



Article

Effect of Saline-Nutrient Solution on Yield, Quality, and Shelf-Life of Sea Fennel (*Crithmum maritimum* L.) Plants

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Abstract: In this study, the effect of salinity (150 mM NaCl) compared to a control (9 mM NaCl) on growth, quality and shelf-life of fresh-cut sea fennel was evaluated. For that, sea fennel plants were cultivated in a hydroponic floating system and the sea fennel leaves were stored for 12 days at 5 °C. At harvest, leaves from plants grown in salinity had a lower content of NO₃⁻, K⁺ and Ca²⁺ and an increased Cl⁻ and Na⁺ concentration when compared to the control. There was a positive effect in the aerial part with increased fresh weight due to salt stress, but a reduction in the root biomass. During storage, weight loss and colour changes were not significant while leaves' firmness was higher for control and increased during storage, probably due to lignification. Microbial growth (psychrophiles, yeast and moulds and enterobacteria) was higher at harvest for control and increased during storage, with no differences between treatments after 12 days at 5 °C. Sensory quality was similar for both treatments but leaves from NaCl treatment had a salty taste that was easily detected by panelists. These results show that saline-nutrient solution applied in hydroponics is a suitable system for sea fennel growth. It gives a slightly salty but high-quality product, acceptable as a "ready-to-eat" vegetable.

Keywords: salinity; microbial growth; sensory quality; floating system; ready-to-eat



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1. Introduction

Sea fennel (*Crithmum maritimum* L.), also known as crest marine, marine fennel, samphire, and rock samphire [1], is a halophyte species, the sole one of the *Crithmum* genus [2,3], which belongs to the *Apiaceae* family.

This species is widespread in the Mediterranean coasts as well in the Canary Islands [2] and along the Atlantic coast of Portugal, England, Wales and Southern Ireland [4].

Being a perennial halophyte species, it is able to grow in sand hills or on rocky cliffs and is remarkably productive under saline conditions to exploit seawater, coastal lands, and other marginal areas otherwise useless [5], without requiring huge allocation and depletion of freshwater resources [6].

Its distinguishing sensory attributes in terms of taste, odour and colour has historically always found applications in culinary Mediterranean tradition and the food industry [1,7], and in some countries (e.g., Italy) its use is so long and rooted in time that such a product is included in the "List of Traditional Agri-Food Products" of the Italian Department for Agriculture [1]. Sea fennel importance is not limited only to the culinary uses (mainly as an appetizer), but also as carminative, diuretic or for treating obesity [8]. In addition, it is rich in several biologically active compounds (ascorbic acid, iodine, carotenoids,

flavonoids, organic acids, phenolics, etc.) [9], exerting beneficial effects against oxidative or mutagenic mechanisms, and pathogenic bacteria [10], which is important for their healthy properties [11,12].

Apart from its use as a fresh product, Renna et al. [1] proposed sea fennel to be used in dried form with different techniques of drying, as this could lead to an “industrial production on a large scale and also to diversify local food through a micro-scale production”.

Similar to other halophyte species, sea fennel has developed mechanisms to tolerate high salinity levels, particularly by accumulating Na^+ and Cl^- into the vacuoles [13]. Furthermore, Jiménez-Becker et al. [14] observed that sea fennel has the capacity, compared to other halophytes, to reduce the uptake of Cl^- , which results in a lower concentration within the leaves and to an increase in the concentration of soluble sugars and proline, in particular at high salinity levels (300 mM of NaCl).

Yet, even if products of halophytes species are produced more and more and sold in the markets worldwide, sea fennel may be still considered as a wild edible plant, since it has not undergone a structured programme for its genetic improvement and cultivation, even if it could be easily domesticated and engineered to exploit its beneficial elements content [15,16]. Recent knowledge suggests that sea fennel shows good potential as an emerging crop, despite studies on its cultivation techniques being limited [17]. A floating system seems to be particularly appropriate for baby leaf vegetable production since it allows precise control of plant nutrition and the maximisation of yield and quality of the product. Thus, Giménez et al. [18] demonstrated that the above system is a suitable method for growing *C. maritimum*. It is well known that cultivation conditions influence the quality of the raw material and therefore can modify its physiological behaviour and suitability for fresh-cut processing [19]. We hypothesise that any preharvest condition that stresses a plant, such as the salinisation of the nutrient solution, could affect the quality and shelf-life of the sea fennel, particularly increasing the phytochemicals of the plant.

