Anais da Academia Brasileira de Ciências (2012) 84(3): 865-870
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
www.scielo.br/aabc

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

MARCELO G.M. D'OCA[1], JOAQUÍN A. MORÓN-VILLARREYES[1], JULIANA S. LEMÕES[1] and CÉSAR S.B. COSTA[2]

[1]Escola de Química e Alimentos, Laboratório Kolbe de Síntese Orgânica,
Universidade Federal do Rio Grande (FURG), Av. Itália, km 08, 96203-900 Rio Grande, RS, Brasil
[2]Instituto de Oceanografia, Laboratório de Biotecnologia de Halófitas,
Universidade Federal do Rio Grande, Av. Itália, km 08, 96203-900 Rio Grande, RS, Brasil

Manuscript received on May 31, 2011; accepted for publication on October 17, 2011

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-ω6 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-ω3 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, Zerai et al. 2010). Glassworts are members of three closely related genera (*Salicornia*, *Sarcocornia* and *Arcthronemum*) of the subfamily Salicornioideae (family Chenopodiaceae) and share similar anato-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

Correspondence to: César S.B. Costa
E-mail: docosta@furg.br

S. bigelovii has been successful in the U.S., Mexico, Saudi Arabia, the United Arabic 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 tocopheros 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 (± 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 ± 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 Nº. 1 and Nº. 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-ω6 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-ω3 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-ω3, 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-ω3, 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 Nº. 1 standard injected in GC-FID) was not found in *S. ambigua* seed oil.

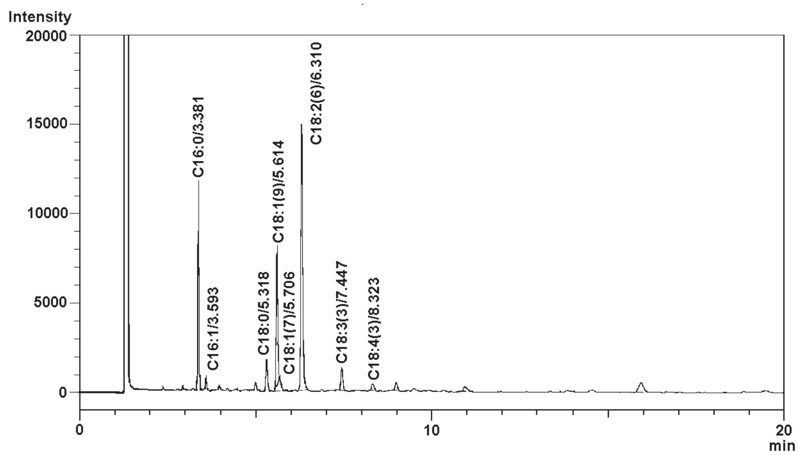

Figure 1. Gas chromatography (GC) of seed oil from South American perennial glassworts (*Sarcocornia ambigua*).

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 (%)	
		S. ambigua	*S. bigelovii* (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		92.9a	-
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.

ACKNOWLEDGMENTS

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) under grant n. 574600/2008-6.

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-ω6 (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-ω3 (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.

REFERENCES

Agoramoorthy G, Chandrasekaran M, Venkatesalu V and Hsu MJ. 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz J Microbiol 38: 739-742.

Alonso MA and Crespo MB. 2008. Taxonomic and nomenclatural notes on South American taxa of *Sarcocornia* A. J. Scott (Chenopodiaceae). Ann Bot Fennici 45: 241-254.

Anwar F, Bhanger MI, Nasir MKA and Ismail S. 2002. Analytical characterization of *Salicornia bigelovii* seed oil cultivated in Pakistan. J Agric Food Chem 50: 4210-4214.

Augusto-Ruiz W, Lepsen L and Cárdenas VOC. 2000. Caracterização da fração mineral das sementes e talos da *Salicornia gaudichaudiana* Moq. In: Resumos do Congresso Regional de Iniciação Científica e Tecnológica em Engenharia - XV CRICTE, Rio Grande (RS): FURG, 27 p.

Austenfeld FA. 1986. Nutrient reserves of *Salicornia europaea* seeds. Physiol Plant 68: 446-450.

