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PHYSICOCHEMICAL PROPERTIES, FATTY ACID PROFILE AND ANTIOXIDANT ACTIVITY OF PEANUT OIL

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Abstract

The oil from seeds of 4 pea nut (*Arachis hypogaea* L.) varieties: Golden, Bari 2000, Mongphalla, and Mongphalli 334 cultivated in arid zones, was subjected to the comparative evaluation of its physicochemical properties, fatty acid profile and antioxidant activity. Pea nut seeds were found to be a rich source of crude fat (45.09-51.63 g/100 g dry weight). The physicochemical properties of the oil were investigated as specific gravity (0.915±0.008-0.918±0.008), acid value (3.96±0.22-4.95±0.71 mg KOH/g oil), saponification value (226.40±3.59-246.56±2.04 mg KOH/g oil) and unsaponifiable matter (3.20±0.23-4.20±0.04 g/100 g oil). The higher amounts of unsaturated fatty acids (82.06-85.93%) were found to be present in each variety. A significant variation ($p<0.05$) was observed among the varieties regarding crude oil content, saponification value, oleic/linoleic (O/L) ratios, phenolic acid content and total antioxidant content. Golden was found to be high in oil content, O/L ratio, antioxidant profile and DPPH· scavenging activity but low in iodine value.

Introduction

Nuts are a good source of oil containing higher unsaturated fatty acids (UFAs) to saturated fatty acids (SFAs) ratio (Sabate, 2003). Willett (1998) reported that higher body weight is associated with higher percentage of fat in the diet. This evidence has been proved as controversial after a number of nut feeding studies in well controlled dietary conditions as well as in free living people using self selected diets. In the light of these studies an inverse association has been found to be present between frequency of nut consumption and body weight (Sabate, 2003). In other studies it has been proved that the frequent nut consumption have beneficial effects on serum lipids and lipoproteins in lowering the rates of coronary artery disease (Kris-Etherton *et al.,* 1999; Sabaté *et al.,* 2001). It is not only the quantity but also the quality of fat in diet which can be helpful in minimizing the risk of coronary heart disease (CHD). Not only the low fat diet, but also the high fat diet containing high unsaturated fatty acids (UFAs)/saturated fatty acids (SFAs) ratio, may tend to decrease the LDL cholesterol level. The SFAs and the trans-fatty acids have been found to increase the LDL cholesterol level. Hence, instead of reducing overall fat intake, the replacement of SFAs and trans-fatty acids by MUFAs and PUFAs may reduce the development of CHD (Frank *et al*., 1997; Curb *et al*., 2000; Frank *et al*., 2001; Lichtenstein, 2003).

The pea nut, often called as "The King of Oilseeds", is botanically known as *Arachis hypopgaea* and belongs to family Leguminosae, which is also called Fabacae. The pea nuts differ in the quantity as well the quality of oil. These differences in the pea nut oil may be due to several factors *i.e*. genotype, the level of maturity of the seed, season and geographical area of production (Brown *et al.,* 1975). About 80% of the total fatty acid content of pea nut oil constitutes unsaturated fatty acids mainly oleic acid and linoleic acid (Ahmed & Young, 1982). Thus the chemistry and quality of pea nut oil mostly depend on the oleic to linoleic ratio. The studies by Millar *et al*., (1987) observed that the oil containing high UFAs/SFAs ratios are thermodynamically more stable and may be heated to high temperatures. Jakson *et al*., (1978) reported that the oil containing higher content of MUFAs fatty acids (oleic acid) are more stable to oxidative damage during refining and storage. On the other hand, Kratz *et al*., (2002) suggested that the linoleic acid, a PUFA, having two double bonds is more susceptible to oxidative rancidity than oleic acid as well as the saturated fatty acids. But Linoleic acid, being an essential fatty acid, also plays a beneficial role in human health in lowering the total blood cholesterol and LDL levels. The long term stability of pea nut oil may also be associated with the antioxidant substances (tocopherols and polyphenols) present in pea nut oil as the minor components.

