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Analytical Characterization of *Salicornia bigelovii* Seed Oil Cultivated in Pakistan

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Seeds of *Salicornia bigelovii* (hybrid variety sos-10) were collected from five coastal areas of Pakistan on the Arabian Sea. Hexane-extracted oil content was 27.2−32.0%. Results of other physical and chemical parameters of the extracted oil were as follows: iodine value, 128.0−130.5; refractive index (40 °C), 1.4680−1.4695; unsaponifiable matter, 1.63−2.00%; saponification value, 178.6−189.0; density (30 °C), 0.9036−0.9074. Tocopherols (α, γ, and δ) in the oil ranged up to 200 mg/kg. The *S. bigelovii* seed oil was found to contain high levels of linoleic acid (74.66−79.49%) and less oleic acid (12.33−16.83%). Saturated fatty acids, palmitic and stearic acids, ranged from 7 to 8.50% and from 1.24 to 1.69%, respectively. Linolenic acid ($C_{18:3}$ ω−3) was found within the range of 1.50−2.31%. The induction period (Rancimat, 20 L/h, 120 °C) of the crude oil was 1.40−1.70 h. Specific extinctions at 232 and 270 nm were 1.90−2.40 and 0.40−0.62, respectively. Many parameters of *S. bigelovii* seed oil were quite compatible with those of safflower oil.

KEYWORDS: ***Salicornia bigelovii*; analytical characteristics; high linoleic; tocopherols**

INTRODUCTION

Salicornia is a wild as well as a cultivated annual shrub grown in the vicinity of coastal areas. It is indigenous to the Arabian Sea coasts of Pakistan and India on the margin of salt lakes. It is also found abundantly on the northern shore of the island of Ceylon (Sri Lanka) (*1*, *2*).

The commonly found *Salicornia* species are (i) *Salicornia indica* [syn. to *arthrocenemum* (Chenopodiaceae)], (ii) *S. brachiata*, (iii) *S. bigelovii*, (iv) *S. uropeae*, (v) *S. perenis*, and (vi) *S. disarticulate*. *Salicornia indica* is a gregarious and herbaceous species wildly distributed on the saline wet soil of Pakistan along Lyari, Clifton, Las Bela, and Mastung (*1*). Another variety, *S. uropaea*, can be compared favorably with other green vegetables in chemical composition, especially minerals, essential amino acids, and protein content. These plants can be used effectively as animal feeds, and amended meal can also be a source of poultry feed, replacing soybean meal (*1*, *3*−*6*). There have been some reports in the literature on the composition, nutrient content, and medicinal and food values of these halophytes (*3*, *4*, *7*).

S. bigelovii is a leafless annual salt-marsh plant with green jointed and succulent stems that ultimately form terminal fruity spikes in which seeds are borne (*5*, *8*). In subtropical regions, it may grow to be a large, upright plant, 50 cm tall, with most of the seed spikes on the upper one-third of the plant. *S. bigelovii* emerged as a potential seawater oilseed crop from a screening of wild halophytes and was selected for seawater field trials including determination of seed yield and analysis (*5*, *9*−*12*). This is one of the numerous sources of the alkaline earth sajji used in medicine and art (*13*). Its native pickle leaves and young shoots are utilized as greens at a time of scarcity. In Pakistan, trials of *S. bigelovii* as an oilseed crop are under investigation in the coastal environment of the Arabian Sea in the Sindh and Baluchistan provinces.

In view of the cardinal role of dietary fats in human health and disease (*14*−*16*), the chemical analysis and, particularly, the fatty acid composition of oil used for domestic consumption have become a research priority of lipid chemistry (*17*−*21*). Such work has not been reported previously on this variety grown in subcontinental countries, especially in Pakistan. In this context, the analytical characterization of oil from *S. bigelovii* seeds that were grown recently in the coastal areas of Pakistan has been made to assess its potential as an edible oil. The results are reported in the present study.

