Analytical Characterization of Hemp (*Cannabis sativa*) Seed Oil from Different Agro-ecological Zones of Pakistan

Farooq Anwar[a],*, Sajid Latif[a], and Muhammad Ashraf[b]

Departments of [a]Chemistry and [b]Botany, University of Agriculture, Faisalabad–38040, Pakistan

ABSTRACT: Cold-pressed oil content of *Cannabis sativa* (hemp) seeds from three different agro-ecological zones of Pakistan ranged from 26.90 to 31.50%. Protein, fiber, ash, and moisture content were found to be 23.00–26.50, 17.00–20.52, 5.00–7.60, and 5.60–8.50%, respectively. Results of some other physical and chemical parameters of the oil were as follows: iodine value, 154.00–165.00; refractive index (40°C), 1.4698–1.4750; density (24°C), 0.9180–0.9270 mg mL^{-1}; saponification value, 184.00–190.00; unsaponifiable matter, 0.70–1.25%; and color (1-in. cell), 0.50–0.80 R + 27.00–32.00 Y. The induction period (Rancimat, 20 L h^{-1}, 120°C) of the nondegummed and degummed oils ranged from 1.35 to 1.72 h and from 1.20 to 1.49 h, respectively. Specific extinctions at 232 and 270 nm were 3.50–4.18 and 0.95–1.43, respectively. The hemp oils investigated were found to contain high levels of linoleic acid, 56.50–60.50%, followed by α-linolenic, oleic, palmitic, stearic, and γ-linolenic acids: 16.85–20.00, 10.17–14.03, 5.75–8.27, 2.19–2.79, and 0.63–1.65%, respectively. Tocopherols (α, γ, and δ) in the nondegummed oils were found to be 54.02–60.40, 600.00–745.00, 35.00–45.60, respectively, and were reduced to 29.90–50.00, 590.00–640.00, and 30.40–39.50 mg kg^{-1}, respectively, after degumming. The results of the present analytical study, compared with those found in the typical literature on hempseed oils, showed *C. sativa* indigenous to Pakistan to be a potentially valuable nonconventional oilseed crop of comparable quality.

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KEY WORDS: Agro-ecological zones of Pakistan, analytical characterization, *Cannabis sativa*, fatty acids, oxidative stability, tocopherols.

Cannabis is classified into the family Cannabaceae and has three main types: *C. sativa*, *C. indica*, and *C. ruderalis*. *Cannabis sativa* (the Latin term for "useful hemp") is characterized by long, thin flowers and spiky leaves. The plant is considered to be native of Western and Central Asia (Russia, China, India, Pakistan, and Iran). It is particularly naturalized in the sub-Himalayan tract in India, where it grows abundantly in the wastelands from Punjab east to Bengal and Bihar and south to Deccan (1). In Pakistan, hemp (*C. sativa*, locally known as "bhang") grows wild and is cultivated in some regions of the Potwar Plateau, the North West Frontier Province (NWFP), and the northern areas of Pakistan (2). The plant has also been cultivated commercially in Europe and in parts of China, Japan, and the United States for its fiber.

Hemp is of economic and pharmaceutical importance throughout the world. A low (0.3%) δ-9-tetrahydrocannabinol (THC) form of industrial hemp is legal to grow, and the global market for low-THC hemp is valued at $100–2000 million annually (3).The hemp foliage and leafed branches have been used as a sedative and narcotic drug known as Herba cannabis (4). The narcotic drugs obtained from the hemp plant include bhang or hashish, ganja, and charas (1). Essential oil obtained from hemp contains volatile compounds, mainly monoterpenes, sesquiterpenes, and other terpenoid-like compounds. Hemp essential oil can be used for cosmetics, as an additive to foods, and in aromatherapy (5). It has pesticidal applications as well.