The antioxidants are the substances that can prevent the oxidation of easily oxidizable substances. These substances have the ability to trap the free radicals produced as a result of different metabolic processes and protect the lipids, proteins and nucleic acids from the oxidative damage (Frei *et al.,* 1988). Recently, pea nut oil has been found to be effective antioxidant to reduce the elevated levels of glucose, glycosylated hemoglobin, vitamin E, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides, glucose-6-phosphatase and fructose-1,6-bisphosphatase activities in the diabetic rats. On the other hand increased levels of hemoglobin, vitamin E, reduced glutathione and hexokinase activity have been observed in the diabetic rats fed on pea nut oil (Ramesh, & Pugalendi, 2006).

In the previous studies we investigated the biochemical composition and some phytochemicals in the seeds of 4 pea nut (*Arachis hypogaea* L.) varieties namely, Golden, Barri 2000, Mongphalla, and Mongphalli 334 cultivated in arid zones of Pakistan (Shad *et al*., 2009). A careful survey of literature reveals that a little work has been done and limited guidelines are available on the physicochemical characteristics, fatty acid profile and antioxidant properties of oil of these pea nut varieties. Presently was to carry out the systematic analysis of the said parameters to compare the oils of these pea nut varieties. The results obtained may provide guidelines for industrialists and manufacturers who are seeking for the right varieties for their products.

Materials and Methods

Mature pods of 4 pea nut varieties namely, Golden, Bari 2000, Mongphalla and Mongphalli 334 recommended for cultivation in arid zone and irrigated regions of Pakistan, were collected from BARI Research Institute Chakwal, Punjab, Pakistan. The seeds and pod shells were separated manually. The mature and healthy seeds were ground and stored in glass containers for analysis.

Content and Physicochemical properties of oil: Extraction of the oil was done by soxhlet apparatus using n-hexane as solvent. Density of the oil was determined by using pycnometer and its specific gravity was calculated by following formula:

Specific gravity = Density of oil/Density of water

The acid value and saponification value were determined by following the standard method as described in British Pharmacopeia (Anon., 1973). The iodine values (IVs) were calculated from fatty acid composition (Hashim *et al.*, 1993) using the following formula:

IV = (% Oleic × 0.8601) + (% Linoleic × 1.7321)

The determination of unsaponifiable matter was carried out by using the method as given in US Pharmacopeia (Anon., 1985).

Fatty acid profile

Preparation of fatty acid methyl esters (FAMEs): FAMEs were prepared by standard IUPAC method 2.301 (Anon., 1979). Briefly, sample of raw seed oil (100 mg) was accurately weighed, placed in 50 mL round bottom flask, followed by the addition of 1 N methanolic potassium hydroxide solution (0.5 mL). The samples were refluxed at 70˚C for 20 minutes. After cooling, hexane and water (10 ml of each) were added. The mixture was vortex mixed for 15 minutes and the upper phase containing the FAMEs was recovered and analyzed by Gas chromatography (GC).

Gas chromatographic conditions: FAMEs were analyzed on a Perkin Elmer Gas Chromatograph (model 8700), fitted with a non-bonded biscyanopropyle siloxane stationary phase, polar capillary column SP-2340 (602 × 0.25 mm), 0.2 µm film thickness and a Flame Ionization Detector. Nitrogen (Oxygen-free) was used as a carrier gas at a flow rate of 3.5 mL min^{-1}. Other conditions were as follow: initial oven temperature, 130˚C; ramp rate, 4˚C/minute; final temperature, 220˚C; injector temperature, 260˚C; detector temperature, 270˚C; temperature hold, 2 minutes before the run and 17 minutes after the run. A sample volume of 1.0 µL was injected. FAMEs were identified by comparing their relative and absolute retention times to those authentic standards of FAMEs obtained from Sigma Chemicals Company. All of the quantification was done by a built in data-handling program provided by the manufacturer of the gas chromatograph (Perkin Elmer). The data were transferred on an Epson LX-800 printer attached to the instrument through an RS-232-C port. The fatty acid composition was reported as a relative percentage of the total peak area.

