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Research Article

Analysis of oilseed of Halophytic species: *Atriplex griffithii, Haloxylon ammodendron, Salicornia europaea, Salsola yazdiana*

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Abstract: Seeds of *Atriplex griffithii, Haloxylon ammodendron, Salicornia europaea* and *Salsola yazdiana* were analyzed to determine their potential as sources of edible oil. The quantity of total oil varied from 13.8% in *Atriplex griffithii* to 20.9% in *H. ammodendron*. The proportion of unsaturated fatty acids were higher (62-73.8%), with the highest values of α-linoleic acid (18.6%), linoleic acid (28.6%) and oleic acid (19.7%) in the seeds of *A. griffithii, H. ammodendron* and *S. europaea*, respectively. Results of physicochemical evaluation of the extracted oils ranged as follows: iodine values, 99.8-106.5 (g I_2/100 g); saponification value, 188-283 (mg KOH/1g of oil); peroxide value, 9-13 (meq/kg) and refractive index, 1.4750- 1.4761. Amongst these oilseeds, *S. europaea* (containing 73.8% unsaturated fatty acids but not erucic acid) was the highest in quality for human consumption followed by *H. ammodendron.*

Keywords: *Atriplex griffithii, Haloxylon ammodendron, Salicornia europaea, Salsola yazdiana,* Halophytes, Oilseed.

1. Introduction

Environmental stresses such as drought, cold and salt restrict crop production worldwide. Amongst these, salinity is a growing threat to agriculture, with a significant component of salt stress being generated by human activity. It is estimated that about 43% of Earth's land have the characteristic of arid and semi-arid lands and 98% of the water is saline (Hendricks and Bushnell, 2008). Geographically, much of Iran is arid or semi-arid and salinization of soil and water resources is a serious threat in many parts of the country (Siadat, 1997), limiting agricultural production. Since, the majority of conventional crops are sensitive to salinity, overcoming the harmful effects of salt demands alternative approaches to agricultural production for the human population (edible oil is an important product). Oil is important in human nutrition as a source of energy and of fatty acids. A group of special crop plants such as corn, sunflower, canola, soya, palm, olive and coconut has been used as common sources of edible oils yet. However, rapid growth of the population, particularly in the developing countries, has increased demand for vegetable oil, but it cannot meet domestic production. For example, in the case of Iran, a considerable proportion (averagely 95%) of the edible oil requirement is provided through imports (Saeedi and Sediqi, 2008). In Iran, the area of saline lands is estimated to be 27 million ha in addition to several hundred kilometers of salty coastal zones of the Persian Gulf. With this in view, introducing novel sources for edible oil from plants that can be irrigated by seawater, would reduce the vegetable oil imports as well as using the maximum potential of national resources. Halophytes are salt-tolerant plants that utilize saline water without harmful effects on growth and reproduction (Flowers and Colmer, 2008). A number of reports have been published suggesting the utilization of oilseeds halophytes as acceptable sources of edible oil (O'Leary *et al.*, 1985; Glenn *et al.*, 1991; Cui *et al.*, 2010; Wang *et al.*, 2012; Shahi *et al.*, 2014) with high enough quality compared to the traditional oilseed crops (Wang *et al.*, 2012). For instance, Glenn *et al.*, (1991) have reported the seeds of *Salicornia bigelovii* (a halophytic species) contained by up to 33% oil with high amount (>73%) of linoleic acid. On the other hand, Weber *et al.*, (2001) recognized the level of unsaturation ranging from 85% to 90% in five halophytic species in the Great Basin desert of North America. They found *Suaeda moquinii,* a highly salt

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tolerant species, had the quality oil as good as that of the edible oils such as olive and canola. There are more reports on other halophytes such as *Crithmum maritimum* and *Zygophyllum album* (Zarrouk *et al.*, 2003), *Kosteletzkya virginica* (He *et al.*, 2003), *Nitraria siberia*, *Suaeda salsa*, *Chenopodium glaucum* and *Descurainia sophia* (Yajun *et al.*, 2003), *Arthrocnemum indicum*, *Alhagi maurorum*, *Cressa cretica*, *Halopyrum mucronatum*, *Haloxylon stocksii* and *Suaeda fruticosa* (Weber *et al.*, 2007), which all contained good quality oil. Another reports are about the oilseeds of *Suaeda aralocaspica* which contain 93% unsaturated fatty acids, with linoleic (>68%) and oleic acid (>20%) (Wang *et al.*, 2012) and *Salicornia herbacea* which contains six unsaturated fatty acids (87%) with dominance of Linoleic acid (59.7%) (Shahi *et al.*, 2014).

