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Fatty acids composition in seeds of the South American glasswort *Sarcocornia ambigua*

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ABSTRACT

Sarcocornia ambigua (Michx.) M.A. Alonso & M.B. Crespo is the most widely distributed species of the perennial genus of glasswort in South America, and it shows great biotechnological potential as a salt-water irrigated crop. Qualitative and quantitative compositions of fatty acids were determined in the seeds of *S. ambigua* that were cultivated in southern Brazil. Hexane extraction of the seed oil from *S. ambigua* yielded 13% of total lipids. The GC-FID (Gas Chromatography Flame Ionization Detector) analysis of the hexane extracts showed five prominent peaks for the seed oil: 42.9 wt.% linoleic- ω 6 acid (18:2), 20.4 wt.% palmitic acid (16:0), 18.5 wt.% oleic acid (18:1), 4.5 wt.% stearic acid (18:0) and 4.0 wt.% linolenic- ω 3 acid (18:3). The sum of the saturated palmitic and stearic acids (24.8%) in *S. ambigua* seed oil exceeded values cited for commercial oils use, as well as the seed oil from the cultivated annual glasswort *Salicornia bigelovii*. No undesirable fatty acid components were found in *S. ambigua* seed oil, and it could be recommended for animal consumption or biofuel production.

Key words: *Sarcocornia*, seed, perennial crop, saline water, biodiesel, palmitic acid.

INTRODUCTION

Glassworts or marsh samphires are small succulent shrubs native to coastal marshes, mangroves and salt deserts, as well as a potential new seed-oil crop for direct irrigation with salt water (Glenn et al. 1991, Costa 2006, Zeraï et al. 2010). Glassworts are members of three closely related genera (*Salicornia*, *Sarcocornia* and *Arctonemum*) of the subfamily Salicornioideae (family Chenopodiaceae) and share similar anatomo-morphological and physiological traits

to survive in environments with a deficit of water and soils with a high salt content (Shepherd et al. 2005, Davy et al. 2006, Alonso and Crespo 2008). The most successfully cultivated glasswort is the annual *Salicornia bigelovii*. Seeds of *S. bigelovii* may contain 26-33% of fatty acids (El-Mallah et al. 1994, Glenn et al. 1998, Anwar et al. 2002), exceeding levels of traditional oil-seeds such as cotton (15-24%) and soybean (17-21%) in the U.S., Brazil and Asia (El-Mallah et al. 1994, Anwar et al. 2002). The average annual seed production of this species (*S. bigelovii*) varies from 2.0 (Glenn et al. 1998) to 3.7 tonnes/hectare/year (Clark 1994). The cultivation of

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S. bigelovii has been successful in the U.S., Mexico, Saudi Arabia, the United Arab Emirates, Egypt, Eritrea and Pakistan (Clark 1994, El-Mallah et al. 1994, Anwar et al. 2002, Zerai et al. 2010). Several researchers have emphasized the importance of halophyte oil as a potential source of polyunsaturated fat and biodiesel supply for growing global demands (Glenn et al. 1998, Ruana et al. 2008).

Little is known about the seed-oil-production potential of perennial glassworts. *Sarcocornia* species have seeds with high fatty acid content (Shepherd et al. 2005) and the ability to sprout from shoots after cutting their terminal reproductive spikes (Costa 2006, Davy et al. 2006). In comparison to intensive annual seed crops, perennial crops do not need annual replanting from seeds, have a potentially shorter growing season, require fewer farm subsidies and generate less environmental degradation (Glover et al. 2007). *Sarcocornia ambigua* (Michx.) M.A. Alonso & M.B. Crespo is the most widely distributed species of this genus in South America, occurring from the coast of Venezuela to the mouth of the Plata River in Argentina (Isacch et al. 2006, Alonso and Crespo 2008). An experimental crop of *S. ambigua* in north-eastern Brazil, irrigated with saline effluent from shrimp farms, yielded an average of 8.9 fresh weight tonnes/hectare after three months of cultivation (Costa 2006, 2011).