In our vision, sea fennel has the potential for more extensive cultivation and for the ready-to-eat market as a baby-leaf vegetable, due not only to its organoleptic characteristics but also to its richness in terms of health-promoting compounds and its suitability for cultivation in saline conditions, an important aspect for the Mediterranean environment. This aspect would be crucial since soil salinity is currently the most important environmental stress limiting crop production in arid and semi-arid areas [20], and, in the near future this trend is expected to worsen [21]. For this purpose, we evaluated the effect of the salinity level of the nutrient solution in a floating system on the growth, quality, and shelf-life of *C. maritimum* during a storage period.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The experiment was conducted in an unheated greenhouse covered with thermal polyethylene located at the Experimental Agro-Food Station, Technical University of Cartagena (UPCT; lat. 37°41' N; long. 0°57' W), using seeds provided by Semillas Cantueso, obtained in Dunas de Artola, (Málaga). Sowing was carried out manually into “styrofloat” trays of 60 cm × 41 cm containing peat. The trays were placed in a growth chamber at 20 °C for 5 days and then transferred to flotation beds, floating on fresh tap water with an electrical conductivity (EC) of 1.1 dS m⁻¹ and a pH of 7.8. Aeration was provided using a blow pump connected to a pipe trellis positioned at the bottom of each flotation bed. Each level of treatment was carried out in 135 cm × 125 cm × 20 cm beds located at three places inside a greenhouse for all the experiments. A week after transferring to the floating beds, the plants were thinned, leaving a plant density around 400 plants m⁻², and the nutrient solution was added to the water and adjusted to EC 2.7 dS m⁻¹ and the pH to 5.8 [22]. After 30 days, NaCl was added to the nutrient solution to half of the plants to reach a concentration of 150 mM, while the other half was set as control treatment (9 mM of NaCl). The EC and temperature of the nutrient solution were monitored during the growing cycle

using sensors (CS547 Campbell Scientific Inc., Logan, UT, USA). Harvesting was carried out when plants had four–five pairs of leaves.

2.2. Analysis at Harvesting Time

Shoot fresh weight (FW), leaf area, specific leaf area (SLA) and root growth parameters were measured on 10 plants in each tray. Leaf area was measured using a leaf area meter (LICOR-3100 C; LICOR Biosciences Inc., Lincoln, NE, USA). Root length, area, and volume, and the number of branches were determined using a Winrhizo LA 1600 root counter (RegentInc., Quebec, QC, Canada) from pictures taken of each root system by a double-pass scanner incorporated in the counter. The dry weight (DW) of the shoot was determined by drying in an oven at 60 °C until constant weight.

At harvesting, the following biochemical parameters were measured in the sea fennel leaves: ions content, total phenolics and flavonoids content and total antioxidant capacity. The ions content was determined and quantified following the method described by Lara et al. [23] in the sea fennel leaves. Ions were extracted in triplicate per treatment. The extraction of 0.2 g of dry leaf samples of each treatment was carried out with 50 mL distilled water and continuous agitation in an orbital shaker (Stuart SSL1, Stone, UK) for 45 min at 110 rpm at 50 °C. Ion concentrations were determined by ion chromatography using a Metrosep A SUPP 5 column (Metrohm AG, Zofingen, Switzerland) at a flow rate of 0.7 mL min⁻¹ for anions and a Metrosep C 2-250 column at a flow rate of 1.0 mL min⁻¹ for cations, following the manufacturer's instructions.

The total phenolic content was determined using the Folin–Ciocalteu colorimetric method, modified by Everette et al. [24]. A 50 µL aliquot of the methanolic extract supernatant was mixed with 50 µL of Folin–Ciocalteu reagent and 750 µL of H₂O. The solution was incubated for 5 min and 150 µL of Na₂CO₃ was added. Then, it was incubated at room temperature for 2 h in darkness, after which the absorption at 765 nm was measured (HP 8453, Hewlett Packard). The measurement was expressed as mg gallic acid (GA) kg⁻¹ FW. Each one of the three replicates was analysed in triplicate (instrumental replicate).

The total antioxidant capacity of the leaves was evaluated in terms of their ability to deactivate the DPPH radical according to Brand-Williams et al. [25], with the modifications described by Lopez-Marín et al. [26]. Briefly, a solution of 2,2-diphenyl-1-picrylhydrazil (DPPH) in methanol was prepared. A 25 µL aliquot of the extract supernatant was mixed and 600 µL of DPPH stock solution added. The homogenate was shaken vigorously and kept in darkness for 15–20 min at room temperature. The absorbance at 517 nm was measured in a spectrophotometer (HP 8453, Hewlett Packard). The measurement was expressed as mg DPPH reduced kg⁻¹ FW.