In the present study, four endemic species of halophytes growing in Iran were investigated for their potential as a source of edible oil; all were in the Amaranthaceae. *Atriplex griffithii* is a salt-tolerant species which endemically grows in both inland and coastal marshes and deserts (Khan *et al.*, 2000). *Haloxylon ammodendron* (saxaul) plants range in size from a large shrub to a small tree, 2-8m (rarely 12m) tall. The flowering period is from March to April and the seed is 1.5mm in diameter: saxaul is highly drought-resistant. *Salicornia europaea* is a fleshy maritime annual. Seeds are cylindric-ovoid, ca. 1.5mm in diam. *Salsola yazdiana* is a shrub whose fruit is spherical with a spiral embryo and no perisperm.

2. Experimental Procedures

2.1. Seed collection and preparation

Seeds of *Atriplex griffithii* Moq., *Haloxylon ammodendron* (Meyer) Bunge, *Salicornia europaea* agg. and *Salsola yazdiana* Assadi were purchased from Pakan-Bazr company (Isfahan, Iran).

2.2. Oil extraction and fatty acid analysis

Dried seeds were ground with a Wiley Mill and extracted three times with methanol and chloroform (1:2 v/v). The percent oil in the seeds was determined by weight. Fatty acids in oil extracts were methylated using potassium methoxide. The methylated fatty acids were separated by capillary gas chromatography (Hp GC 6890) and identified by GC mass spectrometry (Hp MS 5973). Identification of the components was assigned by comparison of their retention times of the fatty acid methyl esters with the known standard mixture.

2.3. Iodine value determination

A sample of oil (0.4g) was dissolved in 20ml chloroform. Iodine bromide solution (25.0ml) was added and after standing for 30 min. (Protected from light, with shaking) saturated potassium iodide solution (20ml; 15%) and 100ml of water were added to the mixture and shaken. After that, the liberated iodine was titrated with 0.1M sodium thiosulfate. Blank determination was also carried out. Iodine value was calculated from the formula (Council of Europe, 2004):

$$\text{Iodine value} = (V_2 - V_1) \times \frac{1.269}{M}$$

Where "V_1" is the volume (ml) of 0.1M sodium thiosulfate consumed for titration in the sample test; "V_2" is the volume (ml) of 0.1M sodium thiosulfate consumed in the blank test; "M" is the amount (g) of the sample.

2.4. Measurement of saponification value

Potassium hydroxide ethanol (25.0ml of 0.5M) was mixed with 2.0g oil sample and gently heated and shaken for 30 minutes. After cooling, the solution was titrated against 0.5M HCl before the test liquid solidified. A blank test (without sample) was performed 3 times to obtain mean values of a titration volume of 0.5M hydrochloric acid.

$$\text{The Saponification value (mg KOH/1g of oil)} = \frac{(V_2 - V_1) \times RF \times C_1 \times K_1}{\text{Weight of oil (g)}}$$

Where, "V_1" is the titration volume (ml); "V_2" is the blank level (25.029ml); RF is the Reagent (HCl) factor (1.006); "C_1" is the concentration conversion coefficient (28.05mg/ml); "K_1" is the unit conversion coefficient (1).

2.5. Peroxide value determination

The extracted oil (5.00g) was dissolved in chloroform-glacial acetic acid (30ml of 2:3 v/v) and 500µl of saturated potassium iodide solution added and then the mixture was shaken for 1 min. in the dark, before the solution was stirred with 30ml distilled water. After titration against 0.01M sodium (a blank test was carried out under the same conditions) the peroxide value was calculated as (Council of Europe, 2004):

$$PV = (V_1 - V_0) \times \frac{10}{m} [meq/kg]$$

Where "V_1" is the volume of thiosulfate solution required to titrate the sample [ml]; "V_0" is the volume of thiosulfate solution required to titrate the blank determination [ml]; "m" is the mass of sample [g].

2.6. Measurement of Refractive Index

The refractive index of chemical compounds is considered important because it indicates characteristic physical properties (Council of Europe, 2004). We determined the index of the oils using an Abbe refractometer equipped with a sodium lamp (Bausch & Lomb, GD8804, USA).

2.7. Data analysis

Data were expressed as means ± standard error (n=3). Seed oil content was expressed as a percentage of seed dry mass. Fatty acids were expressed as the relative percentage of each individual fatty acid of the total fatty acids available in the sample. All determinations were performed in triplicate.

