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Bioactive compounds and antioxidant activity of three biotypes of the sea asparagus *Sarcocornia ambigua* (Michx.) M.A.Alonso & M.B.Crespo: a halophytic crop for cultivation with shrimp farm effluent

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ABSTRACT

Commercially available sea asparagus of the genera *Salicornia* and *Sarcocornia* have shoots rich in minerals and bioactive compounds. However no selection of varieties with desirable nutritional shoot properties are available. In the present study, three biotypes of the sea asparagus *Sarcocornia ambigua* (Michx.) M.A.Alonso & M.B.Crespo cultivated with saline effluent from a shrimp tank were evaluated for total and individual phenolic compounds, L-ascorbic acid, chlorophyll content and antioxidant capacity (ABTS assay) of fresh vegetative and reproductive (with seeds) segments of their shoots. Total phenolic content of biotypes ranged, on average, from 745 to 1586 mg GAE 100 g^{-1} fw. The flavonoids kaempferol and quercetin, followed by gallic acid and other hydroxybenzoic acids were the major phenolic compounds found in *S. ambigua*. Highest individual contents of phenolic compounds occurred in the reproductive segments of the red biotype (ranging from 186 to 365 mg 100 g^{-1} fw). The green biotype showed higher average values of Chl *a* and Chl *a* + *b* (up to 223 and 283 μg g^{-1} fw, respectively) than the other biotypes. Biotypes showed similar contents of L-ascorbic acid with higher concentration in vegetative tissues (46 to 65 mg 100 g^{-1} fw). Shoot antioxidant activity ranged between 3.4 and 4.9 mmol TEAC 100 g^{-1} fw, and was positively correlated with the gallic acid content ($r = 0.752$) and total phenolic acids ($r = 0.670$). Within biotype, reproductive tissues were richer in total phenolic content and antioxidant capacity. Different colour biotypes of *S. ambigua* rich in bioactive compounds may be selected with desirable shoot properties for potential for use as additives in the food industry.

1. Introduction

Cultivated halophytes of the genera *Salicornia* and *Sarcocornia* (Amaranthaceae, subfamily Salicornioideae) have succulent young shoots that are sold as "Samphire" or "Sea asparagus". They are commonly irrigated with salt water or shrimp farm saline effluent, and have become an important source of forage, by-products rich in protein and carbohydrates, edible oil, and food supplements for humans (Davy et al., 2001; Glenn et al., 2013; Kim et al., 2014; Ventura et al., 2015; Pinheiro et al., 2017). At present, commercially available *Salicornia* spp. is comprised of a collection of ecotypes of various origins that were selected from breeding programs undertaken with *Salicornia bigelovii* aiming to increase seed weight and oil seed yield (Glenn et al., 2013). Similar breeding programs have been developed for *Sarcocornia* species in the Middle East but to date, no selected varieties or crops of *Salicornia* or *Sarcocornia* with desirable nutritional shoot properties are available (Ventura et al., 2011, 2015). There is potential to select biotypes for desired characteristics. For example, green and red biotypes of *Salicornia herbacea* occur in Korea. Addition of 1% red biotype dry powder minimized colour changes of reduced-salt cooked sausages compared to the green biotype powder which improved emulsion stability and textural properties of the meat product (Kim et al., 2014).

The genetic make-up of the halophytes determine their physiological tolerance to high salinity and their production of bioactive metabolites, mostly associated with antioxidant systems and repair mechanisms (Han and Kim, 2003; Oh et al., 2007; Kong et al., 2008; Kim et al., 2011; Duarte et al., 2013). The main pigments associated with the reddish colour of *Salicornia* and *Sarcocornia* shoots are betacyanins and other phenolic compounds (Davy et al., 2001; Costa et al., 2006; Davy et al., 2006; Duarte et al., 2013). All these compounds have demonstrated anti-stress properties and are associated with the red colour of shoots of stress tolerant plants (Davy et al., 2001; Costa et al., 2006; Kong et al., 2008; Soares et al., 2008; Severo et al., 2011; Duarte et al., 2013; Pinheiro et al., 2017). Different concentrations of these compounds in the various colour shoot biotypes could result in varying antioxidant properties.

