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Comparison of Physico-Chemical Properties Between Two Varieties of Peanut (*Arachis hypogaea* L.) Seed Oil from Pakistan

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The present research work was aimed to evaluate and quantify the physico-chemical attributes between two varieties of peanut (*Arachis hypogaea* L.) seed oil. The oil yield from two locally grown peanut varieties namely var. Banki and 334, using Soxhelt extraction method, was found to be 34.62 and 32.43 %, respectively. The seed kernel of the peanut varieties tested contained fiber, ash and protein 3.70, 3.90, 2.30, 2.50, 24.62 and 26.19 %, respectively. The physico-chemical attributes of the extracted oils were found to be refractive index (40 ºC) 1.4623, 1.4536; free fatty acids 2.65, 3.55 % as oleic acid; peroxide value 2.50, 3.50 meq/kg; iodine value 93.45, 91.96 g of I/100 g; saponification value 193.20, 188.00 mg of KOH/g of oil; unsponifiable matter 1.20, 1.50 %; *p*-ansidine value 1.54, 1.87, respectively. The colour of the tested oils varied from 1.42-1.51R + 14.00-15.00 Y. The seed oils mainly contained γ-tocopherol (709.1-712.2 mg/kg) followed by α-tocopherol (173.9-193.2 mg/kg) and δ-tocopherol (7.1-8.4 mg/kg). The peanut seed oils were characterized by high level of oleic acid (46.10-47.70 %) followed by linoleic acid (27.60-30.40 %), palmitic acid (12.70-13.50 %) and stearic acid (5.10-5.70 %). Generally, most of the physico-chemical attributes of the peanut seed oils varied insignificantly between the two varieties tested.

Key Words: Peanut oil, Physico-chemical attributes, Tocopherols, HPLC, Fatty acids, Oxidation parameters.

INTRODUCTION

The oilseed industry is one of the most rapidly growing agricultural enterprises globally, in particular, in semi-tropical and tropical agricultural regions, providing highly nutritious human food and animal feed. Several conventional and non-conventional oil seed crops are grown including palm, olive, cotton, sunflower, canola, sesame, safflower, soybean among others[1].

Peanut (*Arachis hypogaea* L.) belonging to Fabaceae family, is a legume native to South America but now cultivated in diverse environmental and agro-climatic conditions in six continents between latitudes 45°N and 35°S[2]. It has esteemed position in commercial venture due to its distinctive flavour. The low yield of this crop is mainly attributed to unreliable rainfall patterns with frequent droughts, lack of high yielding adapted cultivars, damage by diseases and pests, poor agronomic practices and limited use of inputs[3]. Peanut seeds yield non-drying edible oil (up to 50 %) of good composition like olive oil. Peanut seed oil is used for cooking and margarines production[4], as well as surfactants-cleansing and cosmetics agent *etc*.[5]. It is comprised of *ca.* 80 % unsaturated fatty acids with oleic acid ($C_{18:1}$), an average of 50 % and linoleic acid ($C_{18:2}$) around 30 % of the total fatty acids[6].

Taking into consideration the growing interest in nutritional and functional properties of oil[7], much attention has been paid to characterize the oil extracted from conventional and non-conventional oil seed crops. Peanut is relatively a new crop in Pakistan and is cultivated mostly as nut crop rather than major oil seed crop. Up till now, no earlier reports are available on the analytical characterization of the oil extracted from locally grown two common varieties of peanut seeds. The present study was an attempt to evaluate and compare the physico-chemical properties including the tocopherols and fatty acids composition between the seed oils derived from two commonly grown peanut cultivars in Pakistan.

EXPERIMENTAL

The fully mature peanuts of the two varieties (Banki & 334) were procured from Ayub Agricultural Research Institute (AARI), Jhang Road, Faisalabad, Pakistan. Both the varieties

were grown under the same environmental conditions. The seed coats were removed manually to recover peanut seed kernels, the recovered kernels were subjected to conditioning in a hot air oven (60 ºC) for 0.5 h.