3. Results

3.1. Oil analysis in *Atriplex griffithii*

In *A. griffithii*, the quantity of oil was 13.9% dry mass, and the amount of seven saturated and seven unsaturated fatty acids were 38% and 62%, respectively (Tables 1 & 2). Saturated fatty acids ranged from 3.11% to 9.12%. In this species, monounsaturated fatty acids were a greater level (35.7%) compared to other unsaturated fatty acids. The predominant fatty acid was α-Linoleic (C18:3 / 18.55%), followed by Eicosenoic acid (C20:1 / 12.8%) (Table 1). Iodine, saponification, and peroxide values were 100.9 (g I_2/100 g), 260 (mg KOH/1g of oil) and 13 (meq/kg), respectively. The refractive index of the oil was 1.4752 (Table 3).

3.2. Oil analysis in *Haloxylon ammodendron*

Data analysis showed the total oil in the seeds of *H. ammodendron* was 20.9% (Table 2). The seeds contained four saturated and six unsaturated fatty acids which were 37.17% and 65.83% of total oil (Table 1 & 2). Linoleic acid (C18:2) was the most abundant unsaturated fatty acid (28.63%) followed by Eicosenoic acid (C20:1 / 10.78%). The percentage of polyunsaturated fatty acids (including α-Linolenic acid and Linoleic acid) was 37.96%. In this species, Iodine, saponification, and peroxide values were 106.5 (g I_2/100 g), 235(mg KOH/1g of oil) and 9 (meq/kg), respectively. The refractive index of the seed oil was 1.4750 (Table 3).

3.3. Oil analysis in *Salicornia europaea*

This species contained six different saturated (26.2%) and five unsaturated fatty acids (73.8%) (Table 1 & 2). Total oil content in *S. europaea* was estimated at 18%. Oleic acid (C18:1) was the main unsaturated fatty acid (19.69%) in seeds of *S. europaea* followed by Nervonic acid (C24:1 / 16.49%). The only polyunsaturated fatty acid was recognized as α-Linolenic acid (10.57%). Physical and chemical characteristics of the extracted oil were: Iodine value, 99.8 (g I_2/100 g); saponification value, 188 (mg KOH/1g of oil); peroxide values, 9.5 (meq/kg); Refractive index, 1.4750 (Table 3).

3.4. Oil analysis in *Salsola yazdiana*

In *S. yazdiana*, five saturated (32.65%) and seven unsaturated (67.35%) fatty acids were identified; total oil content was 14.9% (Table 1 & 2). The major fatty acid was Eicosenoic acid (C20:1 / 20.13%) followed by γ-Linolenic acid (C18:3 / 15.7%) as a polyunsaturated fatty acid. In this seed, physicochemical properties of the extracted oil were obtained as Iodine value, 101.7 (g I_2/100 g); saponification value, 283 (mg KOH/1g of oil); peroxide values, 10 (meq/kg); Refractive index, 1.4752 (Table 3).

Table 1. Saturated/ Unsaturated fatty acid fractions (%) in the oilseeds of halophytes.

Fatty acids		*Atriplex griffithii*	*Haloxylon ammodendron*	*Salicornia europaea*	*Salsola yazdiana*
Saturated					
Arachidic acid	*C20:0*	3.11±0.12	3.35±0.10	0	5.81±0.19
Behenic acid	*C22:0*	8.30±0.11	0	0	0
Heneicosylic acid	*C21:0*	9.12±0.16	0	0	0
Lignoceric acid	*C24:0*	0	5.91±0.32	4.36±0.23	9.10±0.02
Nonadecylic acid	*C19:0*	4.12±0.13	0	0	0
Palmitic acid	*C16:0*	0	18±0.02	5.9±0.42	12.03±0.34
Margaric acid	*C17:0*	0	6.91±0.18	4.15±0.13	0.73±0.01
Myristic acid	*C14:0*	0	0	5.66±0.25	0
Pentacosylic acid	*C25:0*	3.41±0.22	0	3.13±0.09	0
Stearic acid	*C18:0*	3.15±0.13	0	3±0.32	4.98±0.18
Tricosylic acid	*C23:0*	6.79±0.18	0	0	0
Unsaturated					
α-Linoleic acid	*C18:3*	18.55±0.34	0	0	0
α-Linolenic acid	*C18:3*	0	9.33±0.08	10.57±0.22	0
Eicosenoic acid	*C20:1*	12.8±0.12	10.78±0.27	14.39±0.05	20.13±0.14
Erucic acid	*C22:1*	2.5±0.17	4.55±0.15	0	6.80±0.03
γ-Linolenic acid	*C18:3*	0	0	0	15.70±0.1
Linoleic acid	*C18:2*	0	28.63±0.17	0	8.16±0.06
Linolenic acid	*C18:2*	7.76±0.11	0	0	0
Nervonic acid	*C24:1*	4.52±0.08	3.65±0.4	16.49±0.32	7.47±0.37
Oleic acid	*C18:1*	8.56±0.13	0	19.69±0.14	6.90±0.29
Palmitoleic acid	*C16:1*	7.31±0.27	8.89±0.12	12.66±0.33	2.19±0.05

