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Nutritional Characterization and Storage Ability of *Salicornia ramosissima* and *Sarcocornia perennis* for Fresh Vegetable Salads

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Abstract: The aim of this work was to study the potential of two halophytes for fresh salads. *Sarcocornia perennis* (SAR) and *Salicornia ramosissima* (SAL) plant tips were harvested in May and July, and stored at 4 °C for 14 days. At harvest, mineral analyses (Na, K, Mg, Ca, P, N, Fe, Zn, Cu, Pb and Cd), chlorophyll content, protein, β-carotene and ascorbic acid were performed. Color (CIELab), phenolics, antioxidant activity and microbial contamination were measured at harvest and after 14 days storage at 4 °C, and a taste panel evaluated the products. SAL had generally slightly higher values of vitamin C, phenols and carotenoids than SAR. In SAR after 14 days of storage, the levels of phenols and the antioxidant activity were higher than at harvest, both in May and July. The opposite was observed for SAL. In this case, only the antioxidant activity was higher after 14 days of storage. Microbial contamination was very low, not reaching even half of critical limits. Both halophytes had good nutritional value, being suitable for use as fresh salads which can be stored for up to 14 days at 4 °C. SAR seems to be a better source of antioxidants, Ca and Fe than SAL and has less general quality and nutritional value changes from harvest time through cold storage. Fresh tips of both halophytes showed better appearance and nutritional quality when harvested in the early season (May) than at the end of the season (July).

Keywords: halophytes; antioxidants; quality; minerals; storage; flavor

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1. Introduction

Soil salinity is a serious environmental hazard in agriculture. Around 20% of the world's agricultural lands and nearly 50% of all irrigated land are affected by soil salinity. Furthermore, continuous sea-level rise in a warming world threatens increased salinity in coastal lowlands [1]. Some of those areas are naturally saline or have become salinized as a result of improper irrigation practices.

Halophytes, plants that grow naturally in salt marshes are used in some parts of the world to spice food by replacing salt. Some studies report the use of halophytes cultivated for reclaiming salt-affected soil in arid-zone irrigation areas [2,3]. Some halophytes as Salicornia (*Salicornia ramosissima* J. Woods (Chenopodiaceae)) (SAL) and Sarcocornia (*Sarcocornia perennis* (Miller) A. J. Scott (Chenopodiaceae)) (SAR) have been introduced into the European market as vegetables with leafless shoots resembling green asparagus [4,5].

Due to their habits of growth, they can be cultivated in salinized soils where other vegetable crops cannot be cultivated.

In Portugal, the Ria Formosa is a line of sand dunes and islands extending 60 km along the southern coast (Algarve) covering approximately 18,400 hectares, being a valuable and privileged habitat for all kinds of fauna and flora. Amongst the vegetation, attention has been drawn to the typical marshland plants adapted to the excessive salinity of the salt marshes [4]. Among the halophytes that naturally grow in some salt marsh areas of the Algarve are two edible plants: SAL and SAR are halophyte genera suitable for vegetable production [5–7]. Barreira et al. [8] have reported good nutritional and antioxidant properties of *Salicornia ramosissima* and *Sarcocornia perennis* grown naturally in the salt marshes of the Ria Formosa.

Salicornia spp. are consumed today in northern European countries as fresh or cooked gourmet foods, as garnishes or side dishes for meat and fish courses [5,9]. Known as sea asparagus, glasswort, samphire and pickleweed, *Salicornia* can be eaten raw, cooked, or pickled. As a fresh vegetable, it can be harvested in the vegetative phase. Its green tips provide a good source of minerals, protein, amino-acids and vitamins [6,9,10]. SAL is an annual apparently leafless plant with green, jointed, succulent stems (internodes) that ultimately form terminal spikes in which seeds are borne [11].

Although less studied, *Sarcocornia* spp. produce similar succulent shoots to that of *Salicornia* spp. which can be used for food [5,6]. SAR is a small perennial, prostate to erect, halophytic sub-shrub with succulent, articulated stems often rooting at the nodes and forming clumps. The perennial habit separates the genus from all species of *Salicornia.* Plants of SAR grow on the margins of marshes at Blakeney Point, Norfolk, England, that are rich in some minerals and ascorbic acid [12].

To our knowledge, nutritional and sensory qualities of SAL and SAR, as well as their shelf life ability, have not been extensively studied. The main objective of the present work was to evaluate the nutritional value and general quality acceptance of these two halophytes for food (as fresh for salads, spicing, substituting the salt) at two collection times (May and July), as well as their storage ability. With the results, the intention is to promote their use as new, healthy and economically valuable vegetable crops, which can even be cultivated in soils where other vegetable plants cannot grow due to high salinity.

