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Sea Purslane as an Emerging Food Crop: Nutritional and Biological Studies

Arona Pires [1], Sílvia Agreira [1], Sandrine Ressurreição [1], Joana Marques [2], Raquel Guiné [3], Maria João Barroca [1,2] and Aida Moreira da Silva [1,2,*]

[1] Polytechnic of Coimbra, Coimbra Agriculture School, Bencanta, 3045-601 Coimbra, Portugal; arona@esac.pt (A.P.); sagreira@esec.pt (S.A.); sandrine@esac.pt (S.R.); mjbarroca@esac.pt (M.J.B.)
[2] Unidade de I&D Química-Física Molecular, Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal; marques.jt@uc.pt
[3] CERNAS Research Centre, Department of Food Industry, Polytechnic of Viseu, 3504-510 Viseu, Portugal; raquelguine@esav.ipv.pt
* Correspondence: aidams@esac.pt

Abstract: Halophyte plants are highly adapted to salt marsh ecosystems due to their physiological and ecological characteristics. *Halimione portulacoides* (L.) Aellen is one abundant halophyte shrub that belongs to a *Chenopodiaceae* family and Caryophyllales order and is found on sandy or muddy coastlines and salt marshes. In this study, the leaves of sea purslane (*H. portulacoides*) grown in Figueira da Foz (Portugal) were characterized at nutritional and mineral concentration. Moreover, different methanolic extracts were obtained from the leaves, and the antioxidant activity was assessed by several methods. From a nutritional point of view, this halophyte plant may be considered a good source of dietary fiber, protein, natural minerals such as calcium, magnesium, manganese, copper, and potassium. The primary sugar found in leaves of sea purslane is maltose, followed by sucrose, glucose, and fructose. Finally, leaves showed a high content of phenolic compounds and considerable antioxidant activity. The novel products butter and pasta enriched with powder dried leaves of *H. portulacoides* revealed the plant's potential to be used as a salt substitute and a good alternative to enhance the sensory characteristics of products, with additional health benefits. The nutritional characteristics and the phytochemical value highlight *H. portulacoides* as a potential candidate crop in saline agriculture and to be used as a new vegetable, especially as a premium food in the novel "salty veggies" market or as a kitchen salt substitute.

Keywords: halophyte; sea purslane; minerals; antioxidant activity; novel ingredient

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Citation: Pires, A.; Agreira, S.; Ressurreição, S.; Marques, J.; Guiné, R.; Barroca, M.J.; Moreira da Silva, A. Sea Purslane as an Emerging Food Crop: Nutritional and Biological Studies. *Appl. Sci.* **2021**, *11*, 7860. https://doi.org/10.3390/app11177860

Academic Editors: Francesco Cappello and Magdalena Gorska-Ponikowska

Received: 9 July 2021
Accepted: 24 August 2021
Published: 26 August 2021

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1. Introduction

The increase of the world population leads to increased agricultural production to obtain the necessary amount of food to feed everyone on the planet. Furthermore, global warming and freshwater reduction lead to increased land salinity and dryness [1]. Thus, innovations related to agricultural practices, type of value chain, and products have been developed to increase food availability for the population and to shift toward more sustainable food systems, either in the dominant food system regime or in alternative niches. An example of a new strategy is the introduction of non-conventional plant-based foods [2]. Regarding this issue, the interest in natural ingredients with good nutritional and functional components properties that can replace synthetic ones has been increasing in order to develop promising functional foods [2]. On the other hand, the loss of agro-biodiversity has been an incentive to the introduction in agrosystems of innovative crops with high-value biochemical composition and adaptability to climate change and soil salinities. In this context, halophyte plants are extremely adapted to salt marsh ecosystems due to their physiological and ecological characteristics (support at least 11.7 g L^{-1} of NaCl), allowing them to live and grow in places with very high salt concentrations, where

most plants are unable to survive [1,3]. Furthermore, the leaves of some halophyte plants are rich in bioactive molecules, such as lipophilic compounds and phenols, including flavonoids [3,4]. Hence, the scientific community has tried to understand the importance of halophyte plants for human consumption, creating a market-positioning strategy as added-value components for the pharmaceutical and nutraceutical industries and also for the gastronomic area through the improvement of foods' organoleptic properties [3,5].

The perennial *Halimione portulacoides* (L.) Aellen, sea purslane ("gramata branca" or "beldroega do mar" in Portuguese), is a halophyte shrub that belongs to a Chenopodiaceae family and Caryophyllales order. It is found on sandy and muddy coastlines and salt marshes around the coasts of North Africa, Southwest Asia, and Europe [5,6]. In Europe, it is one of the most productive and abundant species in salt marshes. This plant is dispersed along the coast of the Iberian Peninsula, and the prevalence in the Portuguese estuaries is at Tagus and Ria de Aveiro salt marches [7–10]. With a controversial taxon background, *Atriplex portulacoides* is also acknowledged as a senior synonym of *Halimione portulacoides* in the International Plant Names Index [11]. This plant is characterized as a shrub, reaching up to 1.5 m in height (Figure 1). It is a monoecious, protruding-ascending species, with a silvery-gray color and with stems that are often radiant. The leaves are decussate, speared, hollowed out, whole, and fleshy. The inflorescence features unisexual flowers, and the fruit is sessile [12].



Figure 1. *Halimione portulacoides* (L.) Aellen, sea purslane.

The potential of sea purslane in healthy and functional food products has recently been highlighted by the similarity of its fatty acid composition with the *S. ramosissima*, which is a promising functional food with a renewed interest as a food and pharmaceutical product [4,13]. The lipophilic fraction of *H. portulacoides* leaves from estuarine environments of Portugal is mainly composed of long-chain aliphatic acids (e.g., octacosanoic, triacontanoic, oleic, hexadecanoic, and linoleic) and alcohols (e.g., octacosanol, hexacosanol, and triancontanol) (both in the C16–C30 range) while containing smaller amounts of sterols, such as schottenol, sitosterol, and sitostanol. Furthermore, the environmental stresses induce in a plant the synthesis of a wide range of phenolic compounds, such as sulfated flavonoids, particularly derivatives of isorhamnetin-sulfate and carotenoids (such as zeaxanthin, β-carotene, lutein, auroxanthin, violaxanthin, and antheraxanthin) [11,14–18]. These confer important biological properties, such as antioxidant, anti-inflammatory, anti-trombotic, and anti-cancerogenic activities [4,11,18]. Furthermore, *H. portulacoides* leaves are a good source of protein and important dietary minerals, namely Mg, K, Ca, Fe, Mn, Cu, and Zn [9,11,19–21]. The nutritional and biochemical profiles of the plant responsible for its positive effects on human health increase its interest as a new vegetable, especially as a