Clark A. 1994. Samphire: from sea to shining seed. Saudi Aramco World 45(6): 2-9.

Costa CSB. 2006. A *Salicornia*: uma planta que pode ser utilizada no cultivo integrado com o camarão. Panor Aquic (Braz) 98: 28-33.

Costa CSB. 2011. Restoration of coastal habitats in Brazil using native salt marsh plants. In: Greipsson S (Ed), Restoration Ecology, Sudbury (MA. U.S.A.): Jones and Bartlett Publishers, p. 333-338.

Davy AJ, Bishop GF, Mossman H, Redondo-Gómez S, Castillo JM, Castellanos EM, Luque T and Figueroa ME. 2006. Biological Flora of the British Isles, n. 244. *Sarcocornia perennis* (Miller) A. J. Scott. J Ecol 94: 1035-1048.

D'Oca MGM, Viêgas CV, Lemões JS, Miyasaki EK, Morón-Villarreyes JA, Primel EG and Abreu PC. 2011. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. Biomass Bioenergy 35: 1533-1538.

Eganathan P, Subramanian HMS, Latha R and Rao CS. 2005. Oil analysis in seeds of *Salicornia brachiata*. Ind Crops Prod 23: 177-179.

El-Mallah MH, Turui T and El-Shami S. 1994. Detailed studies on seed oil of *Salicornia* SOS-7 cultivated at the egyptian border of Red Sea. Grasas Aceites 45(6): 385-389.

Epagri. 2008. Panicêutivo-Você sabe o que significa? Agropecuária Catarinense 21(2): 13.

Glenn EP, Brown JJ and O'Leary JW. 1998. Irrigating crops with seawater. Sci Am 279(2): 76-81.

Glenn EP, O'Leary JW, Watson MC, Thomas TL and Kuehl RO. 1991. *Salicornia bigelovii* Torr.: an oilseed halophyte for seawater irrigation. Science 251: 1065-1067.

Glover JD, Cox CM and Reganold JP. 2007. Future farming: A return to roots? Sci Am 297: 82-89.

Guil JL, Torija ME, Gimenez JJ and Rodriguez I. 1996. Identification of fatty acids in edible wild plants by gas chromatography. J Chromatogr A 719: 229-235.

IMAI H, KINOSHITA M AND OHNISHI M. 2004. Chemical characterization of glycerolipds and cerebrosides in halophytic plant, *Salicornia europaea* L. J Oleo Sci 53(7): 337-341.

ISACCH JP, COSTA CSB, RODRÍGUEZ-GALLEGO L, CONDE D, ESCAPA M, GAGLIARDINI DA AND IRIBARNE OO. 2006. Association between distribution pattern of plant communities and environmental factors in SW Atlantic saltmarshes. J Biogeogr 33(5): 888-900.

METCALFE LD, SCHMITZ AA AND PELKA JR. 1966. Rapid preparation of fatty acid esters from lipids for gas liquid chromatography. Anal Chem 38(3): 514-515.

MOTA-URBINA C. 1990. El cultivo de la *Salicornia*, una nueva oleaginosa que se riega con agua del mar. Claridades Agropecuarias 3: 1-3.

RUANA CJ, LIA H, GUOB YQ, QINB P, GALLAGHERC JL, SELISKARC DM, LUTTSD S AND MAHYE G. 2008. *Kosteletzkya virginica*, an agroecoengineering halophytic species for alternative agricultural production in China's east coast: Ecological adaptation and benefits, seed yield, oil content, fatty acid and biodiesel properties. Ecol Eng 32(4): 320-328.

SHEPHERD KA, MACFARLANE TD AND COLMER TD. 2005. Morphology, anatomy and histochemistry of Salicornioideae (Chenopodiaceae) fruits and seeds. Ann Bot (Lond) 95: 917-933.

WEBER DJ, ANSARIB R, GULB B AND AJMAL KHANB M. 2007. Potential of halophytes as source of edible oil. J Arid Environ 68: 315-321.

ZERAI DB, GLENN EP, CHATERVEDI R, LU Z, MAMOOD AN, NELSON SG AND RAY DT. 2010. Potential for improvement of *Salicornia bigelovii* through selective breeding. Ecol Eng 36: 730-739.