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ANALYTICAL CHARACTERIZATION OF RICE (*ORYZA SATIVA*) BRAN AND BRAN OIL FROM DIFFERENT AGRO-ECOLOGICAL REGIONS

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In view of growing need and consciousness about the nutritional and functional properties of vegetable oils, physico-chemical characterization of non-conventional oil seed crops is of much concern to cope the existing challenges. The present research work was aimed to characterize rice (*Oryza sativa*) bran oil from different covariant agricultural regions of Punjab, Pakistan. The investigated rice bran was found to contain oil, protein, fiber and ash content in the range of 13.92-19.81, 15.30-17.60, 7.64-9.65 and 7.50-9.50%, respectively. Physical and chemical parameters of the hexane-extracted rice bran oils were as; density (40°C) 0.909-0.921g/cm^3, refractive index (40°C) 1.4586-1.4596, iodine value 103.75-113.15 g of I_2/100 g of oil, peroxide value 2.47-3.20 meq/kg, acid value 40.39-45.01%, *p*-anisidine value 2.99-3.50, conjugated diene contents 2.01-3.11 and conjugated triene contents 0.89-1.01. Furthermore the rice bran oil was found to contain $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ as major fatty acid and α, γ and δ tocopherols in its composition. Rice bran and rice bran oil has many physio-chemical, nutritional and compositional properties fairly comparable to those with the conventional vegetable oils; being used as important constituents of human diet and other commercial purposes.
Keywords: Non-conventional oils, oxidative stability, *Oryza sativa*, rice bran oil, fatty acids

INTRODUCTION

Rice (*Oryza sativa*) is a member of *Gramineae* family. More than 500 million metric tons of milled rice is produced, per year worldwide, constituting more than a quarter of all cereal grains (Iqbal *et al.,* 2005). Rice represents a staple food group for many cultures and has provided the nutritional basis for mankind since antiquity. People of Asia, South America, much of Africa, portions of Europe, the near East and North America depend upon rice for daily sustenance (Anwar *et al*., 2005).

Rice bran is a huge agricultural waste of rice polishing industry, produced during milling of brown rice. Although, it has been recognized as an excellent source of vitamins and minerals, but underutilized as human food. Research conducted during the last two decades has shown that rice bran is a unique complex of naturally occurring antioxidant compounds (Iqbal *et al.,* 2005; Moldenhauer *et al.,* 2003). It contains high valued protein, fat and dietary fiber. In addition to phytonutrients, vitamins minerals and medicinally important antioxidant, γ-oryzanol (natural mixture of ferulic esters) rice bran has been reckoned as a potential source of edible oil (Iqbal *et al.,* 2005; Moldenhauer *et al.,* 2003; Chatha *et al.,* 2006). Rice bran oil, a miracle product obtained from outer brown layer of rice, is reported to be 15–20%, depending upon the degree of milling, variety and other agroclimatic factors (Anwar *et al*., 2005; Chatha *et al.,* 2006). Rice bran oil in its natural form, contain several constitutes of potential significance in diet and health (Anwar *et al*., 2005; Nicolosi *et al*., 1994; Sharma, 2002). Oil from any single source has not been found to be suitable for all purposes, as oils from different sources generally differ in their composition that necessitates the exploitation of new sources of oils (Ramadan and Morsel, 2002).

Pakistan is a big rice producer and a huge quantity of rice bran is produced annually as agricultural waste. Until now, a comprehensive characterization and comparison of rice bran oil of Basmati Super kernel and Basmati 385 indigenous to different regions of Pakistan has not been reported. So, the present research work was designed for the analytical characterization of rice bran oil of two commonly available varieties (Super kernel and 385) indigenous to different agro-ecological regions of Punjab, Pakistan.

MATERIALS AND METHODS

All the chemicals and reagents used in the present research work were of analytical grade and procured from E. Merk (Darmstadt, Germany) and Sigma-Aldrich Chemical Co. (St. Louis, MO).