Antioxidant properties

Total phenol contents: The Phenolic compounds from the pea nut oil (0.5 g) were extracted in methanol (5 mL) by inversion for 10 minutes. The contents of total phenolic compounds in the methanolic extract were determined with Folin-Ciocalteu reagent using (+) catechin as standard (Naczk & Shahidi, 1989). The methanolic extract (1 mL) was mixed with Folin-Ciocalteu reagent (1.5 mL). After 3 min, 20% Na_2CO_3 solution (2.5 mL) was added and the mixture was diluted up to 10.0 mL with distilled water. The solution was allowed to stay at room temperature. After 45 minutes the mixture was centrifuged and absorbance was measured at 745 nm. Total phenolic content (TPC) was calculated from linear regression equation obtained from catechin standard curve (r = 0.991):

TPC = (Absorbance of the sample + 0.031) / 0.031

The measurement was conducted in triplicate and the average results were expressed as catechin equivalents (mg/100 g oil).

Determination of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity: The antioxidant activity of pea nut oil was determined with some modifications in the method described by Moreno *et al*., (1998). The oil sample (0.1 g) was dissolved in *n*-hexane, the volume was made up to 1 mL and 40µM DPPH· solution prepared in *n*-hexane (2.9 mL) was added to the sample. The solutions were prepared preferably in hexane because the methanolic solution of DPPH· causes turbidity in the reaction mixture and hence interferes in the results. A decrease in absorbance at 517 nm was recorded at 5 min intervals up to 30 min. The linear regression equation obtained from a calibration curve (r = 0.9985), using different concentrations of DPPH·, was used to calculate the remaining DPPH· concentration in the reaction mixture as:

$$A_{517}\ nm = 0.046\ [DPPH\cdot] + 0.059$$

The percent [DPPH·] remaining was calculated as:

$$[DPPH\cdot]_R\ (\%) = 100\times\ [DPPH\cdot]_T/[DPPH\cdot]_0.$$

where $[DPPH\cdot]_T$ is the concentration of DPPH· at 30 minutes time and $[DPPH\cdot]_0$ is the initial concentration.

The amount of total antioxidants (TAO) present in the crude oil was determined by mixing oil (0.1 g) with hexane (0.9 mL) followed by the addition of 40 µM DPPH solution (3 mL). The solution was allowed to stand for 30 minutes at room temperature and the absorbance was recorded at 517 nm. The amount of TAO was expressed as Trolox equivalent (mg/100 g of oil). A kinetic study was also conducted to evaluate the free radical scavenging properties of pea nut oil using stable DPPH·. The percentages of remaining DPPH· were plotted against different time intervals to obtain the initial

time at which the samples reduced the maximum concentration of DPPH·.

Statistical analysis: The results were expressed as mean ± standard deviation of the 3 replicates. Data were statistically analyzed by one way analysis of variance (ANOVA; $p<0.05$) The SPSS statistical software (version 17.0) was used to separate the means by Tukey's multiple range tests.