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Sarcocornia species native to South America have biotechnological and biomass production potential. *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 (Alonso and Crespo, 2008; Medina et al., 2008; Costa and Herrera, 2016). In Brazil, experimental crops of *S. ambigua* irrigated with saline shrimp farm effluent in the states of Ceará, Rio Grande do Sul and Santa Catarina produced high yields (Costa, 2011; Costa and Herrera, 2016; Pinheiro et al., 2017). Effluents from marine aquaculture contain toxic organic compounds, antibiotics and heavy metals, which may be absorbed by halophytes, and this possibility needs to be investigated and the risk for human and animal health evaluated before selling halophytes for consumption. For example, copper and zinc concentrations in shoots of *S. ambigua* cultivated with shrimp farm effluent were lower than in *Salicornia* species grown in their native salt marshes and commercial vegetables (Doncato and Costa, 2017).

Three biotypes with contrasting shoot colour originating from the estuary of Patos Lagoon (RS, Brazil) were detected during cultivation of *S. ambigua* (Doncato and Costa, 2017). Sympatric plants with dark green shoots (green), green shoots with pink tips towards the branches (pink) and deep purplish-red shoots (red) are easily distinguishable during the fruiting period. Additionally, the red shoot biotype shows more branching, with more downward-hanging branches than the green shoot biotype, which has an upright branching growth form. The pink biotype has an intermediate growth form. The occurrence of green and red shoot biotypes of this species has been previously described in southern Brazil (Freitas and Costa, 2014), as well as in Bahía Tacuatí, Venezuela (Medina et al., 2008). Differences in biometrics among pure lineages of *S. ambigua* biotypes are maintained between consecutive generations and consequently the heritability of these traits (Doncato and Costa, 2017).

Little is known about pigments and secondary metabolites such as phenolic compounds composition of *S. ambigua* shoots. Chlorophyll *a* concentration of *S. ambigua* shoots can be inhibited by irrigation with 50% diluted seawater and high UV-B exposition (Costa et al., 2006). When the major phenolic compounds in methanol extracts of *S. ambigua* shoots collected from two localities of south Brazil were characterised, 15 phenolic compounds, including five flavonoids and eight phenolic acids were identified (Bertin et al., 2014). The total phenolic content in *S. ambigua* shoots was 41 mg 100 g^{-1} fw, when cultivated in a greenhouse under constant flow of seawater (Pinheiro et al., 2017). Both the studies reported moderate antioxidant activity (measured by DPPH method) in methanol extracts of *S. ambigua* with Bertin et al. (2014) reporting higher values for samples collected in their natural habitat. However, different extraction methods were used, the biotype of sampled plants was not described and only vegetative shoots of most common green plants were analysed. Additionally, flavonoid and phenolic acid extraction can be carried out using methanol as well as other polar solvents such as hot water, ethanol, acetone or ethyl acetate, either alone or in combination (Qian et al., 2004; Soares et al., 2008). Information on solubility of bioactive compounds is vital for elucidating the functional chemical composition, as well as to suggest methods for extraction, preservation and consumption of a new crop and/or a health beneficial herb.

The purpose of this study was to evaluate potential differences in the contents of bioactive compounds (phenolic compounds, chlorophylls and L-ascorbic acid) and the antioxidant capacity of mature shoots of different colour biotypes of *S. ambigua*, cultivated with saline effluent from a shrimp farm in southern Brazil.

