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STUDY OF ANTIOXIDANT ACTIVITY IN EXTRACTS FROM FRESH AND DRIED HALOPHYTE PLANTS

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Abstract

Salicornia macrostachya Moric. and *Sarcocornia perennis* subsp. *alpini* belong to the same family- Chenopodiaceae - and share morphological and organoleptic characteristics. The visually appealing in terms of freshness, color, particular taste, nutritional values and health benefits are recognized by their consumers as attributes that warrant their gourmet status. The objective of this work was to study the effect of incubation time on antioxidant capacity of these plants. Moreover, the work investigates the antioxidant activity of the extracts of the two halophyte plants and evaluates the effect of air drying the plants at 40 ^{0}C and the procedure of concentration the extracts before evaluating the antioxidant activity, measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

The two halophyte plants, *Salicornia macrostachya* Moric., and *Sarcocornia perennis* subsp. *alpini* were collected from Portuguese salt pans, in the central region of Portugal, and the aerial parts were used as raw material. The drying of plants was performed in a pilot tray drier at 40 ^{0}C and air velocity of 1.5 m/s, for approximately 3 days. The DPPH free radical scavenging of the methanolic extracts was analysed in extracts obtained from fresh and dried plants. The determination of the antioxidant activity was made with the DPPH radical.

The results showed that, in summary, and regarding the DPPH assay, the incubation time of 15 minutes is enough to measure the DPPH scavenging activity in halophyte extracts. Furthermore, the convective air-drying process at 40 ^{0}C showed to be an appropriate process to increase the shelf life of the halophyte plants and the antioxidant capacity of the halophyte plants, as a response to the stress provoked by the heat and humidity drying conditions. The plant extracts showed significant antioxidant potential, with high radical scavenging activity. The extracts prepared from *Salicornia macrostachya* and *Sarcocornia perennis* had, respectively, values of IC_{50} equal to 1.09 and 1.42 mg/mL. However, the antioxidant activity of extracts obtained from dried plants increased much more since the IC_{50} decreased to around 0.6 mg/mL, regardless of the halophyte plant.

Both halophyte plants are a valuable source of natural antioxidants and nutrients for use in food. Besides, their crude extracts may represent a valuable source for developing novel food products (antioxidant-enriched foods), and/or table salt substitutes that satisfy the desires of consumers in terms of health benefits and sensorial acceptance.

Key words: *Salicornia, Sarcocornia, Extraction, Drying, Concentration, Antioxidant activity*.

1. Introduction

Salicornia L. (Chenopodiaceae) is a genus of annual, apparently leafless halophytic herb, that has succulent stems and frequently appears in saline areas associated

with coastlines, tidal floodways', and salt lakes [1]. The Salicornia species have been collected since ancient times as food, given their medicinal qualities and high salt contents. Notwithstanding the growing interest over the past decade in cultivating crops under saline conditions, until today those plants have been gathered regularly from wild populations and sold at local farmers' markets. Nowadays it is much appreciated as a gourmet product in Europe though much more pronounced in Asian countries, where it is used in fresh salads and pickles. In the dried and milled form, it is used as a salt substitute. Salicornia genus is also well known for its applications as an additive in the production of glass and soap [2], as medicinal herbs [3], and also in some appliances in human and domestic animals diet [4].

The glasswort *Sarcocornia perennis* is a globally distributed halophyte being found on every continent with the exclusion of Antarctica. This plant is a perennial macrophyte acting as the dominant species on intermediate and high levels of tidal salt marshes in the South West Atlantic. It is considered an "extreme" halophyte that often grows in bare sediments where it forms roughly circular patches [5]. This plant is characterized by succulent, articulated and leafless stems. The erect to decumbent, aerial stems, reaching up to 30 cm and the main branches are prostrated, and rooting often occurs at the stem nodes. The main stem and some of the lateral branches terminate in flowering spikes. The photosynthetic aerial shoots are dull green to shiny green, later turning yellowish to red or orange brown [6]. Other morphological characteristics of this genus include their succulent stem, opposite vestigial and long connate leaves clothing the internode, and flowers hidden in cavities in the inflorescence axis [7].

Sarcocornia perennis is a halophyte that differs from the closely related Salicornia by presenting a distinct perennial growth rhythm and differs also in the flower arrangement, although having flowers of more or less equal height in the cymes [8]. Recent molecular analysis of halophytes from the Iberian Peninsula region (south-west Spain) has allowed identifying subspecies of *Sarcocornia perennis*: ssp. *perennis*, ssp. *alpini* (Lag.), *Sarcocornia fruticosa*, the hybrid between the latter two, and *Arthrocnemum macrostachyum* [6].