2. Materials and Methods

2.1. Plant Material and Treatments

The plants of *Salicornia ramosissima* J. Woods (SAL) and *Sarcocornia perennis* (Miller) A. J. Scott (SAR) were collected in the salt marshes of Ria Formosa at west-Olhão, Algarve region, Portugal. Plant species were identified based on Davy et al. [11]. and Castroviejo et al. [13]. The first harvest was in mid-May, at the beginning of the collection season and the second at the beginning of July.

Plants were collected and immediately transported to the Postharvest Lab at the University of Algarve within 30 min. In the lab, tips (6–8 cm youngest fully expanded branches) were separated, washed with tap water and left to dry at room temperature for 2 h. Then, samples of approximately 150 g of each species were immediately prepared for analyses (harvest time) or for cold storage. For cold storage, fresh tips were placed in polyethylene foam trays (21.5 cm × 13.5 cm × 3 cm) covered by a film of linear low-density polyethylene (10 µm thick) at 4 ± 0.5 °C for 14 days. Each tray comprised a replicate. At harvest and after 14 days of storage, plant tissues were analyzed in quadruplicate.

For the determination of mineral composition, chlorophyll, β-carotene, ascorbic acid and crude protein contents, samples from both harvest times were used. The other quality parameters including color, phenolics, flavonoids, hydroxycinnamic acid derivatives, antioxidant activity (TEAC, ORAC assays) and microbial growth were measured at harvest and after 14 days of storage from both May and July harvests.

For measurements which could not be done immediately, plants were frozen in liquid nitrogen and stored at −80 °C or dried at 70 °C until at constant weight.

2.2. Determination of Minerals

For mineral analyses, one gram of dry plant material was put in a porcelain crucible, then in an oven (muffle type furnace, Thermolyme 1500) at 500 °C for 7 h. The porcelain crucible with the ashes were subsequently subjected to a slow warming and then 10 mL HCl 37% (Riedel-de Haen, Seelze, Germany) was added until a yellowish appearance of mineral material and release of white smoke had occurred. The samples were filtered using Whatman N°. 42 paper and the volume adjusted to 100 mL in a volumetric flask for subsequent determination of sodium, potassium, phosphorus, magnesium, calcium, zinc, copper, lead and cadmium.

Nitrogen was measured by the Kjeldahl method according to AOAC [14]. Sodium and potassium were determined by flame photometry [14]. Phosphorus was measured by a UV-VIS spectrophotometer according to the method described in AOAC [14]. Contents of magnesium, calcium, iron, zinc and copper were measured by flame atomic absorption spectrophotometry in air-acetylene flame using a Perkin Elmer Aanalyst 800, and lead and cadmium by electrothermal atomic absorption spectrometry (Analyst 800 Atomic Absorption Spectrometer, Perkin Elmer, Waltham, MA, USA) according to AOAC [14].

2.3. Biochemical Composition Determination

β-Carotene was measured following slight modification using the spectrophotometric method of Reyes et al. [15]. Carotenoids were extracted from 1 g fresh samples by homogenizing with 20 mL of acetone:ethanol (1:1) containing 200 $mg \cdot L^{-1}$ butylated hydroxytoluene (BHT). The homogenate was filtered, washed with the solvent and diluted to 20 mL using the extraction solvent. Extracts were transferred to a glass container, 10 mL hexane were added, then shaken and allowed to stand. After 15 min, 10 mL of milliQ water was added, samples vigorously shaken, and separation of phases allowed to take place for 30 min. The absorbance of the upper phase was measured at 470 nm in a Shimadzu 160-UV spectrophotometer. Carotenoids were quantified as β-carotene using a standard curve of β-carotene dissolved in hexane in concentrations ranging from 0.003125 to 0.05 $mg \cdot mL^{-1}$.

Crude protein content was calculated from total nitrogen content determined as described above using a conversion factor of 6.25.

2.4. Color Determination

Color was recorded with a Chroma meter CR-300 series (CE Minolta, Osaka, Japan) on the tips that were placed together in plastic trays, and quantified by CIE (L *, a *, b *) color space. The lightness (L *) value ranges from black = 0 to white = 100, a * changes from green (negative values) to red (positive values), and b * from blue (negative values) to yellow (positive values) [16]. The a * and b * coordinates were converted to chroma (C *) (degree of departure from grey toward pure chromatic color) by the formula $C^* = (a^{*2} + b^{*2})^{1/2}$ and Hue angle (h°) (0° = red-purple, 90° yellow, 180° = bluish-green, 270° = Blue) by the formula h° = arctangent b */a *.