Results and Discussion

The content and the physicochemical characteristics of oil extracted from the pea nut seeds have been presented in Table 1. The oil was pale yellow in colour and in liquid state at room temperature. The content of crude oil in the pea nut seeds ranged from 45.09 to 51.63 g/100 g of dry weight. A significant variation ($p<0.05$) in the crude oil content was observed among the four varieties. Mongphalli 334 contained highest content of crude oil followed by Golden and Mongphalla while Bari 2000 was lowest in these contents. The crude oil content of these pea nut varieties were found to be higher than those reported by Fasoyiro *et al*., (2006) but comparable to those reported by Asibu *et al*., (2008). No significant difference in the specific gravity values (0.915±0.008-0.918±0.008), acid value (3.96±0.22-4.95±0.71 mg KOH/100 g oil), and unsaponifiable matter (3.20±0.23-4.20±0.04 g/100 g oil) was found to be present among the investigated pea nut varieties. The iodine values and saponification values ranged from 70.16 to 86.02 g/100g oil and 226.40±3.59 to 246.56±2.04 mg KOH/100 g oil respectively. These two parameters were found to be statistically different ($p<0.05$) among the varieties. Mongphalla showed higher iodine value while saponification value was found to be higher in Mongphalli 334. The colour, specific gravity value and saponification value were comparable but acid values and un-saponifiable fibre contents were low as compared to those investigated earlier (Onyeke & Acheru, 2002). The iodine value was comparable but saponification value and unsaponifiable matter was also found to be higher than those reported in literature (Ozcan & Seven, 2003**)**. The saponification values were also higher than those reported in recent work on pea nut oil (Falade *et al.*, 2007). The saponification value and acid values were found to be higher but the iodine values were found to be low as compared to those investigated in the chickpea oil (Shad *et al.*, 2009). Higher saponification value suggests the applicability and suitability of oil extracted from the present pea nut varieties for soap formation.

Table 1. The content and physicochemical characteristics of pea nut oils.

Components	Golden	Bari 2000	Mungphalla	Mungphalli 334
Crude oil (g/100 g seeds)	45.09 ± 0.8[*b]	49.18 ± 2.10[a**]	51.63 ± 0.23[a]	50.42 ± 1.02[a]
Specific gravity	0.918 ± 0.008[a]	0.917 ± 0.005[a]	0.915 ± 0.008[a]	0.917 ± 0.007[a]
Acid value (mg KOH/100 g oil)	4.50 ± 0.65[a]	3.96 ± 0.22[a]	4.95 ± 0.71[a]	4.29 ± 0.43[a]
Saponification value (mg KOH/100 g oil)	242.48 ± 2.10[a]	226.40 ± 3.59[b]	243.10 ± 3.24[a]	246.56 ± 2.04[a]
Unsaponifiable matter (g/100 g oil)	4.20 ± 0.04[a]	3.20 ± 0.23[a]	4.00 ± 1.05[a]	3.80 ± 0.76[a]
Iodine value (g/100 g oil)	70.16	81.08	86.02	80.69

[*] Means ± standard deviation

[**] The means followed by the same letter, in each row, are not significantly different at $p \leq 0.05$, using Tukey's multiple range test

Fig. 1 represents the GC profile of fatty acids present in the oil of 4 different pea nut varieties. Six fatty acids [palmitic (C-16:0), stearic (C-18:0), oleic (C-18:1), linoleic (C-18:2), linolinic (C-18:3), and γ-linoleic acid (C-18:2)] were identified in all of the 4 pea nut varieties. Mongphalli 334 also contained a trans fatty acid (Elaidic acid) in a minute quantity. The results reveal that all of the 4 pea nut varieties contained relatively high percentage of UFAs as compared to SFAs (Table 2). Among UFAs, high MUFAs/PUFAs and O/L ratios were observed in each variety. Fig. 2 shows a comparative demonstration of various fatty acids in different varieties. It is clear from the figure that all of the varieties contained oleic acid, a MUFA, as the major UFAs. Linoleic acid, a PUFA, was investigated as the second major UFA in each variety. Oleic acid content was found to be high in Golden followed by Bari 2000, Mongphalla and low in Mongphalli 334. The linoleic acid, on the other hand, was found to be high in Mongphalla followed by Mongphalli 334, Bari 2000 and low in Golden.

Table 2. The fatty acid Profile of pea nut oil.