EXPERIMENTAL PROCEDURES

Materials. The seeds of *S. bigelovii* (hybrid variety sos-10), cultivated at the trial fields of Phitti Creek (coastal areas of the Arabian Sea near Karachi, Pakistan), were procured from the Sindh Coastal Development Authority (SCDA), Karachi, Pakistan. Five coastal sites were selected, and three samples of seeds from each harvest were collected. Seeds were tiny, light to dark brown in color. Safflower seeds were acquired from the Agriculture Research Institute (ARI), Tandojam, Sindh, Pakistan. All reagents (analytical and HPLC) used were from E. Merck or Sigma Aldrich. Pure standards of tocopherols [*dl*-α-tocopherol, (+)-δ-tocopherol, and γ-tocopherol] and fatty methyl esters were obtained from Sigma Chemical Co. (St. Louis, MO).

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Extraction of Oil. The seeds (1 kg) of each batch of *S. bigelovii* were taken, sieved, and flaked by rolling to a thickness of 0.03 cm and then fed to a Soxhlet extractor fitted with a 2-L round-bottom flask and a condenser. The extraction was done on a water bath for 4 h with 1.25 L of *n*-hexane. The solvent was distilled off under vacuum in a rotary evaporator. Except for a small quantity (for tocopherol and Rancimat analysis), the recovered oil from different batches was further degummed.

Degumming of Oil. The oil to be degummed was heated (70 °C) on a water bath. Water was added to a final volume of 18% and mixed well with a glass rod. After cooling, the mixture was centrifuged (3000 rpm) for 10 min in an automatic refrigerated centrifuge (Sorvall RC-3).

The degummed oil was dried over anhydrous sodium sulfate, filtered, and kept in separate sealed bottles under refrigeration (0−4 °C) before use. The same procedure was executed for extraction of oil from safflower seeds.

Analysis of Oilseed Residues. The oilseed residues (meal) after extraction of oil from the seeds were analyzed for protein, fiber, and ash content. Protein content was determined according to a semiautomated FOSFA official method (*22*). Samples of meal were digested for 10 min with a digestion mixture of sulfuric acid/hydrogen peroxide/potassium sulfate, using mercuric oxide as a catalyst. The final endpoint in the ammonia titration was measured photometrically.

Fiber content was determined according to the ISO method (*23*): 2.5 g of finely ground meal was weighed and defatted by extraction with 15 mL of *n*-hexane. The test portion was boiled with sulfuric acid solution (0.255 mol/L) followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol/L), followed by separation, washing, and drying. The dried residue was weighed and ashed in a muffle furnace at 600 °C, and the loss in mass was determined. Ash content was determined according to the ISO method (*24*): 2.0 g of the test portion was taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace at 550 °C, until constant mass was achieved.

Analysis of Extracted Oils. *Physical and Chemical Parameters of the Oil.* Determination of density, refractive index, iodine value, saponification value, and unsaponifiable matter of the extracted oil was carried out by various standard AOCS methods (*25*). The color of the oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, U.K.) using a 1 in. cell. Specific extinctions at 232 and 270 nm were determined using a Perkin-Elmer model Lambda 2 spectrometer. Samples were diluted with isooctane to bring the absorbance within limits (0.2−0.8), and $\epsilon^{1\%}_{1cm(\lambda)}$ was calculated following the method of the IUPAC (*26*).

Oxidative Stability. An automated Metrohm Rancimat model 679, capable of performing at a temperature range of 50−200 °C, was used for the determination of susceptibility to oxidation of the degummed and non-degummed oils (*27*, *28*). Briefly, oil (2.5 g) was carefully weighed into each of the six reaction vessels and analyzed simultaneously. The conductometric cells were filled with deionized water up to a volume of 65 mL. The temperature of analysis was selected as 120 °C, and filtered, dried air was allowed to bubble through the hot oil at the rate of 20 L/h. Processing and evaluation of the experimental data were performed from the break points of the plotted curves, and induction periods of the samples were recorded automatically.

Tocopherol Content. Tocopherols (α, γ, and δ) analysis was carried out by high-performance liquid chromatography (HPLC) following the method of Thompson and Hatina (*29*) with slight modifications. One gram of oil was accurately weighed and made up to volume with heptane in a 10 mL volumetric flask wrapped in foil to inhibit oxidation. A Hitachi L-6200 HPLC unit coupled with a Hitachi F-1050 fluorescence detector was used. A 25 μL sample was injected into an analytical column (250 × 4.9 mm) packed with Lichrosorb SI 605 (5 μm), which was fitted with a 50 mm × 50 mm guard column. A mobile phase of dry heptane/water-saturated heptane/2-propanol (50.0/49.0/1.0) was used at the rate of 1.2 mL/min. Detection was performed at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Pure standards of α-, γ-, and δ-tocopherols were used for identification and calibration. A Hitachi Chromatointegrator model D-2500 with a built-in computer program for data handling was used for the quantification.

