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Characteristics of Oil from Seeds of 4 Mungbean [*Vigna radiata* (L.) Wilczek] Cultivars Grown in Pakistan

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Abstract Mungbean is a widely consumed legume globally. This study was carried out for detailed characterization of oils from mungbean seeds from four indigenously cultivated varieties, as very little information is available on the oil composition of mungbean seeds and inter-varietal variation in oil composition. The oil content was relatively low (2.1-2.7%). The investigated physiochemical parameters included refractive indices (RI) at 40 °C (1.4673-1.4698), relative density (0.9580-0.9618), iodine value (IV) (111.4 -117.1), saponification value (SV) (173.1-181.7 mg KOH/g) and unsaponifiable matter (UM) (13.8–15.01%). Phospholipids and triglycerides were the dominant lipid fractions followed by monoglycerides. Linoleic acid and oleic acid were the dominant fatty acids (FA). Characterization was also made by TLC. Tocopherol analysis demonstrated highest content of γ -tocopherol among its isomers, while α -tocotrienol was present in highest amount in all studied cultivars, among its isomers. Results from most of the parameters revealed significant ($P \le 0.05$) differences among the cultivars. The findings of the study reveal mungbean [Vigna radiata (L.) wilczek], to be a potentially valuable legume crop with comparable nutritional quality oil among all the cultivars.

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Introduction

The mungbean [Vigna radiata (L.) wilczek] has been grown in the Indo-Pak subcontinent since ancient times, where the diet is mostly cereal-based [1] and is a well studied plant for its nutritional value and medicinal importance. It is often praised as a "green pearl" because of its richness in protein, starch, minerals, vitamin B, and amino acids. It is consumed as a seed, sprout, or in processed forms that include cold jellies, noodles, cakes, and brews. It is used as *dhal*; to make curries; sweet and salty soups; is broiled and toasted with onion, chili and salt; in sweet and salt pongal (rice preparation); and patties and sweets of different kinds [2, 3]. Because of its high protein content, easy digestibility and being almost free from flatulence-causing factors; it is preferred for feeding babies and convalescents. Furthermore, it is also consumed in many forms: as boiled dry bean, bean sprouts, green beans, noodles and bean cakes [4-6]. It is also used in traditional medicines. Its seed and soup is a rich source of alkaloids, coumarin, and phytosterin that play an important role in promoting the physiological metabolism of human beings and animals. In traditional Chinese medicine, parts of the mungbean plant are used for treating various ailments; including hepatitis, gastritis, uraemia, toxicosis, red dysentery, cholera, corneal opacity and macula. Seeds are used externally and internally for paralysis, rheumatism, liver complaints, and cough syrups. The roots are considered to be a narcotic [7].

The oil content, including essential fatty acids (FA), is the third important organic component of mungbean. The

mungbean cannot be described as an oil-bearing seed: as the oil content of mungbeans, grown in different parts of the world, is reportedly relatively low on average. Mungbean oil consists of tocopherols and tocotrienols [8]. Both these constituents have their own nutritional and medicinal importance. The main function of α -tocopherol is that of being a radical chain-breaking antioxidant in membranes and lipoproteins as well as in foods [9]. Due to its antioxidant potential and various functions at the molecular level, it is believed to reduce the risk of cardiovascular diseases and of certain types of cancer [10, 11]. γ -Tocopherol has been reported to be more potent than α-tocopherol in decreasing platelet aggregation, LDL oxidation, and delaying intra-arterial thrombus formation [12, 13]. The antioxidant properties of tocotrienols have been reported to be significantly higher than those of tocopherols and may have biologically important properties such as inhibition of cholesterol biosynthesis [14] and are discussed in the context of reducing the risk of breast cancer [15]. Hence concurrent administration of various tocopherols and tocotrienol may result in increased antioxidant, antitumor and hypocholesterolemic potential [16].

It is an important short-duration pulse crop of Kharif (the autumn harvest) season in Pakistan with wide adaptability, low input requirements, and the ability to improve the soil by fixing atmospheric nitrogen. The total area of mungbean cultivation during 2003-2004 in Pakistan was 255.9 thousand acres with a total production of 1.40 million tonnes and an average yield of 550 kg/ha [17]. Area, production and productivity of mungbean in Pakistan have begun to show positive growth and farmers are recognizing mungbean as an important cash crop and also its export potential. This has been brought about by the development of new mungbean cultivars with higher yields, improved adaptation to local agroclimatic conditions and better resistance against different stresses, by the expansion of export markets, and through a keener appreciation of the benefits of crop rotation and alternative cropping systems.