***Collection and stabilization of rice bran*:** Rice bran samples of super kernel and 385 were collected from rice polishing industries from different regions (Chiniot, Gujranwala and Bahawalnagar) of Pakistan in airtight polyethylene bags and further authenticated and identified. Stabilization of rice

bran was carried out according to the method of Malekian *et al.* (2000). A microwave oven with 550 W, out put power was used for the stabilization of rice bran. One hundred grams of rice bran sample was packed in a polyethylene microwave-safe bag and subjected to microwave heating in a preheated oven for 3 min at 120°C and cooled down at room temperature overnight. This procedure was repeated three times to ensure the stabilization.

***Extraction of oil*:** The extraction was executed on water bath for 8-9 h with 300 mL of n-hexane. After extraction, the solvent was distilled off under vacuum in a rotary evaporator (Rotary Vacuum Evaporator N.N. Series equipped with an Aspirator and a Digital Water Bath SB-651; Eyela, Tokyo, Japan) at 45°C and further made moisture free with sodium sulphate. The extracted oil was then stored in refrigerator until used for further analysis.

***Analysis of rice bran residue*:** Rice bran residues after the oil extraction were analyzed for protein, fiber and ash contents. Protein contents were determined according to AOAC (Association of Official Analytical Chemist) official method (1984) using microkjeldhal apparatus. Fiber and ash contents were determined according to ISO method (International Standards Organization, 1981)

***Analysis of extracted rice bran oil*:** Physico-chemical characteristics of oil were evaluated using the parameters like density, refractive index, iodine value, saponification value, unsaponifiable matter and colour according to standard IUPAC methods (1987).

***Determination of oxidative state of rice bran oils*:** The oxidative quality of the oil was assessed by following the oxidation parameters like free fatty acid content, peroxide value, conjugated diene and triene contents and *p*-anisidine value. All of these tests were performed according to standard IUPAC methods (1979).

***Tocopherol contents*:** Tocopherol analysis was performed by High Performance Liquid Chromatography (HPLC) following the method described by Lee *et al*. (2003). An HPLC pump equipped with a Hypersil, BDH C_{18} column and a UV/Vis (Model Sykem, Germany) detector was used. The temperature of the column was maintained at 30°C in a thermostated column compartment. The chromatographic separation was performed by isocratic elution with a mixture of acetonitrile and methanol (65:35, v/v) at a flow rate of $1mL.min^{-1}$.

***Fatty acid composition*:** IUPAC standard method was followed for the preparation of fatty acid methyl esters (FAMEs) of rice bran oil to be analyzed for its fatty acid composition by Gas Chrmatograph (Model 17-A, SHIMADZU) fitted with an SP-2330 (SUPELCO, Inc. Supleco Park, Bellefonte, PA 16823-0048 USA) methyl lignoserate-coated (0.2µm) polar capillary coloumn (30×0.32mm), an FID detector and oxygen free nitrogen gas (carrier gas) at 5mL/min flow rate. Other conditions adjusted were as follow; Initial column temperature, 180°C ; ramp rate, 5°C/min; final column temperature, 220°C; injector temperature, 230°C; detector temperature, 250°C. FAMEs were identified by comparing their retention times with of authentic standards and all the quantification were done by a Chromatography Station for Windows (CSW32) data handling program.

All the experiments were carried out in three replicate and analyzed in triplicate and results has been presented as mean±SD. Furthermore, the data obtained was analyzed by statistical technique ANOVA (Steal and Torrie, 1992).

RESULTS AND DISCUSSION

***Proximate composition of rice bran*:** Table 1 shows the proximate composition of rice bran from different agroecological regions of Pakistan. The oil contents of rice bran from different regions were found in the range of 13.92–15.23 and 19.76–19.81% from super kernel and 385, respectively. The lowest oil contents were obtained from super kernel bran belonging to Bahawalnagar and the highest from 385 belonging to Gujranwala. Statistical analysis showed significant differences ($P \leq 0.05$) in the oil contents of different varieties of rice bran from different regions of Punjab. The variations in the yield of oil of different varieties of rice bran from different agro-ecological regions might be attributed to the diversity of natural soil texture of their derivation and other man-made cumulative effects (Anwar *et al.,* 2005).