Halophytes biosynthesize different primary and secondary metabolites, such as vitamins, terpenoids and phenolics [9].

Some studies have proposed that some species of Salicornia, like *S. herbacea* extracts, are rich in bioactive substances, such as phytosterols, polysaccharides and phenolic compounds, mainly flavonoids and phenolic acids [10, 11]. Owing to their composition, these halophyte plants show important biological properties, such as antioxidant, anti-inflammatory, hypoglycaemic and cytotoxic activities [3, 9 - 12].

Despite the wide distribution of *Sarcocornia*, this has been scarcely studied and the works found were especially focused on biomass, nutrient cycling or decomposition. However, in *Sarcocornia ambigua* were identified high levels of sodium, followed by potassium, magnesium and calcium, as well as phenolic compounds, including coumarin, phenolic aldehyde, phenolic acids and flavonoids [13, 14]. Important total polyphenol, β-carotene, ureides and high total shoot lipid contents, which included an omega-3 fatty acid, were also found in *Sarcocornia fructicosa* ecotypes [8]. The findings showed that *Sarcocornia* leafy vegetables may attract additional interest as an alternative source of polyunsaturated fatty acids and cytoprotective and antioxidant effects on the human diet.

The crops *Salicornia* and *Sarcocornia*, with their extreme salt tolerance and long history of plant's traditional uses, will contribute to the most promising strategies for sustainable agriculture in marginal environments and have been recognized as one of the most promising crops that could be potentially brought in to human or animal food productions [15].

The objective of this work was to study the antioxidant activity of two halophyte plants found in the Portuguese salt pans (*Salicornia macrostachya Moric.* and *Sarcocornia perennis* subspecies (ssp.) *alpini* (Lag.) Castrov.) and evaluate how this varied by drying the plants as opposed to the fresh state and also by varying the extracts (analysed directly after being obtained or submitted to further processing).

2. Materials and Methods

2.1 Samples

Two halophyte plants, *Salicornia macrostachya* Moric. and *Sarcocornia perennis* subsp. *alpini* (Lag.) Castroviejo were analysed in the present study.

The halophyte plants were collected, on April 2015, from salt pans of Figueira da Foz, in the central region of Portugal on the coast to the Atlantic Ocean (40° 6'42.5627"N8°49'59.7034"W). The moisture contents of the halophyte plants were 92.3% (w/w) and 84.24% (w/w), respectively, for *Salicornia* and *Sarcocornia*. The initial moisture content of both plants was evaluated by weight loss in a drying chamber set to a constant temperature of 105 ^{0}C and at atmospheric pressure.

The aerial parts of the raw plant material were stored at a temperature of 5 ^{0}C until used.

2.2 Drying

The drying of aerial parts of the halophyte plants was performed in a pilot tray dried at 40 ^{0}C and air velocity of 1.5 m/s, during approximately 3 days.

2.3 Extraction

Before the extraction, the plants (fresh and dried) were milled and 50 g (in a dry basis) of sample were used as extraction sample. The dried grounded samples were stored in a sealed container at room temperature.

The extraction from fresh and dried samples was performed in three successive steps, with methanol solutions, with a ratio of plant/solvent of 1 (dry basis) : 10 (w/w). Each extraction step was performed during 2 days at room temperature and with continuous stirring. The obtained extracts were combined, filtered through a Whatman (Grade 4) filter paper and half of each extract was concentrated under reduced pressure (R-200 rotary evaporator, Buchi, Switzerland), thus allowing to obtain samples called direct extract and processed extract. While the fresh sample was only evaluated in the direct extract for the dried samples both direct and processed extracts were analysed. The methanolic extract concentration (mass/volume of extract) was determined measuring the mass of a known volume of extract after drying at 65 ± 1 ^{0}C until constant weight. Different extract concentrations (ranging from 12.5 g/mL to 5000 µg/mL) were obtained through the initial methanolic extract. Similar concentration samples were also prepared by dilution of concentrate residue.

Different extracts were storage at 5 ^{0}C, within a bottle involved by aluminium foil.

2.4 Evaluation of antioxidant activity

The antiradical activity of the plant extracts was determined using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Dias *et al*., [16], with small modifications. Briefly, 100 µL of methanolic extract solution at different concentrations was mixed to 100 µL of DDPH methanolic solution (the solution should afford an Abs_{515nm} value in the 0.9 - 1 interval) in a well of a 96-well micro-plates.