Pea nut varieties	SFAs (% of total fat)	UFAs (% of total fat)	MUFAs (% of total fat)	PUFAs (% of total fat)	UFAs/ SFAs	MUFAs/ PUFAs	O/L
Golden	15.54	82.48	74.21	8.27	5.31	8.97	19.79
Bari 2000	12.64	85.93	70.26	15.67	6.80	4.48	5.89
Mongphalla	13.42	85.48	64.58	20.90	6.37	3.09	3.67
Mongphalli 334	16.31	82.06	60.73	21.33	5.03	2.85	3.53

Oleic acid is a MUFA and high intake of diets enriched with MUFA may also protect against atherosclerosis, lower serum cholesterol levels by diminishing oxidative stress and inflammatory mediator while promoting antioxidant defence (Moreno & Mitjavila, 2003). A significant decrease in the total plasma cholesterol and LDL cholesterol by the use of diets rich in MUFAs has been reported (Berry *et al*., 1992). The low-fat diets and the diets containing high levels of MUFAs are equally effective in lowering serum cholesterol levels (Psaltopoulou *et al*., 2004). MUFAs, relative to carbohydrate, increase HDL cholesterol levels

and decrease plasma triglyceride levels. Thus, the CVD risk can be managed with reference to the diets higher in MUFA keeping in the limits of SFA recommendation and compromise weight control (Kris-Etherton, 1999). A number of in human studies showed that MUFAs rich diets may decrease the plasma LDL cholesterol level when compared to PUFAs or carbohydrate-rich diets (Wahrburg *et al*., 1992; Mata *et al*., 1992; Sirtori *et al*., 1992). But the studies on rats showed that diets containing high UFAs/SFA and PUFAs/MUFAs ratio increases the level of very low density lipoprotein in plasma but reduces the effect of dietary cholesterol in elevating the triglycerides level in liver (Changa *et al.*, 2004). The presence of high amounts of UFAs as compared to SFAs, make pea nut suitable for nutritional application. Golden, being high in oleic acid but low in linoleic acid content, showed highest O/L ratio and was found to be promising regarding the protection against CVD risk.

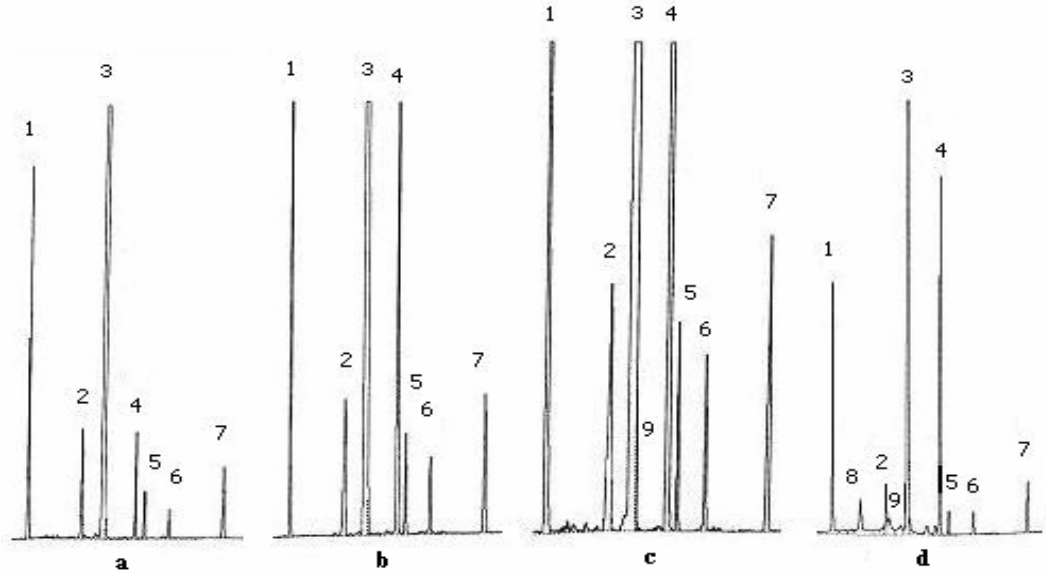

Fig. 1. Gas chromatographic profile of fatty acids in oil of 4 pea nut varieties
a: Golden, b: Bari 2000, c: Mongphalla and d: Mongphalli 334
1: Palmitic (C-18:0), 2: Steric (C-18:0), 3: Oleic (C-18: 1), 4: Linoleic (C-18: 2), 5: Linolenic (C-18: 3), 6: γ-Linoleic (C-18: 2), 7: Unidentified, 8: Elaidic (C-18: 2-trans) and 9: Unidentified.