2.5. Microbiological Analysis

The microbiological quality of SAR and SAL samples was determined by counting aerobic mesophilic bacteria, psychrotrophic bacteria (NP-4405 2002) [17] and yeasts and molds (ISO 21527-2) [18]. Ten grams of each sample were transferred to 90 mL of peptone water (Oxoid) and homogenized. Decimal dilutions were prepared using the same diluent. Chloramphenicol Glucose Agar (Biokar, Paris, France) was used to determine the counts of yeasts and molds and Plate Count Agar (Biokar, Paris, France) was used to determine aerobic mesophilic bacteria and psychrotrophic bacteria. The incubation temperature of agar plates for yeasts and molds was 25 ± 1 °C for 48–72 h, 30 ± 1 °C for 24–72 h for aerobic mesophilic bacteria, and 6.5 ± 1 °C for 5 to 10 days for psychrotrophic bacteria. Results were expressed as Log_{10} Colony Forming Unit (CFU) per gram fresh weight.

2.6. Extraction of Phenols

Extraction of phenols was performed by sonication of one gram of dry tips of SAL and SAR in 7 mL of an ethanol:water (75:25) solution in an ice bath for three minutes using a VC300 Vibracell sonicator (Sonics and Materials, Newtown, CT, USA) with a 20 kHz frequency. After sonication, the samples were centrifuged for 5 min at 2000× *g* and 20 °C, and the supernatant was removed and kept at −20 °C until determination of total phenols.

2.7. Determination of Different Phenolic Classes

Aqueous ethanol (95% *v*/*v*; 1 mL) containing 0.1% hydrochloric acid was added to the extract (1 mL) in a 10-mL volumetric flask, and the volume was made up to 10 mL with 2% hydrochloric acid. The absorbance was measured at 280 nm to determine total phenols using gallic acid as standard, at 320 nm to determine hydroxycinnamic acid derivatives using caffeic acid as standard, and at 360 nm to estimate flavonoids using quercetin as a standard [19].

Determination of Total Phenols by Folin–Ciocalteau Method

A modified version of the Singleton and Rossi [20] method was used for the quantification of total phenols. To the diluted extracts (0.2 mL) was added 0.8 mL of sodium carbonate solution (20%) and 1 mL of Folin–Ciocalteau reagent. The tubes were shaken for homogenization and kept for 30 min at room temperature. The absorbance was read at 765 nm. Results are expressed by reference to a regression curve as milligrams gallic acid equivalents (GAE) per 100 g dry weight.

2.8. Ascorbic Acid and Chlorophyll Content

Ascorbic acid was determined by the indophenol titration method. Fresh material (15 g) was macerated in 45 mL of a solution of oxalic acid (1%) in a mortar. The filtered solutions (10 mL) were titrated with 2,6-dichlorophenol indophenol solution (0.05%) until a faint pink color persisted for more 5 s [14].

Chlorophyll was extracted from 1 g fresh tissue macerated with 5 mL calcium carbonate acetone solution (0.24% in acetone 80%) as reported by Charles et al. [21]. Absorbance was read at 665 and 649 nm.

2.9. Trolox Equivalent Antioxidant Capacity (TEAC)

The determination of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^+$) radical scavenging was carried out as reported by Dorman and Hiltunen [22]. Briefly, the $ABTS^+$ radical was generated by the reaction of an aqueous solution of ABTS (7 mM) (Fluka) with $K_2S_2O_8$ (2.45 mM) (Acros Organics) in the dark for 16 h and adjusting the $Abs_{734\,nm}$ to 0.7 at room temperature. Samples (10 µL) were added to 1490 µL $ABTS^+$, the absorbance at 734 nm was read at time 0 (A_0) and after 6 min (A_1). The percentages of inhibition were obtained from the equation $[(A_0 - A_1/A_0) \times 100]$. The water-soluble derivative of vitamin E, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Fluka) was used as a reference standard, and the results were expressed as mmol Trolox per 100 g dry weight.

2.10. Oxygen Radical Absorbance Capacity (ORAC)

The Oxygen Radical Absorbance Capacity (ORAC) method used, with fluorescein (FL) as the fluorescent probe, was that described by Ou et al. [23]. As the ORAC assay is extremely sensitive, the samples must be diluted appropriately in MilliQ water before analysis to avoid interference. In each well of a 96-well microplate, 150 µL of fluorescein working solution and 25 µL of sample previously diluted, blank (75 mM phosphate buffer), or standard (Trolox) were placed. The plate was incubated in a preheated (37 °C) BioTek Synergy™ 4 Hybrid Microplate Reader for 10 min with a previous shaking of 3 min. 2,2′-Azobis-2-methyl-propanimidamide dihydrochloride (AAPH) was added to each well of the plate. The final volume of the assay was 200 µL. The microplate was shaken for 10 sec,

and fluorescence was read every minute for 90 min at excitation of 485 nm and emission of 527 nm. ORAC values were calculated according to a previous report [24]. Briefly, the net area under the curve (AUC) of the standards and samples was calculated. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the three measurements for each concentration. Final ORAC values were calculated using the regression equation between Trolox concentration and the net AUC and were expressed as µmol Trolox per 100 g dry weight.

