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Microbial and Sensory Evaluation of Halophytes Cultivated in a Soilless System Under Different Salinities

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Abstract

The increasing interest in halophytes as sustainable crops and their potential functional properties highlights the need to understand how cultivation conditions affect their quality for human consumption. The present study aimed to evaluate the influence of salinity on the microbial quality of the halophytes *Disphyma crassifolium*, *Inula crithmoides*, *Mesembryanthemum nodiflorum*, and *Suaeda maritima*, cultivated using a soilless system under different salinities. The sensorial quality of *D. crassifolium* and *S. maritima* was also assessed by an experienced panel of culinary chefs. The microbial quality was measured by counting aerobic microorganisms (30 °C and 6.5 °C), fungi, *Escherichia coli*, and coagulase-positive staphylococci. Salinity increase caused a concentration-dependent salt accumulation in the plants, triggering a rise in the microbial populations, namely aerobic and filamentous fungi on *D. crassifolium* and *I. crithmoides* and psychrotrophic microorganisms on *S. maritima* ($p < 0.05$). Except for *M. nodiflorum*, plants cultivated at the highest salinity (465 mM) had levels of filamentous fungi higher than 3 Log CFU/g. Concerning aerobes, plants presented a satisfactory microbiological quality (<6 Log CFU/g) even when cultivated at high salinity (465 mM), and yeasts, *E. coli*, and staphylococci were never detected. *D. crassifolium* and *S. maritima* cultivated at intermediate salinities were preferred by the sensory evaluation panel. In conclusion, soilless system cultivation produces plants adequate for consumption, considering their microbial and sensorial quality.

Keywords: safe production; *Disphyma crassifolium*; *Inula crithmoides*; *Mesembryanthemum nodiflorum*; *Suaeda maritima*; soilless systems

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1. Introduction

Halophytes are plants adapted to thrive in environments with high salinity, such as coastlines, salt marshes, and mangroves [1,2]. The ability of halophytes to survive in extreme salinity is attributable to their morphophysiological strategies, such as salt exclusion, salt elimination by the root system, salt excretion through specialised organs (salt glands or salt bladders), and diluting ions using succulence mechanisms. The halophytic adaptation

to salinity is also associated with some biochemical mechanisms, such as (a) ion homeostasis, involving various channels and transporters, to prevent salt entrance to the roots, and vacuolar compartmentalisation of ions; (b) the osmotic adjustment attained through uptake of inorganic ions, and the de novo synthesis of organic osmolytes, to maintain turgor pressure and organellar volume within the growing plant cells; and (c) efficient antioxidant defence mechanisms to protect plants from oxidative stress resultant from the accumulation of reactive oxygen species (ROS) produced under high salinity [3,4]. These adaptations allow halophytes not only to survive but to flourish in conditions that are inhospitable to most crops, but they may also interfere with the nutritional, microbial, and sensorial attributes, namely in their taste and texture, when these plants are used as foods. Recent research has highlighted the important ecological roles of halophytes, particularly in coastal ecosystems, where they contribute to erosion prevention, bioremediation of saline environments through pollutant removal, and the restoration of degraded soils via phytodesalination [2,5–7].

Among the most studied halophytes are *Disphyma crassifolium* (Aizoaceae), a low-growing annual succulent with round leaves and pink to violet flowers; *Inula crithmoides* (Asteraceae), a perennial succulent with fleshy green to yellow leaves reaching up to 1 m in height; *Mesembryanthemum nodiflorum* (Aizoaceae), an annual plant forming horizontal clumps with tender, salty, and acidic leaves; and *Suaeda maritima* (Amaranthaceae), characterised by branched stems and sessile curved leaves that turn red with age [8–12]. These species are commonly found in saline marshes and saltmarsh zones of the Mediterranean, as well as in regions such as Macaronesia and the Middle East [2,12]. These plants are increasingly recognised for their ecological resilience and economic potential in saline agriculture [5,13]. Their ability to thrive in marginal soils makes them keystone species for rehabilitating degraded coastal and arid ecosystems, stabilising sediments, and reducing soil salinity [3,7,14]. Economically, these plants offer sustainable alternatives for regions facing freshwater scarcity, with some species already commercialised in markets around the Mediterranean [15]. The integration of halophytes into soilless cultivation systems presents an opportunity to optimise yield, reduce land competition, and address global food security challenges intensified by climate change [16].

Beyond their ecological benefits, halophytes possess significant nutritional and medicinal value [17]. They are rich in bioactive compounds, including vitamins, carotenoids, polyphenols, omega-3 fatty acids, and flavonoids, some of which have demonstrated antihypertensive and antimicrobial effects [1,2,17,18]. For example, *D. crassifolium* is noted for its antioxidant activity; *I. crithmoides* contains relevant levels of vitamins B1, B6, β-carotene, and lutein; *M. nodiflorum* is a good source of vitamins A, C, and B6 and carotenoids, and *S. maritima* is associated with a wide range of metabolites and high antioxidant potential [2,11,18–20]. These nutritional attributes have led to increased interest in the agricultural production, commercialisation, and consumption of halophytes, both as functional foods and as novel ingredients in gourmet cuisine.

While the agronomic potential and phytochemical profiles of halophytes have been widely studied, their microbial quality, particularly under controlled cultivation conditions, such as soilless systems is rather unknown. Most research has focused on their growth and nutritional value, with limited attention given to how salinity regimes influence microbial populations in edible halophytes [2,12,17,21,22]. This is a significant concern, as halophytes, like all plants, are naturally colonised by diverse microbial communities, including bacteria, viruses, and fungi, which are usually non-pathogenic. However, the microbial quality of food is of concern, not only from the food safety point of view but also due to the spoilage associated with the decrease in shelf-life, which may result in vast commercial losses. The growth of microbial populations in foods leads to the deterioration of nutritional quality

and is associated with the alteration of organoleptic properties. Notably, there are no established microbiological criteria specifically for halophytes under European Union food legislation, despite their increasing use as raw or cooked vegetables.