The total flavonoids content was evaluated according to Meda et al. [27]. The procedure consisted of mixing 50 µL of extract, 300 µL of methanol and 350 µL of a 2% AlCl₃ dilution in methanol. After a 15 min incubation in darkness at room temperature, the absorbance at 430 nm was measured. The measurement was expressed as mg rutin kg⁻¹ FW.

2.3. Postharvest Product Handling and Analysis

Leaves free from defects were sanitised in a cold room (10 °C) by immersion in a solution containing 100 ppm NaClO and 0.2 g L⁻¹ citric acid (2 min, 5 °C, pH 6.5). Then, they were rinsed with tap water (2 min, 5 °C) and finally excess of water was removed by a salad spinner (30 s). Twenty g of leaves were placed in polypropylene (PP) baskets (170 mm × 120 mm × 40 mm) and thermo-sealed on the top with a 25 µm thick film-oriented polypropylene (OPP). Three replicates for each irrigation treatment and storage time (processing day and after 6 and 12 days) were prepared and stored in darkness at 5 °C. Each sampling day, and before opening the baskets, atmosphere composition within the package was measured. For that, a 0.5 mL sample of the headspace was withdrawn with a gas-tight syringe and O₂ and CO₂ concentrations were determined by a gas chromatograph (7820A GC Agilent Technologies, Waldbronn, Germany). The gas

chromatograph conditions were: oven at 80 °C, injector and detector at 250 °C, using H₂ and air as gas carriers at 35 mL min⁻¹ and 350 mL min⁻¹, respectively. A stainless-steel column packed with PorapakQ (1/8", 80/100 mesh size; Supelco Inc., Bellefonte, PA, USA) was used.

Microbial growth (mesophilic and psychrophilic aerobic bacteria, enterobacteria, and yeast and mould growth) was determined using standard enumeration methods. Samples of 1 g poured into a sterile stomacher bag (model 400 Bags 6141, London, UK) were homogenized with a 10 mL sterile peptone saline solution (pH 7; Scharlau Chemie SA, Barcelona, Spain) for 10 s in a masticator (Colwort Stomacher 400 Lab, Seward Medical, London, UK). For the enumeration of each microbial group, 10-fold dilution series were prepared in 9 mL of sterile peptone saline solution. Mesophilic, enterobacteria, and psychrotrophic were pour plated, and yeast and mould were spread plated. Media (Scharlau Chemie, Barcelona, Spain) and incubation conditions were as follows: plate count modified agar (PCA) for mesophilic and psychrotrophic aerobic bacteria (30 °C, 48 h and 5 °C for 7 days, respectively); violet red bile dextrose agar for enterobacteria (37 °C, 48 h); and rose Bengal agar for yeasts and moulds (3–5 days, 22 °C). All microbial counts were reported as log colony forming units per gram of product (log CFU g⁻¹). Each of the three replicates was analyzed by duplicate. The presence of *Listeria monocytogenes* was monitored according to the Regulation EC 1441/2007.

Weight loss was calculated as the difference between the initial weight of the samples at the beginning of storage and their final weight after 6 and 12 days. To normalize data, weight loss values were expressed as percentage of the initial value.

Firmness was measured at 22 °C using a texturometer (Brookfield, Canada). A compression test was carried out with a blade (1 mm width) at a force of 90 g and a speed of 10.0 mm s⁻¹ to reach a leave deformation of 0.5 mm. Results were expressed in g.

Leaf colour was determined on three points of each replicate using a colorimeter (Minolta CR-400 Series, Ramsey, NJ, USA). Tristimulus parameters (L*, a*, b*) of the CIE Lab system were used to calculate the Hue angle = arctan (b*/a*) and chroma (C*) = [(a*)² + (b*)²]^{1/2}.

2.4. Sensory Quality Panel

Sensory quality was analysed according to international standards (ASTM 1986) in a standardised room (UNE-EN ISO 8589 2007) equipped with ten testing boxes. Samples coded with three random digit numbers were served at room temperature. Still mineral water was used as palate cleanser. Evaluation was performed by 10 trained judges on day 0 and after 6 and 12 days of storage at 5 °C.