2.11. Sensory Panel

Sensory evaluation was performed by a taste panel with 20 semi-trained panelists. Panel members were selected from university staff members, who were familiarized with this test, and were asked to evaluate the appearance, color, saltiness, texture, succulence, aroma, taste and overall liking on the base of a 5-point hedonic scale: 1-dislike definitely; 2-dislike mildly; 3-neither like nor dislike; 4-like mildly; 5-like definitely. Taste panels were performed at the beginning of the experiment and after 14 days of storage at 4 °C.

2.12. Statistical Analysis

Statistical analysis was carried out with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Analyses of variance (ANOVA) was performed on the data and, when applicable, Duncan's Multiple-Range Test ($p < 0.05$) for comparisons among means was done. Each mean was calculated from four replications.

3. Results and Discussion

3.1. Plant Species Charahterization

3.1.1. Mineral Composition

Mineral composition of *Sarcocornia perennis* (SAR) and *Salicornia ramosissima* (SAL) are presented in Table 1. Contents of Na, K, Mg, Ca and *p* were major minerals, followed by Fe, Zn and Cu which were at minor levels. The values of contaminants Cd and Pb were found at very low levels in the samples.

Table 1. Mineral composition of *Sarcocornia perennis* and *Salicornia ramosissima* grown naturally on the salt marshes of Ria Formosa, Algarve, Portugal. Values represent the mean ± standard error of four replicates.

Element	Units (DW)	Sarcocornia	Salicornia	Significance Level [z]
Sodium (Na)	g·100 g^{-1}	16.00 ± 0.12	17.44 ± 0.24	n s.
Potassium (K)	g·100 g^{-1}	1.11 ± 0.024	1.06 ± 0.051	n s.
Magnesium(Mg)	mg·100 g^{-1}	498.70 ± 0.09	499.01 ± 0.62	n s.
Calcium (Ca)	mg·100 g^{-1}	310.11 ± 0.65	271.43 ± 7.36	0.006
Phosphorus (*p*)	mg·100 g^{-1}	220.01 ± 2.06	230.91 ± 3.81	n s.
Iron (Fe)	mg·100 g^{-1}	35.11 ± 2.49	19.67 ± 2.09	0.001
Zinc (Zn)	mg·100 g^{-1}	2.12 ± 0.16	3.39 ± 0.52	0.005
Copper (Cu)	mg·100 g^{-1}	1.17 ± 0.10	1.31 ± 0.15	n s.
Lead (Pb)	mg·100 g^{-1}	0.10 ± 0.03	0.09 ± 0.02	n s.
Cadmium (Cd)	mg·100 g^{-1}	0.04 ± 0.01	0.05 ± 0.02	n s.

[z] n.s. indicates it was not significant.

Na concentration predominated in the plant mineral composition and amounted to 16 and 17% of dry weight of SAR and SAL, respectively, without significant differences ($p < 0.05$). Values of sodium were higher than those reported for other halophytes [5,8,9,25]. The highest sodium concentration detected in our samples may be partly explained by the fact that both plant species were grown in salt marshes in sites which were sometimes submersed in sea water.

For the same mineral elements, our results showed no significant differences between SAR and SAL except that Ca and Fe were higher in SAR and zinc was higher in SAL

(Table 1), as reported by Barreira et al. [8]. K, Mg, Ca, P and Fe were higher in our study than for *Salicornia bigelovii* [9] and *Sarcocornia fructicosa* and *Salicornia persica* [5]. However, K and Zn were similar to, and Mg, Ca, P and Fe were higher than, that of *Salicornia europaea* [25]. The values of Cu (1.17–1.31 mg 100 g^{-1}), Pb (0.10–0.09 mg 100 g^{-1}) and Cd (0.04–0.05 mg 100 g^{-1}) were very low and not harmful for consumers as reported in the Codex Stan−150 [26].

3.1.2. Biochemical Composition

Chlorophyll, β-carotene, ascorbic acid and crude protein contents in fresh tips of SAR and SAL are depicted in Table 2. Chlorophyll and ascorbic acid concentrations, although slightly higher in SAL than in SAR, did not show significant differences ($p < 0.05$) (Table 2). However, β-carotene and crude protein were significantly higher in SAL than in SAR. Barreira et al. [8] reported no significant differences between these 2 species in total carotenoids and a protein content higher in SAL, confirming our results.

Table 2. Biochemical composition of *Sarcocornia perennis* and *Salicornia ramosissima* grown naturally on the salt marshes of Ria Formosa, Algarve, Portugal. Values represent the mean ± standard error of four replicates.