All reagents (analytical and HPLC grade) used in this study, were obtained from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Pure standards of tocopherols (DL-α-tocopherol, (+) δ-tocopherol, (+) and γ-tocopherol) and fatty acid methyl esters (FAMEs) were from Sigma Chemical Co. (St. Louis, MO).

Extraction of oil: For oil extraction, seeds kernels (100 g) were ground using a commercial grinder and then placed in a paper thimble and fed to a Soxhelt apparatus connected with a water condenser and a round-bottomed flask. The extraction was performed on a water bath for 6 h with *n*-hexane (b.p. 65-68 ºC). After the extraction was accomplished, the excess of the solvent was removed under reduced pressure using a rotary vacuum evaporator (EYELA, N.N. Series, Rikakikai Co. Ltd., Tokyo, Japan).

Analysis of oilseed residues: Sample of meal (residue left after extraction of oil) was analyzed for protein, fiber and ash contents. Protein estimation was followed by the Association of Official Analytical Chemists (AOAC) standard method 976.06[8]. Fiber content was determined according to the ISO method[9]. Ash content was determined following the ISO method[10].

Analysis of extracted oils: Density, refractive index, iodine value, peroxide value, acidity, saponification value and unsaponifiable matter of the extracted peanut oils were determined according to AOCS methods[11]. A Lovibond Tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom) with 1-inch cell was used for the determination of colour coordinates. Specific extinctions, representing conjugated dienes and conjugated trienes, were calculated from the absorbance data at 232 and 270 nm, respectively using a spectrophotometer (U-2001, Hitachi Instrument Inc. Tokyo, Japan). The samples of the tested oils were dissolved in isooctane to maintain the absorbance within the appropriate limits (0.2-0.8) and ($\varepsilon_1^{1\%}{}_{cm(\lambda)}$) was calculated following IUPAC method[12]. In order to calculate *p*-anisidine value, the oil was analyzed using IUPAC protocol[12]. The oil sample, in *iso*-octane, was reacted with *p*-anisidine reagent forming a coloured complex, the absorbance of which was recorded at 350 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc. Tokyo, Japan).

Tocopherols composition: Tocopherols (α, γ and δ) were quantified according to a reported method[13] with slight modifications using NP-HPLC. Oil sample (1 g) dissolved in heptane was diluted up to 10 mL in a volumetric flask. HPLC unit (Hitachi L-6200) coupled with a Hitachi F-1050 fluorescence detector was used. A 20 µL of sample was introduced into column (Lichrosorb SI-60: 250 mm × 4.6 mm; Supelco Inc.), packed with LiChrosorb SI 605 (5 µm), connected to 50 mm × 50 mm (I.D.) guard column with He-Pellosil packing. The solvents (heptane, water-saturated heptane and 2-propanol) were mixed in a ratio of 50.0:48.5:1.5 to constitute a mobile phase which was pumped through the column at a flow rate of 1 mL/min. The detection of tocopherol isomers was done at 290 nm (excitation wavelength) and 325 nm (emission wavelength). Pure standards solutions of tocopherols (α, γ and δ) were prepared and used for identification and quantification purposes. Quantification was carried out using Chromato-integrator (Hitachi, model D-2500) fitted with a built-in computer program for data handling.

Gas chromatographic fatty acids (FAs) analysis: The oil sample was derivatized into fatty acid methyl esters (FAMEs) according to standard IUPAC method[12] and analyzed on a Shimadzu gas chromatograph model 17-A, fitted with a methyl lignoserate coated (film thickness 0.20 µm) SP-2330 (SUPLECO, Inc., Bellefonte, PA) polar capillary column (30 m × 0.25 mm) and a flame ionization detector (FID). Pure nitrogen gas (purity 99.99 %) was used as carrier gas at a rate of 5 mL/min, other conditions used were: initial oven temperature 180 ºC, ramp rate 5 ºC/min, final temperature 220 ºC (initial and final hold up time 2, 10 min, respectively), injector temperature 230 ºC and detector temperature 240 ºC. Pure standards of FAMEs were used for identification and quantification purposes. The quantification was made by a Chromatography Station for Windows (CSW32) data handling software (Data APEX Ltd., Czech Republic). The FA composition was reported as a relative percentage of the total peak area of the standards.