premium food in the novel "salty veggies" market [13]. Furthermore, its high productivity in saline conditions and even in arid lands and its resistance to different environmental stresses render *H. portulacoides* a good candidate for exploitation in sustainable and saline agriculture. However, similar to most halophytes, relevant issues related to their cultivation remain to be defined, such as the effects of the season, geographical location, and the morphology of the environment on their nutritional quality and salinity content [1]. An adaptative trait of *H. portulacoides* is the capacity to concentrate seawater metal cations beneficial to human health. However, in addition to naturally occurring metals, halophytes could accumulate heavy metals derived from the human contamination of salt marshes' sediments [11,22]. The high concentration of toxic heavy metals existent in some lagoon environments and European estuaries have an impact on the macro and micro nutritional composition of *H. portulacoides* [11]. Therefore, the location from which the plants are harvested for food use constitutes a crucial factor for their nutritional quality and eventual toxicity. However, by itself, this is no evidence of toxicity of *H. portulacoides*, since more than 90% of toxic metals are retained in the plant's below-ground organs [23,24]. According to Cabrita et al. [24], the low concentration of mercury in the aerial parts of the plant are attributed to metal release by leaves and stems, probably via stomata.

Although *H. portulacoides* is an almost forgotten traditional food, its use as food (raw or cooked) and forage dates back thousands of years. Indeed, the use of this plant in human culture comes from the Early Neolithic period, as evidenced by the finding of this plant amongst ancient carbonized remains of food in northern Holland [11]. In Italy, *H. portucaloides* is traditionally used raw in salads or cooked in some recipes based on fish [11]. Moreover, its buds can be preserved in vinegar [25]. Nowadays, the fresh leaves of this alimurgical wild edible species have been used in gourmet preparations [11]. Sea purslane's visually appealing aspect in terms of freshness and color are attributes that potentiate its usage in a broad range of foods, namely to garnish dishes. However, sea purslane may be used not only as a fresh product but also as a dried herb. Hence, powdered dried leaves, which improve the product's availability and shelf-life, could represent an excellent alternative to creative reinterpretations of traditional foods. Moreover, it can be incorporated into a broad range of foods, developing functional foods that are a trend in the food industry driven by consumer's acceptance and awareness of their positive health effects. The dry halophyte leaves can be a natural salt substitute and a good alternative to develop innovative traditional products with peculiar flavor and color traits enriched with antioxidants compounds.

As referred above, the chemical, mineral, and bioactive compounds related to the composition of the salt marsh sediments where the plant lives are not yet fully known. Hence, to use *H. portucaloides* as food in safe conditions, a nutritional, biochemical, and mineral characterization of the plant leaves is essential. To the best of our knowledge, the nutritional, biochemical, and mineral composition of *H. portucaloides* of the salt marsh of Figueira da Foz (Portugal) has not yet been characterized. Moreover, this is the first time that it is developed into enriched products with sea purslane dried leaves. In this context, the present study aimed to evaluate the macro and micro nutritional compounds and phytochemical value of *H. portulacoides* concerning its potential to be used in the development of new functional food ingredients. Furthermore, to incentivize the cultivation of *H. portulacoides*, we proposed two novel products, pasta and butter, enriched with powdered dried leaves. In addition, a sensory evaluation of the products was performed by a set of consumer panelists.

2. Materials and Methods

2.1. Plant Material

H. portulacoides (L.) Aellen leaves were collected at Armazéns de Lavos (40°06′43″ N 8°49′59″ W), Figueira da Foz salterns, Portugal, in July 2019. The nutritional and mineral profile was evaluated in the fresh leaves, and the biological profile was evaluated in grinded freeze-dried (in a CoolSafe 100-9 Pro Freeze Dryer, Labogene, Denmark) leaves.

The powdered samples were stored at room temperature and protected from light until further use.

2.2. Chemicals

2,2′-Azino bis(3 ethylbenzothiazoline 6 sulfonic acid) diammonium salt (≥98%), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (97%), 2,2-diphenyl-1-picrylhydrazyl, 2,4,6-tris(2-pyridyl)-s-triazine (≥99%), 5,5′-dithiobis(2-nitrobenzoic acid) (99%), acetylcholinesterase from Electrophorus electricus (electric eel), acetylthiocholine iodide (≥99.0%), aluminum chloride ($AlCl_3$, for synthesis), ammonium acetate (≥98%), butylated hydroxytoluene (≥99%), copper(II) chloride ($CuCl_2$, for synthesis), ethylenediaminetetraacetic acid (≥98.5%), ferrozine (97%), galantamine hydrobromide, gallic acid (≥98.0%), iron(II) chloride ($FeCl_2{\cdot}4(H_2O)$, ≥99%), linoleic acid (≥99%), neocuproine (≥98%), quercetin (≥95%), sodium carbonate (≥99.5%), sodium dihydrogen phosphate ($NaH_2PO_4{\cdot}2(H_2O)$, ≥98%), thiobarbituric acid (≥98%), trichloroacetic acid, TRIS (≥99%), Trolox (97%), tryptic soy agar, Tween®® 80, β-carotene (≥93%), as well as solvents (of analytical grade) were obtained from Merck (Oeiras, Portugal). Acetic acid (glacial p.a.) was purchased from Pronalab (Sintra, Portugal), the Folin–Ciocalteu's reagent, HCl (35%) and iron(III) chloride ($FeCl_3{\cdot}6(H_2O)$) (≥98%) from Panreac (Barcelona, Spain), and potassium persulfate (99%) and sodium phosphate dibasic (Na_2HPO_4, ≥99%) from Honeywell (Carnaxide, Portugal).

2.3. Nutritional Composition Analysis

The Association of Official Analytical Chemists (AOAC, 1997) methodologies were used to determine the chemical properties of *H. portulacoides*. Moisture content (method 930.04), ashes (method 930.05), crude protein (method 978.04) using a nitrogen conversion factor of 6.25, total lipids (method 930.09), and dietary fiber (AOAC 985.29) and crude fiber (method 930.10) were determined. The carbohydrate content was determined from the difference between 100 and the sum of the percentages of moisture, ashes, crude protein, dietary fiber, and total lipid contents.

The Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 was used for calculation of the energy values (expressed in kcal/100 g and kJ/100 g) [26].

Quantification of sugars was performed by high-performance liquid chromatography with refractive index detection (HPLC-RI), consisting of an LC1110 high-pressure pump (GBC, Australia), LC-100 oven (Perkin-Elmer, USA), refraction 830-RI (Jasco, Japan), and an HC-75 Ca ++ 305 × 7.8 mm column (Hamilton, Energy Way, Reno, NV, USA). The mobile phase used was ultrapure water (Direct-pure, 10 Uv) with traces of sodium azide, with a flow rate of 0.6 mL/minute, at 80 °C. The quantification was performed by BioUltra standards (Sigma-Aldrich, St Louis, MO, USA). The data were collected by an Interface Hercule Lite (JMBS) and processed by the software Borwin Chromatography Software, version 1.5, build 16 by Jasco-Borwin (Japan).