Considering the growing interest in the consumption and commercialisation of halophytes, this study investigated how different salinity levels in soilless cultivation systems affect not only the growth but also the microbiological and sensory quality of *D. crassifolium*, *I. crithmoides*, *M. nodiflorum*, and *S. maritima*. The research focused on the microbiological quality, including the presence of *Escherichia coli* and coagulase-positive *Staphylococcus* in these plants. In addition, the study examined the sensory properties of *D. crassifolium* and *S. maritima*, aiming to identify salinity ranges that balance food safety and consumer appeal, while the sensory characteristics of *I. crithmoides* and *M. nodiflorum* have already been described in a previous study. By bridging aspects of agricultural research with microbiological quality and sensory evaluation, this work aims to advance the practical utilisation of halophytes, providing evidence-based strategies for their safe and appealing production in controlled environments.

2. Materials and Methods

2.1. Experimental Setup and Halophyte Species

The study took place in a 200 m^2 polyethylene greenhouse at RiaFresh facilities (Portugal) from early spring to mid-autumn (March to October) under controlled conditions of temperature and relative humidity (%RH). The four species studied, *D. crassifolium*, *M. nodiflorum*, *I. crithmoides*, and *S. maritima*, were grown in a closed, soilless system, with seeds directly sown in the substrate within honeycombed trays. All halophyte species were developed under natural photoperiod and temperature. Figure 1 shows the species studied and cultivated in the greenhouse.

Figure 1. Images of the four halophyte plant species studied: (**a**) *Suaeda maritima*, (**b**) *Disphyma crassifolium*, (**c**) *Inula crithmoides*, and (**d**) *Mesembryanthemum nodiflorum*.

2.2. Growth Conditions

Six distinct salinity treatments (35, 110, 200, 275, 350, and 465 mmol L^{-1} NaCl) were administered across three cultivation tables, each containing three randomly distributed trays.

Each tray comprised one replicate per species, occupying about 0.18 m^2. The salinity levels were defined considering the range between a semi-saline medium (<40 mmol L^{-1} NaCl) and the salinity of seawater (630 mmol L^{-1} NaCl). Individual tanks with added NaCl dissolved in the nutrient solution represented each salinity level.

Nutrient solutions were prepared in 500 L tanks using fresh water from a well. Their composition (proprietary to RiaFresh®) ensured balanced nutrition with all essential macro- and micronutrients, and no deficiencies were observed throughout the trial. An air pump and diffusion system provided continuous aeration. Simulated tidal irrigation involved two daily floods. Solution monitoring included daily pH (Hanna Instruments Portugal, Póvoa de Varzim, Portugal) and electrical conductivity (EC, dSm^{-1}, Hanna Instruments Portugal, Póvoa de Varzim, Portugal) measurements, with nitrate concentration (NO_3^-, mg L^{-1}) assessed twice a week, following the procedure described by Hoather and Rackham [23]. Briefly, filtered samples were acidified and analysed for NO_3^- by absorption at 220 nm (corrected at 275 nm), using a UV–visible spectrophotometer (UV-160 A, Shimadzu, Kyoto, Japan). Nutritive solutions were refilled twice a week and completely replaced at least once a month.

Harvesting was performed when each species' stem reached at least 20 cm. Pruning shears were used, and the fresh weight of collected biomass was immediately recorded. Productivity was quantified based on harvested aerial part fresh weight per area and time after sowing (g m^{-2} day^{-1}). Relative productivity per salinity level was calculated as a percentage variation compared to the lowest salinity (35 mmol L^{-1}). For each treatment and replicate (at least six replicates of each cultivation condition), an aboveground biomass sample was collected, refrigerated, frozen (−20 °C), and freeze-dried before milling in a planetary ball mill (Retsch-PM 100, Retsch GmbH, Haan, Germany). Powdered samples were stored in a desiccator until analysis. The electrical conductivity (EC) was measured to translate the salty flavour of samples using a conductivity meter (Hanna Instruments Portugal, Póvoa de Varzim, Portugal).

2.3. Microbial Quality Evaluation

The microbial quality of the halophytes, *Disphyma crassifolium*, *Inula crithmoides*, *Mesembryanthemum nodiflorum*, and *Suaeda maritima*, cultivated using a soilless technique in three different concentrations (35, 200, and 465 mM of NaCl) was evaluated through the enumeration of aerobic microorganisms at 30 °C and 6.5 °C, filamentous fungi and yeasts, *Escherichia coli*, and coagulase-positive staphylococci, according to the International Organization for Standardization (ISO) standards ISO 4833-1:2013 [24], ISO 17410:2019 [25], ISO 21527-1:2008 [26], ISO 16649-2:2001 [27], and ISO 6888-1:2003 [28], respectively. For each analysis, 10 g of each plant was aseptically collected, mixed with 90 mL of sterile Ringer solution (Oxoid, Basingstoke, UK), and homogenised for 2 min (Model 400 Circulator, Seward, Norfolk, UK). Serial dilutions were performed using a sterile Ringer solution. The aerobic microorganisms at 30 °C were determined after the incorporation of aliquots of 1 mL in Plate Count Agar (PCA) (Scharlau, Barcelona, Spain) and incubated at the mentioned temperature for 3 days. The psychrotrophic aerobic microorganisms (6.5 °C) were determined by spread-plating aliquots of 0.1 mL onto the surface of the PCA, following incubation for 10 days.

Filamentous fungi and yeasts were counted on Dichloran Rose Bengal Chloramphenicol Agar Petri dishes (Scharlau, Barcelona, Spain) incubated at 25 ± 1 °C for 2 to 5 days. To enumerate *E. coli*, aliquots of 1 mL were inoculated in Chromocult Triptone Bile X-glucuronide Agar (TBX) (Merck, Darmstadt, Germany), following incubation at 44 °C for 24 h. The enumeration of the coagulase-positive staphylococci was performed by inoculating Baird-Parker medium (Oxoid) with 0.1 mL of the dilutions, followed by

incubation at 37 °C during 24–48 h, according to the ISO 6888-1 [28]. The Staphylase Test (Oxoid) was used to test for the coagulase reaction of typical and atypical colonies. Aliquots of the dilutions were inoculated in duplicate, for all determinations.