Fatty Acid Composition. Fatty acid methyl esters were prepared according to standard IUPAC method 2.301 and analyzed on a Perkin-Elmer gas chromatograph model 8700 fitted with a polar capillary column SP-2340 (60 m × 0.25 mm) and a flame ionization detector. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. Other conditions were as follows: initial oven temperature, 130 °C; ramp rate, 5 °C/min; final temperature, 220 °C; injector temperature, 260 °C; detector temperature, 270 °C; and temperature hold, 3 min before the run and 6 min after the run. A sample volume of 1.0 μL was injected, and the total analysis time was 20 min. Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of authentic standards of fatty acid methyl esters obtained from Sigma Chemical Co. All of the quantification was done by a built-in data handling program, provided by the manufacturer (Perkin-Elmer) of the gas chromatograph. 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.

Table 1. Analysis of Oilseeds[a]

constituent	*S. bigelovii*	safflower
oil (%)	29.70 ± 2.04 (27.2–32.0)	34.03 ± 1.70 (31.9–36.7)
protein (%)	33.07 ± 1.67 (31.00–35.51)	31.64 ± 2.00 (28.52–35.00)
fiber (%)	5.79 ± 1.50 (4.80–7.99)	7.70 ± 1.47 (6.05–9.80)
ash (%)	6.06 ± 1.10 (5.60–8.10)	6.58 ± 1.50 (5.02–8.11)

[a] Values are means ± SD calculated as percentage of dry seed weights for five *S. bigelovii* and four safflower seeds analyzed individually in triplicate. For details see text. Parentheses show range of the data.

Table 2. Physical and Chemical Characteristics of Degummed Oils[a]

determination	*S. bigelovii*	safflower
iodine value (g of I/100 g of oil)	129.50 ± 1.10 (128.0–130.5)	134.20 ± 1.40 (132.0–137.0)
refractive index (n_D 40 °C)	1.4680 ± 0.005 (1.4670–1.4690)	1.4690 ± 0.004 (1.4676–1.4699)
density (24 °C) (mg/mL)	0.9054 ± 0.001 (0.9036–0.9074)	0.9073 ± 0.001 (0.9050–0.9100)
saponification value (mg of KOH/g of oil)	183.72 ± 4.44 (178.6–189.0)	189.0 ± 3.99 (186.1–195.5)
unsaponifiable matter (%)	1.78 ± 0.145 (1.63–2.00)	1.40 ± 0.139 (1.30–1.64)
color (red units)	1.56 ± 0.24 (1.40–1.90)	2.00 ± 0.20 (1.70–2.10)
color (yellow units)	35.00 ± 2.96 (30.00–40.30)	40.00 ± 3.57 (35.00–50.00)

[a] Values are means ± SD for five *S. bigelovii* and four safflower oils analyzed individually in triplicate. For details see text. Parentheses show range of the data.

RESULTS AND DISCUSSION

To evaluate vegetable oils, *S. bigelovii* seeds, from five different trial fields (coastal sites), and safflower seeds from four different harvests were individually extracted. Values given in **Tables 1−5** are means ± SD of five *S. bigelovii* and four safflower oils, analyzed individually in triplicate. Ranges are given in parentheses.

The hexane-extracted oil content of seeds of *S. bigelovii* crops cultivated at coastal sites of the Arabian Sea was found to be 29.2% (**Table 1**). Oil content varied among the seed samples collected from five different trial fields analyzed individually, ranging from 27.2 to 32.0%, and oil concentration was high in

Table 3. Determination of Oxidative State of Oils[a]

determination	*S. bigelovii*	safflower
$E^{1\%}_{1cm(\lambda_{232})}$	2.10 ± 0.19 (1.90–2.40)	2.37 ± 0.24 (1.80–2.50)
$E^{1\%}_{1cm(\lambda_{270})}$	0.50 ± 0.08 (0.40–0.62)	0.66 ± 0.07 (0.54–0.78)
oxidative stability (ndmo)	1.70 ± 0.08	1.59 ± 0.07
Rancimat method (h)	(1.60–1.85)	(1.45–1.68)
oxidative stability (dmo)	1.40 ± 0.0.06	1.50 ± 0.06
Rancimat method (h)	(1.32–1.48)	(1.40–1.57)

[a] Values are means ± SD for five *S. bigelovii* and four safflower oils analyzed individually in triplicate. For details see text. Parentheses show range of the data. (ndmo), non-degummed oil; (dmo), degummed oil.