To our knowledge, no data has been reported on the functional properties of the oil obtained from the seeds of the mungbean cultivars grown in Pakistan. The exploration of newer dietary sources of bioactive compounds, having profound health benefits given by essential fatty acids, is focus of research among food scientists. The present study is also a part of this series of investigations. The objective of the present study is the physiochemical characterization of the oil of commonly cultivated mungbean cultivars in Pakistan. The information obtained will be useful for consumers, nutritionists, and agricultural scientists alike. The physical and biochemical data derived will also be helpful for researchers in future for further studies. These analytical findings will provide a national database for this valuable legume crop, which has not been explored so far.

Experimental Procedures

Materials and Reagents

The seeds of four improved, high yielding, disease resistant mungbean cultivars namely Chakwal Mung-97, Chakwal Mung 2006, AZRI-Mung-06, and NM-2006, grown and harvested under similar environmental and agroclimatic conditions, during the years 2006-2007, were procured from the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The other growing conditions included: latitude 31° 30°N; longitude 73° 10°E and altitude 213 m above sea level, with an average annual rainfall of less than 400 mm and average temperatures between 26 and 28 °C during the day and 15 and 20 °C during the night. Three samples of each variety were collected from each experimental plot out of three chosen for this study to fulfill the requirements of statistical analysis $(3 \times 3 \times 1)$, thus making n = 9 for one cultivar. Results were averaged and data was reported as means \pm SD. After removing immature and damaged seeds, seeds of all the cultivars were divided into groups for storage in stainless-steel containers at 4 °C before analyses. The solvents (Fisher Scientific, Loughborough, UK) used were of analytical grade and were not further purified.

Extraction

The mungbean samples were ground to flour with an IKA[®] all basic mill (IKA Works Inc., Wilmington, NC, USA) and were passed through a 60-mesh sieve. The seed powder was extracted with a mixture of *n*-hexane/2-propanol (3:1, V/V) in a Soxhlet apparatus (6 h).

General Properties

The color and state of the oil were noted, at room temperature, by visual inspection. Determination of density, refractive index (RI), iodine value (IV), peroxide value (PV), acidity, *p*-anisidine value (p-AV), saponification value (SV), and unsaponifiable matter (UM) of the extracted oil was carried out by standard IUPAC methods 2.101, 2.102, 2.205, 2.201, 2.504 and 2.202, respectively, for the analysis of fats and oils [18].

TLC of Lipids

The total lipids of mung bean samples were fractionated by a reported method [19] using silica gel, G 60 Merck type 5721 and 20 cm × 20 cm glass plates with 0.25 mm thickness. The developing solvent system was *n*-hexane: diethyl ether: acetic acid glacial (80: 20: 2, V/V/V). The separated fractions of total lipids were visualized by exposure to iodine vapor in a closed chamber after drying. All lipid fractions were identified on thin layer plates by comparing their R_f values with those of known lipid standards. For quantitative analysis, the different lipid fractions were scanned by using Shimadzu TLC-Scanner (C-S-910). The area under each peak was measured by the triangulation method [20]. The percentage of each component was calculated with regard to the total area as follows by a reported procedure [21]

% Component = Area of each peak/Total peaks area $\times 100$

Fatty Acid Profile

Fatty acid methyl esters (FAMEs) were prepared according to the standard IUPAC method 2.301 and analyzed on a SHIMADZU gas chromatograph model 17-A with flame ionization detector (FID). Separation was done on a capillary column SP 2330 (30 m \times 0.32 mm \times 0.25 $\mu m;$ Supelco; Bellefonte, Pa., USA.). Nitrogen was used as a carrier gas at a flow rate of 3.0 ml/min. Column temperature was programmed from 180 to 220 °C at the rate of 3 °C/min. Initial and final temperatures were held for 2 and 10 min, respectively. Injector and detector were kept at 230 and 250 °C, respectively. A sample volume of 1.0 µl was injected with the split ratio of 1:75. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards. The quantification was done by a Chromatography Station for Windows (CSW32) data handling software (Data Apex Ltd. CZ-158 00 Pague 5, the Czech Republic). The fatty acid composition was reported as a relative percentage of the total peak area.