The range of rice bran oil content (13.92–19.81%) in the present work is quite comparable to that (15–20%) reported from Koria (Lee, 2002) and Bangladesh (Absar *et al.,* 1998). Anwar *et al.* (2005) also reported the oil contents of different varieties of rice bran indigenous to Pakistan in the range of 14.70–19.10% that is strongly agreeable to the present findings. However, the super kernel variety of rice bran from Bahawalnagar was found to be lower in oil content to that of reported from Korea and Pakistan as well. Such minor variations in the oil contents with in the countries might be attributed to the possible change in environmental and agro-ecological conditions of the regions (Ibrahim *et al.,* 1994). This range of oil content, determined in the present analysis of different varieties of rice bran from different regions of Pakistan, was generally found to exceed from those of cottonseeds (10-12%), indigenous to Pakistan and mango kernel grown in India (Rossel, 1991). This range of oil content was somewhat comparable to that of cottonseed (15–24%) grown in the United States, Brazil, China and some other Asian and European countries (Rossel, 1991).

The crude protein contents of two varieties of rice bran from different regions of Punjab were in the range of 15.3–17.6%. The lowest and highest protein contents were investigated in the rice bran of super kernel and 385 of Chiniot. Statistical analysis showed significant differences ($P \leq 0.05$) in the protein contents of different varieties of rice bran from

Table 1. Proximate composition of rice bran from different agroecological regions

Rice Bran	Oil contents (%)	Protein contents (%)	Fiber contents (%)	Ash contents (%)
Super Kernel (Chiniot)	14.75 ± 0.48[b]	17.6 ± 0.42[a]	7.64 ± 0.06[a]	9.11± 0.06[a]
Super Kernel (Gujranwala)	15.23 ± 0.42[b]	16.9 ± 0.39[a]	7.99 ± 0.07[a]	9.21 ± 0.08[a]
Super Kernel (Bahawalnagar)	13.92 ± 0.39[b]	17.4 ± 0.41[a]	8.47 ± 0.05[a]	9.50 ± 0.07[a]
385 (Chiniot)	19.76 ± 0.60[a]	15.3 ± 0.37[a]	8.96 ± 0.07[a]	7.50 ± 0.05[b]
385 (Gujranwala)	19.81 ± 0.37[a]	16.2 ± 0.29[a]	9.50 ± 0.05[a]	7.60 ± 0.09[b]
385 (Bahawalnagar)	18.79 ± 0.41[a]	16.5 ± 0.40[a]	9.65 ± 0.04[a]	8.00 ± 0.07[b]

Values are mean ± SD of triplicate samples analyzed in triplicate. Different letters in superscript showing significant differences among varieties

different regions of Pakistan. The fiber and ash contents of two varieties of rice bran from various agro-ecological regions of Pakistan were in the range of 7.64–9.65 and 7.50–9.50%, respectively. The lowest and highest fiber and ash contents among different varieties of rice bran were found in super kernel of Chiniot & 385 of Bahawalnagar and 385 of Chiniot & super kernel of Bahawalnagar, respectively. Statistical analysis showed significant differences ($P \leq 0.05$) in the values of fiber and ash contents of different varieties of rice bran from different regions of Pakistan. These variations might be attributed to the different agroclimatic conditions, soil texture and methods of cultivation. The protein, fiber and ash contents as determined in the present analysis could not be compared as there are no previously reported data of rice bran to compare the results with the present work.

***Physico-chemical properties of rice bran oils*:** The results of different physico-chemical parameters are given in Table 2. The values reported for iodine, refractive index, density, saponification value and unsaponifiable matter of different rice bran oils were in close agreement with those reported in the literature (Rossel, 1991). Iodine values of two varieties of rice bran oils were found in the range of 103.75–113.15g I_2/100 g of oil. Rice bran oil from super kernel variety was found to be more unsaturated than that of 385 variety. The degree of unsaturation of rice bran oil from these two varieties was in close agreement with that of reported by Anwar *et al.* (2005), i.e. 112.40 and 105.10 g I_2/100 g of oil for super kernel and 385, respectively. The degree of unsaturation of investigated varieties of rice bran oil lies with in range of corn (103–128), cottonseed (99-119), mustard (92–125) and sesame oil (104–120) but lower than the soybean (120-143) and linseed (155-205) and sunflower oil (110–143) (Rossell, 1991).

