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Characterization and Compositional Studies of Oil from Seeds of Desi Chickpea (*Cicer arietinum* L.) Cultivars Grown in Pakistan

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Abstract The physiochemical properties and fatty acid (FA) composition of oil from seeds of four desi chickpea cultivars, grown in Pakistan, were investigated. The oil content was relatively low (5.88-6.87%). The physiochemical parameters determined included refractive indices (RI) at 40 °C (1.48-1.49), relative density (0.95-0.96), iodine value (IV) (111.87–113.69), acid value (AV) (2.55–2.73 mg KOH/g), saponification value (SV) (183.98–185.64 mg KOH/g), unsaponifiable matter (UM) (2.99-3.71%), peroxide value (PV) (3.97-6.37 mequiv/ Kg), p-anisidine value (p-AV) (5.39-8.74), and oxidation value (OV) (13.67-22.34). Linoleic acid and oleic acid were the dominant FAs. Results from most of the parameters revealed significant (P < 0.05) differences among the cultivars. The findings reveal Desi chickpea (Cicer arietinum L.), indigenous to Pakistan, to be a potentially valuable legume crop with comparable nutritional quality oil.

Keywords Composition · Oil · Desi chickpea · Cultivars · Pakistan

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Introduction

The chickpea (*Cicer arietinum* L.) is a well studied plant for its nutritional value [1]. It is a good source of carbohydrates, minerals and trace elements [2]. In addition, it is a cheap source of high quality protein in the diets of millions in developing countries, who cannot afford animal protein for balanced nutrition. As food, chickpea seeds are eaten fresh as green vegetables, parched, fried, roasted, and boiled, as snack food, sweetmeats and condiments. More than a dozen food preparations such as dhal, flour (besan), hummus, and parched gram (putanas) are made from chickpeas [3].

Oil content, including essential fatty acids (FA), is the third important organic component of chickpeas [1]. The chickpea (Cicer arietinum L.) cannot be described as an oil-bearing seed; as the oil content of chickpeas (Cicer arietinum L.), grown in the different parts of world, reportedly is relatively low on average. The chickpea (Cicer arietinum L.) oil consists of tocopherols, tocotrienols and sterols [4-6]. All these constituents have their own nutritional and medicinal importance. 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 [7–9]. Phytosterols have a lowering effect on cholesterol levels in humans [10] and they also display anti-inflammatory, antibacterial, antifungic, antiulcerative and antitumor activities [6, 11, 12]. All these factors have contributed to place the cultivation of chickpea (Cicer arietinum L.) at the same economic level as that of cereals with the added value that chickpea cultivation is more environment-friendly, as it adds to soil fertility by symbiotic nitrogen fixation.

Chickpea is the most produced legume crop in Pakistan [13]. The total cultivated area in 2003–2004 for chickpea was 963,000 ha with crop production of 675,000 tons [14]. The chickpea crop is grown in Pakistan under three cropping systems, the rain fed system constituting 88% of the total chickpea growing area, where chickpea is grown as a sole crop or mixed with other crops; the rice-based system, constituting 11% of the total growing area, where the crop is grown on residual moisture after rice; and the irrigated system, constituting only 1% of the total area [15]. Based on an informal survey of producing areas, 90% of the chickpea grown in Pakistan is of desi type, and only 10% is of kabuli type. Punjab and NWFP provinces of Pakistan are the major producers of chickpea, constituting 87 and 7% of the area for chickpea cultivation, respectively [16].

In recent years, chickpea production in Pakistan has increased substantially. This has been brought about by the development of new chickpea cultivars with higher yields, improved adaptation to local agroclimatic conditions and better acceptability through improved nutritional status such as FA and ANF (Anti-nutritional factor profiles), 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 chickpea cultivars grown in Pakistan. The composition of total FAs is often the only information provided in studies of chickpea oil. The objective of the present study is to physiochemically characterize the oil of commonly cultivated desi chickpea (*Cicer arietinum* L.) cultivars of Pakistan.

Experimental Procedures

Materials and Reagents

The seeds of four desi chickpea (*Cicer arietinum* L.) cultivars namely Balksar 2000, CM98, Dasht, and Winhar 2000, grown and harvested under similar environmental and agroclimatic conditions, for 3 years, i.e. 2004–2006, were procured from the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Seeds of each variety, i.e. 500 g, were collected for three years, grown at four different locations and analyzed in triplicate $(1 \times 3 \times 3)$. Results were averaged and data was reported as mean \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 chickpea 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, 2.501, 2.201, 2.504 and 2.202, respectively, for the analysis of fats and oils [17]. Oxidation value (OV) was calculated from Holm's equation, OV = p-AV + 2 (PV), while theoretical flavor scores (*F*) were obtained from equation F = 7.7-0.35 (OV) [18].