Hempseed is rich in vitamins A, C, and E; minerals; and β-carotene and is claimed to have exceptional nutritional value (6). It contains 20–25% protein, 20–30% carbohydrates, 25–35% oil, 10–15% insoluble fiber, and a rich array of minerals, particularly phosphorus, potassium, magnesium, sulfur, and calcium along with modest amounts of iron and zinc, the latter of which is an important enzyme co-factor for human FA metabolism (7). Hempseed has long been used as a food ingredient or crushed for oil and meal. Like soy flour, hempseed flour contains adequate protein for the vegetarian diet. The flour, which is characterized by a nutty flavour, adds a unique culinary and healthy twist to baking. Hempseed, in addition to its nutritional value, has demonstrated positive health benefits, including lowering cholesterol and high blood pressure. It has been consumed in food and folk medicinal preparations, or used as a feed for birds and fish (3,7).

Hempseed oil has a good taste and offers various advantages over other vegetable oils. It is considered to be perfectly balanced in regard to the ratio (3:1) of two PUFA essential for human nutrition, linoleic and linolenic acids. Additionally, because of the high amount of PUFA and the presence of γ-linolenic acid, hempseed oil is ideal as an ingredient in light body oils and lipid-enriched creams, known for their ability to penetrate the skin (3). Currently, hemp oil is used for personal care products such as lotions, moisturizers, shampoos, and lip balms. This highly polyunsaturated oil has uses similar to that of linseed oil (e.g., as fuel for lighting, in printer's inks, as a wood preservative), but also has been used as a raw material for detergents and soaps (3,7). The quality of hemp oil is currently under investigation to improve the economic and environmental performance of this

*To whom correspondence should be addressed.
E-mail: fqanwar@yahoo.com

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nonconventional crop through innovative uses of its components or by-products (3).

The availability of hempseed is expected to increase owing to a renewed demand for hemp fiber to make paper and clothing. The versatility of the hempseed lends itself to the development of numerous products for the food, cosmetic, therapeutic, functional food, and nutraceutical industries. Climatic conditions or the date of harvest may influence the maturity of the seeds. Hempseed production and its properties vary widely, depending mainly on the harvest date and on the agroclimatic and geographical conditions in which it is grown. Grigoryev (8) reported variations in the content of hempseed oil and in the tocopherol and FA profile of oils from different locations in Russia.

The ever-increasing demand for vegetable oils, coupled with current awareness about the nutritional and functional role of fats in human diet, has made it essential to characterize additional vegetable oil sources to be explored as specialty oils. Hence, a great deal of interest is currently being focused on the possibility of exploiting the vast numbers of less-familiar plant resources. Many reports on some lesser known nonconventional seeds and fruits indicate that they could be good sources of nutrients for both humans and livestock (9).

Although Pakistan is an agrarian country, its indigenous vegetable oil resources do not meet its domestic requirements. Consequently, enormous quantities of oil are now imported to meet local demands. Because hemp is distributed in Pakistan on a wide scale, great potential exists to explore hempseed oil from Pakistan as a specialty oil. A wide variety of parent rock and soil types are found in Pakistan, which might exert considerable influence on the composition and properties of hempseed oil. To date, a full characterization of the hempseed oil indigenous to Pakistan has not yet been reported. In this context, as a part of our ongoing systematic characterization of nonconventional oilseed crops (10,11), we have assayed hemp (*C. sativa*) seeds from their natural habitats in Pakistan and conducted a comprehensive analysis. The primary objective of the present study was to conduct a detailed analysis and investigate the physicochemical characteristics of hempseed oil native to three different agro-ecological zones in Pakistan. Our analytical findings were correlated with those of literature reports to accelerate efforts to establish a global database for this valuable oilseed crop.

EXPERIMENTAL PROCEDURES

Materials. Hemp (*C. sativa)* seeds (2.0 kg of each sample) were assayed from three different provenances in Pakistan. Three samples of dry seeds from wild hemp from each of three agro-ecological regions, i.e., Lahore (Wagah, Punjab; sample LHR), Jehlum (Sarai Alimgir, Punjab; sample JHL), and Swat (approx. 75 miles from Saidu Sharif near Kalam Marghazar, NWFP; sample SWT), were harvested. The seeds were identified and authenticated by Dr. Muhammad Ashraf Chairmaim, Department of Botany, University of Agriculture, Faisalabad. All reagents (analytical and HPLC) used were from E. Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Pure standards of tocopherols [DL-α-tocopherol, (+)-δ-tocopherol, (+)-γ-tocopherol] and FAME were obtained from Sigma Chemical Co. (St. Louis, MO).

