Original Article

Variations of quality characteristics among oils of different soybean varieties

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Abstract A study was carried out to evaluate the variation of quality attributes among oils from different soybean varieties (Bovender special, Foster and F-8827). Oils were extracted using n-hexane as solvent. Results indicated that contents of seed oil among the tested varieties varied from 15.85% to 19.49%, moisture 8.4–10.2%, protein 41.67–45.64%, fiber 6.6–7.6% while ash 5.5–6.9%. The physical and chemical characteristics among the tested oils varied as: color (4.2–5.3R + 40–50Y), iodine value (119–128 g of I/100 g of oil), refractive index (1.4590–1.468), density (0.8698–0.8712 g/cm 3 at 36 °C), free fatty acid content (0.39–0.67% as oleic acid), saponification value (181–187 mg KOH/g) and unsaponifiable matter (0.42–0.74%). The oxidation parameters including peroxide value, conjugated dienes and conjugated triene were recorded as 1.80–2.64 meq/kg, 0.41–0.65 and 1.50–1.91, respectively. The fatty acid composition showed the presence of palmitic acid (11.00–13.50%), stearic acid (3.02–4.90%), oleic acid (22.60–24.00%),...
1. Introduction

Vegetable oils being an important ingredient of our diet act as a source of essential fatty acids and nutrition and can be extracted from a variety of plant seeds such as soybean, cotton, sesame, sunflower, safflower, palm, corn and canola (Anwar et al., 2005a).

One of the important oil seed crops namely soybean (Glycine max L.) is a member of the Leguminosae family [sub-family Papilionoideae]. The plant is annual, generally grows to a height of 20–180 cm, and has white or lilac flowers and pods. The pods, which usually contain two or three seeds, are formed in the leaf axils. There are several varieties of soybean with yellow, green, brown or black seeds and cultivars. Soybean is essentially a subtropical crop but can also be grown in tropical to temperate regions up to temperatures as high as 50 °C. Soybean like other legumes is useful for crop rotation due to the ability to fix nitrogen from the air (Anonymous, 1994).

Protein and oil are the two important seed constituents that make soy bean (Glycine max) an important crop. It contains 40–42% high quality protein and 18–22% oil comprising up of 85% unsaturated fatty acid. Soybean not only contains high quality protein, but the protein content is also much higher than that of other plant foods. This valuable bean also contains about 12% carbohydrate (Yaklich et al., 2002). Soy protein is valued as a healthy protein due to containing a balanced proportion of all of the important and essential amino acids required by the human body (Potter et al., 1998). It is the only vegetable source that contains a complete protein. It contains all the nine essential amino acids that a human body cannot synthesize and which must be obtained from foods. In fact soybeans can provide two fold more proteins as compared to any other vegetable crop or grain (Anonymous, 2015). The soybean flakes obtained after extraction of oil can be further processed into protein meals and protein isolates for animal and human use. Hydrolyzed soybean protein (HSP) can be used in soups, sauces, gravies, spice blends, canned and frozen vegetables, meats and poultry as a flavor enhancer. Moreover, the textured soy flour is widely used as a meat extender (Anonymous, 1994).

The health attributes of soy have been linked to the isoflavones naturally occurring in soy in conjunction with the soy protein (Potter et al., 1998). Soybean contains two primary isoflavones namely genistein and diadzein. A third isoflavone, glycitin is also reported in small amounts. Typically, genistein is found at the highest level in soy foods (Messina and Loprinzi, 2001).

It is assumed that the oil yield and physicochemical properties of oils are not only varied in relation to different species and different agro-climatic regions rather such variations can also be seen within the oils of different varieties/cultivars of the same species. Some recent reports revealed considerable inter-varietal variations the chemical composition of seed oils of okra and watermelon (Anwar et al., 2011a,b).

World production of soybean (Glycine max) in 2014/15 was over 319.36 million metric tons, with 108.01 million metric tons of this production coming from U.S., where soybean plantings on an annual basis are over 33.61 million ha. Soybeans are desired on the marketplace as a valuable source of protein and oil (USDA, 2015). Although soybean as an oil seed crop was introduced in Pakistan along with sunflower a few decades back, yet it could not make its place in the country. Since its average yield per hectare under local agro-climatic set up is also quite low, its production remained small. Soybean, as an oilseed crop, is under research and production trials in several parts of Pakistan. Several attempts have been made over the decades to introduce soybean for commercial planting but no encouraging success was achieved. Presently, it is grown on a very small area, in Punjab, Sindh and Khyber Pakhtunkhwa provinces of Pakistan (FAO, 2013).