Statistical analysis: All the experiments were carried out in triplicate and the results thus produced were statistically analyzed using statistical software STATISTICA 5.5 (Stat Soft Inc., Tulsa, Oklahoma, USA). Statically significant difference was taken at probability value of $p < 0.05$. All the data are tabulated as mean values ± standard deviation obtained from triplicate determinations.

RESULTS AND DISCUSSION

In the present study, the solvent (*n*-hexane) was used for oil extraction from peanut seed kernels. Peanut seed kernels were found to give an oil yield in the range 32.43-34.62 %. The oil content (34.62 %) of var. Banki was found to be slightly higher ($p > 0.05$) than that of var. 334 (32.43 %) (Table-1). The oil content (32.43-34.62 %) from peanut seeds kernels determined in this study was found to be higher than the value (20.8 %) reported in literature[14] from Nigeria and lower than the value (44.09 %) reported by Ozcan and Seven[15] from Turkey. The differences in the oil yield among different regions might be attributed to the variations of the varieties, farming environment, ripening stage, the harvesting time of the seeds and the extraction method used[16]. The oil content (32.43-34.62 %) of peanut seeds of the tested varieties in the present analysis was found to be higher than those of conventional oilseed crops:cotton (15.0-24.0 %), soybean (17.0-21.0 %), while it was within the range of those of mustard (24.0- 40.0 %) and safflower (25.0-40.0 %) seeds[17]. Analysis of the meal (left after the extraction of oil) revealed the seed protein content 24.62-25.19 %, crude fiber (3.70-3.90 %) and ash (2.30-2.50 %). The protein content (24.62-25.19 %) from peanut seeds of tested varieties was found to be lower than that (36.93 %) reported by Ozcan and Seven from Turkey[15]. The present study suggests that peanut seed meal can be used in chicken feed and animal feed as a source of protein. The ash content (2.30-2.50 %) estimated in this study was comparable to the value

TABLE-1
PROXIMATE ANALYSIS OF PEANUT SEED KERNEL OF DIFFERENT VARIETIES

Parameters	Banki	334
Oil yield (%)	34.62 ± 0.98[a]	32.43 ± 0.69[a]
Protein content (%)	24.62 ± 0.41[a]	26.19 ± 0.78[a]
Crude fiber (%)	3.70 ± 0.15[a]	3.90 ± 0.13[a]
Ash content (%)	2.30 ± 0.07[a]	2.50 ± 0.08[a]

Values are mean ± SD for three replicates. Different superscript letters within the same row indicate significant ($p < 0.05$) differences of means between the tested varieties.

(2.05) reported in the literature[15]. The digestibility level can be deduced from the crude fiber content of the meal. Food items having higher fiber content are less digestible, palatable and nutritionally less important.

The presently extracted seed oils were evaluated for physico-chemical attributes (Table-2). The oil produced from both the varieties (Banki and var.334) was found to have colour index of 1.42R + 14.00Y, 1.51R + 15.00Y and density 0.92 and 0.93 mg/mL, respectively. The saponification value of extracted oils was found to be 188.00-193.25 mg of KOH/g of oil which showing a good agreement with the value (187-196 mg of KOH/g of oil) reported in the literature[18]. The unsaponifiable matter (1.20-1.50 %) estimated in the present analysis was found to be higher than the value (0.99 %) reported from Turkey[15]. The refractive index (1.4536-1.4623) of tested oils is in good agreement with the values present in the literature[15,19]. The free fatty acid (FFA) value (2.65-3.55 %) of the oils was found to be higher when compared with the value (0.98) reported from Turkey[15]. Iodine value of oil gives an idea about the number of unsaturation sites present in the oil. The iodine value (91.96-93.45 g of I/100 g of oil) of investigated oils was found to be higher than that of the value (82 g of I/100 g of oil) reported in the literature[15]. The results of all the evaluated physico-chemical attributes of var. 334 are comparable to those of var. Banki except unsponifiable matter and free fatty acid (FFA).