Tocopherol and Tocotrienol Contents

Tocopherols were determined by HPLC with reported procedure [22]. The HPLC system of Merck Hitachi

(Darmstadt, Germany) consisted of pump L-6000: F-1000 fluorescence spectrophotometer set to excitation wavelength 295 nm and emission wavelength 330 nm; D-2000 integrator: Lichrospher 100 Diol pre-column with particle size 5 µm, length 4 mm, and internal diameter 4 mm; Lichrospher 100 Diol pre-column with particle size 5 µm, length 250 mm and internal diameter 4 mm; Rheodyne 7125 injector and a 20 µl sample loop. The system was operated at 21 ± 1 °C, with mobile phase *n*-hexane/tertbutyl methyl ether 94.4:5.6 V/V at a flow rate of 1.2 ml/ min. The sample size ranged from 5 to 20 µl of extracted oil. A calibration curve was prepared by injecting known concentrations of standard tocopherols. The concentration of tocopherols and tocotrienols in the samples were calculated from equations derived from individual tocopherol standards.

Statistical Analysis

All the analyses were carried out in triplicate and the results are expressed as mean values \pm SD. *P* values were determined to evaluate the differences among the cultivars at 95% CI. Analysis was carried out by using the "MSTATC" statistical computer package [23].

Results and Discussion

In view of global industrialization, ever-increasing demand and interest of people for low cholesterol diets, and scientific awareness regarding the nutritional and functional properties of food, compositional characterization of legume crops like mungbean is of great importance in the development and commercialization of such crops particularly for countries like Pakistan, where these crops occupy a major area of cultivation.

The oil contents and different physical properties of oils from mungbean seeds from different cultivars are presented in Table 1. The oil yields for the four cultivars ranged from 2.1% for Chakwal Mung-97 to 2.7% for Chakwal Mung-2006. The results revealed that the

 Table 1 Oil content and physiochemical properties of oils from seeds of mungbean cultivars

Attribute	Chakwal mung-97	Chakwal mung 2006	AZRI-mung-06	NM-2006
		Chakwar mang 2000	Tiziti mung 00	1001 2000
Oil contents	$2.7^{\rm a} \pm 0.3$	$2.1^{b} \pm 0.2$	$2.1^{b} \pm 0.1$	$2.5^{\mathrm{a}}\pm0.1$
Relative density (40 °C) (g/cm ³)	$1.0^{\rm ns} \pm 0.0$	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Refractive index (40 °C)	$1.5^{\rm ns} \pm 0.3$	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.4
Unsaponifiable matter (% w/w)	$15.0^{\rm ns} \pm 0.1$	14.4 ± 0.1	13.8 ± 0.9	14.9 ± 0.9
Saponification values (mg KOH/g)	$173.1^{ns} \pm 2.3$	181.7 ± 2.5	179.0 ± 5.6	175.1 ± 6.4
Iodine values (Wijs method)	$117.1^{ns} \pm 4.4$	114.3 ± 3.2	115.4 ± 2.3	115.7 ± 5.1

Data are means $(n = 9) \pm SD$ (n = 9), (P < 0.05), Mean values with different letters in rows differ significantly

Lipid fractions (%)	Chakwal mung-97	Chakwal mung 2006	AZRI-mung-06	NM-2006
Phospholipids	$32.3^{ns} \pm 3.4$	31.6 ± 2.9	30.9 ± 4.0	32.1 ± 3.7
Monoglycerides	$08.6^{\rm b} \pm 0.1$	$07.2^{c} \pm 0.2$	$09.3^{\rm a} \pm 0.1$	$7.4^{\rm c} \pm 0.1$
1,2&2,3-diglycerides	$05.6^{\circ} \pm 0.4$	$06.7^{\rm b} \pm 0.3$	$07.2^{\rm a} \pm 0.3$	$06.8^{\mathrm{ab}}\pm0.2$
Sterols	$05.7^{\rm ab} \pm 0.6$	$05.9^{\rm a} \pm 0.4$	$5.0^{\mathrm{b}}\pm0.7$	$05.6^{\mathrm{ab}}\pm0.6$
1,3-diglycerides	$02.8^{\rm b} \pm 0.3$	$03.4^{\rm a} \pm 0.2$	$03.0^{\rm b} \pm 0.2$	$02.8^{\rm b}\pm0.2$
Free fatty acids	$08.4^{a} \pm 0.3$	$08.0^{\rm b} \pm 0.3$	$08.2^{\mathrm{ab}}\pm0.2$	$08.2^{\mathrm{ab}}\pm0.1$
Triglycerides	$30.1^{ns} \pm 2.2$	29.9 ± 3.9	30.3 ± 3.8	30.4 ± 3.0
Hydrocarbons + sterol esters	$06.7^{b} \pm 0.6$	$07.4^{\rm a} \pm 0.5$	$06.2^{b} \pm 0.2$	$06.6^{b} \pm 0.4$