Soybean varieties namely, Bovender special, Foster and F8827, investigated in the present study were grown at Ayub Agricultural Research Institute, Faisalabad, Pakistan for adaptive studies. As the oil yield and the physicochemical properties and attributes of the oils can vary among different varieties of oil seeds with respect to their genetic makeup (Clemente and Cahoon, 2009; Hudson, 2012; Ma et al., 2015), so a need exists to investigate such variations with regard to different soybean cultivars. Until now, a full characterization and comparison of the quality attributes of the oils produced from seeds of mentioned locally cultivated soybean varieties has not yet been investigated. The main objective of the present study was to conduct a detailed analysis and to assess the variations in physicochemical characteristics of soybean seed oils of different varieties cultivated in Pakistan. The main theme behind carrying out this study was to convey information to the local growers and industrialists about the physicochemical attributes of the above varieties thus helping them in selection of the appropriate variety for cultivation and industrial processing at regional level.

2. Materials and methods

2.1. Chemicals, standards and reagents

Chloroform, oxalic acid, acetic acid, phenolphthalein, hydrochloric acid, potassium iodide, n-hexane, sodium sulfate, potassium hydroxide, sodium thiosulfate, potassium peranganate, boric acid, carbon tetrachloride, iso-octane, iodine, sodium hydroxide, methanol, sulfuric acid, p-anisidine and pure standards of fatty acid methyl esters (FAMEs) used in the present study were purchased from E-Merck and/or Sigma–Aldrich Chemical Corporation (St. Louis, MO, USA).
2.2. Soybean seed samples and oil extraction

The seeds of three varieties (Bovender special, Foster and F-8827) of soybean used in this study were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. The average weight of seeds was recorded to be 0.12–0.20 g. The seeds were crushed and placed in a Pyrex glass Soxhlet extractor, attached with a water condenser and a Pyrex round bottomed flask (500 mL capacity). Extraction was carried out using a water bath with n-hexane as extraction solvent. After the oil extraction, the extra solvent was removed under vacuum in a rotary evaporator machine (EYELA, N. N. Series fitted with an Aspirator and a Digital Water Bath SB-651, Japan) at 45 °C.

2.3. Analysis of oilseeds residues

The oilseed residues were analyzed for protein, fiber and ash contents. The crude protein was estimated according to ISO method-5983 (ISO Standards, 1981) using micro Kjeldhal apparatus. Fiber contents were determined according to the official method (AOAC, 1990) while ash contents by the ISO method – 749 (ISO, 1977).

2.4. Analysis of oils physicochemical parameters

The extracted oils were analyzed for saponification value, unsaponifiable matter, color index, iodine value, conjugated dienes and trienes, refractive index, density, peroxide value and unsaponifiable matter, according to the standards were placed in various test tubes. Five ml of 0.5 M methanolic NaOH solution was then added and allowed to heat on a steam bath for 5 min until they globalized into solution. Ten ml of BF3 in methanol was added to each sample in the test tube and the mixture was again boiled for 2 min. Each of the mixture containing the fatty acid methyl esters (FAME) was extracted with 30 ml petroleum ether (b.p.40–60 °C) and 20 ml saturated NaCl solution in a separating funnel. The lower layer of aqueous methanol was separated, drained off and discarded, while the upper petroleum ether layer was discarded, while the upper petroleum ether layer was collected and concentrated. The concentrated extract recovered was re-extracted by repeating the same procedure. The hexane layers produced from both the extractions were pooled and evaporated to dryness under nitrogen streaming. The concentrated extract recovered was re-dissolved in one mL of mobile phase by vortexing for 30 s and then finally transferred to an HPLC sample vial. A 20 μL prepared sample was injected into a Supelcosil LC-Si column (250 × 4.6 mm, Supelco Inc.). A mobile phase consisting of ethyl acetate/acetic acid/hexane (1:1:98, v/v/v) was used at a flow rate of 1.5 mL/min. Detection of three tocopherol compounds was done at 295 nm with a UV detector. The unknown tocopherols were identified by comparing their retention times with those of pure standards of α-, γ- and δ-tocopherols from Sigma–Aldrich. These compounds were quantified on the basis of matching of peak areas of the pure standards based on an external standard method using a D-2500 Hitachi Chromato integrator.