Table 2. Analysis of seed oil in the halophytes.

Constituent	*Atriplex griffithii*	*Haloxylon ammodendron*	*Salicornia europaea*	*Salsola yazdiana*
Total oil (%)	13.8	20.9	18	14.9
Saturated fatty acid (%)	38	37.17	26.2	32.65
Monosaturated (%)	35.69	27.87	63.22	43.49
Polysaturated (%)	26.31	37.96	10.57	23.86
Total Unsaturated (%)	62	65.83	73.8	67.35
Unsaturated/Saturated (%)	1.63	1.77	2.82	2.06

Table 3. Physicochemical characterization of seed oil in the halophytes.

Determination	*Atriplex griffithii*	*Haloxylon ammodendron*	*Salicornia europaea*	*Salsola yazdiana*
Iodine value (g I_2/100 g oil)	100.9±5.46	106.5±3.38	99.8±4.12	101.7±3.26
Saponification value (mg KOH/1g of oil)	260±4.3	235±6.25	188±2.19	283±4.19
Peroxide value (meq/ kg)	13±0.58	9±0.61	9.5±0.27	10±0.79
Refractive index	1.4752±0.002	1.4761±0.007	1.4750±0.004	1.4752±0.006

4. Discussion

Total oil content of the seeds used in the present study (*Atriplex griffithii*, *Haloxylon ammodendron*, *Salicornia europaea* and *Salsola yazdiana*) ranged from 13.8 to 20.9%. Different oil content has been reported in halophytes in previous studies (O'Leary *et al*., 1985; Weber *et al*., 2007). For instance, Weber *et al.*, (2001) extracted oil from a low of 10% in *Kochia scoparia* to 26% in *Suaeda torreyana* and He *et al.*, (2003) obtained a total oil content of 11% from the seed of *K. virginica.* Yajun *et al.*, (2003) found an oil recovery, ranging from 9 to 35% in four halophytes of their study. In this respect, the total oil content of the species studied here similar to those in previously studied halophytes. By way of comparison, the oil content of traditional oilseed plants (soybean, safflower, sesame, sunflower, etc.) ranged from 19 to 49% (Weiss, 1983; Fayyaz-ul-Hassan *et al.*, 2011; Wang *et al.*, 2012). Data suggest that canola produces the best oil for human consumption; total oil content from canola seed is 40%, with 90% unsaturated fatty acid contents (Declercq and Daun, 1998). Investigations on oilseed of local halophytic species confirm the hypothesis that their oil quality is comparable with conventional edible oils such as those from sunflower and canola. These crops could be grown with brackish water. Halophytes such as *Cakile edentula* (O'Leary *et al.*, 1985) and *Crambe abyssinica* (Mandel *et al*., 2002) have been reported to contain 50% and 60% oil, respectively. *Cakile maritime* is another halophyte plant from Tunisia which contains 25.4-38.8% oil, but because of the presence of 25-35% erucic acid, the oil was appropriate only for the industrial purpose and not suitable for human use (Gandour *et al.*, 2011).

In the present study, the seeds contained a variety of saturated, monounsaturated and polyunsaturated fatty acids (Table 1). The predominant fatty acids were monounsaturated (except for *Haloxylon ammodendron*). According to our data, total unsaturated fatty acids of the studied seeds ranged from 62% to 73.8%. Among the studied species, the ratio of unsaturated to saturated fatty acids was the highest in *S. europaea* (2.82) followed by *Salsola yazdiana* (2.06). It is known that oil quality for human consumption is related to its degree of unsaturation (Ariffin *et al*., 2009). Oil with high unsaturation is regarded to be favorable for the reduction of serum cholesterol and atherosclerosis and may result in the prevention of heart diseases. Unsaturation in the seeds of various halophytes (such as *Halopyrum*, *Haloxylon*, *Suaeda* species) has been reported to range from 54 to 96% (Yu *et al*., 2005; Mu *et al*., 2006; Weber *et al*., 2007; Cui *et al*., 2010; Li and Fan, 2010; Wang *et al*., 2012). Thus, our data compare well with the previous reports from the seeds of halophytes. Moreover, the oils from the seeds of our halophytes were free from any such undesirable component such as erucic acid (i.e. *S. europaea*) or values were low, ranging from 2.5 to 6.8%.