Biochemical Composition	Units (FW)	Sarcocornia	Salicornia	Significance Level [z]
Chlorophyll	mg·100 g^{-1}	24.96 ± 4.11	27.63 ± 4.48	n.s.
β-carotene	mg·100 g^{-1}	5.00 ± 0.50	6.53 ± 0.68	0.018
Ascorbic acid	mg·100 g^{-1}	6.80 ± 0.70	8.60 ± 1.04	n.s.
Crude protein	g·100 g^{-1}	1.28 ± 0.06	1.44 ± 0.08	0.038

[z] n.s. indicates it was not significant.

The chlorophyll, ascorbic acid and β-carotene concentrations in the halophytes from Ria Formosa were similar, and the crude protein values were higher compared with those of *Sarcocornia fructiosa* and *Salicornia bigelovii* [5,9]. Such results show that these halophytes from Ria Formosa (Portugal) are a good source of the antioxidants ascorbic acid and β-carotene.

3.2. Quality Parameters Evolution Through Storage at Two Harvest Seazons

3.2.1. Color Parameters

The color parameters lightness (L *) and Chroma* were higher for SAL in May samples than July samples, with no significant differences between harvest dates for SAR (Table 3). The Hue * value was higher in July than May for both species. This means that in May, SAL tips were lighter (L *) and had more intense green color as determined by higher Chroma *. The Hue* value showed a greener color of the tips from both species in July than in May according to McGuire [16].

After 14 days storage of the fresh tips at 4 °C, tips of both species from the May harvest became significantly lighter (higher L *) than at harvest, while these changes were not noticeable in July samples (Table 3). The H * value, which represents the green color, was maintained through storage in May harvest, but decreased ($p < 0.05$) in July through storage (Table 3). The color intensity (C *) significantly decreased ($p < 0.05$) after 14 days at 4 °C, only for SAL in May harvest.

Color values showed higher intensity of the green tips color in SAL in May than in July, what means more attractive color in May than July and no changes for SAR. This agrees with the sensory evaluation which showed a decreased color appreciation only in SAL from May to July. Color values did not significantly change during 14 days at 4 °C for both species, except SAL of May harvest which decreased color attributes in general. Lu et al. [9] found no changes in color for *Salicornia bigelovii* stored at 2 °C and Gago et al. [4] for SAR and SAL at 1° and 4 °C, for up to 15 days.

Table 3. Color parameters of *Sarcocornia perennis* and *Salicornia ramosissima* grown naturally on the salt marshes of Ria Formosa, Algarve, Portugal, at harvest and after 14 days at 4 °C. Values represent the mean ± standard error of four replicates.

	Sarcocornia		Salicornia	
Parameters	**May**	**July**	**May**	**July**
		At harvest		
Color L *	31.0 ± 0.4 ^{bBz}	30.0 ± 1.5 ^{bA}	37.4 ± 1.6 ^{aB}	32.9 ± 2.2 ^{bA}
Hue *	117.1 ± 1.8 ^{bA}	123.2 ± 1.0 ^{aA}	116.0 ± 0.2 ^{bA}	123.2 ± 0.9 ^{aA}
Chroma *	20.6 ± 0.7 ^{bA}	17.3 ± 1.0 ^{cdA}	25.3 ± 0.6 ^{aA}	19.3 ± 1.6 ^{bcA}
		After 14 days		
Color L *	34.9 ± 2.1 ^{aA}	30.83 ± 1.1 ^{bA}	33.60 ± 1.3 ^{aA}	30.44 ± 0.8 ^{bA}
Hue *	119.2 ± 1.9 ^{aA}	118.93 ± 1.9 ^{aB}	118.8 ± 0.5 ^{aA}	120.56 ± 0.7 ^{aB}
Chroma *	21.2 ± 0.6 ^{aA}	19.15 ± 1.2 ^{bA}	21.66 ± 0.3 ^{aB}	18.56 ± 0.4 ^{bA}

^{z} Values in the same row followed by the same lower case, and in the same column followed by the same upper case for the same parameter, are not significantly different by Duncan's multiple range test ($p < 0.05$).

3.2.2. Phenolics

In general, traditional spectrophotometric assays provide simple and fast screening methods to quantify classes of phenolic compounds in crude plant samples. The Folin–Ciocalteau reagent is widely used for total phenol determination, but other methods are also used, such as a direct reading at 220 and 280 nm for quantification of total phenols. In all of these methods there are always some substances such as proteins, nucleic acids and amino acids that can interfere by absorbing at the same wavelength as that used for quantification of the phenols. In the present work, more than one method was determined in the quantification of total phenols: direct spectrophotometric measurement at 280, 320 and 360 nm measuring phenols, hydroxycinnamic acid derivatives and flavonoids, respectively, and Folin–Ciocalteau for total phenols.