Oil extraction and moisture content. After removing the seed impurities, the oil was extracted by cold pressing. Prior to pressing, samples were dried in a vacuum oven at 60°C to a 4–5% moisture content. For thermal conditioning prior to pressing, dried seeds were cooked at 60°C for 5 min. Pressing was done in a manual hydraulic press for 20 min in the range of 35–50 MPa. Except for a small quantity of oil (for tocopherol and Rancimat analyses), the recovered oil from different batches was further degummed.

Degumming of oils. The oil to be degummed was heated at 70°C on a water bath. Hot water was added to a final volume of 18% and mixed for 10 min with the aid of a glass rod. After cooling, the oil was centrifuged ($1221 \times g$) for 10 min in 100-cm^3 tubes in an automatic refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan) (10). The degummed and centrifuged oil was left in contact (stirred) with anhydrous sodium sulfate for approximately 5 min, filtered through a filter paper by gravity in a vaccum-drying oven (VOC-300 SD; Eyela, Tokyo, Japan) at 50°C, and stored in separate sealed bottles under refrigeration (0–4°C) until used for the Rancimat and tocopherol analyses.

Analysis of oilseed residues. After oil extraction, the hempseed residue was analyzed for protein, fiber, and ash content. The protein content was determined according to a semi-automated FOSFA official method (12). The samples of meal were digested for 10 min with a digestion mixture of sulfuric acid/hydrogen peroxide/potassium sulfate, with selenium dioxide as a catalyst. The final end point in the ammonia titration was measured photometrically.

Fiber content was estimated according to an ISO method (13). A finely ground sample (2.5 g) of meal was weighed and freed from fat by a 15-mL *n*-hexane extraction. The test portion was boiled with sulfuric acid (0.255 mol L^{-1}), followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol L^{-1}), followed by separation, washing, and drying. The dried residue was weighed and ashed in a muffle furnace (TMF-2100; Eyela) at 600°C, and the loss of mass was determined.

Ash content was determined according to an ISO method (14). Two grams of the test portion was taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace at 550°C until a constant mass was achieved.

Analysis of extracted oil. (i) Physical and chemical parameters of oils. Iodine value, refractive index, density, saponification value, and unsaponifiable matter were determined by various standard AOCS methods (15). The color of the oil was determined by a Lovibond tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom) using a 1-in. cell. Specific extinctions at 232 and 270 nm were determined using a Hitachi U-2001 spectrophotometer, model 121-0032. Samples of oil were diluted with iso-octane, and spectra were recorded in the UV region, with absorbance values recorded at 232 and 270 nm,

and $\varepsilon^{1\%}_{1cm}$ (λ) calculated following the standard IUPAC method (16).

(ii) Oxidative stability. An automated Metrohm Rancimat apparatus, model 679, capable of operating over a temperature range of 50–200°C, was used to determine induction periods of the degummed and nondegummed oils. Testing was carried out at 120 ± 0.1°C, and oxidative stability was measured following a procedure described elsewhere (17). Briefly, oil (2.5 g) was carefully weighed into each of six reaction vessels and analyzed simultaneously. Induction periods of the samples were recorded automatically and corresponded to the break point of the plotted curves.

(iii) FA composition. FAME were prepared according to IUPAC method 2.301 and were analyzed on a Shimadzu gas chromatograph, model 17-A, fitted with a methyl lignoserate-coated (film thickness 0.25 µm) SP-2330 polar capillary column (30 m × 0.32 mm; Supelco Inc., Supelco Park Bellefonte, PA), and an FID. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 5.0 mL min^{-1}. Other conditions were as follows: initial oven temperature, 180°C; ramp rate, 5°C min^{-1}; final temperature, 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. FAME were identified by comparing their relative and absolute retention times with those of authentic standards. A data-handling program, Chromatography Station for Windows (CSW32), was used for quantification. The FA composition was reported as a relative percentage of the total peak area.