The results were expressed as Log CFU/g of plant fresh weight. Experimental values are expressed as means ± standard deviation.

2.4. Sensory Analytical Method

Quantitative descriptive analysis (QDA) was employed to evaluate the organoleptic characteristics of *Disphyma crassifolium* and *Suaeda maritima* cultivated at six different salinities (35, 110, 200, 275, 350, and 465 mM NaCl), following the methodology outlined by Lima et al. [12]. In the referenced study, the authors assessed the same sensory attributes for *Inula crithmoides* and *Mesembryanthemum nodiflorum*. The sensory indicators for this study were established through preliminary discussions with a fine-dining chef with large experience using these plants in gourmet cuisine, referencing established descriptors for the evaluation of the halophyte plants. The selected attributes included flavour (salty and bitter taste), texture (crunchiness, juiciness, amount of fibre), physical parameters (appearance, plant length), and overall appreciation. Each attribute was precisely defined prior to the evaluation; for example, "crunchiness" referred to the perceived crispness during mastication, "juiciness" to the release of liquid upon biting, and "amount of fibre" to the perceived fibrousness or stringiness of the sample.

Quantification was performed using a four-point intensity scale specifically constructed for this study. For salty taste, bitter taste, and crunchiness, the scale ranged from "not enough" to "too much"; for the amount of fibre, from "non-existent" to "excessive"; for plant length, from "too short" to "too long"; and for appearance and overall appreciation, from "very bad" to "excellent". These scoring principles were communicated in a detailed guideline document to all panellists before evaluation, ensuring a shared understanding of the criteria.

The tasting panel consisted of ten award-winning fine-dining chefs, with a mean professional experience of sixteen years. All chefs were selected based on their expertise in the use of halophyte plants in gourmet cuisine and their prior experience in sensory analysis. To enhance objectivity and reliability, a calibration session was conducted online before the formal evaluation. During this session, panellists discussed and aligned their understanding of each attribute and the scoring system, using reference samples where possible. This process also served to clarify terminology and ensure consistency in the application of the evaluation criteria according to QDA methodology requirements defined by Stone et al. [29].

To minimise individual preference bias and ensure impartiality, all samples were coded with random three-digit numbers and presented in a randomised order to each chef, ensuring a blind tasting. Chefs were instructed to refrain from discussing their assessments with others during the evaluation and were reminded to base their ratings solely on the defined criteria. Completed questionnaires were submitted online ensuring the confidentiality and independence of responses. The questionnaire itself was reviewed and pilot-tested by one of the chefs to confirm clarity and appropriateness of language. Given the high level of expertise of the panel and the calibration session, no further formal training was deemed necessary. These procedures ensured that the evaluation was rigorous and reproducible, with careful steps taken to control for potential biases and enhance the reliability and objectivity of the sensory data.

2.5. Statistical Analysis

Statistical analysis was performed using Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA). Results were expressed as mean ± standard error of the mean, and experiments were conducted at least in triplicate. Significant differences were assessed by analysis of variance (ANOVA) using the Tukey HSD (honestly significant differences) test, or Duncan's new multiple range test when parameterisation of the data did not prevail.

3. Results and Discussion

3.1. Effects of Salinity on Productivity and Conductivity

Increasing cultivation salinity negatively impacted plant growth (Figure 2a). Similarly to what had been observed with *M. nodiflorum* and *I. crithmoides* [2], the species *D. crassifolium* exhibited a significant decrease (around 50%) in productivity at a salinity of 110 mmol L^{-1}, compared to samples grown at 35 mmol L^{-1}. At the highest salinity tested (465 mmol L^{-1}), productivity dropped to 95%. *S. maritima* displayed similar responses, although slightly less pronounced. Its productivity decreased by 40% with the increase from 35 to 110 mmol L^{-1}, and 86% at 465 mmol L^{-1}. These findings align with previous results by Aghaleh et al. [30] and Lima et al. [2], who observed reduced growth in *Salicornia persica*, *S. europaea*, and *S. ramosissima* at salinities exceeding 100 mmol L^{-1}. Similarly, Lima et al. [12] reported a 57% decrease in relative productivity for *I. crithmoides* when salinity was increased from 35 to 110 mmol L^{-1}. However, *M. nodiflorum* exhibited a contrasting behaviour, achieving maximum productivity between salinities of 35 and 100 mmol L^{-1}. These results indicate that the synergistic response between crop salinity and productivity is specific to each halophyte plant species. Nonetheless, the increase in salt concentration has undoubtedly resulted in a significant escalation in strategies employed to maintain ion and osmotic homeostasis, which, in turn, resulted in high energy expenditures, explaining the observed decline in productivity [31].