A 5-point scale was scored for colour, texture (crispness), flavour, aroma and global acceptance (5: excellent, 4: good, 3: fair, limit of usability, 2: poor; 1: extremely bad) and for defects as off-odours and mechanical damage (5: none; 4: slight; 3: moderate, limit of usability; 2: severe; 1: extreme) [28].

2.5. Statistical Analysis

A randomised complete block design with three replicates (beds) per both treatments, control and salinity, was used in the greenhouse. Each bed had three floating trays of 60 cm × 41 cm. Data were analysed using Statgraphics Plus. Analysis of variance (two-way ANOVA) was performed in which levels of salinity (9 and 150 mM), and storage time (0, 6 and 12 d) were included. When interactions were significant, they were included in the ANOVA, a least significant difference test was performed to compare level of salinity, and storage time. When the variables were measured at harvesting time, only salinity factor was included.

3. Results

3.1. Growth, Yield, and Quality Characteristic of *C. maritimum* at Harvesting

The salinity treatment did not affect the shoot FW and root parameters (Table 1). However, NaCl treatment reduced the leaf area and specific leaf area of *C. maritimum* plants, which indicates that the leaves were thicker when plants were grown with 150 mM NaCl.

Table 1. Influence of salinity treatment (control and 150 mM NaCl) on fresh weight, leaf area, specific leaf area (SLA), total root length, area root, diameter root and volume root of *C. maritimum* at harvest.

Treatments	Shoot Fresh Weight (g plant ⁻¹)	Shoot Dry Weight (g plant ⁻¹)	Leaf Area (cm ² plant ⁻¹)	SLA (m ² kg ⁻¹)	Total Root Length (cm)	Root Diameter (mm)	Root Volume (cm ³)
Control	2.10 ± 0.47 a	0.205 ± 0.005 a	3.43 ± 0.21 b	0.19 ± 0.012 b	112.24 ± 6.31 a	0.37 ± 0.01 a	0.59 ± 0.07 a
150 mM NaCl	2.45 ± 0.42 a	0.235 ± 0.005 a	2.37 ± 0.09 a	0.11 ± 0.005 a	109.92 ± 5.84 a	0.33 ± 0.02 a	0.46 ± 0.05 a

Values are the mean ± SE (*n* = 6). Values in the same column with different letters for each anion differ significantly according to LSD test (*p* < 0.05).

Some differences were observed regarding the contents of anions and cations in the leaf of *C. maritimum* at harvesting (Tables 2 and 3). With regard to anions, the content of nitrate was reduced by 17% in salinity conditions, while the content of chloride was increased by 3.7-fold. Furthermore, the addition of NaCl significantly reduced the content of bromide and sulphate, while the content of phosphate and oxalate was not affected by the salinity. Regarding cations, the content of sodium was found to increase by ca. 500%, whilst potassium, calcium and magnesium ions accumulated to a minor extent when sea fennel was grown in salinity conditions. These results agree with the hypothesis that sea fennel requires salt to grow, and it can tolerate high concentrations of salt. Finally, Cl⁻ content was found to be systematically higher than Na⁺, an imbalance that clearly indicates the existence in sea fennel plants of a regulatory mechanism to retain Na⁺ far away from leaves since it could be a toxic element for the photosynthetic system.

Table 2. The content of anions (NO₃⁻, Cl⁻, Br⁻, PO₄³⁻, SO₄²⁻, C₂O₄²⁻) (mg kg⁻¹ FW) in the leaf of *C. maritimum* under the different treatments (control and 150 mM NaCl) at harvesting.

Treatments	NO ₃ ⁻	Cl ⁻	Br ⁻	PO ₄ ³⁻	SO ₄ ²⁻	C ₂ O ₄ ²⁻
Control	1530.31 ± 586.47 b	1810.97 ± 120.56 a	152.00 ± 2.82 b	947.01 ± 94.14 a	1699.55 ± 32.28 b	88.19 ± 33.59 a
150 mM NaCl	1263.03 ± 19.79 a	6718.18 ± 1029.31 b	125.98 ± 3.46 a	1154.39 ± 142.73 a	671.25 ± 47.66 a	88.70 ± 28.87 a

Values are the mean ± SE (*n* = 6). Values in the same column with different letters for each anion differ significantly according to LSD test (*p* < 0.05).

Table 3. The content of cations (Na⁺, K⁺, Ca²⁺, Mg²⁺) (mg kg⁻¹ FW) in the leaf of *C. maritimum* under the different treatments (control and 150 mM NaCl) at harvesting.