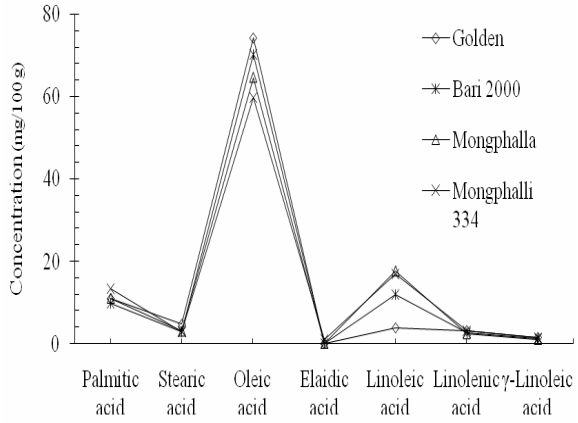

Fig. 2. Comparative description of fatty acids present in oil of four pea nut varieties.

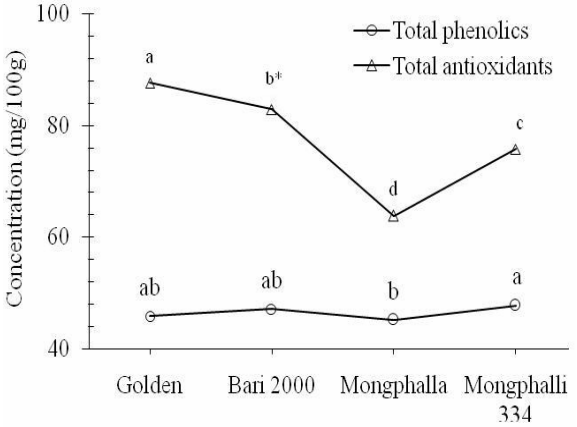

Fig. 3. Total phenolic compounds and total antioxidant profile of oils extracted from pea nut seeds.
*The values expressed with same letter, in each series, are not significantly different at $p \leq 0.05$, using Tukey's multiple range tests.

Linoleic acid having two double bonds is more susceptible to oxidative rancidity than oleic acid and the saturated fatty acids (Kratz *et al.,* 2002). The rancidity of oil is due to the reaction of oxygen with the double bonds of UFAs resulting in the products having unpleasant odor and flavor. Hence, the oil containing higher MUFAs/PUFAs ratio may be recommended in nutritional supplements for better health. It has also been reported that higher O/L ratio and lower IVs indicate the better oil stability and longer shelf life and good quality of oil (Ahmed & Young 1982). In this regard, the present pea nut varieties were found to constitute better fat composition as compared to those reported by other workers (Nelson *et al.,* 2000; Fasoyiro *et al.,* 2006; Asibu *et al*., 2008). Regarding oil stability and shelf life, Golden, being high in O/L ratio and low in IV, was found to be the promising one among the present varieties.