2. Material and methods

2.1. Plant material

In April 2010, at the Marine Aquaculture Laboratory (EMA-FURG, RS, Brazil; 32°12′19″ S, 52°10′45″ W), branches of six month-old *S. ambigua* plants were harvested at ground level from a 10 m by 20 m plot irrigated with saline effluent from a *Litopennaeus vannamei* shrimp tank, stocked with 200 shrimp m^{-2}. Plants were spaced 40 cm apart and watered by filling drainage ditches once daily with 2000 L of effluent. During the growth period, the average values (± standard error; n = 12) of water electrical conductivity, pH, dissolved oxygen, nitrate, ammonium and phosphate were 46.0 ± 3.6 dS m^{-1}, 8.56 ± 0.8, 6.82 ± 1.3 mg L^{-1}, 2.65 ± 1.3 mg L^{-1}, 0.21 ± 0.1 mg L^{-1} and 1.17 ± 0.2 mg L^{-1}, respectively. In the 2-weeks prior to harvest, about 70 mm of rainfall accumulated, maximum daily temperatures ranged between 20–30 °C and average values of electrical conductivity and moisture content at the soil surface of the plot (0–5 cm deep) were 6.0 ± 0.5 dS m^{-1} and 6.8 ± 0.6%, respectively (n = 30; ± standard error). *S. ambigua* plants have a strongly reduce morphology composed of articulated, succulent green cylindrical segments that at maturity carry inconspicuous flowers. The seeds develop inside reproductive (fertile) segments, and are only available after the senescence of shoots. Green, red and pink biotypes were collected at the fruiting stage. Vegetative and reproductive (with seeds) plant segments harvested from the three biotypes were separated *in situ*, placed in vials, frozen in liquid nitrogen, and kept at −20 °C until analysis.

2.2. Quantification of total phenolic compounds

Total phenolic compounds were determined using the Folin–Ciocalteau phenol reagent. The Folin-Ciocalteu method is one the most utilized for quantification of phenolic compounds (e.g. Arruda et al., 2017). Briefly, 1 g fresh weight tissue (fw) sample was ground in a mortar and pestle, and repeatedly extracted with 10 mL deionized water, 80% acidified aqueous methanol solution (0.01% v/v HCl 37%), 80% aqueous ethanol solution, and a mixture of methanol-ethanol-deionized water (40/40/20, v/v/v), respectively. The flasks were capped and placed on an orbital shaker set at 200 rpm for 1 h at room temperature (20 °C ± 3 °C) in the dark. Extracts were then centrifuged at 14,000 × g for 25 min at 4 °C, and the supernatant volume adjusted to 10 mL with the respective extraction solutions. Then 125 μL of each extract was combined with 500 μL distilled water and 125 μL Folin Ciocalteau phenol reagent (2 N). Although the Folin-Ciocalteu reagent is non-specific for phenolic compounds, this reaction is favored by medium alkaline (Singleton and Rossi, 1965). The solution was allowed to rest for 8 min, then 1.25 mL sodium carbonate (7% m/v) and 1 mL distilled water added, adjusting the final volume to 3 mL Then the mixture was allowed to rest for 90 min at room temperature (23 ± 2 °C) in the dark, and absorbance was measured at 760 nm in a U-2010 Hitachi UV/Vis spectrophotometer (Tokyo, Japan). Total phenolic content was expressed in mg gallic acid equivalents per 100 g fresh weight (mg GAE 100 g^{-1} fw). All spectrophotometric analyses were performed in triplicate.

2.3. Identification of individual phenolic compounds

Gallic acid, ρ-hydroxybenzoic acid, ρ-coumaric acid, ferulic acid, caffeic acid, (+)-catechin, (-)-epicatechin, quercetin and kaempferol were determined according to Hakkinen et al. (1998). One milliliter of the extract was hydrolysed using 35 mL acidified methanol (HCl 37%, 15% v/v). The extract was then kept in a water bath at 35 °C for 24 h in the dark, filtered (Whatman n°1), concentrated (rotary evaporator at 40 °C) and re-suspended in 10 mL methanol. Samples were centrifuged (14,000 × *g* for 25 min), filtered through a 0.22 μm Durapore membrane, and an aliquot of 20 μL was analysed by Shimadzu HPLC chromatograph (Kyoto, Japan) equipped with a quaternary pump, on-line degasser, column heater, and diode array detector. Analytical separation was carried out on a Shim-Pak CLC-ODS column (3.9 cm × 150 mm × 4 μm). Chromatographic analyses were performed using a Shimadzu HPLC chromatograph (Kyoto, Japan) equipped with a quaternary pump, on-line degasser, column heater,