2.4. Mineral and Heavy Metal Composition Analysis

For minerals and heavy metals analyses of *H. portulacoides*, a PerkinElmer PinAAcle 900 T Atomic Absorption Spectrometer (USA) was used. The contents of calcium, copper, iron, magnesium, manganese, potassium, sodium, and zinc were quantified by flame atomic absorption spectrometry (FAAS) (ISO 6869:2000) [27]. Cadmium, lead, and chromium were analyzed by graphite furnace atomic absorption spectrometry (GFAAS) (EN 14082:2003) [28]. A Thermo X series II inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the iodine content in *H. portulacoides* (EN 15111:2007). The phosphorus content was determined by spectrophotometry (ISO 6491:1998) [29] with a PG instruments T80+ UV/VIS spectrophotometer (UK). Traces of mercury were analyzed by an AMA254 Mercury Analyzer (Leco, USA).

2.5. Color Coordinates

The color coordinates of the *H. portulacoides* leaves were assessed in the top and bottom sides using a colorimeter (Chroma Meter—CR-400, Konica Minolta, Tokyo, Japan), and they were registered in the CIE Lab color space. The axis for L^* corresponds to brightness and varies from 0 (black) until 100 (absolute white). The chromatic coordinate a^* ranges from green (negative values) to red (positive values), and the b^* coordinate ranges from blue (negative) to yellow (positive). The total color difference (TCD) was calculated using Equation (1), which allows quantifying the overall color difference between dried and fresh leaves, which is this case was the control sample:

$$TCD = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2} \quad (1)$$

where L_0^*, a_0^*, and b_0^* are the color coordinates for the control sample [30].

For evaluation of color, 20 measurements were made in the fresh leaves (top and bottom sides) and in the dried powder.

2.6. Extraction Procedure

H. portulacoides powdered leaves samples (5 g) were extracted with 100 mL of ethanol for 3 h, at room temperature, in a magnetic stirrer. The extract was centrifuged for 10 min at 2500 rpm; then, the supernatant was filtered and stored at a concentration of 5 mg/mL at 4 °C until further analysis.

2.7. Chemical Composition and Antioxidant and Enzymatic Activities

The total phenolic and flavonoid contents (TPC and TFC, respectively) and the determination of the enzymatic (cholinesterase inhibition) and antioxidant activities (DPPH and ABTS radical scavenging methods, β-carotene/linoleic acid bleaching method, lipid peroxidation inhibition, metal chelating ability, reducing power—FRAP and CUPRAC) and enzymatic (cholinesterase inhibition) activities were performed according to modified versions of literature reported methods [31].

2.8. Drying

The drying of *H. portulacoides* fresh leaves (5 kg) was performed in a laboratory scale tray dryer. The drying unit consists of a chamber equipped with six trays (45 × 90 cm), heating elements, and a flow fan with adjustable speed yielding. The leaves were dried at a temperature of 65 °C and air velocity of 1.5 m/s for 22 h. The dried leaves were grinded using a homogenizer and reduced to powder.

2.9. Food Usage Suggestions

2.9.1. Pasta

A basic pasta recipe was prepared (control) with the following ingredients: 100 g of wheat flour T65, one egg, and 1.6 g of salt (control). In enriched pasta, the content of salt was replaced by 7.7 g of powdered dried *H. portulacoides* (equivalent to 1.6 g of salt, assuming that all Na of the dried leaves is linked to NaCl). In both pastas, the ingredients were mixed and kneaded by hand for 15 min and left to rest in the cold, at approximately 4 °C, for 30 min. Then, the paste was prepared and shaped with a home pasta-shaping machine (Figure 2). The machine was adjusted to produce pasta with 1 mm of thickness.



Figure 2. Ingredients and pasta preparation.

The pasta cooking time was 10 min in boiling water (for 100 g of pasta, 2 L of water was used, to which 6 g of salt was added). Cooking was carried out with precision by respecting the standardized protocol ISO 7304-1:2016 [32] in order to make it possible to compare both samples (control and enriched pasta). After cooking, the pasta was poured into a sieve and drained.

2.9.2. Butter

Pasteurized cream (with 40% of fat) was cooled at ±6 °C. After that, cream was mixed in a tank, and the biological ripening was performed at 15 ± 2 °C until reaching a pH between 4.8 and 5.2 by adding a dairy starter culture (*Lactococcus lactis* subsp. cremoris, *Leuconostoc, Lactococcus lactis* subsp. Lactis, and *Lactococcus lactis* subsp. lactis biovar diacetylactis) (FS-DVS Flora Danica). The mixture was introduced in a churning machine. In the churning process, the cream was violently agitated to break down fat globules, allowing the fat to coagulate into butter grains. During churning at a temperature between 8 and 12 °C, the buttermilk was drained off, and butter began to appear in the form of grains.

The grains were washed with pasteurized water to remove any residual buttermilk and milk solids, controlling the butter's moisture content up to 16%. Next, to a portion of butter was added salt to produce butter with 1% of NaCl salt, and to another part was added 1% of dried powdered sea purslane. After salting, the butter was worked vigorously to ensure an even distribution of the salt or sea purslane powder, depending on the case. The butter was packed in vegetal paper and refrigerated at 3 °C until further use.

2.10. Sensory Evaluation

Consumer panelists were recruited from the Coimbra Agriculture School (ESAC) in order to evaluate possible changes in the organoleptic characteristics of pasta and butter resulting from the addition of *H. portulacoides*. To assess the preference for a given product by the tasters, a Product Preference Test was used. Samples of products non-fortified with dried powder of *H. portulacoides* (control) and fortified were presented randomly to 40 tasters. Panelists were placed randomly at room temperature, and water was served to clean their palates prior to proceeding to the next sample.

The organoleptic attributes (color, flavor, texture, appearance, and overall acceptance) of pasta and butter samples (control and enriched with *H. portulacoides*) were evaluated using a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely). Tasters were also

asked to rank products according to their purchasing preference and enquired about the regularity of consumption of pasta and butter.

2.11. Statistical Analysis

Statistical analyses were performed to evaluate if the differences between mean values (color and sensorial attributes) were statistically significant. For comparison of mean values between two groups (samples), the independent samples T-test was used. The results of biological activity were analyzed using one-way ANOVA (for three or more groups) followed by Tukey's post hoc test for statistical comparison between the experimental data. In all cases, the level of significance considered was 5%. The statistical analyses were performed using the software SPSS V26 and GraphPad Prism (GraphPad Software, USA)

The IC50 values were calculated for each extract fitting the results using nonlinear regression analysis in sigmoidal dose–response curves (variable slope).