Seeds of *S. ambigua* are elipsoid in shape and small (0.8 X 1.4 mm) (Alonso and Crespo 2008). The membranous testa of the seed encloses one well-developed embryo covered with short, slightly curved hairs. The embryo with the hypocotyl and radicle folded back on the cotyledons occupies virtually all of the volume of the seed and there has no perisperm. Thus, energy stores within the embryo. Shepherd et al. (2005) showed positive histochemical stains for lipids in the embryo of *Sarcocornia perennis*, and these lipids occupied the cell cytoplasm throughout most of the embryo. Little is known about the composition of *S. ambigua* seeds. Augusto-Ruiz et al. (2000) reported protein and ash

contents of 12% and 7.4% in the seeds of *S. ambigua*, respectively. Saponins, iodine and tocopherols were also found in its seed content (EPAGRI 2008).

S. ambigua has a great biotechnological potential as a salt-water irrigated crop. However, insufficient information is available regarding the oil quality and content in this species. The aim of this work was to determine both qualitative and quantitative compositions of fatty acids in the seeds of *S. ambigua* cultivated in southern Brazil.

MATERIALS AND METHODS

PLANT MATERIAL

In order to determine the composition of the fatty acids and the total lipid content of *S. ambigua*, mature seeds were collected from trial fields at Cassino Beach, which is located in the coastal area of Rio Grande (RS) in southern Brazil. During the growth period, plant plot was irrigated with a saline effluent from a *Litopennaeus vannamei* shrimp tank, and average values (\pm standard deviation) of soil surface (0-5 cm deep) electrical conductivity and moisture content were 5.7 ± 2.9 dS m⁻¹ and $7.8 \pm 3.2\%$, respectively. Prior to extractions, seed samples were kept in the dark at -5 °C for 24 h. The seeds were manually separated, cleaned and washed to remove adhering residues. The seeds were then dried at 50 °C for 24 hours and subsequently weighed and ground. Seed mass averaged 0.10 ± 0.04 mg ($n = 100$). Commercial solvents (Synth, Brazil and Merck, Germany) were used for the extractions. Fatty acid profiles were analyzed in a Shimadzu GC-2010 equipped with a split/splitless injector and Flame Ionization Detector (FID).

OIL EXTRACTION

Triplicate samples of dry seeds (1 g) and hexane (6 mL) were first placed in 15 mL round-bottom flasks equipped with a condenser, then stirred (700 rpm) at 50 °C for 24 hours and subsequently filtered. The organic phase was carefully collected

and the solvent evaporated under reduced pressure. The lipid fraction was dried to constant weight in an oven at 60 °C.

FATTY ACID PROFILES

The derivatization of the lipid fraction of *S. ambigua* was carried out according to Metcalfe et al. (1966). A lipid fraction sample (300 mg) was placed in a test tube, a 3 mL mixture of boron trifluoride-methanol was added and then the test tube and its contents were heated in a water bath at 70 °C for 20 minutes. To recover the fatty acid methyl esters, the derivatized mixture was washed into a separatory funnel with 15 mL of hexane and 20 mL of distilled water. The organic and aqueous phases were then separated. The organic phase containing the fatty esters was dried and the solvent was evaporated at 50 °C. The fatty acid profiles were then determined by GC-FID as follows: Injector: SPL, 250 °C, split injection 1:50, linear velocity mode, flow rate of 1 mL/min, injection volume of 2 µL; Column: RTX-wax (30 m length x 0.25 mm i.d. x 0.25 µm film thickness), oven temperature of 200 °C, with total analysis time of 30 min. and a detector temperature of 260 °C (D'Oca et al. 2011). The identification of fatty acids was performed through a comparison with the retention time of standards. Individual patterns of saturated fatty acids were used from C6:0 to C24:0 in the form of methyl esters. The standard mixtures of methyl esters of PUFA N°. 1 and N°. 3 (Supelco) were also used. The fatty acids quantification was carried out by area normalization.