Different solutions were kept in the dark at room temperature for different incubation time (15 - 60 minutes). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 515 nm in a µQuant MQX200 microplate reader (Biotek, USA) and converted to the $DPPH^{\cdot}$ scavenging activity percentage. A solution of 100 µL of methanol and 100 µL of methanol solution of $DPPH^{\cdot}$ was used as control. Three independent experiments were performed. The percentage of DPPH inhibition was determined for each extract using Equation (1):

$$\text{Inhibition } (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

The DPPH scavenging activity was expressed as IC_{50} values (mg/mL), calculated using a nonlinear regression analysis. Three independent experiments were performed.

3. Results and Discussion

3.1 Estimation of incubation time for DPPH method

Assessments of antioxidant properties of natural compounds are of crucial importance due to their uses in medicine, cosmetics and food. These natural antioxidants are known to minimize the adverse effects of free radicals in the living system and many of them were isolated, characterized and available for various applications. Many of these natural antioxidants are actively considered as prophylactic and therapeutic agents for possible applications for radiation countermeasures combating cancers and age related diseases [17].

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is routinely utilized for the assessment of the antiradical properties of different compounds and considered as one of the standard and easy colorimetric methods for the evaluation of antioxidant properties of pure compounds [18]. However, the use of different materials, solvents (such as ethanol, aqueous acetone, aqueous alcohol and benzene) and time reaction resulted in a variation in the values of reference standards and measured parameters of new antioxidants. Also, the reversibility of reaction between DPPH and antioxidant, reduction of the radical and the different kinetics of the antioxidants may lead to underestimated reading of antioxidant capacity of many antioxidants [18].

Usually, the DPPH radical scavenging by H atom donating antioxidants in literature has been reported by using a fixed reaction time that usually ranged between 20 - 30 minutes, independently of the antioxidant concentration. However, in this range and depending on the antioxidant concentration is not assured that the steady state saturation, i.e. maximum decrease in DPPH radical, was attained. Thus, before analyzing the antioxidant capacity of the plant extracts it is important to assess the reaction time.

In all the performed experiments the solvent used was methanol, since it is mentioned as the most suitable for the DPPH assay [19]. Figure 1 presents the scavenging percentage determined at different reaction times (15, 30 and 60 minutes) and concentration of dried

Sarcocornia (similar studies were done to *Salicornia* extracts and are available upon request). Regarding the results, is expectable a fast kinetic of reaction between the DPPH and the antioxidants present in *Sarcocornia* extracts. From the results obtained, is possible to conclude that in the range of 15 to 60 minutes the scavenging percentage remains constant for each extract concentration. The results further show that the time of 15 minutes was enough to obtain a steady state in absorbance of DPPH radical in the tested range of extract concentrations. However, the time defined to measure the DPPH scavenging activity was fixed at 30 minutes, since this was the common time in different laboratorial protocols.

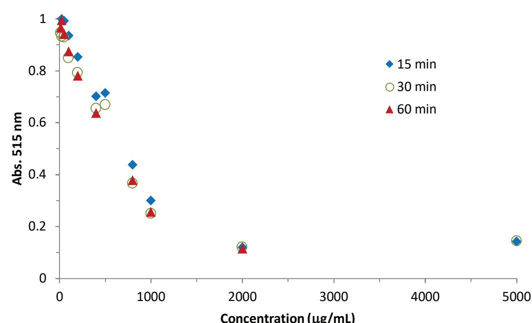

Figure 1. Scavenging of DPPH radical, measured at different times of incubation, for various concentrations of dried *Sarcocornia perennis* extracts

3.2 Effect of drying

Drying is an efficient way of preserving food, owing to the low moisture and water activity, a_w of the dried products. However, drying, and particularly hot air drying, implies an exposure to high temperature for some time, and this may affect the products at the physical or chemical levels. Specifically, polyphenols, which are sensitive to temperature, may be affected by heat treatment, leading to some changes on their content and antioxidant capacity [20]. Thus, to study the effect of drying process at 40 ^{0}C on the antioxidant capacity of both plants the direct extracts of fresh plants and dried plants were analyzed by the DPPH assay.