3. Results and Discussion

3.1. Nutritional Composition

The *H. portulacoides* plant can become important for application in new food products due to its nutritional composition and health-beneficial properties. Table 1 shows the average values of the main chemical components for sea purslane grown in Figueira da Foz (Portugal), expressed in raw and dry matter.

Table 1. Nutritional composition of *Halminione portulacoides*.

Composition	Raw Matter	Dry Matter
Energy (kcal/100 g)	48.03 ± 0.06	218.59 ± 0.30
Moisture (g/100 g)	78.03 ± 0.01	-
Ash (g/100 g)	6.09 ± 0.02	27.70 ± 0.09
Dietary fiber (g/100 g)	8.90 ± 0.01	40.49 ± 0.06
Crude fiber (g/100 g)	4.54 ± 0.01	20.64 ± 0.07
Protein (g/100 g)	2.08 ± 0.02	9.47 ± 0.07
Lipids (g/100 g)	0.46 ± 0.01	2.07 ± 0.05
Carbohydrates * (g/100 g)	4.45 ± 0.01	20.26 ± 0.06

* excluding fiber.

The moisture content of wild *H. portulacoides* evaluated in this work was 78.03 g/100 g. However, the moisture content of the same plant growing in hydroponics conditions was reported as being around 90% [9].

In general, halophyte plants such as *H. portulacoides* have higher ash contents than other edible plants. As an example, *Sarcocornia ambigua* has an ash content of 24.98% (dry matter) [33], contrasting with *Sarcocornia perennis* (43.62%, dry matter) [2], *Arthrocnemum macrostachyum* (31.6%, dry matter), and *Salicornia ramosissima* (29.2%, dry matter) [4]. The ash content is related to the total concentration of minerals. Therefore, the high concentration of ash observed in these sea plants is probably related to their ability to retain the minerals of the seacoast saline soils [4].

The total mineral content (ash) of sea purslane leaves (27.70%, dry matter) was very similar to that reported by Briens et al. (around 28%, dry matter), which were both collected in salt marshes [34]. Sea purslane that grows in saline hydroponic conditions, with different nutrient solutions, presented a leaf mineral content of 36.67% (dry weight) [9].

Plants have a variety of lipids with important biological functions involving plant metabolism. These lipids play structural and signaling roles that are significant in the metabolic regulation, protection, and homeostasis of the cell [13].

The lipid content extractable with petroleum ether was 0.46 g/100 g of raw matter. Custódio et al. [9] reported that the lipid content in leaves was 0.33 g/100 g, and in another study, the same author presented values in the range 0.74–0.94 g/100 g [3]. The lipids in plants act as signalers, energy storage compounds, and hydrophobic barriers for the membrane [35]. In human health, lipids are essential for promoting the absorption

of some vitamins and helping build some tissues [36]. Maciel et al. (2018) presented nineteen different fatty acids in *H. portulacoides* leaves characterized by a high percentage of polyunsaturated fatty acids (PUFA) (approximately 60%) and omega-3 (n-3) (approximately 45%). The percentage of saturated fatty acids (SFA) was around 27%, and monounsaturated fatty acids (MUFAs) represented approximately 12%. In addition, it was referred that the first and second most abundant SFA were C18:3 (n-3) (approximately 43.5%) and C16:0 (19.2%), respectively. The MUFA fatty acid present in the highest amount was C18:1 (n-9)—oleic acid (approximately 10%). The ratio of n-6/n-3 fatty acids (0.32) and the presence of phospholipids and glycolipids of high biological value [13] increase the nutritional value of sea purslane, enabling it to be used as gourmet food with potential health benefits.

Leaves' protein content, 9.47 g/100 g of dry matter, was similar to values reported for halophyte plants such as Sarcocornia perennis subsp. alpini (8.10 g/100 g) and *Sarcocornia perennis* subsp. perennis (6.90 g/100 g) [4]. The recommended intake for adults is 0.8 g of protein per kilogram of body weight [36].

The total carbohydrates of leaves were 4.45 g/100 g (fresh weight) or 20.26 g/100 g (dry matter). The carbohydrates in plants are the main sources of energy and constitute carbon skeletons for organic compounds and storage components [37,38]. In addition, they help to maintain glycemic homeostasis and gastrointestinal integrity. The lowest amount of carbohydrates that humans should consume per day is 130 g [36,39].

H. portulacoides presented higher inorganic matter (ash), lipids, and protein contents than, for example, *Salicornia* spp., which is a halophyte plant suitable for human consumption and considered as a promising functional food [40]. The results highlight that, as other commercially available halophytes, *H. portulacoides* has the potential to be consumed fresh, processed, or used in novel food products with health benefits.

Figure 3 illustrates the four sugars (maltose, glucose, fructose, and sucrose) identified in these plant leaves. The major sugar was maltose with 3.01 $\pm$ 0.08 g/100 g, followed by sucrose with 0.49 $\pm$ 0.02 g/100 g, glucose with 0.30 $\pm$ 0.02 g/100 g, and fructose with 0.21 $\pm$ 0.01 g/100 g, as expressed in raw matter. Among the main soluble sugars, maltose was predominant in leaves of sea purslane, and glucose and fructose were not accumulated significantly in this plant. Total sugars represented 90% of the carbohydrates found in *H. portulacoides* leaves. Custódio et al. [9] reported a content of 0.3 g/100 g (raw matter) of total sugars in sea purslane leaves. According to Briens et al. [34], the amount of carbohydrates in *H. portucaloides* leaves (dry matter) was 127 µmol/g, corresponding to 50 µmol/g of sucrose, 41 µmol/g of fructose, 23 µmol/g of glucose, and 13 µmol/g of other carbohydrates. Based on the capacity to accumulate carbohydrates and (or) nitrogenous solutes, *H. portulacoides* is a species that produces more nitrogenous solutes than soluble carbohydrates under saline stress.

Based on the ratio between carbohydrates (20.26%) and ash (27.70%), which are both expressed in dry matter, *H. portulacoides* could be considered a plant with a high level of inorganic ions and a low content of sugars. Other halophyte plants with this behavior are *Atriplex, Aster, Salicornia,* and *Suaeda* [34]. Moreover, 100 g of fresh leaves provide 48.03 $\pm$ 0.06 kcal of energy, which is higher than the value of 18.5 kcal described by Custodio et al. [9].