(iv) Tocopherol content. Tocopherol (α, γ, and δ) analysis was carried out by HPLC following the method of Thompson and Hatina (18) with slight modifications. One gram of oil was accurately weighed and made up to volume with heptane in a 10-mL volumetric flask wrapped in foil to inhibit oxidation. A Hitachi L-6200 HPLC unit coupled with a Hitachi F-1050 fluorescence detector was used. A 20-µL sample was injected into a LiChrosorb SI-60 column (250 × 4.6 mm; Supelco Inc.) packed with LiChrosorb SI 605 (5 µm), which was fitted with a 50 × 50 mm (i.d.) guard column with He-Pellosil packing. A mobile phase of dry heptane/water-saturated heptane/2-propanol (50.0:48.5:1.5) was used at the rate of 1.4 mL min^{-1}. Detection was performed at an excitation wavelength of 290 nm and an emission wavelength of 325 nm. Tocopherols were identified by comparing the retention times with those of pure standards of α-, γ-, and δ- tocopherols, and were quantified on the basis of peak area percentages of the unknowns with those of pure standards (Sigma Chemical Co.). Quantification was based on an external standard method. A D-2500 Hitachi Chromatointegrator model with a built-in computer program for data handling was used for quantification.

Statistical analysis. Three hemp oilseed samples were assayed from each region and analyzed individually in triplicate; data are reported as means ± SD ($n = 3 \times 3$).

RESULTS AND DISCUSSION

Data for the analysis of hemp (*C. sativa*) oilseeds and extracted oils from three different agro-ecological regions of Pakistan are summarized in Tables 1–5. Values for the present analysis are given as means ± SD for three hemp oilseed samples from each region, analyzed individually in triplicate. The content of cold-pressed oil ranged from 26.90 to 31.50% (Table 1). The oil concentration was highest (31.50%) in the seed samples collected from the vicinity of Swat (wet mountainous region in the NWFP of Pakistan), whereas the seeds assayed from hemp plants grown in the periphery of Lahore (a semiarid zone with hot summers and cold winters, at an altitude approx. 540 ft above sea level) were the lowest in oil content (26.90%). The seed samples harvested from Jhelum, which is located in a salt range on the Potwar Plateau (a semiarid zone, almost humid, with hot summers and cold winters, located at an altitude approx. 1700 ft above sea level) contained 28.20% of oil.

Hemp is naturalized and grows wild in a mild, humid climate with a temperature range of 60–80°F. It is mainly distributed in the NWFP and grows abundantly along the roadsides in the northern regions of Pakistan, but is sparsely cultivated in the Lahore region and other parts of the country. In contrast to the Lahore region, the former region, i.e., Swat, is located at a higher altitude (2500–7500 ft above sea level) in the sub-Himalayan tract of the NWFP, a colder zone in which the average temperature is low and the climate is humid, with mild summers and very cold winters. Furthermore, the soil texture in the Swat region, which covers high mountains interrupted by wide and narrow valley plains and plateaus, ranges from silt loam to silty clays, whereas the soil texture of the Lahore region is

TABLE 1
Analysis of Hemp (*Cannabis sativa*) Seeds[a]

Constituent (%)	Sample LHR	Sample JHL	Sample SWT	Literature: Mölleken *et al.* (19)	Grigoryev (8)	Bagci *et al.* (4)
Oil content	26.90 ± 0.50	28.20 ± 0.35	31.50 ± 0.28	30	30–35	31.79
Protein content	23.00 ± 0.37	26.50 ± 0.45	24.95 ± 0.55	25–30	20–25	NR
Fiber content	20.52 ± 0.71	18.48 ± 0.47	17.00 ± 0.66	NR	NR	NR
Ash content	5.00 ± 0.39	7.60 ± 0.42	6.00 ± 0.40	NR	NR	NR
Moisture content	8.50 ± 0.24	6.82 ± 0.18	5.60 ± 0.28	NR	NR	NR

[a]Values are means ± SD, calculated as the percentage of dry seed weight for three hemp (*C. sativa)* seed samples analyzed individually in triplicate. Sample LHR, sample collected from Lahore; Sample JHL, sample collected from Jhelum; Sample SWT, sample collected from Swat; NR, not reported.