Treatments	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
Control	777.57 ± 54.46 a	3642.49 ± 109.48 b	1108.29 ± 20.91 b	379.10 ± 12.46 b
150 mM NaCl	4639.45 ± 703.02 b	1070.45 ± 345.86 a	532.05 ± 143.54 a	203.42 ± 10.47 a

Values are the mean ± SE (*n* = 6). Values in the same column with different letters for each cation differ significantly according to LSD test (*p* < 0.05).

Salinity significantly reduced the phenolics content in sea fennel leaves at harvesting by 6% but increased the flavonoids content by 10% (Table 4) However, no significant differences were found between treatments with respect to antioxidant capacity.

Table 4. Total phenolics content, total flavonoids, and total antioxidant capacity in leaves of *C. maritimum* under the different treatments (control and 150 mM NaCl).

Treatments	Total Phenolics (mg GA kg ⁻¹ FW)	Total Flavonoids (mg Rutin kg ⁻¹ FW)	Total Antioxidant Capacity (mg DPPH _{reduced} kg ⁻¹ FW)
Control	887.43 ± 11.95 b	1966.89 ± 45.17 a	112.24 ± 6.31 a
150 mM NaCl	833.53 ± 9.42 a	2167.24 ± 22.09 b	109.92 ± 5.84 a

Values are the mean ± SE ($n = 6$). Values in the same column with different letters differ significantly according to LSD test ($p < 0.05$).

3.2. Postharvest Quality

Sea fennel leaves lost water moderately in both treatments during the storage time (data not shown). Particularly, after 12 days of storage, sea fennel leaves obtained from plants grown under salinity presented a higher weight loss (1.24%) than those grown in the control (0.32%).

Leaves' firmness (Table 5) was higher for control than for those grown under salty conditions and increased slightly during storage in both treatments, most likely related to lignification. The leaves of plants grown with NaCl treatment had less turgor due to higher dehydration, presenting lower firmness than the control.

Table 5. Effect of 150 mM NaCl addition in the nutrient solution on *C. maritimum* leaf firmness (mm) at 0, 6 and 12 days of storage at 5 °C.

	Sea Fennel Firmness (g)
Salinity Treatment (A)	
Control	596.27 ± 20.43 b ^x
150 mM NaCl	457.94 ± 28.27 a
Storage (B)	
0 days	494.48 ± 35.26 a ^y
6 days	515.05 ± 34.01 a
12 days	571.80 ± 28.37 a
Significant Differences	
A	***
B	ns
A × B	ns

Asterisks indicates significance at *** $p < 0.001$; ns: non-significant. Different letters in the same column indicate significant differences. Values are the mean ± SE (^x $n = 45$, ^y $n = 30$).

A passive modified atmosphere was generated inside the packages, which was related to the respiration rate of the produce. After 6 days of storage at 5 °C, no differences in O₂ and CO₂ concentration in the atmosphere within the packages were observed between treatments (Figure 1). However, after 12 days of storage, CO₂ concentration was slightly higher and O₂ concentration was moderately lower within the baskets of sea fennel leaves grown with 150 mM NaCl. It could indicate a higher respiration rate for these leaves, probably induced by the pre-harvest stressing conditions of salinity. However, the trend seems to be that both treatments were close to reaching the steady-state and, probably, at that moment differences between treatments would be minimal.

As regards the colour of the sea fennel leaves at harvest, the plants treated with NaCl, presented a luminosity (L* parameter) about 6% higher than the control (Table 6). Due to that, hue values were lower for those leaves than for the control. Salinity slightly affected leaf colouration towards lighter colours. However, it was almost undetected by the sensory panel. The colour parameters did not change significantly over the 12 days of monitoring. Therefore, salinity did not adversely affect the colour, keeping marketability at values that resemble those of the control.

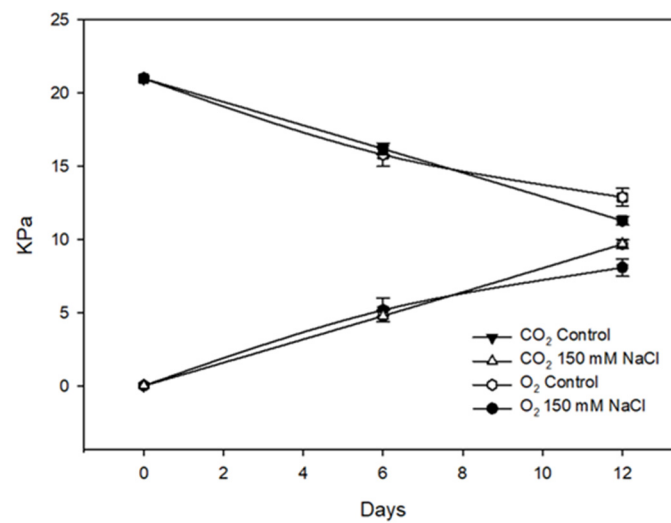


Figure 1. Effect of the salinity of the growing nutrient solution on the atmosphere composition within packages of fresh-cut sea fennel stored for 12 d at 5 °C. Values are the mean \pm SE ($n = 3$).