The values of refractive index of two varieties of rice bran oils from various agro-ecological regions of Pakistan were in the range of 1.4586–1.4596. Statistical analysis showed no significant differences ($P \leq 0.05$) in the values of refractive

Table 2. Physico-chemical properties of rice bran oils from different agroecological regions

Properties	**Super Kernel** (Chiniot)	**Super Kernel** (Gujranwala)	**Super Kernel** (Bahawalnagar)	**385** (Chiniot)	**385** (Gujranwala)	**385** (Bahawalnagar)
Iodine value (g of I_2/100 g of oil)	111.24 ± 0.60[b]	113.15 ± 0.60[a]	109.37 ± 0.60[c]	104.35 ± 0.60[e]	106.01 ± 0.60[d]	103.75 ± 0.60[e]
Refractive index (40°C)	1.4593 ± 0.04[a]	1.4596 ± 0.06[a]	1.4590 ± 0.05[a]	1.4587 ± 0.04[a]	1.4588 ± 0.05[a]	1.4586 ± 0.04[a]
Density (40°C) (g/cm^3)	0.918 ± 0.03[a]	0.920 ± 0.05[a]	0.921 ± 0.04[a]	0.911 ± 0.02[a]	0.909 ± 0.03[a]	0.912 ± 0.05[a]
Saponification value (mg of KOH/g of oil)	182 ± 3.25	180 ± 3.34[a]	181 ± 3.36[a]	187 ± 3.20[a]	186 ± 3.29[a]	185 ± 3.40[a]
Unsaponifiable matter (%)	6.18 ± 0.25[a]	6.16 ± 0.30[a]	6.20 ± 0.23[a]	4.92 ± 0.13[b]	4.99 ± 0.11[b]	5.10 ± 0.10[ab]
Colour (Red unit)	7.45 ± 0.32[a]	7.25 ± 0.41[a]	7.50 ± 0.35[a]	7.00 ± 0.37	7.10 ± 0.40[a]	7.20 ± 0.45[a]
Colour (Yellow unit)	29.50 ± 0.91[a]	30.00 ± 0.75[a]	29.40 ± 0.85[a]	30.50 ± 0.85[a]	30.25 ± 0.65[a]	30.40 ± 0.79[a]

Values are mean ± SD of duplicate samples analyzed individually in triplicate; Different letters in superscript showing significant differences among varieties

indexes of oils of different varieties of rice bran. These values of refractive index of rice bran oils were fairly comparable to those of cocoa butter (1.4560–1.4590), Indian-illip (1.4590-1.4620) and cottonseed oil (1.4580-1.4660) but lower than those of sunflower (1.4670-1.4690) and soybean oil (1.4670-1.4700) (Rossell, 1991).

The density of two varieties of rice bran oils from various agroecological regions of Punjab was in the range of 0.909-0.921g/cm^3. Statistical analysis showed no significant differences ($p \leq 0.05$) in the values of densities of oils from different varieties of rice bran from different regions of Pakistan. These values of densities of rice bran oils were fairly comparable to those of many vegetable oils (Rossell, 1991).