RESULTS AND DISCUSSION

Hexane extraction of the seed oil from *S. ambigua* yielded 13% (+/- 1.6%) of total lipids, which is less than yields reported for seeds of *S. bigelovii* (Mota-Urbina 1990, Glenn et al. 1991, El-Mallah et al. 1994, Anwar et al. 2002), *Salicornia europaea* (Austenfeld 1986) and *Salicornia brachiata* (Eganathan et al. 2005), containing 18-30% of total lipids.

The GC-FID analysis of hexane extracts showed five prominent peaks for the seed oil of *S. ambigua* (**Fig. 1**). The oil was composed of 42.9 wt.% linoleic- ω 6 acid (18:2), 20.4 wt.% palmitic acid (16:0), 18.5 wt.% oleic acid (18:1), 4.5 wt.% stearic acid (18:0) and 4.0 wt.% linolenic- ω 3 acid (18:3) (**Table I**). The oil was rich in polyunsaturated fatty acids, particularly linoleic acid, which has medical significance and, more specifically, the oil contained a small amount of C18:3 linolenic- ω 3, which may result in better oil stability than commercial oils (El-Mallah et al. 1994). For instance, soyabean oil contains up to 6.8% of linolenic- ω 3, and it is less stable due to fast oxidation when compared to *S. bigelovii* seed oil, which has only 1.4% of this fatty acid (El-Mallah et al. 1994). The sum of saturated palmitic and stearic acids (24.8%) in *S. ambigua* seed oil represents a major quantitative difference in comparison to commercial oils, such as canola and olive oil with low saturated acids, and it exceeds values cited for *S. bigelovii* seed oil (7.0-12.4%) (Table I). Intermediate values of saturated acids were found in seeds from *Salicornia brachiata* (16.5%) (Eganathan et al. 2005) and the chenopod *Suaeda fruticosa* (17.0%) (Weber et al. 2007). Similar high concentrations of palmitic acid (21.8-29.4%) were found in seeds of salt flat and coastal dune halophytes (*Arthrocnemum macrostachyum*, *Haloxylon stocksii*, *Alhagi maurorum*, *Cressa cretica* and *Halopyrum mucronatum*) from Asia (Weber et al. 2007), as well as in aerial shoots of *Salicornia europaea* plants (21.6%) (Guil et al. 1996) of which it is a major component of glycerolipids (Imai et al. 2004). Hexane leaf extract of the salt tolerant spurge *Excoecaria agallocha* revealed an elevated amount of palmitic acid (56.0% of total fatty acids), which inhibits yeast growth (*Candida* spp) and possesses antibacterial activity against Gram-positive bacteria (Agoramoorthy et al. 2007). Additionally, as for *Salicornia europaea* (Guil et al. 1996), the toxic erudic acid (C22:1; included in PUFA N°. 1 standard injected in GC-FID) was not found in *S. ambigua* seed oil.

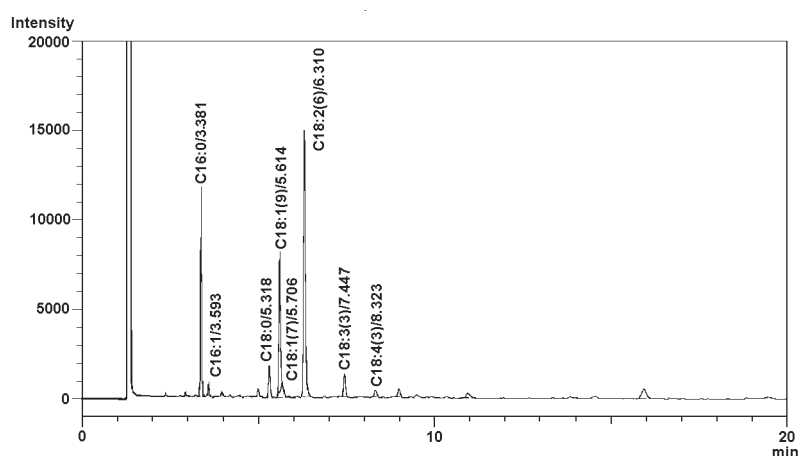


Figure 1. Gas chromatography (GC) of seed oil from South American perennial glassworts (*Sarcocornia ambigua*).