Table 4. Tocopherol Content of the Non-degummed Oils[a]

determination	*S. bigelovii*	safflower
α-tocopherol (mg/kg)	200.00 ± 13.05 (165.50–230.01)	356.40 ± 18.00 (298.30–400.70)
γ-tocopherol (mg/kg)	74.60 ± 4.20 (50.94–100.30)	95.60 ± 5.00 (70.81–120.42)
δ-tocopherol (mg/kg)	63.00 ± 5.00 (40.10–79.80)	40.50 ± 3.31 (25.43–76.04)

[a] Values are means ± SD for five *S. bigelovii* and four safflower oils analyzed individually in triplicate. For details see text. Parentheses show range of the data.

Table 5. Fatty Acid Composition (Percent) of Oils[a]

fatty acid	*S. bigelovii*	safflower
$C_{16:0}$	7.52 ± 0.24 (7.00–8.50)	6.70 ± 0.25 (6.03–7.81)
$C_{18:0}$	1.45 ± 0.07 (1.24–1.69)	2.50 ± 0.10 (2.05–3.00)
$C_{18:1}$	13.42 ± 0.56 (12.33–16.83)	12.30 ± 0.70 (9.50–15.70)
$C_{18:2}$	75.50 ± 2.04 (74.66–79.490	78.00 ± 3.50 (73.60–80.04)
$C_{18:3}$	1.98 ± 0.09 (1.50–2.30)	

[a] Values are means ± SD for five *S. bigelovii* and four safflower oils analyzed individually in triplicate. For details see text. Parentheses show range of the data.

those seeds harvested from extreme coastal environments and where the irrigation water from seawater wells was supplemented with nitrogen fertilizers. This small variation in oil yield of seeds may be the result of the difference between the upper limit of salt tolerance currently exhibited by crop plants and that required to tolerate seawater salinity (*7*, *30*). Other factors involved may be the coastal desert, provision of urea and ammonium nitrate fertilizers, and annual seasonal cultivation pattern (*5*, *31*).

The range of oil content of *S. bigelovii* seeds determined in the present work was in close agreement with that reported by Glenn et al. (*5*) for cultivated *S. bigelovii* Torr plants derived from trials conducted at Puerto Penasco, Sonora, Mexico, an extreme desert environment of the northern Gulf of California. The average oil content of the seeds exceeds those of two conventional oilseed crops, cotton (15.0−24.0%) and soybean (17−21%) grown in the United States, Brazil, and Asia (*32*), but is slightly lower than that of safflower seeds.

The analysis of oilseed residue revealed a high protein content of the seeds, ranging from 31.00 to 35.51%, whereas fiber and ash contents were low, 4.80−7.99 and 5.60−8.10%, respectively. The present analysis showed the meal to be a rich source of protein, which could be added to chicken diets as a source of calories and protein and may replace soybean meal for the local poultry industry. There are reports in the literature of deactivation of saponins suspected to be in the meal by the soaking of seeds in 1% NaOH before extraction, similar to saponin detoxification by NaOH treatment in kochia seeds (*33*). The unamended meal may be suitable for ruminants, which are less sensitive to saponins than poultry (*6*).

The results of some of the physical and chemical parameters of the extracted oils are given in **Table 2**. The values determined for refractive index (1.4680) and density (0.9054 mg/mL) were in close agreement with those of safflower oil, whereas iodine value (129.5 g of I/100 g of oil) and saponifiation value (183.72 mg of KOH/g of oil) of the former were lower than those of the latter.

The *S. bigelovii* seed oil had more unsaponifiable matter (1.78%) than safflower oil but was superior in color measurement (1.6 R + 35 Y) (after degumming) to degummed safflower oil. The intensity of the color of vegetable oils depends mainly upon the presence of various pigments such as chlorophyll, which are effectively removed during degumming, refining, and bleaching steps of processing. The vegetable oils with minimum values of color index are good for edible purposes.