TABLE-2
PHYSICO-CHEMICAL CHARACTERISTICS OF PEANUT SEED KERNEL OIL OF DIFFERENT VARIETIES

Parameters	Banki	334
Refractive index (40 ºC)	1.4623 ± 0.004[a]	1.4536 + 0.003[a]
Color (red unit)	1.42 ± 0.04[a]	1.51 + 0.05[a]
Color (yellow unit)	14.00 ± 0.48[a]	15.00+0.45[a]
Density (mg/mL) (24 ^{0}C)	0.92 ± 0.05[a]	0.93+0.03[a]
Saponification Value (mg of KOH/g of oil)	193.25 ± 4.58[a]	188.00 ± 3.92[a]
Unsaponificable matter (%)	1.20 ± 0.06[b]	1.50 ± 0.04[a]
Iodine value (g of I/100 g of oil)	93.45 ± 2.79[a]	91.96 ± 1.98[a]
Free Fatty Acid (% as oleic acid)	2.65 ± 0.08[b]	3.55 ± 0.11[a]

Values are mean ± SD for three replicates. Different superscript letters within the same row indicate significant ($p < 0.05$) differences of means between the tested varieties.

Peanut seed oil was found to exhibit good oxidative stability (Table-3). The oxidative deterioration and purity of the oil can be assessed by the magnitude of specific extinctions at 232 and 270 nm in terms of conjugated dienes and conjugated trienes, respectively[20]. The investigated seed oils (Banki and 334) showed specific extinctions 2.41, 2.11 at 232 nm and 0.19, 0.21 at 270 nm, respectively revealing no considerable variation between the two varieties tested. Peroxide value of var. Banki and 334 was found to be 2.5 and 3.5 meq/kg, respectively showing significant ($p < 0.05$) difference between the varieties tested. *p*-Anisidine value (1.54) of var. Banki was significantly ($p < 0.05$) different from var. 334 (1.87). No earlier reports are available in the literature on the oxidative state of peanut oil, with which we can compare the data of our present findings.

TABLE-3
OXIDATIVE STATE OF PEANUT SEED KERNEL OIL OF DIFFERENT VARIETIES

Parameters	Banki	334
Conjugated diene $\varepsilon_1^{1\%}{}_{cm\,(\lambda 232)}$	2.41 ± 0.08[a]	2.11 ± 0.06[a]
Conjugated triene $\varepsilon_1^{1\%}{}_{cm\,(\lambda 268)}$	0.19 ± 0.04[a]	0.21 ± 0.06[a]
p-Anisidine value	1.54 ± 0.05[b]	1.87 ± 0.04[a]
Peroxide value (meq/kg)	2.50 ± 0.08[b]	3.50 ± 0.11[a]

Values are mean ± SD for three replicates. Different superscript letters within the same row indicate significant ($p < 0.05$) differences of means between the tested varieties.