Figure 2 shows the antioxidant capacity of methanolic extracts of fresh and dried plants of *Salicornia macrostachya* and *Sarcocornia perennis*. For both plants, the results show that in case of smaller concentrations the scavenger of DPPH radical increases almost linearly and reaches a plateau near 2 mg/mL, which corresponds to 90% of free radical scavenging.
Furthermore, from de results of the inhibition of

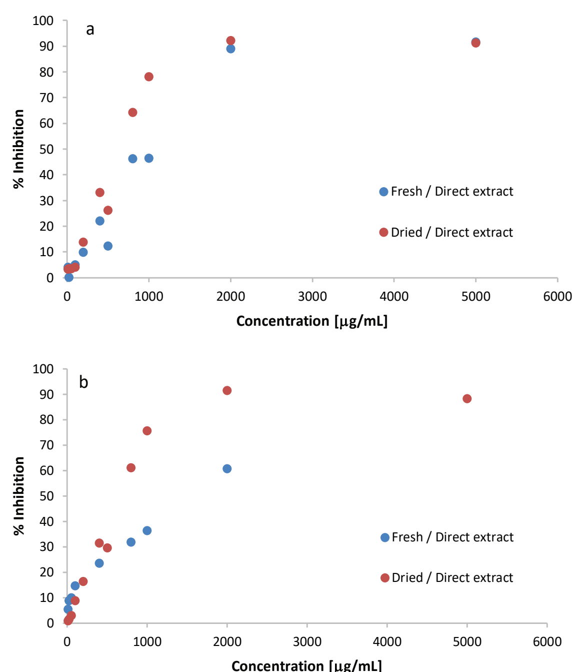

Figure 2. DPPH free radical scavenging activity of the extract of (a) *Salicornia macrostachya* and (b) *Sarcocornia perennis* plants measured in direct extract of fresh and dried plants for different concentration of extract

DPPH radicals it is possible to conclude that drying temperature at 40 ^{0}C allows an increase on the percentage of inhibition of DPPH of *Salicornia macrostachya* and *Sarcocornia perennis* extracts. In fact, some recent studies have shown that dried plant materials (including air-drying) contain the same or even higher antioxidants and antioxidant activity when compared to their fresh state [21, 22]. Besides, the dried plants would present higher values of antioxidant capacity since the drying process might make the tissue more brittle, which in turn results in rapid cell wall breakdown during the milling that could release more bioactive compounds into the solvent during extraction step. Thus, the use of these halophytes' plants in dry state instead fresh state can be a good alternative to develop enriched products.

3.3 Effect of extracts evaporation under vacuum

Part of the direct extract obtained from the dried plants was evaporated under vacuum. The residual extracts were resuspended in methanol to a final concentration of 5,000 µg/mL. Different concentrations of extract were prepared from stock solution, trough dilutions.

Figure 3 presents the percentage of inhibition of *Salicornia* and *Sarcocornia* plant extracts prepared from resuspension of the residues obtained through

evaporation under vacuum (named processed extracts).

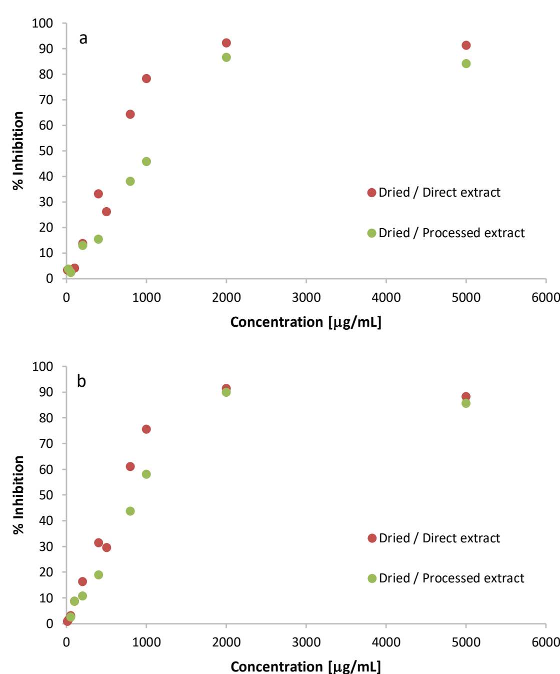

Figure 3. DPPH free radical scavenging activity of dried (a) *Salicornia macrostachya* and (b) *Sarcocornia perennis* plants measured in the direct extracts and in the extracts prepared from the residue of vacuum evaporation

The results in Figure 3 show a similar trend for both plants, in a way that for higher concentrations there were no differences in the antioxidant activity measured in the direct or processed extracts. However, for lower concentrations of the extract, i.e., for more diluted extracts the antioxidant activity was higher if the sample analysed was the direct extract without further processing. This indicates that the effect of the suspension procedure used may have a nefarious effect in case of the less concentrated extracts, i.e., with lower amounts of compounds with antioxidant capacity. This effect has not been yet reported in literature.