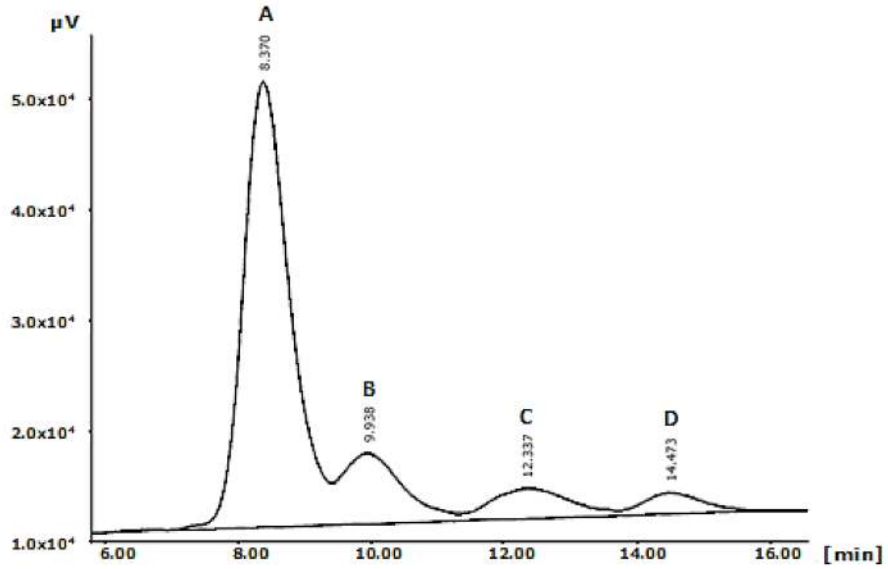


Figure 3. Chromatogram of *Halimione portulacoides* sugars. A: maltose, B: sucrose, C: glucose, and D: fructose.

3.2. Color

Since the sea purslane leaves have a slightly different color on the top and bottom, the color coordinates were evaluated on both sides. The values for the *L**, *a**, and *b** coordinates on the top fresh leaves were 49.74, −7.20, and 6.48, respectively. The values for the bottom of the fresh leaves were similar: 49.98, −7.36, and 7.32, respectively for *L**, *a**, and *b**. The fresh leaves showed a silvery–gray color.

The color parameters (*L**, *a**, and *b**) for fresh leaves and dried powder of *H. portulacoides* are presented in Figure 4. Comparing the color parameters of the dried samples with those obtained for fresh leaves, it was possible to conclude that drying induced a rise in *L** and *b** color parameters, indicating an increase in the lightness and yellowness of the dehydrated plant. These differences were statistically significant for the color coordinates *L** and *b** ($p < 0.0005$ in both cases) but not significant for *a** ($p = 0.832$).

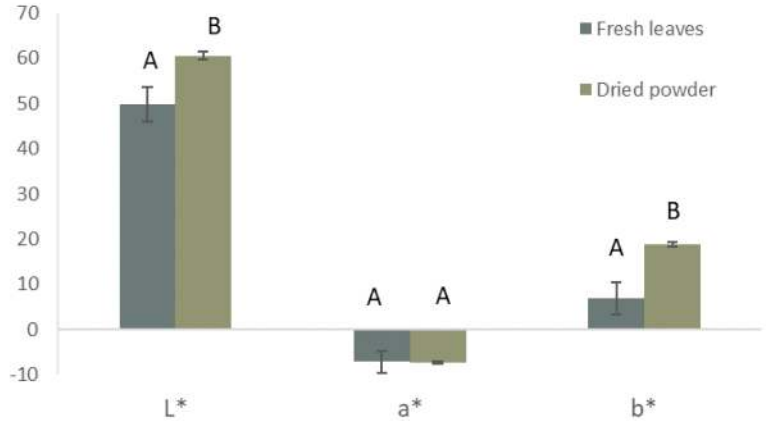


Figure 4. Color parameters of fresh leaves and dried powder of *H. portulacoides*. Bars with the same letter are not statistically different for each of the color coordinates. The color used for each series in the graph is the estimated real color of sea purslane in the fresh and dried states.

The total color difference ΔE, which is a combination of the *L* *, *a* *, and *b* * values, is a colorimetric parameter extensively used to characterize the variation of color in food during processing. The color difference had a value of 16.1 for the leaves dried at 65 °C, being mainly attributed to differences in lightness and yellowness parameters.

3.3. Mineral and Heavy Metal Composition

Due to the saline environment, halophytes, in general, have higher contents in minerals than other edible plants [41]. The mineral content of *H. portulacoides* leaves, obtained by atomic absorption spectrometry, is shown in Table 2.

Table 2. Mineral composition (mg/100 g raw matter) and the mineral intake (%) provided by 100 g of fresh leaves of *H. portulacoides*.

Composition	Raw Matter mg/100 g	Intake Provided by 100 Fresh Leaves (%)
Sodium, Na	1799.38 ± 13.98	78.0
Potassium, K	314.97 ± 0.89	9.5
Calcium, Ca	168.02 ± 0.63	24.0
Magnesium, Mg	67.24 ± 0.29	28.0
Phosphorus, P	40.41 ± 1.58	7.3
Iron, Fe	2.18 ± 0.15	7.6
Manganese, Mn	1.51 ± 0.04	73.7
Zinc, Zn	0.64 ± 0.05	7.8
Copper, Cu	0.21 ± 0.02	16.8
Iodine, I	0.011 ± 0.004	7.3

The intake of minerals provided by 100 g of fresh leaves of *H. portulacoides* was estimated from the average RNIs (Recommended Nutrient Intakes) for adult females and males in the European Union (when applicable) (adapted from [42–45]).

The most abundant minerals were sodium, potassium, calcium, magnesium, and phosphorus, followed by iron, manganese, zinc, copper, and iodine with minor concentration. Phosphorus, calcium, magnesium, sodium, potassium, and iron are essential minerals for human health [37]. Zinc, copper, manganese, chromium, and nickel are necessary in residual concentrations in the human diet [37].

In general, a distinctive property of the halophyte plant is its exceptionally high sodium content. In fact, the amount of sodium in fresh leaves of *H. portucaloides* is around 1.8% (raw matter). The sodium consumption per day should not exceed 2300 mg [46] and, consequently, the intake of 100 g of fresh *H. portulacoides* corresponds to 78% of the daily value recommended for sodium. Sodium is considered an essential nutrient, but its excessive consumption is associated with several pathologies such as hypertension and cardiovascular disease [4]. Hence, the sodium concentration in leaves is acceptable for human intake, but special care must be taken to not exceed the daily dose recommended by the FDA [46].

The content of sodium (8.19 g/100 g, dry matter) was similar to the value 7.82 g/100 g (dry matter) found by Custódio et al. [9] for leaves of *H. portulacoides* and *Salicornia* species (Table 3), which are succulent shoots highly appreciated in gourmet cuisine due to their salty taste. The level of sodium accumulation in plant tissues depends on the availability of elemental nutrients concentrations, namely nitrogen, in saline environments [22,47].

Potassium and calcium are other minerals in high concentration in this plant, respectively, 1433.64 and 764.77 mg/100 g (dry matter). In dry matter, the potassium concentration in leaves of *H. portulacoides* was similar to that in *Salicornia bigelovii* but much higher than *Sarcocornia perennis alpini* or *Salicornia ramosissima* [48]. Leaves of *H. portulacoides* presented much higher calcium contents than *Sarcocornia* and *Salicornia* species (Table 3). Amongst the halophyte plants, the *H. portulacoides* plant is a good source of calcium and potassium.