The range of saponification values (180 - 187 mg of KOH/g of oil) and unsaponifiable matter (4.92-6.20%) of different varieties of rice bran oil from different agroecological regions of Punjab were in close agreement with of reported in literature (Rossell, 1991). Statistical analysis showed significant differences ($p \leq 0.05$) in the values of saponification and unsaponifiable matter of oils of different varieties of rice bran from different regions of Pakistan. These variations in physical and chemical properties of the investigated oils of different rice cultivars may be attributed to the source and milling system of rice polishing industries (Anwar *et al.,* 2005). Saponification values of the investigated varieties of rice bran oil were comparable to those of castor bean (176-187 mg of KOH/g of oil), mustard seed (170-184 mg of KOH/g of oil), shea nut (178-190 mg of KOH/g of oil) and nutmeg butter (170-190 mg of KOH/g of oil) but lower than those of sunflower (188-194 mg of KOH/g of oil) and soybean oil (188-195). The unsaponifiable matter of the investigated varieties of rice bran oil was comparable to that of shea nut (4-8%), but higher than those of sunflower (0.3-1.3%), cottonseed (0.5-1.5) and soybean oil (0.5-1.6%) (Rossell, 1991).

The colour index of crude rice bran oil of super kernel (7.45R + 29.50Y, 7.25R + 30.00Y and 7.50R + 29.40Y) and 385 (7.00R + 30.50Y, 7.10R + 30.25Y and 7.20R + 30.40Y) from different agro-ecological regions of Pakistan was in good agreement with that of reported by Anwar *et al.,* (2005) for super kernel (7.50R + 30.00Y) and 385 (7.00R + 30.50Y) but poor in colour measurement than 386 (4.5R + 25.00Y). Statistical analysis showed non-significant differences ($P \leq 0.05$) in the colour index of oils from different varieties of rice bran from different regions of Pakistan. The value of colour index of fully refined soybean and cotton seed oil (2.00R + 2.50Y) was superior to the rice bran oil (Rossel, 1991). The intensity of colour of vegetable oils depends mainly on the presence of various pigments such as chlorophyll, which are effectively removed during degumming, refining and bleaching steps of oil processing. The vegetable oils with minimum value of colour index are more suitable for edible and domestic purposes (Anwar *et al.,* 2005).

***Oxidative status of rice bran oils*:** The investigated varieties of rice bran oils from different agroecological regions of Pakistan exhibited very good oxidative status as indicated by the determinations given in Table 3. Free fatty acid (FFA) contents of super kernel and 385 rice bran oils of Chiniot, Gujranwala and Bahawalnagar were found 43.97, 45.01, 43.01 and 40.39, 41.90, 42.75%, respectively. Statistical analysis showed significant differences ($P \leq 0.05$) in the FFA contents of oils from different varieties of rice bran. FFA contents of rice bran oils are very high and not comparable to any other conventional vegetable oils like sunflower, soybean and canola. These high FFA content might be attributed to the high temperature of extraction method. Solvent extraction method using hexane as solvent requires high temperature, which produces very high amount of FFA due to thermal agitation, is not favourable for extraction of rice bran oil for edible purposes.

Table 3. Determination of oxidative state of rice bran oils from different agroecological regions

Properties	**Super Kernel** (Chiniot)	**Super Kernel** (Gujranwala)	**Super Kernel** (Bahawalnagar)	**385** (Chiniot)	**385** (Gujranwala)	**385** (Bahawalnagar)
Free fatty acid (%) (as oleic acid)	43.97 ± 1.25^{a}	45.01 ± 1.20^{a}	43.01 ± 1.17^{ab}	40.39 ± 1.27^{b}	41.90 ± 1.22^{b}	42.75 ± 1.19^{ab}
Peroxide value (meq/kg)	3.20 ± 0.05^{a}	2.99 ± 0.06^{b}	3.10 ± 0.04^{ab}	2.53 ± 0.03^{d}	2.47 ± 0.04^{d}	2.80 ± 0.05^{c}
Para anisidine value $^{1\%}\varepsilon$ 1cm (λ 350)	3.50 ± 0.03^{a}	3.40 ± 0.04^{a}	3.46 ± 0.05^{a}	2.99 ± 0.04^{b}	3.01 ± 0.03^{b}	3.10 ± 0.04^{b}
Conjugated diene $^{1\%}\varepsilon$ 1cm (λ 232)	2.01 ± 0.02^{d}	2.30 ± 0.03^{c}	2.23 ± 0.02^{c}	3.11 ± 0.04^{a}	2.90 ± 0.02^{b}	3.00 ± 0.03^{ab}
Conjugated triene $^{1\%}\varepsilon$ 1cm (λ 268)	0.90 ± 0.02^{c}	0.89 ± 0.02^{c}	0.96 ± 0.02^{b}	1.00 ± 0.02^{a}	0.99 ± 0.02^{a}	1.01 ± 0.02^{a}