3.4 DPPH radical scavenging (IC_{50}) of extracts

As shown in Figure 4, the extracts of both halophyte plants showed a high radical scavenging activity. This was defined by IC_{50} that refers to the concentration of extract necessary to decrease the initial concentration of DPPH free radicals by 50%. The values of IC_{50} for extracts obtained from fresh plants and analysed directly on the extract of Salicornia and Sarcocornia are, respectively, 1.09 mg/mL and 1.42 mg/mL. These plants live in environments with high UV radiation and salinities and these stressful conditions often lead to the production of radical oxygen species. Therefore, the plants response to oxidative stress by producing antioxidant compounds [23]. However, the results in Figure 4 also show that the antioxidant activity increased sharply on the extracts obtained from the dried plants, since the IC_{50} decreased to around 0.6 mg/mL, regardless of the halophyte plant. The drying of food products, when at mild temperatures, has been proven to increase the amount of antioxidant compounds, as a response to the stress provoked by the heat and humidity conditions used in the drying process. Additionally, drying may lead to a higher permeabilization of cell walls in the material, thus allowing to extract more compounds as compared with the fresh sample [24]. This is true if the drying temperature is not too high, in which case the phenolic compounds with antioxidant activity start to degrade [25].

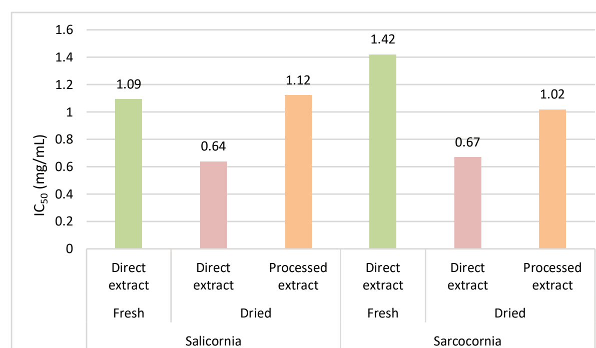

Figure 4. DPPH free radical scavenging activity (IC_{50}) of *Salicornia macrostachya* and *Sarcocornia perennis* plants

Ethanolic extracts of the dried *S. perennis* subsp. *alpini* (dried in an oven 40 ^{0}C for 4 days), collected in Castro Marim (south of Portugal) presented an $IC_{50\text{-}DPPH}$ of 11.5 mg/mL [26], revealing much lower antioxidant activity than the same halopythe plant collected in Figueira da Foz (centre of Portugal). Also, the extracts of dried *Salicornia macrostachya collected in* Figueira da Foz have a much higher antioxidant activity (IC_{50} = 0.64 mg/mL) as compared with the *Salicornia ramosissima* (IC_{50} = 5.69 mg/mL) collected in Castro Marim [26].

However, even for the same halophyte species, the nutritional value and bioactive compounds of Salicornia and Sarcocornia are very much influenced by geographical locations and soil conditions, such as salinity [8]. Indeed, the methanolic extract obtained from freeze dried *Sarcocornia perennis* of Korba (Tunisia), has a value of IC_{50} of 0.403 mg/mL [27].

4. Conclusions

- This work allowed concluding that for the determination of the antioxidant activity of the halophyte plants studied using the DPPH method, and extraction time of time of 15 minutes is enough to obtain a steady state in absorbance of DPPH radical

in the extracts up to extract concentration of 5,000 µg/mL.

- Furthermore, the convective air-drying process at 40 ^{0}C showed to be an appropriate process to preserve the halophyte plants and to increase their antioxidant activity, as a response to the stress provoked by the heat and humidity conditions inside the drying chamber.

- The plant extracts showed significant antioxidant potential, with high radical scavenging activity. The extracts prepared from *Salicornia macrostachya* and *Sarcocornia perennis* had, respectively, an IC_{50} of 1.09 and 1.42 mg/mL. However, the antioxidant activity of extracts obtained from dried plants increased much more since the IC_{50} decreased to around 0.6 mg/mL, regardless of the halophyte plant.

- These findings highlight the potential of these halophytes as a valuable source of natural antioxidants and nutrients for different applications in the food industry.

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