Copper, zinc, and iron are essential micronutrients necessary in chloroplast reactions, enzyme systems, protein synthesis, and hormone growth [49]. Iron is an essential mineral and cofactor in the synthesis of neurotransmitters, as well as an important constituent of proteins involved in oxygen transport and metabolism [19]. Concentrations of iron (9.92 mg/100 g of dried matter) in leaves of sea purslane are similar to the halophyte *Salicornia bigelovii* Torr [48].

Zinc concentration in *H. portulacoides* leaves (2.93 mg/100 g in dry matter) is higher than the value reported by Reboredo et al. (1.94 mg/100 g in dry matter) [50].

Iodine is a vital nutrient for human health, since it regulates thyroid function. Low iodine intake is responsible for thyroid disorders [51]. The iodine content (0.05 mg/100 g of dry matter) presented in sea purslane leaves was higher than in cereals and grains (ranging from 0.0016 to 0.039 g/100 g), meat (0.0034–0.034 g/100 g), dairy products (0.047–0.069 g/100 g), fresh fruit (0.00018 g/100 g), fresh vegetables (0.0036 g/100 g), leafy vegetables (salad) (0.0236 g/100 g), mushrooms (0.021 g/100 g), or nuts (0.0218 g/100 g), which are all expressed in dry matter [52].

Although foods of marine environment such as marine fish and seaweed are the major suppliers of iodine [53], *H. portucloides* represents an important source of iodine. The oral intake of iodine recognized for adequate nutrition in human adolescents and adults is 150 mg per day [52]. The ingestion of 100 g of fresh leaves corresponds to 7.3% of the daily values recommended for iodine [43].

In general, *H. portulacoides* leaves are a good source of minerals such as Ca, Mg, Mn, and Cu. It should be noted that 100 g of fresh leaves provide values of 24% and 28%, respectively, for calcium and magnesium, 74% for manganese, and 17% for copper.

Table 3. Mineral composition (dry matter) of *Halimione portulacoides* and other halophyte plants.

Composition	*H. portulacoides* Present Study	*Sarcocornia perennis alpini* [4]	*Salicornia ramosissima* [4]	*Salicornia bigelovii* Torr [43]
Na (mg/100 g)	8190.18 ± 35.10	6430 ± 90	8990 ± 50	8618 ± 613
K (mg/100 g)	1433.64 ± 4.07	1030 ± 10	892 ± 23	1520 ± 69
Ca (mg/100 g)	764.77 ± 2.86	263 ± 1	486 ± 5	535 ± 17
Mg (mg/100 g)	306.06 ± 1.32	703 ± 4	943 ± 8	1019 ± 52
P (mg/100 g)	183.93 ± 2.68	-	-	155 ± 9
Fe (mg/100 g)	9.92 ± 0.67	128 ± 5	153 ± 2	8.64 ± 0
Mn (mg/100 g)	6.87 ± 0.16	6.52 ± 0.03	20.4 ± 0. 4	-
Zn (mg/100 g)	2.93 ± 0.22	2.52 ± 0.01	6.87 ± 0.01	3.5 ± 0.12
Cu (mg/100 g)	0.94 ± 0.08	-	-	0.79 ± 0.12
I (mg/100 g)	0.05 ± 0.02	-	-	-
Cd (µg/100 g)	89.02 ± 0.47	19 ± 0.00	nd	8.63 ± 0.00
Pb (µg/100 g)	17.91 ± 0.55	131 ± 2	145 ± 2	17.27 ± 8.64
Hg (µg/100 g)	6.98 ± 0.10	-	-	-
Cr (µg/100 g)	-	492 ± 11	524 ± 5	-

nd: not detected; -: not presented. Adapted from [4,48,54].

Cadmium, lead, and mercury, considered human carcinogens [55], were detected in *H. portulacoides*. According to the Commission Regulation (EC) n° 466/2001 of 8 March 2001, the maximum content of cadmium in leafy vegetables, fresh herbs, celery, and all cultured mushrooms is 0.2 mg/kg (20 µg/100 g of fresh weight), and the maximum lead content in brassica, leafy vegetables, fresh herbs, and all mushrooms is 0.3 mg/kg (30 µg/100 g of fresh weight) [56]. Thus, the concentrations of toxic metals were below the legislated values and there was a much lower concentration of lead than in *Sarcocornia perennis alpini* and *Salicornia ramosissima* from Castro Marim (Algarve, south of Portugal) (Table 3) [4]. The different amounts of these contaminants are dependent on the level of contamination of sediments in salt marshes, since halophytes have the ability to accumulate metals such as Zn, Cr, Pb, Ni, and Cd, among others. However, metal concentrations found in the above-ground tissues of halophyte plants such as *Salicornia fruticosa* and *Salicornia maritima* from Tagus and Guadiana estuaries (Portugal) were up to four orders of magnitude lower than in below-ground parts, confirming metal retention in their roots and a residual upward translocation [54,57,58].

Mercury is a dangerous pollutant due to its high toxicity, making it a major threat to coastal ecosystems [24]. As other metals, the mobility of mercury is greater in the roots, and only a small part is translocated to the above-ground parts of the plant [10]. Hence,

the mercury concentration of above-ground tissues can be 174 to 545-fold times lower than that of the roots [10].

In fact, the content of mercury in *H. portulacoides* leaves (1.53 μg/100 g of raw matter) is around 33 times lower than the limit values defined for fishery products such as fish (50 μg/100 g) [56]. Moreover, the content of mercury in *H. portulacoides* leaves (under study) collected in Figueira da Foz (Portugal) is lower than the values reported for the same plant tissues (6–14 μg/100 g) collected in Laranjo Bay salt marsh (Ria de Aveiro, Portugal), during April 2003 and April 2004 [10]. The higher concentration of mercury in sea purslane leaves of Ria Formosa can be attributed to its high level of mercury contamination, being one the most mercury-contaminated systems in Europe [10].

The content of heavy metals (cadmium, lead, and mercury) found in *H. portulacoides* leaves was much lower than the values that could be considered dangerous to human health, making the leaves a safe product.

3.4. Total Phenolic and Flavonoid Content

Halophytes are rich in highly bioactive phytochemicals [15], particularly in phenolic compounds, which are abundantly present in the human diet, and to which are attributed important antioxidant properties, their intake being associated with a decreased risk of development of oxidative stress-related diseases [14]. Flavonoids are one of the most important families of phenolic compounds, thus contributing to their health benefits, namely as anticancer and chemopreventive agents [17]. Therefore, the measurement of the phytochemical composition of an extract in terms of total phenolic and flavonoid content can be used to estimate its antioxidant potential. Regarding the TPC of the *H. portulacoides* extract, the results presented in Table 4 support the advantage of using ethanol as extraction solvent, since a higher content of phenolic compounds was obtained when compared with reported extractions using hexane, chloroform, methanol [16], ethyl acetate, and water [59]. Moreover, the TPC for the *H. portulacoides* extract is in agreement with previous results obtained for this plant harvested in the same region of Portugal [18]. The TFC is higher than those reported for other halophytes, namely *Ipomoea pes-caprae* [60], which can be attributed to the rich content of sulfated flavonoids, particularly derivatives of isorhamnetin-sulfate [18], which proved to possess higher antioxidant activity than α-tocopherol [61].