Table 2 Percentage composition of lipid classes in the oils from seeds of mungbean cultivars

Data are means $(n = 9) \pm SD$ (n = 9), (P < 0.05), Mean values with different letters in rows differ significantly

cultivars differed significantly with each other as far as total oil contents are concerned. The low yields reported here are in partial agreement with previously reported values (0.8–2.9%) [8, 21]. The variation in oil content within the countries and species is attributed to the environmental and geological conditions of the regions [24]. The low oil yields obtained were also supported with the view that legumes are generally not oil-bearing crops [25]. The oil is of greenish color and is liquid at room temperature (29.0 \pm 1 °C). The liquid character was also reported earlier for chickpea seed oil [26].

The range of relative densities (Table 1), 0.9580-0.9618, was slightly higher than the relative densities for groundnut (0.91–0.92), soybean (0.92–0.92) and *Phaseolus vulgaris* (0.94–0.98) oils [25] but in close proximity to each other suggesting similarities in the textures of the oils. The refractive indices (RI) fall within a close range, from 1.4670 to 1.4691. Although this range is comparable with the RI for *P. vulgaris* (1.47–1.48), grape seed oil (1.47– 1.48) and soybean (1.47–1.48); the legume with the highest oil content [27]. These relatively high RI are an indication of substantial unsaturation in the oils of the studied mungbean cultivars. Bulk chemical properties such as acid

Table 3 Fatty acid composition (relative %) of oil from mungbean seeds

Fatty acid	Chakwal mung-97	Chakwal mung 2006	AZRI- mung-06	NM-2006
C16	$24.9^{ns}\pm2.2$	24.0 ± 2.0	24.2 ± 1.9	24.7 ± 1.6
C18	$6.1^{\mathrm{ns}}\pm0.6$	5.9 ± 0.2	6.2 ± 0.4	6.1 ± 0.8
C20	$1.3^{ m ns}\pm0.4$	1.6 ± 0.4	1.3 ± 0.3	1.2 ± 0.2
C22	$2.2^{ns}\pm0.1$	2.3 ± 0.3	2.1 ± 0.2	2.2 ± 0.3
C24	$1.4^{ns}\pm0.2$	1.3 ± 0.2	1.2 ± 0.1	1.4 ± 0.1
C18:1	$5.4^{b} \pm 0.1$	$5.8^{\rm a}\pm0.2$	$5.5^{ab}\pm0.2$	$5.6^{ab}\pm0.4$
C18:2	$37.2^{ns}\pm4.5$	36.9 ± 5.1	37.1 ± 3.7	37.3 ± 3.9
C18:3	$21.8^{ns}\pm0.0$	21.6 ± 0.1	21.6 ± 0.1	21.5 ± 0.1

Data are means $(n = 9) \pm SD$ (n = 9), (p < 0.05), Mean values with different letters in rows differ significantly

value AV, SV and IV give structural stability, and quality information about oils and fats [25]. The range of SV from 173.1 to 181.7 mg KOH/g indicates the absence of lauric acid in the investigated mungbean seed oils; instead this range is indicative of oils characterized by medium chainlength FA. The range of SV for the mungbean cultivars is lower than those of olive (184-196 mg KOH/g), soybean (189–195 mg KOH/g), P. vulgaris (172.2–196.6 mg KOH/g) and sunflower seed (188-194 mg KOH/g) oils [25]. The UM ranged from 13.8 to 15.01 1% W/W (Table 1) and it was revealed that cultivars had significant amount of UM. These values are bit higher than UM pattern observed for low oil-bearing seeds [25] but are in line with mungbean determined earlier (14.8-16.6%) [28, 29]. The IV ranged from 111.4 to 117.1 (Wij's method) (Table 1). These values were higher than the IV for groundnut oil (80-106, Wij's method) and Phaseolus vulgaris (80.5-92.3, Wij's method) oils [25], but in agreement to those reported earlier for chickpea and mungbean [26, 29].

Neutral lipids, dominated by TG, were the predominant lipid compounds in the oils (Table 2). The TAG was the predominant component in the neutral lipids, however significant amounts of sterols and sterol esters indicated that sterols constitute a prominent component of the UM. The phospholipid content was quite significant as polar lipid component. Similar findings have been reported earlier [21].