Separation of the lipid classes, in each oil sample, was accomplished by adsorption column chromatography using florisil (7% H₂O, W/W; Saarchem Pty. Ltd., Muldersdrift, Republic of South Africa) and gradient elution as: hydrocarbons (*n*-hexane, 100%), sterol esters (*n*-hexane/ether, 95:5% V/V), triacylglycerol (TAG) plus free fatty acid (TAG + FFA) (*n*-hexane/ether, 85:15% V/V), free sterols (*n*-hexane/ether, 75:25% V/V), diacylglycerol (DAG) (*n*-hexane/ether, 50:50% V/V), monoacylglycerol (MAG) (ether/methanol, 90:10% V/V), glycolipids (acetone, 100%) and phospholipids (methanol, 100%) [19].

Separation of Acylglycerols

Triacylglycerol (TAG), diacylglycerol (DAG) with free fatty acid (FFA) (DAG + FFA), and monoacylglycerol (MAG) in the oil samples were further separated by gradient elution on silica gel (Saarchem Pty. Ltd.) using benzene (100%), benzene/ether (90:10% V/V), and ether (100%), respectively [19].

Fatty Acid Profile

Fatty acid methyl esters (FAMEs) were prepared according to a standard IUPAC method 2.301 and analyzed on a SHIMADZU gas chromatograph model 17-A, SP-2330 (SUPELCO Inc. Supelco Park Bellefonte, PA, 16823– 0048, USA) employing a polar capillary column (30 m \times 0.32 mm), coated with a methyl lignoserate (0.25 µm film thickness), and a flame ionization detector. Oxygen free nitrogen was used as a carrier gas at a flow rate of 5.0 mL min⁻¹. Other conditions were: initial oven temperature, 180 °C; ramp rate, 5 °C min⁻¹ to a final temperature of 220 °C; injector temperature, 230 °C; detector temperature, 250 °C; and temperature hold, 2 min before the run and 10 min after the run. A sample volume of 1.5 µL was injected. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards (Sigma, St. Louis, MO, USA; 99% purity specific for 168 GLC). A data-handling program, Chromatography Station for Windows (CSW32) was used for the quantification. The FA composition was reported as a relative percentage of the total peak area.

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% C.I. Analysis was carried out by using the "MSTATC" statistical computer package [20].

Results and Discussion

The oil contents and different physical properties of oil from desi chickpea seeds from different cultivars are presented in Table 1.

The oil yields for the four cultivars ranged from 5.88% for Dasht to 6.87% for Winhar 2000. The results revealed that the cultivars differed significantly with each other as far as total oil contents are concerned. The low yields reported here are in close agreement with previously reported values (4.95–5.20%) [5]. The variation in oil content within the countries and species is attributed to the environmental and geological conditions of the regions [21]. The low oil yields obtained were also supported with the view that legumes are generally not oilbearing crops [22]. The oil is of brownish yellow color and is liquid at room temperature (29.0 \pm 1 °C). The

liquid character is also reported earlier for groundnut seed oil [23].

The range of relative densities (Table 1), 0.95–0.96, 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 [22] 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 1.48 to 1.49. Although this range is comparable with the RI for *Phaseolus vulgaris* (1.47–1.48) and grapeseed oil (1.47–1.48), the range is higher than the RI for soybean (1.47–1.48) and groundnut (1.46–1.46) oils; the two legumes with the highest oil content [24]. These relatively high RI are an indication of substantial unsaturation in the oils of the studied chickpea cultivars.

Bulk chemical properties such as acid value AV, SV, IV, PV, and p-anisidine value (p-AV) give structural stability, and quality information about oils and fats [22]. These values are reported in Tables 2 and 3. The range of AVs (2.55-2.73 mg KOH/g) resemble closely to that reported earlier for chickpea (2.4-2.6 mg KOH/g) seed oil [24] but lower than AVs reported for *Phaseolus vulgaris* (11.0–19.2 mg KOH/g) seed oil [22]. These relatively lower AVs might render the process of refining a bit easier [25]. The range of SV from 183.98 to 185.64 mg KOH/g indicates the absence of lauric acid in the investigated chickpea seed oils, instead this range is indicative of oils characterized by medium chain-length FAs. Indeed, the range of SV for the chickpea cultivars is quite similar to those of olive (184–196 mg KOH/g), soybean (189– 195 mg KOH/g), Phaseolus vulgaris (172.2-196.6 mg KOH/g) and sunflower seed (188-194 mg KOH/g) oils [22].