Values are mean ± SD of duplicate samples analyzed individually in triplicate; Different letters in superscript showing significant differences among varieties

The peroxide value (meq/kg of oil) which measure the hydroperoxide oxidation products of oils (Chatha *et al*., 2006), were quite low i.e. super kernel and 385 varieties of Chiniot, 3.20 & 2.53; super kernel and 385 of Gujranwala, 2.99 & 2.80; super kernel and 385 of Bahawalnagar, 3.10 & 2.47 meq/kg. Statistical analysis showed significant differences ($P \leq 0.05$) in peroxide values of oils of different varieties of rice bran. These values are fairly comparable to those of reported by Anwar *et al.* (2005). However, these values are higher than those of refined, bleached and deodorized corn and low erucic rapeseed oil, i.e. 0.5 & 2.0 meq/kg (Rossell, 1991).

The *p*-anisidine value which measure the aldehydic secondary oxidation *p*roducts of oils (Chatha *et al.,* 2006), were found in the range of 2.99-3.50, i.e. super kernel and 385 verieties of Chiniot, 3.50 & 2.99; super kernel and 385 of Gujranwala, 3.40 & 3.01; super kernel and 385 of Bahawalnagar, 3.10 & 3.46, respectively. Statistical analysis showed significant differences ($P \leq 0.05$) in *p*-anisidine value of oils of different varieties of rice bran native to different agro-ecological regions of Pakistan. These values are fairly comparable to those of reported by Anwar *et al.* (2005. These values are also in agreement with those of most of conventional and non-conventional vegetable oils reported in the literature (Anwar *et al.,* 2003; Rossell, 1991).

The investigated varieties of rice bran oils also exhibited very good oxidative status in terms of measurement of conjugated diene (CD) and conjugated triene (CT), determined as specifc extinctions at 232 and 268nm respectively. The specifc extinctions at 232 and 268nm, which revealed the oxidative stability and purity of oil (Yoon et al., 1985), were found to be quite low, i.e. the CD and CT contents of super kernel and 385 of Chiniot, Gujranwala and Bahawalnagar were 2.01, 3.11& 0.90, 1.00; 2.30, 3.00 & 0.89, 0.99; 2.23, 2.90 & 0.96, 1.0, respectively. These findings are in close agreement with those of reported by Anwar *et al.* (2005) for different varieties of rice bran oil indigenous to Pakistan. These values are also comparable with those of many conventional and non-conventional vegetable oils (Anwar *et al.,* 2003).

***Tocopherol composition of rice bran oils*:** Table 4 shows the content of different tocopherols in the non-degummed (Crude) rice bran oils as determined by HPLC in the High Tech. Laboratory of Botany Department. The level of α tocopherol, γ tocopherol and δ tocopherol in super kernel and 385 from different regions was found in the range of 271.46-285.00 & 164.25–180.67, 78.86–83.00 & 117.59–121.36, 73.10–76.23 mg/Kg, respectively. It is depicted from the results that the amount of α tocopherol and δ tocopherol in super kernel rice bran oil was significantly ($P \leq 0.05$) higher than that of 385, whereas, the amount of γ tocopherol in 385 rice bran oil was significantly ($P \leq 0.05$) higher than that of super kernel. These amounts of different tocopherols in the investigated varieties of rice bran oils were in close agreement with those of reported for the same varieties of rice bran oils indigenous to Pakistan (Anwar *et al.,* 2005).