The above characteristics of *S. bigelovii* seed oil could not be compared with the literature because there are no reports available on detailed characterization of this oil. These properties of the investigated oil, which are not so significantly different from those in safflower oil, were in good agreement with those of sunflower and soybean oils, which are mainly used for domestic cooking in Pakistan (*34*). The present data also support the usefulness of the *S. bigelovii* seed oil for edible as well as industrial uses.

S. bigelovii seed oil also exhibited good oxidative stability as indicated by the determinations shown in **Table 3**. The specific extinctions at 232 and 270 nm, which revealed the oxidative deterioration and purity of the oils (*35*), were similar for *S. bigelovii* and safflower oils.

The induction period (Rancimat; 20 L/h, 120 °C), which is a characteristic of the oxidative stability of oils and fats (*36*), of the non-degummed *S. bigelovii* seed oil was 1.70 h, indicating fair stability. After degumming, the induction period of the oil was decreased to 1.40 h, a reduction of 9.67% in oxidative stability, which could be attributed to the degumming process. There are no previously reported data to compare the results of specific extinctions and induction period with our present work.

Table 4 shows the content of different tocopherols in the non-degummed oils as determined by HPLC. Tocopherols were analyzed in crude non-degummed oils, because most of the steps involved during processing or storage reduce the level of tocopherols (*37*). The levels of α-tocopherol, γ-tocopherol, and δ-tocopherol in the *S. bigelovii* seed oil were 200.00, 74.60, and 63.00 mg/kg, respectively. The contents of α-tocopherol, γ-tocopherol, and δ- tocopherol in the safflower oil were 356.24, 95.60, and 40.50 mg/kg, respectively, which was well in line with the values for most of the edible oils reported in the literature (*37*).

The concentration of δ-tocopherol, which has a greater antioxidant activity than either γ- or α-tocopherol (*38*), in the *S. bigelovii* seed oils was significantly higher than in the safflower oil and, thus, would be expected to contribute to good oxidative stability and protection during storage and processing. Although the contents of γ- and α-tocopherols in the *S. bigelovii* seed oil were lower than those of safflower oil, they were still at a fairly high level to protect against oxidation. As with many of the other traits, there were no previously reported data on the tocopherol content of *S. bigelovii* seed oil.

Table 5 shows the fatty acids composition (mean ± SD; range) of the investigated oils. The sum of the saturated palmitic and stearic acids in the *S. bigelovii* seed oil was 8.97% (7.52% palmitic acid and 1.45% stearic acid). Monounsaturated fatty acid (oleic acid) was found at 13.42% (12.33−16.83). The oil was rich in polyunsaturated fatty acids, particularly linoleic acid ($\omega-6$), which is of great medical importance. The total 18-C polyunsaturated fatty acid content was 77.48%, most of which was $C_{18:2}$ ($\omega-6$ or *n*-6, 75.50%), with a small amount of $C_{18:3}$ ($\omega-3$ or $n-3$, 1.98%). Both of these polyunsaturated fatty acids had been a research priority and gained attention in biomedical science during the past decade (*16*, *39*−*41*). The present fatty composition of *S. bigelovii* seed oil was found to be in very close agreement with that of high-linoleic safflower oil, which showed that the former oil falls in the high-linoleic category. The only significant difference was a small amount of $C_{18:3}$ (1.98%) in the *S. bigelovii* oil, which was absent in the safflower oil. The fatty acid composition of this potential oilseed crop was also in good agreement with that reported by Glenn et al. (*5*). Austenfeld (*42*) had also found a similar fatty acid profile in *S. europaea* L.

Pakistan imports millions of tons of edible oils and seeds, resulting in an enormous amount of foreign exchange being spent every year. As Pakistan has a vast coastal boarder along the Arabian Sea, *S. bigelovii* appears to be a potentially valuable new oilseed crop for the coastal deserts, yielding a useful oil high in essential fatty acids of great biological importance. Our present investigations revealed that *S. bigelovii* seed oil indigenous to Pakistan has very good potential for edible purposes as well as for developing nutritionally balanced blended formulations with other high-oleic oils. The great demand for oilseeds coincides with the functional and nutritional properties of this oil, which is well positioned to meet the environmental and technical needs of the country.

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NOTE ADDED AFTER ASAP POSTING

An author's name was incorrectly given in initial Web posting on June 20, 2002. The correct name is given in this posting.

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