2.7. Tocopherol analysis

In the HPLC experiment, the samples for tocopherol (α, γ, and δ) analysis were prepared based on the method described in current protocols in “Food Analytical Chemistry” (Wrolstad, 2003). A known quantity of oil (0.1 g) was placed in a test tube (16 × 25 cm), containing ascorbic acid (0.05 g), 5 mL of 90% ethanol and 0.5 mL of 80% aqueous KOH solution. The contents of the tube were mixed well by vortexing the tube for 30 s. The tube was capped after flushing with nitrogen and then placed/incubated in a water bath (70 °C) for 30 min with periodical vortexing. After incubation the tube was removed from the water bath and cooled down by placing in an ice bath for 5 min. After that 3 mL of deionized water and 5 mL n-hexane were added to the tube and it was vortexed again for 30 s followed by centrifugation at 1000g for 10 min at room temperature. The upper hexane layer was collected in another test tube. The aqueous layer and the residue thus obtained (recovered) was re-extracted by repeating the same procedure. The hexane layers produced from both the extractions were pooled and evaporated to dryness under nitrogen streaming. The concentrated extract recovered was re-dissolved in one mL of mobile phase by vortexing for 30 s and then finally transferred to an HPLC sample vial. A 20 μL prepared sample was injected into a Supelcosil LC-Si column (250 × 4.6 mm, Supelco Inc.). A mobile phase consisting of ethyl acetate/acetic acid/hexane (1:1:98, v/v/v) was used at a flow rate of 1.5 mL/min. Detection of three tocopherol compounds was done at 295 nm with a UV detector. The unknown tocopherols were identified by comparing their retention times with those of pure standards of α-, γ- and δ-tocopherols from Sigma–Aldrich. These compounds were quantified on the basis of matching of peak areas of the pure standards based on an external standard method using a D-2500 Hitachi Chromato integrator.

2.8. Statistical analysis

Data were statistically analyzed to find out significant differences of parameters among varieties analyzed by using MSTATC program (version 2.10).
3. Results and discussion

The physicochemical examination of oils is mainly made from the stand point of their edible as well as industrial uses. The quality of vegetable oils can be judged by the knowledge of their physical and chemical characteristics.

3.1. Percentage oil yield

The percentage yield of hexane-extracted oil content from different varieties of soybean (Bovender special, Foster and F-8827) seeds was found to be in the range of 15.85–19.49%. A significant variation was observed for oil content among the soybean seed samples analyzed. The oil content (19.84%) was considerably higher for F8827 variety and lower (15.85%) in the seeds of variety Bovender special. The oil content is a quantitative trait whose variability is conditioned with genetic difference between the varieties (Vrataric et al., 1994; Vollmann et al., 2000). The oil content in soybean seeds from different varieties, ranging from 15.85–19.49%, was comparable with the findings of (Maestri et al., 1998) and (Al-Kahtani, 1989) who reported the oil content in different genotypes of soybean to be 198 to 267 g/kg and 15.84–21.35%, respectively. The range of oil content of soybean seeds, recorded in the present work, was also in close agreement with that reported by Anjum19 for different soybean varieties. The present oil content of soybean was lower as compared with two other oilseed crops: Cucurbita pepo (35%) and sunflower hybrids (36–39%) investigated by (Younis et al., 2000) and (Shahbaz and Hassan, 2000), respectively.

3.2. Analysis of soybean seed residues

Analysis of oil seed residues revealed a high protein content for the seeds, ranging from 41.675% to 45.645%. Bovender special variety had the highest protein content, while the Soybean variety F-8827 contained the least amount of protein. The protein content investigated presently was comparable (40.57–47.60%) with that reported by (Al-Kahtani, 1989) but higher than (37.6–38%) that investigated by (Yoshida et al., 2006). Compared with some conventional oil seed crops, the protein content of the presently analyzed soybean samples was higher than those of safflower (20–22%), sunflower (16.5–19.6%) and cotton seed (19.40%) as reported in the literature (Pritchard, 1991). As expected, the oilseed samples high in oil content were generally found to be lower in protein, fiber and ash contents. Interestingly soybean based protein is readily digestible and is primarily composed of edestin and albumin, which are the key components of human blood plasma. The crushed soybean by product is suitable for animal feed and as a cheaper source of calories (Anwar et al., 2002).

Fiber and ash contents of soybean seeds samples were found to be in the range of 6.6–7.6% and 5.5–6.9%, respectively. There was no significant variation in fiber and ash contents of soybean seeds among the varieties tested. Ash content was comparable to the previous findings for soybean varieties which were 5.81–6.54% (Al-Kahtani, 1989). Fiber and ash contents in the present analysis were also comparable to that of Moringa seeds (6.8–7.5% and 5.9–7%) reported by (Anwar et al., 2005b) as well as to those of most of the vegetable oils reported earlier by (Pritchard, 1991). The crude fiber is an indication of the relative digestibility and bulkiness of foods. The crude fiber is indigestible matter in food stuff, it is made up of cellulose, lignin and nitrogenous matter. Feeds that are rich in fiber are less digestible and therefore, less nutritious than those lower in fiber according to the findings of (Anjum, 1993).