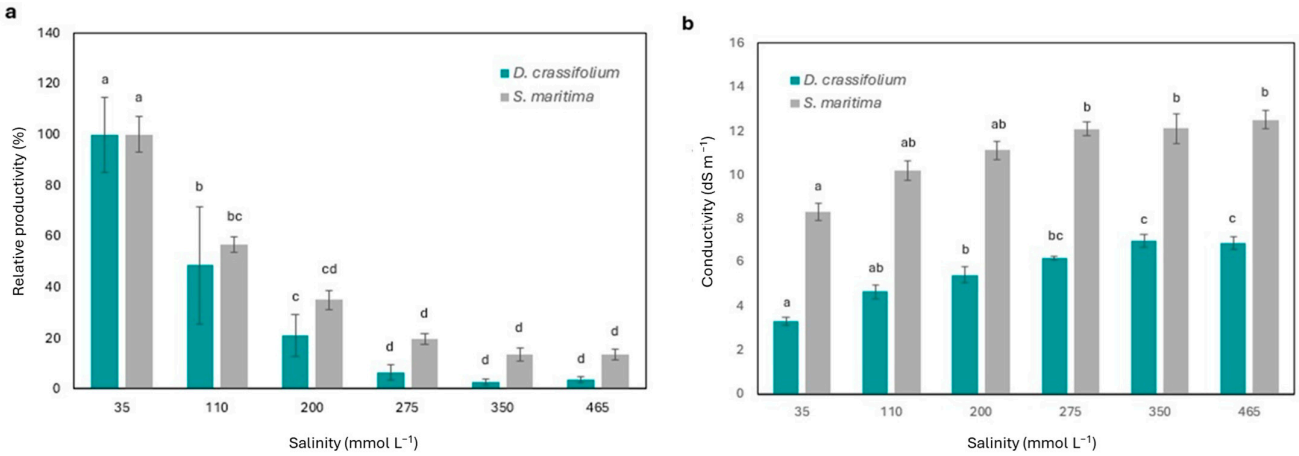

Figure 2. Relative productivity (**a**) and conductivity (**b**) of *D. crassifolium* and *S. maritima* in salinities between 35 and 465 mmol L^{-1}. Bars of the same colour labelled with different letters are significantly different ($p < 0.05$). SEM error bars were used for statistical comparisons between experimental treatments.

The conductivity of both species (Figure 2b) showed a slight increase from 110 mmol L^{-1} with stabilisation at higher salinities (275 to 465 mmol L^{-1}), which shows an acclimation linked with the ability to regulate salt concentration in the tissues.

3.2. Microbial Quality

The effect of increasing salinity on the microbial quality of the halophytes *Disphyma crassifolium*, *Inula crithmoides*, *Mesembryanthemum nodiflorum* and *Suaeda maritima* are shown in Table 1.

Table 1. Microbial quality of halophyte plants studied.

Halophyte Species	Microbial Groups (Log CFU g/plant $^{-1}$)	Salinity (mmol L^{-1})		
		35	200	465
D. crassifolium	Aerobic microorganisms at 30 °C	2.00 ± 0.06 ^{aA}	3.08 ± 0.05 ^{bB}	3.61 ± 0.05 ^{cC}
M. nodiflorum		2.98 ± 0.03 ^{aB}	3.26 ± 0.12 ^{aB}	3.06 ± 0.08 ^{aB}
I. crithmoides		4.54 ± 0.20 ^{aC}	5.18 ± 0.14 ^{bC}	5.38 ± 0.05 ^{bD}
S. maritima		2.65 ± 0.03 ^{bB}	2.48 ± 0.01 ^{aA}	2.60 ± 0.06 ^{abA}
D. crassifolium	Aerobic microorganisms at 6.5 °C (psychrotrophics)	2.00 ±0.01 ^{aA}	2.35 ± 0.49 ^{aA}	3.07 ± 0.16 ^{aB}
M. nodiflorum		2.35 ± 0.49 ^{aA}	2.15 ± 0.21 ^{aA}	2.97 ± 0.10 ^{aB}
I. crithmoides		2.35 ± 0.49 ^{aA}	2.50 ± 0.28 ^{aA}	2.15 ± 0.21 ^{aA}
S. maritima		2.48 ± 0.01 ^{aA}	2.80 ± 0.14 ^{abA}	3.14 ± 0.20 ^{cB}
D. crassifolium	Filamentous fungi	2.45 ± 0.21 ^{aAB}	3.80 ± 0.03 ^{bA}	4.38 ± 0.05 ^{cC}
M. nodiflorum		2.15 ± 0.21 ^{aA}	1.24 ± 1.75 ^{aA}	2.30 ± 0.43 ^{aA}
I. crithmoides		2.63 ± 0.21 ^{aAB}	3.00 ± 0.06 ^{aA}	3.11 ± 0.01 ^{aAB}
S. maritima		3.03 ± 0.11 ^{aB}	3.02 ± 0.09 ^{aA}	3.29 ± 0.02 ^{aB}
D. crassifolium	Yeasts	≤2	≤2	≤2
M. nodiflorum		≤2	≤2	≤2
I. crithmoides		≤2	≤2	≤2
S. maritima		≤2	≤2	≤2

Different uppercase letters in the same column indicate significant differences ($p < 0.05$) between halophyte species at each salinity, and different lowercase letters in the same line indicate significant differences ($p < 0.05$) between salinity levels for each species (≤2 Log CFU/g, indicates below the detection limit).

The increase in salinity of the nutritive solution was accompanied by a general rise in the microbial populations studied, namely the total aerobic and filamentous fungi on *D. crassifolium* and *I. crithmoides* and the psychrotrophic population on *S. maritima* ($p < 0.05$). The psychrotrophic population was less abundant when compared to the values of the mesophylls. This rise in microbial populations in conditions of salt stress was also observed in *Salicornia ramosissima* [2]. As mentioned above, the increase in the cultivation salinity of the nutritive solution during the growth of the four plants implies the adoption of ionic and osmotic homeostasis strategies, which are certainly different in the plants studied. *D. crassifolium* loses succulence and increases osmotic metabolite (e.g., proline) production due to salt increase [32]. Similarly, *I. crithmoides* increases the production of osmolytes, such as glycine betaine, and sugars (arabinose, fructose, and glucose). Still, it also activates a K^+ transportation system, which may contribute to its salt tolerance [33]. *M. nodiflorum*, similarly to other *Mesembryanthemum* species, accumulates salt and water in epidermal bladder cells that act as a water storage reservoir, which helps them to maintain ion sequestration and homeostasis in the leaves [34]. Finally, *S. maritima* accumulates inorganic ions and proline to adapt to saline conditions, decreasing its transpiration rate and improving its water use efficiency [35,36]. These different responses to salt stress may result in different degrees of colonisation by microbial populations, as seen in this work.