The level of different tocopherols (α, γ and δ) in both varieties (Banki and 334) of peanut seed oils are given in Table-4. An appreciable amount of γ-tocopherol (193.2 and 173.9 mg/kg) was detected in Banki and 334 peanut seed oils, respectively showing significant ($p > 0.05$) difference between the varieties analyzed. The concentration of α-tocopherol was highest among others with contribution at 712.2 and 709.1 mg/kg, respectively while a small amount of δ-tocopherol (7.1-8.4 mg/kg) was also present. The present α-tocopherol (193.2 and 173.9 mg/kg), which has the superior vitamin E potency[1], was found to be lower than that reported in peanut seed oil (387.0 mg/kg) by Ozcan and Seven from Turkey[15]. When compared with common vegetable oils, the level of γ-tocopherol (173.9-193.2 mg/kg) estimated in the extracted peanut seed oils was found to be lower than that of cotton (338 mg/kg), maize (282 mg/kg), sunflower (670 mg/kg) and higher than that of soybean (99.5 mg/kg) oils[1]. The amount δ-tocopherol (7.1-8.4 mg/kg) determined in present study was higher than those of cotton (3.3 mg/kg), sunflower (0.6 mg/kg) and lower than those of maize (54 mg/kg) soybean seed oil (421 mg/kg)[1]. The contents of γ-tocopherols (709.1-712.2 mg/kg) in the seed oil of tested peanut varieties were found to be higher than those of cotton (429 mg/kg), sunflower (11 mg/kg) but lower than those of maize (1034 mg/kg) and soybean seed (1021mg/kg) oils[1]. Due to lack of availability of the reports on tocopherols (γ and δ) of peanut seed oil, we could not compare the results of our present finding with those of literature.

TABLE-4
TOCOPHEROLS CONTENT OF PEANUT SEED KERNEL OIL OF DIFFERENT VARIETIES

Tocopherols	Banki	334
α-Tocopherol (mg/kg)	193.2 ± 0.67[a]	173.9 ± 0.52[a]
γ-Tocopherol (mg/kg)	712.2 ± 2.45[a]	709.1 ± 2.38[a]
δ-Tocopherol (mg/kg)	7.1 ± 0.02[b]	8.4 ± 0.03[a]

Values are mean ± SD for three replicates. Different superscript letters within the same row indicate significant ($p < 0.05$) differences of means between the tested varieties.

The fatty acid (FA) composition of peanut seed oil is given in Table-5. The contents of total saturated fatty acids in the extracted oils from tested varieties (Banki and 334) were found to be 21.9 and 21.4 followed by mono-unsaturated fatty acids 50.5, 48.3 and poly-unsaturated fatty acid 27.6 and 30.4, respectively. The concentration (21.4-21.9 %) of the total saturated fatty acid in peanut (*A. hypogaea*) seed oil was slightly higher than the values reported for different species of peanut including *Arachis sylvestris* (17.4 %), *Arachis pintoi* (18.4 %), *Arachis villosa* (19.5 %) and *Arachis stenosperma* (19.2 %) from Argentina[21]. The presently studied seed oils contained oleic acid ($C_{18:1}$; n-9) as a dominant mono-unsaturated fatty acid (46.10-47.70) and palmitic ($C_{16:0}$) as a dominant saturated fatty acid (12.70-13.50). An appreciable amount (27.70 %) of linoleic acid ($C_{18:2}$; n-6) and small amount of 2.80 % of gadoleic acid ($C_{20:1}$), was also recorded. The level of oleic acid (46.10-47.70) determined in the present analysis was found to be higher than those of cotton (19.0), soybean (21.7), maize (32.2) and sunflower seed (20.4) oil[1]. Vegetable oils containing high-oleic acid content are gaining considerable importance due to their higher stability and nutritional value[7,22]. The levels of saturated and unsaturated fatty acids of var. Banki were found to be insignificantly different ($p > 0.05$) when compared with var. 334.

TABLE-5
FATTY ACID COMPOSITION OF PEANUT SEED KERNEL OIL OF DIFFERENT VARIETIES

Fatty acids (%)	Banki	334
$C_{16:0}$	13.50 ± 0.38[a]	12.70 ± 0.39[a]
$C_{18:0}$	5.70 ± 0.18[a]	5.10 ± 0.50[a]
$C_{18:1}$	47.70 ± 1.85[a]	46.10 ± 1.38[a]
$C_{18:2}$	27.60 ± 1.20[a]	30.40 ± 1.21[a]
$C_{20:0}$	2.70 ± 0.09[b]	3.60 ± 0.11[a]
$C_{20:1}$	2.80 ± 0.08[a]	2.20 ± 0.06[b]

Values are mean ± SD for three replicates. Different superscript letters within the same row indicate significant ($p < 0.05$) differences of means between the tested varieties.