TABLE I

Fatty acid composition (percent) of seed oil from South American perennial glasswort (*Sarcocornia ambigua*) and commercial annual glasswort (*Salicornia bigelovii*) (RSD<5%).

| Fatty Acids | | Amount (%) | |
|-------------|-------------------------|-------------------|---|
| | | <i>S. ambigua</i> | <i>S. bigelovii</i> ^(1, 2, 3, 4) |
| C16:0 | Palmitic acid | 20.4 | 7.0-9.0 |
| C16:1 | Palmitoleic acid | 1.4 | - |
| C18:0 | Stearic acid | 4.5 | 1.2-3.4 |
| C18:1(9) | Oleic acid | 18.5 | 12.3-16.8 |
| C18:1(7) | 7-cis-octadecenoic acid | 0.6 | 1.0 |
| C18:2 | Linoleic acid (ω6) | 42.0 | 66.9-79.5 |
| C18:3 | Linolenic acid (ω3) | 4.0 | 1.4-2.4 |
| C18:4 | Stearidonic acid | 1.5 | - |
| Total | | 92.9 ^a | - |
| Saturated | | 24.9 | 8.2-13.8 |
| Unsaturated | | 68.0 | 86.2-91.0 |

^a Minor components, not listed, were detected to give 100%.

Reference sources: (1) Mota-Urbina (1990); (2) Glenn et al. (1991); (3) El-Mallah et al. (1994); (4) Anwar et al. (2002).

S. ambigua can be grown in a salt-water irrigated environment, and its seed oil showed a composition similar to that of commercial oils. Apart from some quantitative differences regarding individual fatty acids, particularly saturated acids, no undesirable fatty acid component was found in this halophyte seed oil, and it could be recommended for animal consumption or biofuel production.

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RESUMO

Sarcocornia ambigua (Michx.) M.A. Alonso & M.B. Crespo é a espécie mais amplamente distribuída na América do Sul deste gênero de erva-sal com hábito perene e apresenta um grande potencial biotecnológico como uma cultivar capaz de ser irrigada com água salgada. As composições qualitativas e quantitativas dos ácidos graxos foram determinadas nas sementes de *S. ambigua* cultivada no extremo sul do Brasil. A extração de óleo das sementes com hexano obteve um rendimento de lipídeos totais de 13%. A análise em um sistema cromatográfico GC-DIC (cromatografia em fase gasosa com detecção por ionização de chama) dos extratos de hexano do óleo das sementes mostrou cinco picos proeminentes: 42,9% ácido linoleico- $\omega 6$ (18:2), 20,4% ácido palmítico (16:0), 18,5% ácido oleico (18:1), 4,5% ácido esteárico (18:0) e 4,0% ácido linolênico- $\omega 3$ (18:3). O somatório dos ácidos graxos saturados ácido palmítico e ácido esteárico (24,8%) no óleo de sementes de *S. ambigua* excedeu valores citados para óleos de uso comercial, bem como do óleo da semente da erva-sal de hábito anual *Salicornia bigelovii*, cultivada comercialmente. Não foram encontrados ácidos graxos indesejáveis no óleo da semente de *S. ambigua* e este poderia ser recomendado para o consumo animal ou produção de biodiesel.

Palavras-chave: *Sarcocornia*, semente, cultivar perene, água salgada, biodiesel, ácido palmítico.

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