The highest numbers of aerobic microorganisms were observed in *I. crithmoides* (4.54, 5.18, 5.38 Log CFU g^{-1} in the 35, 200, and 465 mM of NaCl, respectively) and the lowest numbers of filamentous fungi on *M. nodiflorum* (2.15, 1.24, and 2.30 Log CFU g^{-1} in the three concentrations, respectively). In the work of Lima et al. [12], *M. nodiflorum* extracts showed an increasing trend in phenolic production with salt stress, while *I. crithmoides* appeared to be more susceptible, losing the ability to produce phenolic compounds at salin-

ities of 100 mmol L^{-1} or higher. In addition, *M. nodiflorum* showed a significant increase in the antioxidant capacity observed up to a salinity of 275 mmol L^{-1}. The fact that *M. nodiflorum* presents higher phenolic content and higher antioxidant capacity may explain the inferior microbial colonisation observed when compared to *I. crithmoides*, namely regarding aerobic microorganisms at 30 °C and filamentous fungi, as phenolics are known to inhibit the growth of different bacteria and fungi [37].

Yeasts, *E. coli*, and *S. aureus* were not detected on these halophytes in any growing conditions. However, except for *M. nodiflorum*, plants cultivated at the highest salinity (465 mM) showed a level of filamentous fungi higher than 3 Log CFU g^{-1}. Nonetheless, regarding contamination with aerobes, the halophytes studied presented a satisfactory microbiological quality (<6 Log CFU g^{-1}) even when plants were cultivated at the highest salinity tested (465 mM). Overall, plants grown with low salinity levels (35 mM) revealed better microbial quality, being classified as satisfactory when considering aerobic microorganisms grown at 30 °C, and acceptable when considering fungi. Additionally, none of the samples studied contained *E. coli*, so it can be said that the halophytes analysed fulfilled the microbiological criteria of food hygiene according to the European Commission [38,39]. Results of the present study indicate that soilless cultures using low salinities produce plants adequate for consumption, considering their microbial quality. Antioxidative defence systems, especially non-enzymatic antioxidants (like flavonoids, ascorbate, glutathione, and tocopherols) but also enzymatic antioxidants (like superoxide dismutase, catalase, and glutathione reductase), are part of the mechanisms that enable halophytes to thrive in high salt conditions [3]. They may also be implicated in antimicrobial defence systems in some halophyte species/strains such as *M. nodiflorum* and *S. maritima*. On the other hand, different species of halophytic plants certainly develop distinct adaptive strategies depending on their gene pool and the environment in which they grow. In the case of *D. crassifolium*, hydroxybenzoic acids, which are antimicrobial and antifungal agents, were not detected, thus justifying the increase in the number of filamentous fungi, with increasing salinity in the plants studied (Table 1). In addition, this species has been described as having an efficient osmolyte accumulation system [40], and we hypothesise that these osmolytes constitute food reserves for some microbial groups (filamentous fungi). The present study's results indicate that soilless cultures using low salinities produce plants adequate for consumption, considering their microbial quality. However, the growth of the microbial populations during storage was not evaluated, and thus, the shelf-life of plants at different temperatures, including refrigeration, was not evaluated.

3.3. Sensory Properties

To assess the commercial potential of these plants, an organoleptic evaluation was conducted by a trained sensory panel comprising highly acclaimed chefs, many of whom were awarded Michelin stars. This approach aimed to identify the plant with the most desirable sensory characteristics. Employing the quantitative descriptive analysis (QDA) method, the chefs defined the relevant sensory attributes for evaluation. Each chef received a set of plants grown at varying salinity levels and evaluated them based on predetermined parameters, which included saltiness, bitterness, crunchiness, juiciness, fibre content, visual appearance, and plant length. The responses were ranked and statistically analysed, with the results for species *D. crassifolium* and *S. maritima* presented in Figure 3. Results concerning the other two species were previously published in Lima et al. [12].

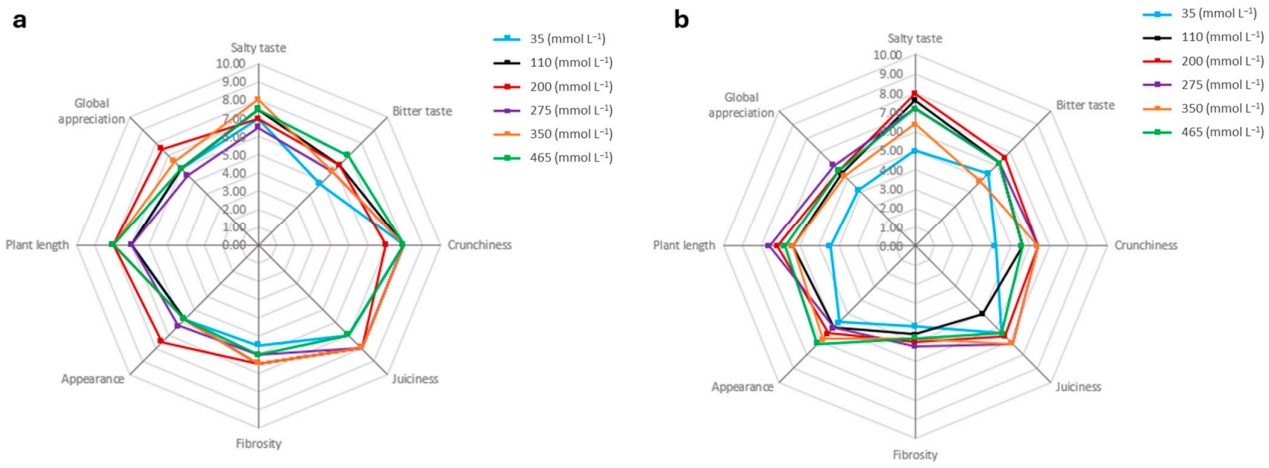

Figure 3. Sensory profile of *D. crassifolium* (**a**) and *S. maritima* (**b**) grown at six different salinity levels determined by QDA test. The distance from the centre point indicates the relative intensity of each attribute, with greater distance signifying a strong.