Conclusion

Generally, most of the physico-chemical parameters of the extracted peanut seed oils were found to be insignificantly ($p > 0.05$) varied between the two tested varieties. The present study revealed that both varieties of peanut seed oil contain high content of oleic acid, good oxidative stability and appropriate level of tocopherols and small amount of saturated fatty acids. Pakistan is an agricultural country blessed with huge plains, land, variety of season and one of the best organized irrigation systems. In such a compatible cultivation regime the production of vegetable oils can be enhanced locally to meet the domestic needs of the country standing at *ca.* 3.5 MMT per annum. As peanut has high oil yield, therefore, the cultivation of this crop over wide scale production can help bridge the significant gap between the local demand and supply.

REFERENCES

1. J.B. Rossell, In eds.: J.B. Rossell and J.L.R. Pritchard, Vegetable Oils and Fats, Analysis of Oils, Fats and Fatty Foods, Elsevier Applied Science, New York, pp. 261-327 (1991).
2. F. Panhwar, Published by Digitalverlag GmbH, Germany (www.chemlin.com) (2005).
3. A.V. Schirack, M. Darke, T.H. Sanders and K.P. Sandeeop, *J. Sensory Studies*, **21**, 428 (2006).
4. H.D. Upadhaya and S.N. Nigam, *Crop Sci*., **39**, 115 (1999).
5. Plessis and Steiman, *Immunol. Allergy Clinics North Am*., **17**, 10 (2004).
6. G.O. Adegoke, K.O. Falade and O.C. Bbalola, *J. Food Agric. Environ*., **2**, 128 (2004).
7. B. Delplanque, *Oil Crops Gras Lipides*, **7**, 467 (2000).
8. Association of Official Analytical Chemists (AOAC), Official Methods of Analysis of the Association of Official Analytical Chemists, AOAC Inc., Virginia, Method, edn. 15, pp. 976.05 (1990).
9. International Organization for Standardization, Animal Feeding Stuffs-determination of Nitrogen and Calculation of Crude Protein Contents, ISO, Geneva, Standard No. 5983 (1981).
10. International Organization for Standardization, Oilseed Residues, Determination for Total Ash, ISO, Geneva Standard No. 749 (1977).
11. American Oil Chemist's Society (AOCS), Official and Recommended Practices of the American Oil Chemists Society, AOCS Press Champaign, edn. 5 (1997).
12. International Union of Pure and Applied Chemistry (IUPAC), Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th revised and Enlarged Edition, Blackwell Scientific, London (1987).
13. J.N. Thompson and G. Hatina, *J. Liq. Chromatogr*., **2**, 327 (1979).
14. G.N. Anyasor, K.O. Ogunwenmo, O.A. Oyelana, D. Ajayi and J. Dangana, *Pak. J. Nutr*., **8**, 269 (2009).
15. M. Ozcan and S. Seven, *Grasasy Y Aceites*, **54**, 12 (2003).
16. M.K. Egbekun and M.U. Ehieze, *Plant Food. Hum. Nutr*., **51**, 35 (1997).
17. J.L.R. Pritchard, Analysis and Properties of Oilseeds, In eds.: J.B. Rossell and J.L.R. Pritchard, Analysis of Oilseeds, Fats and Fatty Foods, Elsevier Applied Science, New York, pp. 80-98 (1991).
18. D. Pearson, Chemical Analysis of Food, J.A. Churchill, London, edn. 8, p. 535 (1981).
19. C.O. Eromosele and N.H. Pascal, *Bioresour. Technol*., **86**, 203 (2003).
20. M.A. Somali, M.A. Bajnedi and S.S. Al-fhaimani, *J. Am. Oil Chem. Soc*., **61**, 85 (1984).
21. N.R. Grosso, V. Nepote and C.A. Guzman, *J. Agric. Food Chem*., **48**, 806 (2000).
22. J. Neuza and G. Lireny, *Cienc. Technol. Aliment*, **18**, 335 (1998).