TABLE 2
Physiochemical Properties of Hemp (*C. sativa*) Seed Oil[a]

Constituent	Sample LHR	Sample JHL	Sample SWT
Refractive index (40°C)	1.4698 ± 0.004	1.4714 ± 0.002	1.4750 ± 0.004
Density (24°C, mg mL^{-1})	0.9180 ± 0.003	0.9235 ± 0.003	0.9270 ± 0.003
Saponification value (mg KOH/g of oil)	190.00 ± 1.86	185.60 ± 1.40	184.00 ± 1.73
Iodine value (g of I/100 g of oil)	154.00 ± 1.65	159.20 ± 1.29	165.00 ± 1.58
Unsaponifiable matter (% w/w)	0.70 ± 0.04	0.94 ± 0.04	1.25 ± 0.05
Color (1-in. cell)			
Red units	0.50 ± 0.10	0.70 ± 0.15	0.80 ± 0.14
Yellow units	27.00 ± 1.30	29.00 ± 1.60	32.00 ± 1.20

[a]Values are means ± SD of three hemp (*C. sativa)* oils, analyzed individually in triplicate. For abbreviations see Table 1.

TABLE 3
Determination of the Oxidative State of Hemp (*C. sativa*) Seed Oil[a]

Constituent	Sample LHR	Sample JHL	Sample SWT
Conjugated diene $\varepsilon^{1\%}_{1cm}$ (λ232)	3.50 ± 0.12	3.62 ± 0.15	4.18 ± 0.15
Conjugated triene $\varepsilon^{1\%}_{1cm}$ (λ270)	0.95 ± 0.06	1.15 ± 0.10	1.43 ± 0.09
Oxidative stability, Rancimat method (h)			
Nondegummed oil	1.72 ± 0.13	1.43 ± 0.10	1.35 ± 0.12
Degummed oil	1.49 ± 0.11	1.17 ± 0.09	1.20 ± 0.14

[a]Values are means ± SD of three hemp (*C. sativa)* oils, analyzed individually in triplicate. For abbreviations see Table 1.

loamy clay. The large variation in seasonal temperature and the soil texture specific to the Lahore region might have been the two major factors contributing to the reduced oil content of hempseed in this region.

The average oil content (28.87%) of hempseed from different agro-ecological regions of Pakistan was slightly lower than that reported by Mölleken *et al.* (19) from Germany (30.00%) and Bagci *et al.* (4) from Turkey (31.79%). However, the oil content of hempseed indigenous to Pakistan was considerably lower than that reported from different cultivation areas of Russia (30–35%) (8). Such variation in oil yield of hempseed across countries might be attributed to the agro-climatic conditions of the regions.

The range (26.90–31.50%) of hempseed oil content from different agro-ecological regions as compared with those of some conventional and nonconventional oilseed crops, was found to exceed those of cottonseed (15.0–24.0%), soybean (17.0–21.0%), and olive (20.0– 25.0%) grown in the United States, Brazil, China, and other Asian and European countries (20). It was comparable to that of *Salicornia bigelovii* (27.2–32.0%) (11) but lower than that of *Moringa oleifera* (33.23–40.90%) (10).

Analysis of the oilseed residue of hempseed from different agro-ecological regions revealed a high protein content, ranging from 23.00 to 26.50%, whereas the fiber, ash, and moisture contents were in the ranges of 17.00–20.52%, 5.00–7.60%, and 5.60–8.50%, respectively. The value of the protein content of the hempseed investigated was comparable to those reported by Grigoryev (8) (20–25%), but lower than those reported by Mölleken (19) (25–30%). Compared with conventional oilseed crops, the protein content of hempseed was higher than those of safflower (20–22%), sunflower (16.50–19.60%), and cottonseed (19.40%) but comparable to those of linseed (24%), sesame seed (20–25%), and mustard seed (25–35%) (20). Hempseed protein

TABLE 4
FA Composition (g/100 g of FA) of Hemp (*C. sativa)* Seed Oil[a]