Table 4. Chemical composition and antioxidant and enzymatic activities of the *H. portulacoides* leaves extract.

Assay	*H. portulacoides* Extract
Chemical composition	
TPC (mg GAE/g extract)	16.10 ± 0.20
TFC (mg QCE/g extract)	26.60 ± 0.80
Antioxidant activity	
DPPH (IC_{50} mg/mL)	3.70 ± 0.40
ABTS (IC_{50} mg/mL)	>5
β-carotene/linoleic acid (IC_{50} mg/mL)	0.15 ± 0.03
Lipid peroxidation (IC_{50} mg/mL)	>5
Metal chelating ability (IC_{50} mg/mL)	2.30 ± 0.50
FRAP (mg TE/g extract)	19.90 ± 1.90
CUPRAC (mg TE/g extract)	44.00 ± 2.30
Enzymatic activity	
AChE inhibition (IC_{50} mg/mL)	>5

GAE, gallic acid equivalents; QCE, quercetin equivalents; TE, Trolox equivalents.

3.5. Antioxidant and Enzymatic Activities

The ethanolic extract of *H. portulacoides* exhibits a higher DPPH activity (IC50 = 3.70 ± 0.40 mg/mL) when compared to data obtained for extracts using other solvents (IC50 > 10 mg/mL) and analogous ABTS scavenging capacity as reported hexane, methanol, and water extracts [16]. When compared to the inhibitory potential of other halophytes, *H. portulacoides* (IC50 = 0.15 mg/mL) shows higher activity than *Suaeda pruinose, Suaeda maritima*, and *Suaeda mollis* (IC50 values of 0.54, 1.42, and 0.54 mg/mL, respectively) [62]. Nevertheless, it is still considerably less active than the antioxidant BHT (IC50 = 0.005 mg/mL) [31]. Regarding the TBARS assay, the *H. portulacoides* extract did not present the capacity to inhibit lipid peroxidation in the range of concentrations tested.

Antioxidants are able to chelate and reduce prooxidant metal ions responsible for the production of ROS, e.g., the ferrous ions that produce free radicals via the Fenton reaction [63]. The *H. portulacoides* extract is more effective in reducing copper (CUPRAC—44.00 mg TE/g extract) than iron (FRAP—19.90 mg TE/g extract). This was already reported for water, methanol, and ethyl acetate extracts of *H. portulacoides*, with FRAP values ranging from 31.59 to 50.06 mg TE/g extract and CUPRAC from 51.83 to 71.21 mg TE/g extract [60].

The acetylcholinesterase inhibition was tested for the *H. portulacoides* extract, though no significant activity was observed in the range of concentrations tested. Previous studies had already reported a very modest activity for water, methanol, and ethyl acetate extracts of *H. portulacoides* against AChE [60]. Nevertheless, these results contrast with those reported for ethanolic extracts of the leaves of other halophytes, such as *Armeria pungens*, with a high activity against AChE (IC50 = 90.3 μg/mL) [64].

The results for the β-carotene bleaching assay were obtained after 2 h of reaction. Values represent the mean ± standard deviation of three independent experiments.

3.6. Sensory Evaluation

The assessors, 30% men and 60% women, were aged between 18 and 63 years old, being on average of 31.25 ± 14.12 years.

The test is presented with two coded samples to evaluate the product attributes in the hedonic scale. Figure 5 shows the control and enriched pasta with *H. portulacoides*.

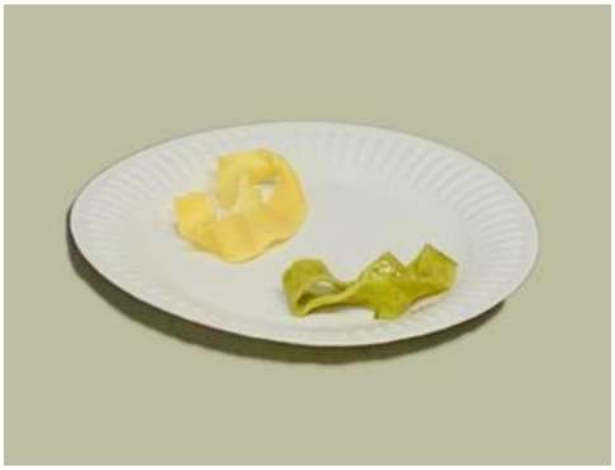


Figure 5. Control (non-enriched) and enriched pasta with *H. portulacoides*.

Figure 6 shows the results of the sensory evaluation of four pasta attributes: color, flavor, texture, and overall acceptance; all were expressed in the scale from 1 (dislike extremely) to 9 (like extremely). Among the sensorial parameters, texture showed the best results (7.2 and 7.7, respectively, for enriched pasta and control pasta). The overall acceptance also had results very close to 7.6 for the pasta without *H. portulacoides* and close to 6.8 for the pasta with *H. portulacoides*. The attributes of color and flavor had,

respectively, scores of 8 and 6.5 for the enriched pasta and around 7.7 and 7.5 for the control pasta. Although the lower scores were obtained for the enriched pasta, its acceptance was reasonable. Statistical analysis showed that the differences between the mean scores for the control and enriched pastas were significant for attributes flavor ($p = 0.004$) and overall acceptance ($p = 0.003$), while for the other attributes (color and texture), there were no significant differences ($p > 0.05$). Figure 6 shows the results of the sensory evaluation of four pasta attributes: color, flavor, texture, and overall acceptance, which are all expressed in the scale from 1 (dislike extremely) to 9 (like extremely). Among the sensorial parameters, texture showed the best results (7.2 and 7.7, respectively, for enriched pasta and control pasta). The overall acceptance also had results very close to 7.6 for the pasta without *H. portulacoides* and close to 6.8 for the pasta with *H. portulacoides*. The attributes of color and flavor had, respectively, scores of 8 and 6.5 for the enriched pasta and around 7.7 and 7.5 for the control pasta. Although the lower scores were obtained for the enriched pasta, its acceptance was reasonable. Statistical analysis showed that the differences between the mean scores for the control and enriched pastas were significant for attributes flavor ($p = 0.004$) and overall acceptance ($p = 0.003$), while for the other attributes (color and texture), there were no significant differences ($p > 0.05$).