Total phenolics showed higher values when determined by the Folin–Ciocalteu method than directly at λ = 280 nm (Table 4). This discrepancy is likely related to the interference of other components with the Folin–Ciocalteau method already reported above. Nevertheless, tips collected in May showed higher phenols and flavonoids in SAL than in SAR [8]. Between harvest dates there was a significant decrease in total phenolics content in both species when phenolics where determined directly at 280 nm (Table 4). However, for the Folin–Ciocalteau method, although values decreased, there were no significant differences for SAR and an increase was noted for SAL (Table 4). The diminution in the total phenols from May to July may be attributed to the higher exposure of plants to UV radiation in July. Some authors have reported that UV treatments of SAL were responsible for a decrease of phenol content [27].

After 14 days storage at 4 °C, the levels of phenols, measured directly at 280 nm, increased significantly ($p < 0.05$) in SAR but not in SAL (Table 4). With the Folin–Ciocalteau method, the increase was significant only for the July harvest of SAR (Table 4). For SAL there was a significant increase in May and a significant decrease ($p < 0.05$) with the July collection (Table 4). Our values were higher than those reported for *Salicornia bigelovii*, *Salicornia persica*, and *Salicornia ramosissima* [27].

Phenols are the most abundant secondary metabolites in plants. The levels of flavonoids were significantly higher in SAL than in SAR. In SAR the levels of flavonoids decreased from May to July, while in SAL they increased (Table 4). Similarly, hydroxycinnamic acid derivatives were higher in SAL than in SAR, but there was a significant decrease from May to July (Table 4). In both species the levels of hydroxycinnamic acid derivatives decreased between harvest dates, to a greater extent than that observed for total phenols and flavonoids (Table 4).

Table 4. Phenolics and antioxidant capacity of *Sarcocornia perennis* and *Salicornia ramosissima* grown naturally on the salt marshes of Ria Formosa, Algarve, Portugal, at harvest and after 14 days at 4 °C. Values represent the mean ± standard error of four replicates.

		Sarcocornia		Salicornia	
Parameters	**Units (DW)**	**May**	**July**	**May**	**July**
		At harvest			
Phenolics (λ = 280 nm)	mg·100 g^{-1}	1319.0 ± 61.0 $^{bB\,z}$	1060.2 ± 26.9 ^{cB}	1514.7 ± 95.9 ^{aA}	904.2 ± 46.0 ^{dA}
Flavonoids (λ = 360 nm)	mg·100 g^{-1}	305.0 ± 20.8 ^{cB}	211.3 ± 11.3 ^{dB}	513.6 ± 43.0 ^{bA}	658.4 ± 22.5 ^{aA}
Hydroxycinnamic acid derivatives (λ = 320 nm)	mg·100 g^{-1}	366.7 ± 71.8 ^{bB}	120.4 ± 8.7 ^{dB}	531.4 ± 65.4 ^{aA}	221.1 ± 12.3 ^{cA}
Phenolics (Folin–Ciocalteau)	mg GAE·100 g^{-1}	3274.4 ± 501.3 ^{bA}	2558.6 ± 434.5 ^{bcB}	2457.1 ± 137.0 ^{cB}	4107.7 ± 56.2 ^{aA}
TEAC	mM Trolox·100 g^{-1}	0.99 ± 0.06 ^{bB}	0.44 ± 0.01 ^{cB}	1.90 ± 0.13 ^{aA}	0.37 ± 0.01 ^{dB}
ORAC	mM Trolox·100 g^{-1}	6.89 ± 0.73 ^{bB}	3.23 ± 0.18 ^{cB}	15.97 ± 0.23 ^{aA}	2.74 ± 0.05 ^{dA}
		After 14 days			
Phenolics (λ = 280 nm)	mg·100 g^{-1}	1509.2 ± 63.3 ^{aA}	1475.6 ± 113 ^{abA}	1341.7 ± 56.7 ^{bA}	771.5 ± 52.5 ^{cA}
Flavonoids (λ = 360 nm)	mg·100 g^{-1}	512.7 ± 17.1 ^{aA}	479.2 ± 55.3 ^{abA}	472.2 ± 16.5 ^{bA}	231.6 ± 20.6 ^{cB}
Hydroxycinnamic acid derivatives (λ = 320 nm)	mg·100 g^{-1}	537.6 ± 26.6 ^{aA}	417.5 ± 51.3 ^{bA}	373.0 ± 18.0 ^{bB}	211.7 ± 15.4 ^{cA}
Phenolics (Folin–Ciocalteau)	mg GAE·100 g^{-1}	3384.2 ± 49.0 ^{bA}	3561.8 ± 25.0 ^{bA}	5074.1 ± 288.2 ^{aA}	3299.5 ± 44.1 ^{bB}
TEAC	mM Trolox·100 g^{-1}	2.54 ± 0.40 ^{aA}	0.75 ± 0.08 ^{cA}	0.93 ± 0.07 ^{bB}	0.77 ± 0.08 ^{cA}
ORAC	mM Trolox·100 g^{-1}	8.84 ± 1.15 ^{abA}	9.06 ± 0.43 ^{aA}	9.55 ± 0.29 ^{aB}	2.84 ± 0.41 ^{cA}

^{z} Values in the same row followed by the same lower case, and in the same column followed by the same upper case for the same parameter, are not significantly different by Duncan's multiple range test ($p < 0.05$).