Despite a wide range in cultivation salinity (35–465 mmol L^{-1}), most sensory attributes of the analysed halophyte plant samples displayed subtle differences. This aligns with previous findings by Lima et al. [12] for *M. nodiflorum* and *I. crithmoides* grown under similar salinity variations, using the same sensory attributes, quantitative descriptive analysis (QDA) method, and trained tasters. As in Lima et al. [12], the most noticeable sensory differences between salt concentration in the cultivating medium were observed for prominent attributes like salty and bitter tastes in both species, although attributes like crunchiness and juiciness were also discriminant of salinity in *S. maritima*. The most pronounced sensory changes occurred in this species, with the plants grown at the lowest salinity tested scoring poorer for all attributes except juiciness. Notably, the "crunchiness" attribute had the lowest average score. These results are supported by other studies that showed that increasing cultivation salinity intensifies saltiness, while lower salinities enhance firmness but reduce juiciness and affect colour [12,41]. Moreover, Martins-Noguerol et al. [42] reported that higher salinity reduces the content of monounsaturated and polyunsaturated fatty acids and proteins, directly impacting the texture, liquid retention, taste, and juiciness of samples grown at the highest NaCl concentration (450 mmol L^{-1}). Common to both tested species was the fact that the intermediate salinities tested had the best scores in "Global Appreciation". The scores of juiciness, crunchiness, and amount of fibre contributed the most to these results. The accumulation of osmolytes and succulence mechanisms to maintain the cell turgor pressure under higher saline conditions are strategies adopted by various halophytic plants for ion and osmotic homeostasis, explaining the general increasing juiciness and crunchiness attributes of plants used in the present work as salinity increases, particularly in *D. crassifolium*. Lima et al. [12] also found that *I. crithmoides* and *M. nodiflorum* grown at intermediate concentrations of NaCl performed better.

In summary, lower salinities applied in soilless cultivation systems provide a more balanced organoleptic profile for halophyte species.

4. Conclusions

This study demonstrated that soilless cultivation of *Disphyma crassifolium*, *Inula crithmoides*, *Mesembryanthemum nodiflorum*, and *Suaeda maritima* ensures microbiological quality (<6 Log CFU/g aerobes; absence of *E. coli*, staphylococci, and yeasts) at salinities up to 465 mM, in accordance with EU food hygiene standards. However, filamentous fungi exceeded 3 Log CFU/g in most species at 465 mM, highlighting salinity-dependent microbial risks. Furthermore, the results indicate that intermediate salinities (200–350 mM NaCl)

optimised the sensory appeal of *D. crassifolium* and *S. maritima*, which were preferred by panellists due to attributes such as texture and flavour, while also maintaining microbial quality. This dual focus on safety and sensory preference establishes 200–350 mM as the ideal range for commercial production, offering a model for scaling up halophyte agriculture in saline environments without compromising food safety or consumer acceptance [38,39].

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to (During the XtremeGourmet project duration (2018–2020), there was no formally constituted Ethics Committee at Algarve University, which is why it was not possible to obtain prior ethical opinion or approval. However, it should be noted that the study was conducted in accordance with the fundamental principles of research ethics, namely: Obtaining informed consent from participants; Guaranteeing the anonymity/confidentiality of the data collected; No collection of sensitive data (e.g., health, religion, sexual orientation); No physical or psychological interventions; Respecting voluntariness and the right to withdraw from the activities at any time. The tasting activities were performed by experienced chefs and were described in the application that was approved by the University of Algarve (10ID00025 - Project N.º 17676-XTREMEGOURMET) and the Regional Coordination and Development Commission that funded the project, and were conducted following institutional requirements with all ethical principles respected, which were subsequently required by the ethics committee created after the end of the project. Therefore, the study did not require formal ethical evaluation at the time it was conducted, but followed the requirements of the institution, legislation, and good practice).

Informed Consent Statement: Informed consent was obtained from all participants prior to their involvement in the study. Verbal consent was obtained instead of written as all participants had already signed a non-disclosure agreement concerning the details of plant cultivation and the experiments conducted. At the time of the study, the University of Algarve did not have an Ethics Committee; therefore, an Ethics Approval Exemption was obtained from the Scientific Council of the Faculty of Sciences and Technology. Nonetheless, the sensory evaluation was carried out in accordance with institutional requirements, with all ethical principles upheld, as stipulated by the Ethics Committee that was later established.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: Miguel Salazar is the CEO and owner of Agro-On, the company that commercializes the plants under study. Agro-On was the promoter of the XtremeGourmet project, whose funding is acknowledged in the "Funding" section. Carla Nunes is an employee of Agro-On. Miguel Salazar contributed to funding acquisition and administration, study conceptualization, data collection, and manuscript review. Carla Nunes also participated in the review of the submitted manuscript.