The content of α tocopherol ranged 164.25–285.00mg/Kg which has greatest vitamin E potency (Rossell, 1991), in the investigated oils was higher than the values reported for palm kernel, coconut and palm oils, while it was well in line to those reported for soybean, groundnut and low and high erucic acid rapeseed oils (Rossell, 1991). The level of γ tocopherol (80.99–121.36 mg/Kg) was significantly higher to those of coconut, palm and sunflower oils, whereas, lower to those of cottonseed, soybean and high erucic rapeseed oils (Rossell, 1991). The concentration of δ tocopherol ranged 37.05–76.23 mg/ Kg, which has greater antioxidant potency than either γ- or α-tocopherol (Tsakin, 1998), in the rice bran oils was comparable to maize oil, whereas, it was appreciably higher to those of palm kernel, coconut, cottonseed, groundnut, palm, sunflower and high and low erucic rapeseed oils (Rossell, 1991), and thus would be expected to contribute good oxidative stability and protection to the oil during storage and processing.

***Fatty acid composition of rice bran oils*:** Table 5 shows the fatty acid (FA) composition of different rice bran oils. The content of total saturated fatty acids (SFA); myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and arachidic ($C_{20:0}$) acid in super kernel and 385 bran oil of different regions were in the range of 1.35-1.50 & 3.99-4.29, 15.94-16.90 & 18.52-19.53,

Table 4. Tocopherol content (mg/Kg) of non-degummed rice bran oils from different agroecological regions

Rice bran	α-tocopherol	γ-tocopherol	δ-tocopherol
Super Kernel (Chiniot)	279.90 ± 8.45^{ab}	80.99 ± 4.44^{b}	73.10 ± 2.34^{a}
Super Kernel (Gujranwala)	285.00 ± 9.73^{a}	83.00 ± 4.71^{b}	76.23 ± 4.12^{a}
Super Kernel (Bahawalnagar)	271.46 ± 8.68^{b}	78.86 ± 5.20^{b}	74.99 ± 3.17^{a}
385 (Chiniot)	173.15 ± 4.47^{cd}	117.59 ± 5.02^{a}	37.05 ± 2.10^{b}
385 (Gujranwala)	180.67 ± 7.38^{c}	120.74 ± 4.26^{a}	40.43 ± 2.14^{b}
385 (Bahawalnagar)	164.25 ± 6.25^{d}	121.36 ± 3.97^{a}	38.95 ± 2.39^{b}

Values are mean ± SD of duplicate samples analyzed individually in triplicate; Different letters in superscript showing significant differences among varieties

2.37-2.65 & 3.91-4.12 and 1.08-1.27 & 0.93–1.00%, respectively. The level of SFA in 385 rice bran oil was higher than that of super kernel. This level of SFA in super kernel and 385 rice bran oil as investigated in the present analysis was closely comparable to that of reported by Anwar *et al.* (2005) for different varieties of rice bran oil indigenous to Pakistan. The level of SFA content of two investigated varieties of rice bran oils was also comparable with those of palm kernel, cottonseed and avocado oil (Rossell, 1991) but varied to those of other commonly grown and edible vegetable oils (Rossell, 1991).

