash	4.2 ± 0.26^{b}	3.6 ± 0.17^{a}	3.4 ± 0.17^{a}
Total protein	14.8 ± 0.26^{a}	15.5 ± 0.50^{ab}	15.9 ± 0.34^{b}
Water soluble protein	9.5 ± 0.30^{a}	10.2 ± 0.20^{b}	9.7 ± 0.26^{ab}
Starch	5.1 ± 0.26^{a}	5.2 ± 0.20^{a}	5.2 ± 0.17^{a}
Crude fibre	5.1 ± 0.26^{b}	4.0 ± 0.20^{a}	4.7 ± 0.17^{b}
Total sugar	1.0 ± 0.20^{a}	1.3 ± 0.10^{a}	1.2 ± 0.20^{a}
Total carbo-hydrate	40.7	40.8	38.2

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

Ash content is regarded a general measure of quality and often is a useful criterion in identifying the authenticity of food, with high ash figure suggesting the presence of an inorganic adulterant (Egan *et al.*, 1981). Total protein content was found to be 14.8-15.9% of which 9.5-10.2% was water soluble; these values for total protein were lower than 17.5% quantified by Mariod *et al.*, (2009) and 28.6% by Achu *et al.* (2005) for the same source. The protein content in the present results was lower than the values, 25.0% (Yanty *et al.*, 2008) for *Cucumis melo* var. *inodorus* and 29.9% (Mian-Hao and Yansong, 2007) for *Cucumis melo* hybrid 'ChunLi' seeds. Crude fibre content was 4.0-5.1%, similar to the value of 4.1% cited by Achu *et al.* (2005), but much lower than 23.3% for *Cucumis melo* var. *inodorus* and 19.0% for *Cucumis melo* hybrid AF-522 seeds (Yanty *et al.*, 2008). No significant differences ($P<0.05$) in mean starch content (5.1-5.2%) and total sugar content (1.0-1.3%) were observed in the sample means. Carbohydrate contents, in the range of 38.2-40.8%, were higher than those of 19.8% for *Cucumis melo* var. *inodorus* and 22.9% for *Cucumis melo* hybrid AF-522 seeds (Yanty *et al.*, 2008).

The findings imply that *C. sativus* seeds may be used as a potentially attractive source of lipids and some common nutrients. The protein content also commends *C. sativus* seeds as a nutritive complement. The present study, moreover, highlights the importance of understanding the cultural context and uses of cultivated plant foods. It may be that not all cultivated plant foods are consumed by all the members of a community. Consumption patterns, for example, can vary by gender or age, or even physiological state (e.g. pregnancy). Nutrient information would be critical to the success of efforts for promoting the wider use of indigenous plant foods as part of a broader program aimed at educating local populations with regard to the nutritional benefits of many cultivated plant foods that exist in their environment.

Conclusion

Improved knowledge on the analysis of *Cucumis sativus* seeds would assist in efforts to achieve industrial application of this plant. The physicochemical constants of the oils studied herein can be helpful in identifying the quality of oil and oil products for commercial exploitation. The quality of *C. sativus* seed oil is comparable to that of other oils and can be utilized in the paint, varnish and ink industries and is also recommended for human consumption after proper refining. In terms of both quantity and quality, all three varieties of *C. sativus*, herein reported, are potentially useful and important nutritional sources. The results agree with the data reported in the literature and these analytical data will also be helpful for the selection of variety.

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