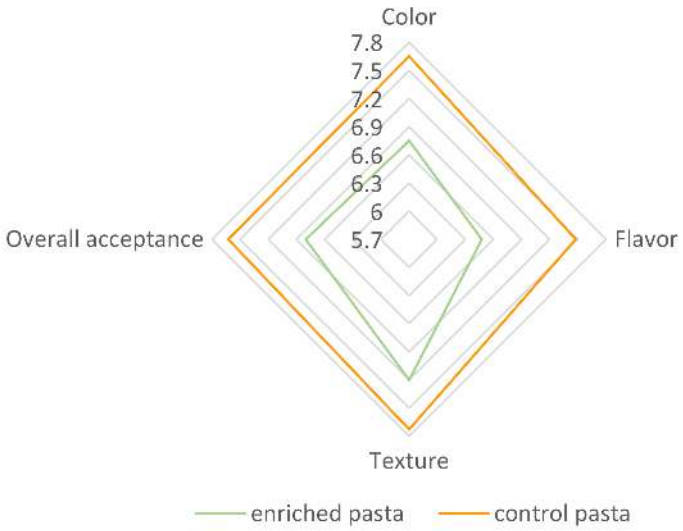


Figure 6. Sensory analysis for control (non-enriched) and enriched pasta with *H. portulacoides*.

When asked about the purchasing preference, 37.5% of the panel members preferred pasta enriched with *H. portulacoides* and 62.5% preferred control pasta.

Figure 7 shows the butter enriched with *H. portulacoides*.



Figure 7. Butter enriched with *H. portulacoides*.

Figure 8 presents the results of the sensory evaluation of six parameters: appearance, color, flavor, scent, texture, and overall acceptance to enriched and control butter. It should be noted that the enriched butter had the higher scores in all organoleptic characteristics. Texture had the higher scores for both butters, but the enriched butter presented the highest value. The overall acceptance and appearance of fortified butter also scored with values of 8.15 in both cases, while the values were, respectively, 7.48 and 7.60 for the butter without *H. portulacoides*. Overall, the tasters preferred the butter with *H. portulacoides* to the control butter. Statistical analysis revealed that significant differences were found between the control and enriched butter samples only for the mean scores for appearance ($p = 0.003$), while for all other attributes, the differences were not significant ($p > 0.05$).

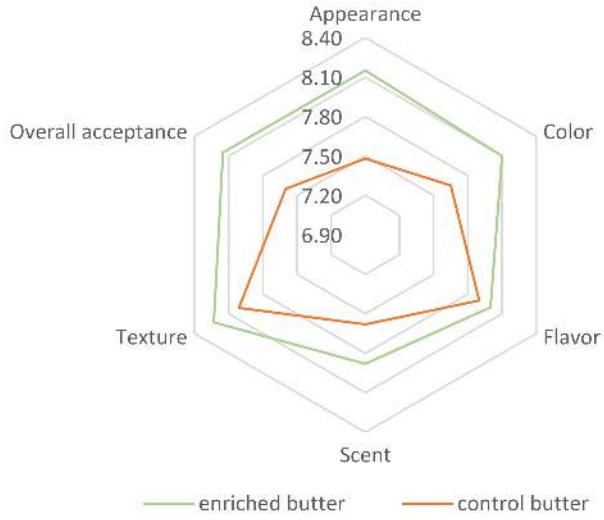


Figure 8. Sensory analysis for control (non-enriched) and enriched butter with *H. portulacoides*.

Moreover, when asked about the purchasing preference, only 7.5% of the panel members referred that they would not buy enriched butter, resulting in a good market acceptance if this product would be available in the market. Although both products had been enriched with dried powder leaves of *H. portucaloides*, their acceptability is different, depending strongly on the type of product.

4. Conclusions

The present work determined the nutritional and mineral profile of *H. portulacoides* leaves collected in the salterns of Figueira da Foz (Portugal) and their biological activity. The exploitation of powder dried leaves as a salt substitute and enhancer of sensory characteristics of foods (pasta and butter) was also assessed.

The halophyte sea purslane plant may be considered a good source of dietary fiber, protein, and lipids, presenting higher concentration of these nutrients than some Salicornia species that are halophyte plants suitable for human consumption and considered as promising functional foods. Moreover, high concentration of minerals such as sodium, potassium, calcium, magnesium, and phosphorous were found in sea purslane leaves. Although they have low concentration of manganese, the ingestion of 100 g of fresh leaves provides 74% of the daily dose recommended for adults.

The *H. portulacoides* leaves extract with the green solvent (ethanol) yielded more phenolic compounds than extractions using other organic solvents and higher content in flavonoids when compared to other halophytes such as Ipomoea pes-caprae. Moreover, an increased antioxidant potential measured by the DPPH and ABTS radical scavenging assays was found compared to the use of other solvents and when compared to other halophytes such as *Suaeda* species. In addition, the *H. portulacoides* leaves extract was more effective in reducing copper than iron, as assessed by the CUPRAC and FRAP assays.

In terms of its use as a novel ingredient, butter and pasta enriched with powder dried leaves of *H. portulacoides* revealed the plant's potential to be used as a salt substitute that enhances the sensory characteristics of products, providing health benefits to the consumers.

Author Contributions: Conceptualization, A.M.d.S. and M.J.B.; methodology, A.P., S.A., S.R. and J.M.; software, J.M. and R.G.; validation, A.M.d.S., M.J.B. and R.G.; formal analysis, A.M.d.S., M.J.B. and R.G.; investigation, A.P., S.R.; resources, A.P., S.A., S.R., M.J.B., J.M., R.G. and A.M.d.S.; data curation, A.M.d.S., M.J.B. and R.G.; writing—A.P., M.J.B. and A.M.d.S.; original draft preparation, A.P., M.J.B. and A.M.d.S.; writing—review and editing, A.M.d.S., M.J.B. and R.G.; visualization, A.M.d.S., M.J.B. and R.G.; supervision, A.M.d.S. and M.J.B.; project administration, A.M.d.S. and M.J.B.; funding acquisition, A.M.d.S. and M.J.B. All authors have read and agreed to the published version of the manuscript."

Funding: This research was funded by the Portuguese Foundation for Science and Technology (UID/MULTI/00070/2019) and from the European Regional Development Fund, through Portugal 2020-POCI-01-0145-FEDER-029305, IDEAS4life—Novos IngreDiEntes Alimentares de Plantas MarítimaS; and Centro 2020-Centro-01-0145-FEDER-000007, Project ReNATURE—Valorization of the Natural Endogenous Resources of the Centro Region.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors would like to thank Gilda Saraiva for the halophyte supply. A very special thanks is owed to David Gomes, Maria Adélia Vaz, and Maria Lurdes Pires for assisting in the manufacture of butter and in the incorporation of dried powder of *H. portulacoides* leaves.

Conflicts of Interest: The authors declare no conflict of interest.

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