Some authors have demonstrated that increased UV radiation, can alter the composition of plant secondary compounds, for example, increasing flavonoid and related compounds as a defense mechanism of plants because they would reduce UV-induced tissue damage [28]. In other species, some authors found that although flavonoids and hydroxycinnamic acids were induced by UV radiation, their formation depended on the developmental stage of the leaf exposed to radiation; hydroxycinnamic acid conjugates were the most important UV-protectant compounds in young rye leaves, whereas flavonoids were the most important UV-protectants in mature rye leaves [29].

From May to July, in the Algarve region the sunlight increases as does UV radiation, and augmentation of the phenol content could be expected. However, between harvest dates there was a general decrease in all phenolic groups, particularly in SAR plants. Our results were in accordance with those reported by Hupel et al. [27] who found a global decrease of phenol content when *Salicornia ramonissima* had been exposed to UV radiation. In our case, a noticeable decrease of hydroxycinnamic acid derivatives may reveal a more mature plant material used in the assay in July, in which the role of these compounds is far much less important in the defense against harmful UV-radiation.

After 14 days storage at 4 °C, SAR showed a significant increase ($p < 0.05$) in both flavonoids and hydroxycinnamic acids derivatives in all treatments, while SAL showed a decrease, except for May flavonoids and July acids which were maintained (Table 4).

The storage of fresh vegetables has resulted, in some cases, in an increase of phenolic content (flavonoids and hydroxycinnamic acid derivatives), within the first days of cold storage [30]. The hypothesis postulated is that the increase may be triggered by postharvest plant stresses, which stimulate the biosynthesis of polyphenols [31]. However, as in our study, storage of lettuce and endive in the dark at 1 °C for 7 days was responsible for a

diminution of total flavonoid glycosides as reported by DuPont et al. [32], or flavonoids in some strawberry cultivars stored at 6 °C [33].

The antioxidant activity as measured by TEAC and ORAC methods was significantly lower in July than in May for both species (Table 4). Interestingly, the decrease was higher in SAL than in SAR, with SAL presenting the highest levels of TEAC and ORAC followed by SAR in May, then by SAR and, finally, SAL in July. The decrease in antioxidant activity between the two harvest dates may be attributed to the general decrease of phenols, such as already reported for *Salicornia ramosissima* when the levels of phenols decreased after exposure to UV radiation [27].

The 14 days storage at 4 °C induced a significant increase ($p < 0.05$) in antioxidant activity as measured by the TEAC and ORAC methods in SAR at both harvest dates and SAL in July, although in SAL TEAC increase was not statistically significant (Table 4). The general increase of antioxidant activity in both samples after 14 days of storage may be related to the highest phenol content also reported in these samples. Interestingly, in the May harvest, antioxidant activity of SAL decreased during cold storage.

3.2.3. Microbial Contamination

Microbial contamination was very low for yeasts and molds and null for bacterial contamination at both harvest times and both species (Table 5). Yeast and molds slightly increased during 14 days storage at 4 °C, except for SAR in the July harvest where the increase was significant (Table 5). However, values were between 2 and 3 Log_{10} CFU·g^{-1}, which are much lower than the critical limit for yeasts and molds (5 Log_{10} CFU·g^{-1}) [34]. In the July harvest there was an increase in bacterial counts after 14 days storage at 4 °C for SAL and SAR (Table 5). However, the bacterial counts, 4 Log_{10} CFU·g^{-1}, were still much lower than the critical limits of 8 Log_{10} CFU·g^{-1} [34].

Table 5. Microbial growth of *Sarcocornia perennis* and *Salicornia ramosissima* grown naturally on the salt marshes of Ria Formosa, Algarve, Portugal, after 14 days at 4 °C. Values represent the mean ± standard error of four replicates.

		Sarcocornia		Salicornia	
Parameters	**Units (DW)**	**May**	**July**	**May**	**July**
		At harvest			
Yeast and Molds	Log_{10}(CFU·g^{-1})	2.74 ± 0.07 $^{bA\ z}$	0.67 ± 0.40 ^{cB}	2.82 ± 0.12 ^{bA}	3.21 ± 0.09 ^{aA}
Psycrotrophic Bacteria	Log_{10}(CFU·g^{-1})	ND	ND ^{B}	ND	ND ^{B}
		After 14 days			
Yeast and Molds	Log_{10}(CFU·g^{-1})	2.30 ± 0.17 ^{dA}	2.64 ± 0.12 ^{cA}	3.92 ± 0.14 ^{aA}	3.58 ± 0.07 ^{bA}
Psycrotrophic Bacteria	Log_{10}(CFU·g^{-1})	ND	3.96 ± 0.13 ^{aA}	ND	4.29 ± 0.31 ^{A}

[z] Values in the same row followed by the same lower case, and in the same column followed by the same upper case for the same parameter, are not significantly different by Duncan's multiple range test ($p < 0.05$). ND = not detectable.