3.3. Moisture contents

Percentage of moisture in seeds of different varieties of soybean (Bovender special, Foster and F-8827) was found to be in the range of 8.4–10.2% as shown in Table 1. These values investigated in the present study were closer to that of soybean seed (5.13–7.68%) reported by (Al-Kahtani, 1989) and for soybean and Xyopia aethiopica reported by (Yoshida et al., 2006) and (Barminas et al., 1999). The so called critical moisture level for the beginning of rapid spoilage is relatively higher in seeds of low oil contents and relatively low for high oil content seeds. Moisture content in the seeds depends upon the maturity and quality of seeds. The moisture contents of seed determine the ability of all seeds to be stored well.

3.4. Physicochemical parameters of oils

The oil color in terms of yellow and red units for different samples of soybean seed oils were in the range of 40–50 Y and 4.0–5.3 R. The intensity of the color of vegetable oils is linked with the presence of different pigments such as chlorophyll and carotenoids which are effectively removed during the processing (de-gumming, refining and especially bleaching) step of oil. The vegetable oils with low color values are better for edible and domestic applications (Table 2).

Values determined for the different physico-chemical attributes of oils were as follows; iodine value (119–128), refractive index (1.4590–1.468) density (0.8698–0.8712 g/cm3 at 36 °C), FFA (0.39–0.67%), unsaponifiable matter (0.42–0.74%), and saponification number (181–187 of KOH/g of oil).

Values for refractive index are comparable with the findings of (Kyenge et al., 2012) who reported RI of 1.470 at 32 °C for soybean oil. Density and refractive indices of investigated oils in the present analysis were in close agreement with some other oilseed crops: Maurritia flexuosa (0.86 g/cm3 at 36 °C and

Table 1 Percentage (±SD) of oil and other constituents of soybean seeds.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Soybean variety</th>
<th>Foster</th>
<th>F-8827</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>15.85 ± 0.3a</td>
<td>16.74 ± 0.25b</td>
<td>19.49 ± 0.32c</td>
</tr>
<tr>
<td>Fiber</td>
<td>6.60 ± 0.24a</td>
<td>7.10 ± 0.23b</td>
<td>7.60 ± 0.23b</td>
</tr>
<tr>
<td>Moisture</td>
<td>10.20 ± 0.22a</td>
<td>9.50 ± 0.19b</td>
<td>8.40 ± 0.16c</td>
</tr>
<tr>
<td>Crude protein</td>
<td>45.64 ± 2.60a</td>
<td>43.66 ± 2.20a</td>
<td>41.67 ± 2.07a</td>
</tr>
<tr>
<td>Ash</td>
<td>5.50 ± 0.36a</td>
<td>6.20 ± 0.32a</td>
<td>6.90 ± 0.26b</td>
</tr>
</tbody>
</table>

Values are mean ± SD of three samples from each soybean variety, analyzed individually in triplicate.
1.46 at 40 °C) investigated by (Albuquerque et al., 2005) and cotton seed oil (1.4580–1.4660) reported by (Rossell, 1991). The saponification number (181–187 mg of KOH/g of oil) of soybean seed oil was almost comparable with the findings of (Kyenge et al., 2012) who reported the saponification value of soybean oil as 189 mg of KOH/g of oil. The values are comparable with some other oils like hemp seed oil (184–190 of KOH/g of oil) and Moringa oil (177.29–184.1 of KOH/g of oil) reported by (Anwar et al., 2006) and (Anwar et al., 2005b), respectively.

The unsaponifiable matter (0.6–0.74%) of the investigated soybean oils in this analysis was close to that of soybean oil 0.55–0.95% as reported by (Al-Kahtani, 1989) and that of soybean seed oil was close to that of soybean oil /C176/C186/C196/C206/C216/C226/C236/C246/C256/C266/C276/C286/C296/C306/C316/C326/C336

It is well known that the iodine value is a measure of degree of unsaturation (double bond content) in vegetable oils principally oleic, linoleic and linolenic acids. High iodine value means that oil is highly unsaturated and could be used for edible purposes and or for industrial applications such as in paints varnishes, and emulsion formulations. The statistical analysis revealed a significant variation in saponification value and unsaponifiable matter among varieties of soybean oil. The iodine value of the oils (119–128 g of I/100 g of oil) was comparable with (128.4) that reported by (Diaz et al., 2006). The specific extinctions at 232 and 270 nm, which represent the oxidative deterioration and purity of oils, were 1.51–1.91 and 0.40–0.65, respectively. A good oxidation state of soybean oils of different varieties