The UM ranged from 2.99 to 3.71% W/W (Table 2) and it was revealed that cultivars differed significantly with respect to UM. These values are consistent with the UM pattern observed for low oil-bearing seeds [22] and are in line for chickpea determined earlier (3.4–4% and 3%) [26, 27]. The UM determined in this work prompted a separate study to investigate the components of the UM.

Neutral lipids, dominated by TAG, were the predominant lipid compounds in the oils (Table 3). The TAG was

Table 1 Oil content and physical properties of oil of desi chickpea cultivars

Chickpea cultivars	Oil contents	Color	Relative density (40 °C) (g/cm ³)	Refractive index (40 °C)
Balksar 2000	6.87 ± 0.33 a	Brown-yellow	0.96 ± 0.01	1.48
CM 98	5.89 ± 0.73 b	Brown-yellow	0.96 ± 0.02	1.48
Dasht	5.88 ± 0.12 b	Brown-yellow	0.95 ± 0.01	1.49
Winhar 2000	6.72 ± 0.09 a	Brown-yellow	0.96 ± 0.02	1.48

Data are expressed as means \pm standard deviations (n = 9) on dry weight basis, values marked by the same letter in same column of same class are not significantly different (P < 0.05)

Chickpea cultivars	Acid values (mg KOH/g)	Iodine values (Wijs method)	Saponification values (mg KOH/g)	Unsaponifiable matter (% w/w)
Balksar 2000	2.67 ± 0.04	112.55 ± 0.72	185.64 ± 0.22	3.37 ± 0.09 b
CM 98	2.73 ± 0.03	111.87 ± 0.19	184.77 ± 0.47	$2.99 \pm 0.02 \text{ c}$
Dasht	2.59 ± 0.03	113.69 ± 0.37	183.98 ± 0.19	3.71 ± 0.07 a
Winhar 2000	2.55 ± 0.07	112.32 ± 0.63	185.33 ± 0.22	$3.10 \pm 0.06 \text{ c}$

Table 2 Chemical characteristics of oil of desi chickpea cultivars

Data are expressed as means \pm standard deviations (n = 9) on dry weight basis, values marked by the same letter in same column of same class are not significantly different (P < 0.05)

Table 3 Percentage composition of lipid classes in the oils from desi chickpea cultivars estimated from adsorption column chromatography

Chickpea cultivars	Neutral	lipids			Polar lipic	Polar lipids		
	HC	TAG	STE + FFA	FST	DAG	MAG	GL	PL
Balksar 2000	0.9	63.2	3.3	2.7	1.6	2.7	0.8	17.8
CM 98	0.3	55.7	2.9	2.3	0.7	2.3	0.1	16.9
Dasht	0.7	57.9	3.0	3.3	1.1	2.5	0.5	19.9
Winhar 2000	0.4	62.4	2.7	2.5	1.4	2.2	0.7	18.3

Values represent the average of two replicate analyses

HC hydrocarbons, STE sterol esters, FST free sterols, GL glycolipids, PL phospholipids, FFA free fatty acids, TAG triacylglycerols, DAG diacylglycerols, MAG monoacylglycerols

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, whereas glycolipids were present only in trace amounts as polar lipid components.

The IV ranged from 111.87 to 113.69 (Wijs method) (Table 2). These values were higher than the IV for groundnut oil (80-106, Wijs method) and Phaseolus vulgaris (80.5–92.3, Wijs method) oils [22], but in agreement to those reported earlier for chickpea [26, 28]. The IV, obtained in this study indicates that the oils contain appreciable level of unsaturated FAs. The seed oils from the chickpea cultivars investigated must thus contain significant amounts of saturated FAs, likely to be palmitic acid (16:0), a common feature of legume seed oils [29], which is confirmed by the FA profile of the chickpea cultivars investigated. The FA composition of the chickpea cultivars investigated (Table 4), despite differences among cultivars, followed the general pattern for legumes [22]; with linoleic and oleic acids as the dominant FAs. 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 FAs required for growth, physiological functions and maintenance [30]. Thus, consumption of the chickpea cultivars, in addition to providing nutrients such as proteins, carbohydrates, and minerals, must also impart some of the widely acclaimed health benefits of these FAs to the indigenous population of Pakistan. The FA composition of the oils (Table 4) largely corroborates measurements of the physicochemical characteristics of the oils (Tables 2, 3). Rather, high RI values are an indication of the presence of considerable amounts of PUFA in the oils [31]. The high content of linoleic acid would increase the susceptibility of the oils to oxidation and hence result in high OV.