References

1. Barreira, L.; Resek, E.; Rodrigues, M.J.; Rocha, M.I.; Pereira, H.; Bandarra, N.; da Silva, M.M.; Varela, J.; Custódio, L. Halophytes: Gourmet Food with Nutritional Health Benefits? *J. Food Compos. Anal.* **2017**, *59*, 35–42. [CrossRef]
2. Lima, A.R.; Castañeda-Loaiza, V.; Salazar, M.; Nunes, C.; Quintas, C.; Gama, F.; Pestana, M.; Correia, P.J.; Santos, T.; Varela, J.; et al. Influence of Cultivation Salinity in the Nutritional Composition, Antioxidant Capacity and Microbial Quality of *Salicornia ramosissima* Commercially Produced in Soilless Systems. *Food Chem.* **2020**, *333*, 127525. [CrossRef] [PubMed]
3. Mann, A.; Lata, C.; Kumar, N.; Kumar, A.; Kumar, A.; Sheoran, P. Halophytes as New Model Plant Species for Salt Tolerance Strategies. *Front. Plant Sci.* **2023**, *14*, 1137211. [CrossRef] [PubMed]
4. Rahman, M.M.; Mostofa, M.G.; Keya, S.S.; Siddiqui, M.N.; Ansary, M.M.U.; Das, A.K.; Rahman, M.A.; Tran, L.S.P. Adaptive Mechanisms of Halophytes and Their Potential in Improving Salinity Tolerance in Plants. *Int. J. Mol. Sci.* **2021**, *22*, 10733. [CrossRef]
5. Vineeth, V.T.; Kumar, S.; Shukla, M.; Chinchmalatpure, A.; Sharma, P.C. Ecological and Economic Potential of Major Halophytes and Salt Tolerant Vegetation in India. In *Abiotic Stress in Plants*; Fahad, S., Saud, S., Chen, Y., Wu, C., Wang, D., Eds.; IntechOpen: London, UK, 2021; ISBN 978-1-83881-063-4.
6. Fatnani, D.; Patel, M.; Parida, A.K. Regulation of Chromium Translocation to Shoot and Physiological, Metabolomic, and Ionomic Adjustments Confer Chromium Stress Tolerance in the Halophyte *Suaeda maritima*. *Environ. Pollut.* **2023**, *320*, 121046. [CrossRef]
7. Grigore, M. *Handbook of Halophytes: From Molecules to Ecosystems Towards Biosaline Agriculture*; Springer: New York, NY, USA, 2020; ISBN 9783030576349.
8. Farrell, C.; Mitchell, R.E.; Szota, C.; Rayner, J.P.; Williams, N.S.G. Green Roofs for Hot and Dry Climates: Interacting Effects of Plant Water Use, Succulence and Substrate. *Ecol. Eng.* **2012**, *49*, 270–276. [CrossRef]
9. Zurayk, R.A.; Baalbaki, R. *Inula crithmoides*: A Candidate Plant for Saline Agriculture. *Arid Soil Res. Rehabil.* **1996**, *10*, 213–223. [CrossRef]
10. Castroviejo, S.; Aedo, C.; Laínz, M.; Muñoz Garmedndia, F.; Nieto Feliner, G.P.J.; Benedí, C. Flora Ibérica: Plantas Vasculares de La Península Ibérica e Islas Baleares. In *Flora Iberica: Real Jardín Botánico*; CSIC: Madrid, Spain, 1997.
11. Mohamed, N.A.F.; Al-Touby, S.S.; Hossain, M.A. Evaluation of Cytotoxic and Antioxidant Activities of Different Polarities Extracts of *Suaeda maritima*. *Biocatal. Agric. Biotechnol.* **2022**, *42*, 102370. [CrossRef]
12. Lima, A.R.; Gama, F.; Castañeda-Loaiza, V.; Costa, C.; Schüler, L.M.; Santos, T.; Salazar, M.; Nunes, C.; Cruz, R.M.S.; Varela, J.; et al. Nutritional and Functional Evaluation of *Inula crithmoides* and *Mesembryanthemum nodiflorum* Grown in Different Salinities for Human Consumption. *Molecules* **2021**, *26*, 4543. [CrossRef]
13. Duarte, B.; Caçador, I. Iberian Halophytes as Agroecological Solutions for Degraded Lands and Biosaline Agriculture. *Sustainability* **2021**, *13*, 1005. [CrossRef]
14. Wungrampha, S.; Rawat, N.; Pareek, A. Survival Strategies in Halophytes: Adaptation and Regulation. In *Handbook of Halophytes*; Grigore, M., Ed.; Springer: Cham, Switzerland, 2021; pp. 1591–1612. ISBN 978-3-030-57635-6.
15. Petropoulos, S.A.; Karkanis, A.; Martins, N.; Ferreira, I.C.F.R. Edible Halophytes of the Mediterranean Basin: Potential Candidates for Novel Food Products. *Trends Food Sci. Technol.* **2018**, *74*, 69–84. [CrossRef]
16. Linekha, R.; Jeno, J.G.A.; Abirami, K.; Yamunadevi, B.; Nakkeeran, E. Halophiles and Their Adaptations: A Comprehensive Review on Recent Progress and Prospects in Biodesalination Applications. *Clean* **2024**, *52*, 2300260. [CrossRef]
17. Correia, I.; Antunes, M.; Tecelão, C.; Neves, M.; Pires, C.L.; Cruz, P.F.; Rodrigues, M.; Peralta, C.; Pereira, D.; Reboredo, F.; et al. Nutritive Value and Bioactivities of a Halophyte Edible Plant. *Plants* **2024**, *13*, 427. [CrossRef] [PubMed]
18. Castañeda-Loaiza, V.; Oliveira, M.; Santos, T.; Schüler, L.; Lima, A.R.; Gama, F.; Salazar, M.; Neng, N.R.; Nogueira, J.M.F.; Varela, J.; et al. Wild vs Cultivated Halophytes: Nutritional and Functional Differences. *Food Chem.* **2020**, *333*, 127536. [CrossRef]
19. Peddi, P.; Ptsrk, P.R.; Rani, N.U.; Tulasi, S.L. Green Synthesis, Characterization, Antioxidant, Antibacterial, and Photocatalytic Activity of *Suaeda maritima* (L.) Dumort Aqueous Extract-Mediated Copper Oxide Nanoparticles. *J. Genet. Eng. Biotechnol.* **2021**, *19*, 131. [CrossRef]
20. Oliveira-Alves, S.C.; Andrade, F.; Sousa, J.; Bento-Silva, A.; Duarte, B.; Caçador, I.; Salazar, M.; Mecha, E.; Serra, A.T.; Bronze, M.R. Soilless Cultivated Halophyte Plants: Volatile, Nutritional, Phytochemical, and Biological Differences. *Antioxidants* **2023**, *12*, 1161. [CrossRef]
21. Giamperi, L.; Bucchini, A.; Fraternale, D.; Genovese, S.; Curini, M.; Ricci, D. Composition and Antioxidant Activity of *Inula crithmoides* Essential Oil Grown in Central Italy (Marche Region). *Nat. Prod. Commun.* **2010**, *5*, 315–318. [CrossRef]
22. Qneibi, M.; Hanania, M.; Jaradat, N.; Emwas, N.; Radwan, S. *Inula Viscosa* (L.) Greuter, Phytochemical Composition, Antioxidant, Total Phenolic Content, Total Flavonoids Content and Neuroprotective Effects. *Eur. J. Integr. Med.* **2021**, *42*, 101291. [CrossRef]
23. Hoather, R.C.; Rackham, R.F. Oxidised Nitrogen in Waters and Sewage Effluents Observed by Ultra-Violet Spectrophotometry. *Analyst* **1959**, *84*, 548–551. [CrossRef]
24. *ISO 4833-1*; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms—Part 1: Colony Count at 30 Degrees C by the Pour Plate Technique. ISO: Geneva, Switzerland, 2013.