The FA composition of the mungbean cultivars investigated (Table 4), despite differences among cultivars, followed the general pattern for legumes [24]; with linoleic and oleic acids as the dominant FA (Table 3).The seed oils from the mungbean cultivars investigated contained significant amounts of saturated FA, (16:0), a common feature of legume seed oils [30], which is confirmed by the FA profile of the mungbean cultivars investigated. The predominance of linoleic and oleic acids, certainly adds an extra dimension to the nutritional value of chickpea seed oil. Linoleic and linolenic acids are the most important essential FA required for growth, physiological functions **Table 4** Tocopherol andTocotrienol content (mg/100 goil) of mungbean cultivars

Tocopherols (mg/100 g of oil)	Chakwal Mung-97	Chakwal Mung 2006	AZRI-Mung-06	NM-2006
α	$10.9^{\rm ns} \pm 0.9$	10.0 ± 0.6	10.5 ± 0.9	10.8 ± 0.3
ß	$0.9^{\mathrm{b}}\pm0.0$	$1.0^{\mathrm{a}} \pm 0.0$	$0.9^{ m bc}\pm 0.0$	$0.8^{\rm c} \pm 0.0$
γ	1,457.8 $^{\rm ns}$ \pm 12.2	$1,457.1 \pm 15.8$	$1,458.0 \pm 16.3$	$1,456.9 \pm 20.3$
δ	$97.6^{ns} \pm 3.3$	96.9 ± 4.2	97.1 ± 2.7	97.3 ± 4.0
Tocotrienols				
α	$2.7^{\mathrm{a}} \pm 0.0$	$2.1^{c} \pm 0.1$	$2.3^{b} \pm 0.0$	$2.8^{\mathrm{a}} \pm 0.0$
ß	$0.8^{b} \pm 0.1$	$0.9^{\rm a} \pm 0.1$	$0.6^{\rm c} \pm 0.0$	$0.8^{\mathrm{b}}\pm0.0$
γ	$1.9^{\rm a} \pm 0.2$	$1.6^{b} \pm 0.1$	$1.5^{b} \pm 0.1$	$1.9^{\rm a} \pm 0.1$
δ	$0.7^{\mathrm{ab}}\pm0.1$	$0.5^{\rm c} \pm 0.0$	$0.6^{\rm bc} \pm 0.1$	$0.7^{\mathrm{a}} \pm 0.1$

Data are means $(n = 9) \pm SD$ (n = 9), (P < 0.05), Mean values with different letters in rows differ significantly

and maintenance [31]. Thus, consumption of the mungbean cultivars, in addition to providing nutrients such as proteins, carbohydrates, and minerals, must also impart some of the widely acclaimed health benefits of these FA to the indigenous population of Pakistan. The FA composition of the oils largely corroborates measurements of the physicochemical characteristics of the oils (Tables 1, and 2). Rather, high RI values are an indication of the presence of considerable amounts of PUFA in the oils [32]. The high content of linoleic acid would increase the susceptibility of the oils to oxidation. A similar FA profile for mungbean oil was reported earlier [21].

Tocopherol and tocotrienol contents are well in line with those reported earlier [8] and each cultivar was found to contain appreciable amounts of these constituents (Table 4).The concentration of δ -tocopherol which has a greater antioxidant activity than either γ - or α -tocopherol, is comparable to the values reported. These relatively higher values would be expected to contribute to excellent oxidative stability and protection to mungbean oil during storage and processing.

This study of mungbean in Pakistan has demonstrated biochemical differences among the cultivar samples. Previous reports also underlined differences, both physical and biochemical, among various cultivars. Despite variation among investigated cultivars with reference to investigated parameters, these mungbean cultivars are comparable in all the studied attributes with those reported throughout the world. The results may therefore offer a scientific basis for use of the seeds, both in human diet and some commercial products and consumption of the mungbean cultivars, in addition to providing such nutrients as proteins, carbohydrates, and minerals, also impart some of the widely acclaimed health benefits of the these oil constituents to the indigenous population of Pakistan. Given the continuing population growth and the still low per capita income of the indigenous population, the prospects for further expanding mungbean utilization appear bright. To achieve this, however, priority needs to be given to mungbean cultivation in national development programs and continued strong support for both basic and applied research on mungbean is required at the national level to maintain the momentum generated by current improvements.

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