The PV (mequiv/kg of oil) and *p*-anisidine value (*p*-AV) measure hydroperoxides and secondary oxidation products, i.e. aldehydes, of the oils, respectively [31]. The PV (Table 5) of the oils from the chickpea cultivars were within the Codex recommended maximum (10 mequiv/kg) for edible oils [24]. The values are in partial agreement to that reported for Phaseolus vulgaris (1.8-10.7 mequiv/kg) oils [22]. However, the p-AV values of 5.39–8.74 anisidine units suggest the presence of significant amounts of secondary oxidation products in the test oil samples. The OV (13.09–22.34) indicates that considerable oxidative activity might be due to either lipoxygenase or autoxidation. The lipoxygenase activity of chickpea oil has also been reported earlier [32]. The OV are comparable with those reported for Phaseolus vulgaris seed oil (11.0-31.2) [22]. All the cultivars differed significantly with respect to PV, p-AV and OV. These relatively higher oxidation values prompted a separate study for lipoxygenase activity of chickpea oil. Table 5 shows theoretical flavor scores (F) of the oil from all chickpea cultivars. Although the equation was developed

Table 4 Fatty acid composition (% w/w) of oil from desi chickpea cultivars

Fatty acid (% in oil) Palmatic (C16:0)	Balksar 2000 19.8 ± 0.1	CM 98 18.9 ± 0.1	Dasht 21.5 ± 0.1	Winhar 2000 17.8 ± 0.1
Palmitoleic (16:1)	0.5 ± 0.0	0.7 ± 0.1	0.3 ± 0.1	0.9 ± 0.0
Stearic (C18:0)	1.5 ± 0.0	1.0 ± 0.0	0.9 ± 0.1	1.8 ± 0.0
Oleic (C18:1)	20.9 ± 0.1	24.4 ± 0.2	21.6 ± 0.2	23.6 ± 0.1
Linoleic (C18:2)	55.2 ± 0.2	53.7 ± 0.1	52.9 ± 0.2	54.2 ± 0.2
Linolenic C18:3)	0.7 ± 0.0	0.3 ± 0.1	1.0 ± 0.1	0.4 ± 0.0
Arachidic (C20:0)	1.4 ± 0.0	1.0 ± 0.0	1.8 ± 0.0	1.3 ± 0.0

Data are expressed as means \pm standard deviations (n = 9) on dry weight basis, values marked by the same letter in same column of same class are not significantly different (P < 0.05)

Table 5 Peroxide values, p-ansidine values, oxidation values and flavor scores of oil of desi chickpea cultivars

Chickpea cultivars	Peroxide value (mequiv/kg)	<i>p</i> -Anisidine value	Oxidation value	Flavor score
Balksar 2000	6.37 ± 0.07 a	8.74 ± 0.62 a	22.34 ± 0.76 a	-0.92 ± 0.03 b
CM 98	4.59 ± 0.03 c	5.69 ± 0.41 c	13.67 ± 0.53 c	2.91 ± 0.07 a
Dasht	$3.97 \pm 0.08 \text{ d}$	5.39 ± 023 c	13.09 ± 0.39 c	3.11 ± 0.06 a
Winhar 2000	5.37 ± 0.05 b	8.09 ± 0.31 b	18.23 ± 0.41 b	1.32 ± 0.04 b

Data are expressed as means \pm standard deviations (n = 9) on dry weight basis, values marked by the same letter in same column of same class are not significantly different

(P < 0.05)

for soybean oil, the flavor scores (-0.92 to 3.11) indicate that the oils from the test chickpea cultivars would receive rather low acceptance as an edible oil without further refinement. The investigated cultivars differed significantly with respect to theoretical flavor scores. The values are comparable to flavor score values reported for *Phaseolus vulgaris* seed oil (-3.2 to 3.9) [22].

Despite variation among investigated chickpea cultivars, with regard to their oil composition, these chickpea cultivars are comparable in all the studied attributes with those reported throughout the world. Pakistan, undoubtedly, has the capacity for large-scale production of chickpea and the area sown for chickpea is expected to increase in the next few years as new higher yielding chickpea varieties are released. The results, may therefore offer a scientific basis for use of the seeds, both in the human diet and other commercial products. These analytical findings will provide a regional database for this valuable legume crop, which has not been explored so far. The data obtained will be useful to both producers and consumers.

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