25. *ISO 17410*; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Psychrotrophic Microorganisms. ISO: Geneva, Switzerland, 2019.
26. *ISO 21527-1*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—Part 1: Colony Count Technique in Products with Water Activity Greater than 0,95. ISO: Geneva, Switzerland, 2008.
27. *ISO 16649-2:2001*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Beta-Glucuronidase-Positive Escherichia Coli. ISO: Geneva, Switzerland, 2001.
28. *ISO 6888-1_2021*; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (Staphylococcus Aureus and Other Species)—Part 1_ Method Using Baird-Parker Agar Medium. ISO: Geneva, Switzerland, 2021.
29. Stone, H.; Sidel, J.; Oliver, S.; Woolsey, A.; Singleton, R.C. Sensory Evaluation by Quantitative Descriptive Analysis. In *Descriptive Sensory Analysis in Practice*; Gacula, M.C., Jr., Ed.; Food & Nutrition Press, Inc.: Scottsdale, AZ, USA, 2004; pp. 24–32, ISBN 9780470385036.
30. Aghaleh, M.; Niknam, V.; Ebrahimzadeh, H.; Razavi, K. Salt Stress Effects on Growth, Pigments, Proteins and Lipid Peroxidation in *Salicornia persica* and *S. europaea*. *Biol. Plant.* **2009**, *53*, 243–248. [CrossRef]
31. Munns, R.; Passioura, J.B.; Colmer, T.D.; Byrt, C.S. Osmotic Adjustment and Energy Limitations to Plant Growth in Saline Soil. *New Phytol.* **2020**, *225*, 1091–1096. [CrossRef] [PubMed]
32. Matabane, R. Tolerance of Salt Marsh Ecotone Species to Salinity and Inundation. Master's Thesis, Nelson Mandela University, Gqeberha, South Africa, 2018.
33. Hassan, M.A.; Chaura, J.; López-Gresa, M.P.; Borsai, O.; Daniso, E.; Donat-Torres, M.P.; Mayoral, O.; Vicente, O.; Boscaiu, M. Native-Invasive Plants vs. Halophytes in Mediterranean Salt Marshes: Stress Tolerance Mechanisms in Two Related Species. *Front. Plant Sci.* **2016**, *7*, 473. [CrossRef] [PubMed]
34. Agarie, S.; Shimoda, T.; Shimizu, Y.; Baumann, K.; Sunagawa, H.; Kondo, A.; Ueno, O.; Nakahara, T.; Nose, A.; Cushman, J.C. Salt Tolerance, Salt Accumulation, and Ionic Homeostasis in an Epidermal Bladder-Cell-Less Mutant of the Common Ice Plant *Mesembryanthemum crystallinum*. *J. Exp. Bot.* **2007**, *58*, 1957–1967. [CrossRef]
35. Choi, S.-C.; Lim, S.-H.; Kim, S.-H.; Choi, D.-G.; Kim, J.-G.; Choo, Y.-S. Growth and Solute Pattern of *Suaeda maritima* and *Suaeda asparagoides* in an Abandoned Salt Field. *J. Ecol. Environ.* **2012**, *35*, 351–358. [CrossRef]
36. Alhdad, G.M. Responses of *Suaeda maritima* to Flooding and Salinity. PhD Thesis, University of Sussex, Brighton, UK, 2012.
37. Lopes, T.F.; Ortigueira, J.; Matos, C.T.; Reis, A.; Francisco, G. Conceptual Design of an Autotrophic Multi-Strain Microalgae-Based Biorefinery: Preliminary Techno-Economic and Life Cycle Assessments. *Fermentation* **2023**, *9*, 255. [CrossRef]
38. European Commission. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, L 338, 1–26, 22 December 2005. Available online: https://eur-lex.europa.eu/eli/reg/2005/2073/oj/eng (accessed on 9 April 2025).
39. European Commission. Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, L 322, 12–29, 7 December 2007. Available online: https://eur-lex.europa.eu/eli/reg/2007/1441/oj/eng (accessed on 9 April 2025).
40. Nikalje, G.C.; Nikam, T.D.; Suprasanna, P. Looking at Halophytic Adaptation to High Salinity Through Genomics Landscape. *Curr. Genom.* **2017**, *18*, 542–552. [CrossRef]
41. Pietrasik, Z.; Gaudette, N.J. The Impact of Salt Replacers and Fl Avor Enhancer on the Processing Characteristics and Consumer Acceptance of Restructured Cooked Hams. *Meat Sci.* **2014**, *96*, 1165–1170. [CrossRef]
42. Martins-Noguerol, R.; Cambrollé, J.; Machilla-Leytón, J.M.; Puerto-Marchena, A.; Muñoz-Vallés, S.; Millian-Linares, M.C.; Millán, F.; Martínez-Force, E.; Figueroa, M.E.; Pedroche, J.; et al. Influence of Soil Salinity on the Protein and Fatty Acid Composition of the Edible Halophyte *Halimione portulacoides*. *Food Chem.* **2021**, *352*, 129370. [CrossRef] [PubMed]