The phenolic compounds are known to possess antioxidant properties and contribute to the oxidative stability of oil (Gomez-Alonso *et al*., 2002). Excellent linear correlations have been reported between the total phenolic (TP) content and antioxidant activity (Javanmardi, *et al.*, 2003; Huang *et al*., 2005; Silva *et al.,* 2007). Fig. 3 displays the results of comparative analysis of TP compounds in the methanolic extracts of pea nut oil and the total antioxidants (TAO) present in the crude oil. Statistically significant variation ($p<0.05$) was observed among the varieties regarding the TP compounds (45.16±0.50-47.74±0.70 mg/100 g oil) and TAO content (63.81±0.80-87.60±0.60 mg/100 g oil). TAO content was found to be highest in Golden followed by Bari 2000, Mongphalli 334 and Mongphalla contained lowest TAO content. TAO content was found to be almost two fold higher as compared to TP content in each variety. The higher value of TAO content as compared to TP content indicates the presence of some compounds other than the phenolic compounds showing antioxidant activities in pea nut oil. Tocopherols are one of the compounds reported to be present in pea nut oil (Hashim *et al*., 1993; Falade *et al*., 2007) which possess antioxidant activity (Frei *et al*., 1988). Since, the oil of each pea nut variety contains a comparable amount of TP compounds, the variation in the TAO contents among the varieties may be attributed to the differences in the other antioxidant compounds present in the pea nut oils. The higher value of antioxidants is evidence for the good stability and better shelf life of the pea nut oils. In this regard, Golden was found to be the best pea nut variety as compared to the others investigated in the present work.

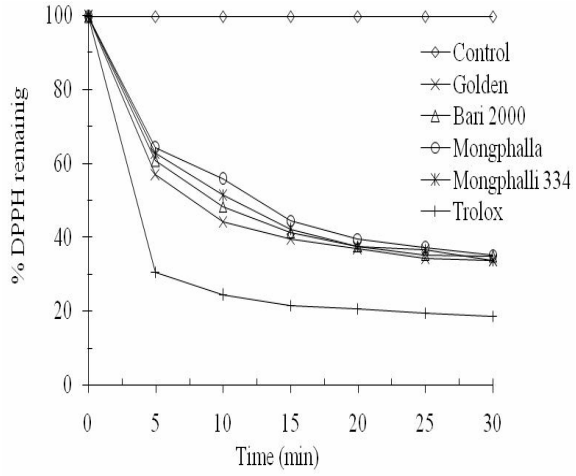

Fig. 4. Reaction kinetics of pea nut oils with 40 µM DPPH radical.

Fig. 4 represents the kinetic behaviour of DPPH free radical scavenging activities of the antioxidants present in crude oil of 4 pea nut varieties as compared to that of Trolox as standard antioxidant. The DPPH free radical was used as substrate to evaluate the antioxidant activity of the oil. The degree of discolouration of the reaction mixture with time is directly related to the percent decrease in the DPPH· concentration due to the hydrogen donating ability of the antioxidant compounds present in the oil (Shimada *et al*., 1992). The analysis results showed that the kinetic behaviour of all the varieties was comparable. A fast decrease in the DPPH· concentration was observed in the first five minutes in each case. After five minutes, a variation in the antioxidant activity was observed among the varieties which may be due to the variation in the quality and quantity of TAO among the varieties. The order of antioxidant activities was: Golden > Bari 2000 > Mongphalla > Mongphalli 334. However, the oils from all of the varieties showed lower antioxidant activities as compared to Trolox.

Conclusion

Higher saponification value indicates the suitability of the oil of present pea nut varieties, particularly Mongphalli 334, for soap industry. The presence of high amounts of unsaturated fatty acids as compared to saturated fatty acids, favours the suitability of the investigated pea nut varieties for nutritional applications especially in lowering the CHD risk. Due to their high O/L ratio, low iodine value, good phenolic composition and antioxidant activity of the oil, all of the 4 varieties could be suggested to possess the better oil stability and desirable nutritional composition. Among the four varieties, Golden shows relatively high O/L ratio, low iodine value and high antioxidant activity of the oil. It is, therefore, concluded that the present pea nut varieties particularly Golden, could be the best choice for the biochemists, food scientists, researchers and manufacturers concerning food and nutrition. The present data could be a source of information for the researchers and manufacturers concerning the pea nut and other legumes. The data may also be helpful for the nutritionist and nut consumers in the selection of the best foods and food products.

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