Some of the physicochemical properties (such as iodine, saponification and peroxide values and refractive index) of the extracted oils were also evaluated in the present study. Iodine value is a measure of the unsaturation of fats and oils: high iodine value indicates high unsaturation of fats and oils. This value can be used to quantify the proportion of double bonds in the oil and considered as a good indicator of the vulnerability of oil to oxidation (Eganathan *et al*., 2006). The iodine value is also used to classify oil in non-drying groups which can be regarded as liquid oil. Oils with iodine value above 125 are classified as drying oils; those with iodine value 110–140 are classified as semi drying oils. Those with an iodine value less than 110 are considered as nondrying oil (Knothe, 2002). The iodine values of our selected species which ranged from 99.8 to 106.5 (g I_2/100 g) indicate all the oils are nondrying oils and at the same level as cotton or mustard oil. Oils that are nondrying oil can be utilized for a longer period of time with less possibility of rancid (Ibeto *et al*., 2012). In our research, the saponification values varied from 188 to 283 (mg KOH/1g of oil). The saponification value is the number of milligrams of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat. The saponification value gives an indication of the

nature of the fatty acid constituent of fat and thus, depends on the average molecular weight of the fatty acid constituent of fat. The greater the molecular weight (the longer the carbon chain), the smaller the number of fatty acids are liberated per gram of fat hydrolyzed and therefore, the smaller the saponification number and vice versa. Saponification value is also used in checking adulteration. The saponification value of oilseeds of *S. europaea* (188mg KOH/1g of oil) compared well to groundnut oil, safflower oil, or sunflower oil and similar to the saponification value in the seeds of *Salicornia bigelovii* (183mg KOH/1g of oil; (Anwar *et al.*, 2002). Yet, it seems there is no consistency of saponification value in all species of *Salicornia*; as Eganathan *et al.*, (2006) reported a higher quantity for this parameter (547.5) in seeds of *Salicornia brachiata.* The saponification values of *H. ammodendron*, *A. griffithii* and *S. yazdiana* were slightly higher, but still comparable with coconut oil (250mg KOH/1g of oil). Seeds with high concentrations of saponins are undesirable for edible purpose, but removing these compounds by washing in running water to make them more acceptable for edible use (Anwar *et al.*, 2002).

Peroxide values obtained from the extracted oils of our selected species ranged from 9-13 (meq/kg). Peroxide value is often used to evaluate the primary lipid oxidation and demonstrate quantity of peroxides formed in fats and oils during oxidation (Gulcan and Bedia, 2007). Increasing peroxide value is considered as an indicator for lipid oxidation. The relatively low level of peroxide value exhibits the presence of antioxidants (such as tocopherols) in the extracted oils. Eganathan *et al.*, (2006) estimated the peroxide value in *S. brachiata* as 46.9 (meq/kg).

The values obtained for refractive index of the present studied species ranged from 1.4750-1.4761 and were in agreement with the values reported for other seed oils such as soybean oil and corn oil (Sodeke, 2005). The refractive index of liquid oil or fatty acids depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation. The refractive index can be used as a quality control technique for finding the adulteration of oils. Triacylglycerols have higher refractive indices than do their constituent free acids. Values of refractive index for different oils generally vary between 1.447 and 1.482 (Shahidi, 2005).

Our results confirmed that the quality of seed oils of halophytes (*Salicornia europaea, Haloxylon ammodendron, Salsola yazdiana*, and *Atriplex griffithii*) was comparable with conventional edible oils and these halophytes are potentially valuable oilseed crops, which could be grown in saline deserts and/or irrigated by seawater.

Overall, amongst these species, *Salicornia europaea* containing 73.8% unsaturated fatty acids but not erucic acid was recognized as the best candidate to be used as a source of edible oil in saline conditions followed by *Haloxylon ammodendron*, which exhibited a higher level of oil percentage but less erucic acid compared to those in *Salsola yazdiana*.

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