				Literature									
FA	Sample LHR	Sample JHL	Sample SWT	Rossell (21)	Aitzetmüller (28)	Deferne and Pate (7)	Callaway *et al.* (23)	Kuhn (25)	Mölleken and Theimer (26)	Orhan *et al.* (6)	Grigoryev (8)	Bagci *et al.* (4)	Oomah *et al.* (3)
16:0	8.27 ± 0.18	5.75 ± 0.16	6.02 ± 0.20	5.60	6.70	6–9	7.00	NR	6.23	8.53	7.05	6.53	6.60
18:0	2.19 ± 0.15	2.79 ± 0.13	2.40 ± 0.10	2.60	2.60	2–3	3.22	NR	2.65	3.06	2.50	2.64	2.70
18:1	14.03 ± 0.14	13.10 ± 0.19	10.17 ± 0.17	10.60	16.40	10–16	15.15	NR	10.22	NR	11.20	15.21	10.10
18:2	56.50 ± 1.00	58.30 ± 1.00	60.50 ± 0.80	59.40	53.10	50–70	56.24	52.00	56.42	54.66	54.40	50.46	54.30
18:3α	16.85 ± 0.45	18.75 ± 0.57	20.00 ± 0.39	19.40	16.10	15–25	15.95	18.00	18.60	33.72	20.78	20.09	19.10
18:3γ	1.65 ± 0.07	1.24 ± 0.05	0.63 ± 0.05	NR	1.10	1–6	0.94	2.00	2.45	2.01	4.75	0.58	3.60
18:4	ND	ND	ND	NR	0.40	NR	0.39	NR	NR	NR	1.40	0.34	NR
20:2	ND	ND	1.00	1.90	0.80	NR	NR	NR	1.02	NR	0.70	0.70	1.40

[a]Values are means ± SD of three hemp (*C. sativa)* oils, analyzed individually in triplicate. ND, not detected; for other abbreviations see Table 1.

TABLE 5
Comparison of Tocopherol in Hemp (*C. sativa*) Seed Oil[a]

Tocopherols ($mg\ kg^{-1}$)	Nondegummed oil			Degummed oil			Literature		
	Sample LHR	Sample JHL	Sample SWT	Sample LHR	Sample JHL	Sample SWT	Grigoryev (8)	Oomah *et al.* (3)	Bagci *et al.* (4)
α	60.40 ± 1.40	41.80 ± 2.00	54.02 ± 0.90	50.00 ± 1.90	29.90 ± 0.90	48.50 ± 1.10	254.70	34.00	42.23
γ	650.00 ± 4.50	600.00 ± 12.90	745.00 ± 10.20	590.00 ± 9.60	570.00 ± 8.70	640.00 ± 8.50	NR	733.00	664.90
δ	45.60 ± 2.50	42.30 ± 1.85	35.00 ± 2.20	39.50 ± 1.40	36.10 ± 1.50	30.40 ± 2.10	148.70	25.00	2.46

[a]Values are means ± SD of three hemp (*C. sativa*) oils, analyzed individually in triplicate. For abbreviations see Table 1.

is readily digestible, as it is primarily composed of edestin and albumin, which are components of human blood plasma (7). However, heat-treating whole hempseed denatures this protein and renders it insoluble, possibly affecting digestibility. The crushed hempseed by-product is suitable for animal feed and as a human staple because of its spectrum of amino acids (which includes all eight of those essential to the human diet) as well as its carbohydrates and the small amount of residual oil (7). The present analysis showed the meal to be a good source of protein, which could be added to human and poultry diets as a source of nutrition and calories. It could also be used as a fertilizer and as a potential animal foodstuff as a source of nutrition and calories, providing value-added by-products.

The moisture content of the hempseed in the present analysis was comparable to those of sunflower (6.0–9.0%), palm (5.9–8.5%), and rapeseed (6.0–9.0%). The fiber content was lower than that of cottonseed (22.6%) but significantly higher than those of soybean (4.8%), sesame seed (12.0%), and sunflower seed (13.2–15.7%) (20).