The investigated bran oils of super kernel and 385 were found to contain a high level of monounsaturated fatty acids (MUFA), i.e. 41.97–43.65 & 39.98–41.02%, respectively. The level of oleic acid ($C_{18:1}$ ω-9) which account for the major fraction of total fatty acids was rather comparable to those of rice bran, palm and allanblackia oils (Rossell, 1991). Our findings are in close agreement regarding the oleic acid content in different varieties of rice bran oils indigenous to Pakistan (Anwar *et al.*, 2005). The content of linoleic acid ($C_{18:2}$ ω-6) in the investigated varieties of rice bran oils was found up to level of 32.68%. The linoleic acid content of super kernel variety was higher than that of 385. The content of linolenic acid ($C_{18:3}$ ω-3) in super kernel rice bran oil was found to be 1.37, 1.50 and 1.30% belonging to regions of Chiniot, Gujranwala and Bahawalnagar, respectively. However, rice bran oil from basmati 385 was found to contain trace amounts of linolenic acid. Statistical analysis showed significant differences ($P \leq 0.05$) in the concentrations of various fatty acids of rice bran oils belonging to different agro-ecological regions of Punjab. The amount of linolenic acid ($C_{18:3}$ ω-3) in the present analysis of investigated varieties of rice bran oils was in close agreement with those of reported by Anwar *et al*. (2005) and Rossell (1991). The concentrations of major fatty acids $C_{18:2}$, $C_{18:1}$, $C_{18:0}$, $C_{16:0}$ of the investigated oils were in close agreement with that reported by Hemavathy and Prabhakar (1987), for the rice bran oils indigenous to India. Whereas, the amount of $C_{16:0}$ and $C_{18:0}$ in rice bran oil of 385 varied to some extent from the findings of some researchers (Hemavathy and Prabhakar, 1987). The fatty acid composition of investigated varieties of rice bran oils were found quite similar in content of $C_{18:1}$ with those of rice bran oil indigenous to Korea (Lee *et al*., 2003). However, the amounts of $C_{18:2}$ were varied to some extent. The present fatty acid analysis of super kernel and 385 rice bran oils from different agro-ecological regions of Punjab showed that oleic acid was the predominant fatty acid followed by linoleic acid and palmitic acid.

The Japan's Ministry of health and welfare suggested fatty acid ratio of saturated/monounsaturated/polyunsaturated for the healthy edible oils as 1:1.5:1. The fatty acid composition of investigated varieties of rice bran oils from different agro-ecological regions of Punjab falls in the recommendations and contains high ratio of monounsaturated to saturated fatty acids. As the rice bran oil is unique in its fatty acid composition and thought to be one of the highest qualities of vegetable oil. It comes into sight that rice bran oil is roughly equal to the other vegetable oils supplying fatty acids requirements for human health but unique in its fatty acid composition. In consideration of this and other investigated properties rice bran oil have much to offer in caring for nutritional and health needs of pets and other animals in addition to human beings. It is concluded from the results of present study that rice bran and rice bran oil has many physio-chemical, nutritional and compositional properties fairly comparable to those with the conventional vegetable oils, being used as important constituents of human diet and other commercial purposes.

Table 5. Fatty acid composition of rice bran oils from different agroecological regions

Fatty Acid	Super Kernel (Chiniot)	Super Kernel (Gujranwala)	Super Kernel (Bahawalnagar)	385 (Chiniot)	385 (Gujranwala)	385 (Bahawalnagar)
C14:0	1.39 ± 0.01	1.50 ± 0.02	1.35 ± 0.01	3.99 ± 0.03	4.29 ± 0.04	4.18 ± 0.03
C16:0	15.94 ± 0.51	16.90 ± 0.65	16.65 ± 0.47	18.87 ± 0.36	19.53 ± 0.48	18.52 ± 0.50
C18:0	2.49 ± 0.10	2.65 ± 0.06	2.37 ± 0.10	3.98 ± 0.13	4.12 ± 0.21	3.91 ± 0.05
C18:1	42.79 ± 1.99	43.65 ± 2.06	41.97 ± 2.10	40.98 ± 0.93	41.02 ± 2.21	39.89 ± 1.50
C18:2	30.34 ± 1.73	32.68 ± 1.54	31.27 ± 1.64	27.59 ± 1.13	29.67 ± 1.58	28.57 ± 1.44
C18:3	1.37 ± 0.01	1.50 ± 0.04	1.30 ± 0.02	tr	tr	tr
C20:0	1.17 ± 0.03	1.27 ± 0.05	1.08 ± 0.04	0.98 ± 0.01	1.00 ± 0.04	0.93 ± 0.02
C20:1	0.99 ± 0.01	1.09 ± 0.05	0.95 ± 0.02	tr	tr	tr
C22:0	---nd	---nd	---nd	---nd	---nd	---nd
C24:0	---nd	---nd	---nd	---nd	---nd	---nd

Values are mean ± SD of duplicate samples analyzed individually in triplicate; ---nd __ not identified and tr __ trace amount

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