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Soybean variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acids (% as oleic acid)</td>
<td>Bovender special</td>
</tr>
<tr>
<td>0.67 ± 0.03b</td>
<td>0.45 ± 0.02b</td>
</tr>
<tr>
<td>Refractive index (40 °C)</td>
<td>1.468 ± 0.07a</td>
</tr>
<tr>
<td>Density (g/cm³) 36 °C</td>
<td>0.8701 ± 0.05a</td>
</tr>
<tr>
<td>Iodine value (g of I/100 g of oil)</td>
<td>119.00 ± 0.05a</td>
</tr>
<tr>
<td>Saponification value (mg of KOH per g of oil)</td>
<td>187.00 ± 0.05a</td>
</tr>
<tr>
<td>Unsaponifiable matter (%)</td>
<td>0.60 ± 0.03b</td>
</tr>
<tr>
<td>Color (1-in cell) Red unit</td>
<td>5.30 ± 0.10b</td>
</tr>
<tr>
<td>Yellow unit</td>
<td>50.0 ± 1.30b</td>
</tr>
</tbody>
</table>

Values are mean ± SD of three samples from each soybean variety, analyzed individually in triplicate.
in the present analysis might be ascribed to higher levels of tocopherol antioxidants which retarded lipid oxidation (Anwar, 2004).

The fatty acid composition of soybean oils from different varieties (Bovender special, Foster and F-8827) is given in Table 4. The contents of saturates such as palmitic acid (C16:0) and stearic acid (C18:0) in the tested soybean oils ranged between 10.01–13.5% and 3.02–4.9%, respectively. These values were slightly lower than the findings of (Al-Kahtani, 1989) who investigated 9.18–12.54% and 4.33–5.23% for palmitic and stearic acids, respectively. The oils also contained an appreciable amount of oleic acid 22.6–24.0%. Linoleic acid (C18:2) was the predominant fatty acid with its content varying between 49.03% and 53.02%. The detected level of C18:2 of soybean oils is slightly lower than that investigated by (Al-Kahtani, 1989) who reported 47.81–58.04% linoleic acid for soybean seed oils from Saudi Arabia. The amount of another valuable polyunsaturated fatty acid namely linolenic acid (C18:3) varied from 6.50% to 8.00%. These amounts are quite comparable with the findings of (Al-Kahtani, 1989) 4.7–6.26% from Saudi Arabia. A small difference can be attributed to the genetic variability among the varieties and the ecological conditions of the countries. The composition of most of the fatty acids varied non-significantly (p > 0.05) among oils of the tested soybean varieties. The concentrations of major fatty acids, investigated in the present study were in close agreement with those reported by (Anjum, 1993) for soybean seed oils from Pakistan.

Soybean oil is a rich source of tocopherols (vitamin E) as reported by (Quek et al., 2007). The contents of tocopherols (α, γ, and δ) in different varieties (Bovender special, Foster and F-8827) of soybean seed oils ranged from 66.50 to 90.74 mg/kg, 907.55–1011.87 mg/kg and 399.81–411.50 mg/kg, respectively (Table 5). The concentration of α tocopherol was slightly lower than that reported in the literature (152 mg/kg) while that of γ and δ tocopherols were higher than that given in the literature (494 and 182 mg/kg, respectively) according to (Gliszczyńska-Świgło et al., 2007) for some Polish soybean varieties. The variations may be due to the genetic and ecological variations.

The contents of α, γ, and δ in the present analysis were higher than that of hemp seed oil reported by (Anwar et al., 2006) but the content of α tocopherols was less than that of S. bigelovii and safflower oils by (Anwar et al., 2002). The content of α-tocopherols was less but δ was higher than that of Moringa oil as studied by (Anwar et al., 2005b). Of the tocopherols, alpha homolog showed the highest vitamin-E activity, where as the δ-isomer exhibited potent antioxidant activity. The analysis revealed the soybean oil to be an exceptional source of δ-tocopherol.

4. Conclusion

The tested three varieties of soybean were found to be quite different on the basis of variation in most of the important physico-chemical characteristics. The difference may be attributed to their different genetic properties. Soybean variety F-8827 showed better nutritional status due to the higher quantity of essential fatty acids like linoleic acid and linolenic acid and tocopherols as compared to other two varieties selected. Data of this study might be useful for oil chemists and breeders for further investigations. At the same time it might be helpful for local soybean growers/farmers and oil producers for the selection of the appropriate soybean variety for cultivation and industrial processing.

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