Various physical and chemical parameters of the extracted hemp oil are given in Table 2. The values determined for iodine value (154.00–165.00 g of iodine/100 g of oil), refractive index at 40°C (1.4698–1.4750), density at 24°C (0.9180–0.9270), saponification value (184.00–190.00 mg of KOH/g of oil), and unsaponifiabe matter (0.70–1.25%) for hempseed oil native to different agro-ecological regions of Pakistan could not be compared with the literature, as no previously reported data on these parameters in hempseed oils were available with which to compare with our present work. The iodine value of hempseed oils was, however, higher than those of cottonseed (99–119 g of iodine/100 g of oil), soybean (120–143 g of iodine/100 g of oil), and sunflower oils (110–143 g of iodine/100 g of oil) but lower than that of linseed oil (155–205 g of iodine/100 g of oil) (21). The refractive index at 40°C was higher than that of most vegetable oils reported in the literature (21). The saponification value was within the range of cottonseed, olive, pumpkin seed, and safflower oils (21). The color (0.50–0.80 R + 27.00–32.00 Y) of the hempseed oils investigated was lower in yellow and red units than those of some other nonconventional vegetable oils (10,11). The intensity of the color of vegetable oils depends mainly on the presence of various pigments, such as chlorophyll and carotenoids, which are effectively removed during the degumming, refining, and bleaching steps of oil processing. Vegetable oils with minimum color index values are more suitable for edible and domestic purposes.

The oxidation parameters of hemp oil native to Pakistan are shown in Table 3. The specific extinctions at 232 and 270 nm, which reveal the oxidative deterioration and purity of the oils (22), ranged from 3.50 to 4.18 and 0.95 to 1.43, respectively. These specific extinction values were somewhat higher that those of some nonconventional oils such as *M. oleifera* and *S. bigelovii* seed oils (11). The average induction period (Rancimat: 20L/h, 120°C), which is a characteristic of the oxidative stability of oils and fats (17), of nondegummed hemp oil from different agro-ecological regions of Pakistan was 1.50 h (range 1.35–1.72 h). After degumming, the induction periods of the oils were decreased to 1.29 h (range 1.20–1.49), a reduction of 14.00% (range 11.11–18.18%) in oxidative stability, which could be attributed to the degumming process. No previously reported data on nondegummed hempseed oil were available with which to compare the induction period results from our present work. Because of the high content of PUFA, hempseed oil is fairly unstable and becomes rancid quickly unless preserved.

Table 4 shows the FA composition of hempseed oils indigenous to Pakistan. The contents of palmitic and stearic acids in the hempseed oils ranged from 5.75 to 8.27 and 2.19 to 2.79%, respectively. The oils were found to contain a high level of unsaturation (89.03–91.39%). The contents of linoleic acid (18:2n-6) ranged from 56.50 to 60.50%, followed by α-linolenic (18:3n-3) and oleic acid (18:1n-9), with ranges of 16.85–20.0 and 10.17–14.03%, respectively. A small amount of γ-linolenic acid (18:3n-6), ranging from 0.63 to 1.65%, was also detected.

The average content (58.43%) of the major FA, i.e., 18:2, was appreciably higher than the values in hempseed oils reported by Rossell (21) and Callaway *et al*. (23), whereas it varied somewhat compared with those in the hempseed oils investigated by Yazicioglu and Karaali (24), Kuhn (25), and Bagci *et al*. (4). The concentration of 18:3α was notably lower than those reported by Yazicioglu and Karaali (24) and Orhan *et al*. (6) but was comparable to the values reported by Kuhn (25), Oomah *et al*. (3), and Mölleken and Theimer (26). Literature reports have shown that hempseed oil contains a variety of FA, with linoleic (18:2) and linolenic (18:3) acids predominating (3,7,27,28). Rumyantseva and Lemeshev (29) reported that 18:2 and 18:3 usually account for approximately 50–70% and 15–25%, respectively, of the total FA content of the hempseed oil.