3.2.4. Sensorial Evaluation

Taste panel scoring was from 1 to 5 (1—dislike definitely; 2—dislike mildly; 3—neither like nor dislike; 4—like mildly; 5—like definitely). The parameters evaluated by panelists showed all of the values above 3, what is considered acceptable to good for both species (Table 5).

The general appreciation by panelists was maintained after 14 days at 4 °C and no significant differences were observed between harvest dates or species (Table 6). This means that there is a potential for SAR and SAL to be part of fresh vegetable salads, being a condiment substitute of salt and can keep their organoleptic characteristics up to 14 days at 0 °C.

Table 6. Sensory evaluation of *Sarcocornia perennis* and *Salicornia ramosissima* grown naturally on the salt marshes of Ria Formosa, Algarve, Portugal, at harvest and after 14 days at 4 °C. Values represent the mean ± standard error of twenty replicates.

		Sarcocornia		Salicornia	
Parameters	**Days**	**May**	**July**	**May**	**July**
Appearance	0	3.55 $^{abA\ z}$	3.70 ^{aA}	3.62 ^{aA}	3.25 ^{bA}
	14	3.25 ^{bA}	3.75 ^{aA}	3.61 ^{aA}	3.47 ^{abA}
Color	0	3.53 ^{aA}	3.80 ^{aA}	3.82 ^{aA}	3.00 ^{bA}
	14	3.28 ^{abA}	3.50 ^{aA}	3.55 ^{aA}	2.92 ^{bA}
Saltiness	0	3.45 ^{aA}	3.33 ^{aA}	3.27 ^{abA}	3.45 ^{aA}
	14	3.00 ^{abA}	3.50 ^{aA}	3.00 ^{abA}	2.92 ^{bA}
Texture	0	3.25 ^{abA}	3.27 ^{aA}	3.30 ^{aA}	3.45 ^{aA}
	14	3.12 ^{abA}	3.25 ^{abA}	3.44 ^{aA}	3.00 ^{bA}
Succulence	0	3.25 ^{aA}	3.53 ^{aA}	3.48 ^{aA}	3.35 ^{aA}
	14	3.47 ^{aA}	3.67 ^{aA}	3.28 ^{aA}	3.33 ^{aA}
Aroma	0	3.15 ^{aA}	3.13 ^{aA}	3.13 ^{aA}	3.20 ^{aA}
	14	3.18 ^{aA}	3.25 ^{aA}	3.00 ^{aA}	3.00 ^{aA}
Taste	0	3.10 ^{bA}	3.40 ^{aA}	3.30 ^{aA}	3.35 ^{aA}
	14	3.29 ^{abA}	3.15 ^{abA}	3.44 ^{aA}	3.50 ^{aA}
Overall liking	0	3.33 ^{abA}	3.45 ^{aA}	3.42 ^{aA}	3.29 ^{abA}
	14	3.23 ^{abA}	3.44 ^{aA}	3.33 ^{abA}	3.16 ^{bA}

[z] Evaluation based on a 5-point hedonic scale: 1—dislike definitely; 2—dislike mildly; 3—neither like nor dislike; 4—like mildly; 5—like definitely. Values in the same row followed by the same lower case, and in the same column followed by the same upper case for the same parameter, are not significantly different by Duncan's multiple range test ($p < 0.05$).

4. Conclusions

Both *Salicornia ramosissima* and *Sarcocornia perennis* from Ria Formosa (Portugal) are new fresh vegetables for human consumption and can be considered as good sources of nutrients such as Mg, K, P, Ca and Fe. Due to their high Na content, those two halophytes can be valuable for spicing fresh vegetable salads or other foods as a substitute for salt in gourmet cuisine. This work showed that both SAL and SAR are also good sources of proteins, vitamins, carotenes and phenols with potential antioxidant activity and consequently with an important nutritional value which is not lost during 14 days cold storage at 4 °C.

SAR seemed to be a better source of antioxidants, Ca and Fe than SAL and had less general quality and nutritional value changes through harvest dates and during cold storage. Fresh tips of both halophytes showed better appearance and nutritional quality when harvested in the early season (May) than at the end of the season (July).

Both *Salicornia ramosissima* and *Sarcocornia perennis* are new healthy and economically valuable vegetable crops, suitable for spicing fresh salads and foods in general, which can be cultivated in soils where other vegetable plants cannot grow due to high salinity.

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