The contents of 16:0 and 18:0 in the hempseeed oils from Pakistan were comparable to those reported by Rossell (21),

Aitzetmüller (28), Deferne and Pate (7), Mölleken and Theimer (26), and Oomah *et al.* (3) but varied from those of Mehmedic (27), Yazicioglu and Karaali (24), Callaway *et al*. (23), and Orhan *et al*. (6). The concentration of 18:1 was slightly higher than the values reported by Rossell (21), Mölleken and Theimer (26), Oomah *et al*. (3), and Grigoryev (8) but lower than those reported by Yazicioglu and Karaali (24), Deferne and Pate (7), Callaway *et al*. (23), and Bagci *et al*. (4). γ-Linolenic acid (1.20%), an unusual FA found in the present analysis, was well in line with the studies by Aitzetmüller (28); was higher than those reported by Bagci *et al*. (4) and Callaway *et al*. (23); and was lower than those reported by Deferne and Pate (7), Kuhn (25), Mölleken and Theimer (26), Orhan *et al*. (6), Oomah *et al*. (3), and Grigoryev (8). There have been reports that except for one variety from Jamaica, all the hemp cultivars contain varying quantities of γ-linolenic acid (26). The occurrence and distribution of γ-linolenic acid in the plant kingdom may have chemotaxonomic significance in some families (30). γ-Linolenic acid is highly appreciated and of considerable interest for its dietary uses and medicinal attributes. γ-Linolenic acid is one of the important FA used as both a health nutrient and a therapeutic agent, and only recently have its potential physiological benefits been extensively investigated (4,30). The high content of EFA of nutraceutical value present in hempseed oil ensures the regulation of membrane fluidity by stabilizing the lipid bilayer, and it might be useful in reducing cholesterol in the blood and thus agonistic against atherosclerosis (31). However, a high degree of unsaturation renders hempseed oil extremely sensitive to oxidative rancidity. Because heat or light accelerates the degradation, the oil is unsatisfactory for frying or baking, although moderate heat for short periods is probably tolerable (7).

Table 5 shows the content of different tocopherols in the nondegummed (crude) and degummed hempseed oils as determined by HPLC. The average levels of α-, γ-, and δ-tocopherol in the nondegummed oils from different agro-ecological regions were 52.07 (range 41.80–60.40), 665.00 (range 600.00–745.00), and 40.97 mg kg^{-1} (range 35.00–45.60 mg kg^{-1}), respectively. After degumming, the contents were reduced to 42.80 (range 29.90–50.00), 600.00 (range 570.00–640.00), and 35.33 mg kg^{-1} (range 30.40–39.50 mg kg^{-1}), reductions of 17.80, 9.77 and 13.77% in the contents of α-, γ-, and δ-tocopherol, respectively. This loss of tocopherol content might be attributed to the oil degumming process, because most of the steps involved during processing and storage reduce the level of tocopherols (21). The content of α-tocopherol in the present analysis was in agreement with the values reported by Bagci *et al*. (4) from Turkey. The contents of α- and δ-tocopherol were considerably lower than those reported by Grigoryev (8) from Russia. However, the concentration of δ-tocopherol was well in line with those reported by Bagci *et al*. (4) and Oomah *et al*. (3). Among the tocopherols, the α-homolog shows the highest vitamin E activity, whereas the δ-isomer exhibits potent antioxidant activity. Our current findings suggest that hempseed oil would be a good source of important tocopherols. As with many of the other traits, no previously reported data on the tocopherol contents of degummed hempseed oils are available in the literature.

The results of the present analysis showed that the fatty acid composition of hempseed oil native to Pakistan falls in the category of high linoleic and α-linolenic acids and contains a ratio of 18:2n-6 to 18:3n-3 of 3.15:1.0, which is quite close to the recommended nutritional ratio of 3:1. Hemp grows wild over vast areas of the upper Punjab, the NWFP, and the northern areas of Pakistan; thus, it appears to be a potentially valuable crop, yielding a useful oil that might be an acceptable substitute for high-linoleic oils such as soybean, sunflower, corn, and cottonseed oils as dietary fats. Our present investigations revealed that hemp oil indigenous to Pakistan could be utilized successfully as a valuable source of EFA (18:2n-6, 18:3n-3) of nutraceutical value. It could also be used in the preparation of various food commodities because of its nutritional and therapeutic attributes, and could be used with other high-oleic vegetable oils for the preparation of nutritionally balanced oil blends.

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