Table 6. Effect of 150 mM NaCl addition in the nutrient solution on *C. maritimum* leaf colour parameters at 0, 6 and 12 days of storage at 5 °C.

	L	a	b	HUE	Chroma
Salinity Treatment (A)					
Control	37.20 \pm 0.66 a ^x	−11.31 \pm 0.39 a	18.32 \pm 1.01 a	121.98 \pm 1.08 b	22.26 \pm 0.76 a
150 mM NaCl	39.14 \pm 0.40 b	−10.94 \pm 0.27 a	20.62 \pm 0.55 a	118.01 \pm 0.70 a	23.35 \pm 0.54 a
Storage (B)					
0 days	38.51 \pm 0.62 a ^y	11.29 \pm 0.42 a	20.25 \pm 1.12 a	119.43 \pm 1.96 a	21.09 \pm 0.94 a
6 days	37.95 \pm 1.07 a	10.84 \pm 0.36 a	18.98 \pm 1.28 a	120.04 \pm 1.14 a	23.58 \pm 0.56 a
12 days	38.04 \pm 0.66 a	11.26 \pm 0.49 a	19.18 \pm 0.94 a	120.51 \pm 1.04 a	22.71 \pm 0.98 a
Significant Differences					
A	*	ns	ns	*	ns
B	ns	ns	ns	ns	ns
A \times B	ns	ns	ns	ns	ns

Asterisk indicates significance at * $p < 0.05$, ns: non-significant. Different letters in the same column indicate significant differences. Values are the mean \pm SE (^x $n = 9$, ^y $n = 6$).

Microbial load (psychrophiles, yeast and moulds and enterobacteria) was higher at harvest for control leaves and increased during storage at 5 °C. *Listeria* was not detected in any treatment. The results in Table 7 show that there was a significant interaction between salinity treatments and storage for psychrophilic bacteria, enterobacteria and yeast and mould counts.

Psychrophilic bacteria counts were significantly higher in control leaves at harvest, but after 6 days of storage, there were no significant differences between treatments (Figure 2). A similar trend was found for Enterobacteria.

The sensory quality, even when decreasing, was acceptable for both treatments at the end of storage (Table 8). The most important changes were observed in texture and freshness, mainly related to a lower crispness associated with the water loss. The leaves obtained from salinity had a salty taste which was not observed in the control. However, this hint of salt was not unpleasant. The samples did not present strange smells in any case.

Table 7. Psychrophilic bacteria, mesophilic bacteria, enterobacteria and yeast and moulds counts (log CFU g⁻¹) of *C. maritimum* leaves after different salinity treatment (control and 150 mM NaCl) and storage at 5 °C for 0, 6 and 12 days.

	Psychrophilic Bacteria (log UFC g ⁻¹)	Mesophilic Bacteria (log UFC g ⁻¹)	Enterobacteria (log UFC g ⁻¹)	Yeast and Moulds (log UFC g ⁻¹)
Salinity Treatment (A)				
Control	5.81 ± 0.28 b ^x	5.40 ± 0.31 a	5.15 ± 0.38 b	3.89 ± 0.18 b
150 mM NaCl	5.25 ± 0.45 a	5.24 ± 0.36 a	3.90 ± 0.98 a	3.34 ± 0.12 a
Storage (B)				
0 days	4.23 ± 0.44 a ^y	4.05 ± 0.11 a	1.89 ± 0.83 a	3.09 ± 0.16 a
6 days	5.99 ± 0.08 b	5.61 ± 0.09 b	5.61 ± 0.13 b	3.82 ± 0.10 b
12 days	6.38 ± 0.01 b	6.30 ± 0.01 c	6.21 ± 0.07 c	3.84 ± 0.21 b
Significant Differences				
A	*	ns	***	**
B	***	***	***	**
A × B	*	ns	***	ns

Asterisk indicates significances at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns: non-significant. Different letters in the same column indicate significant differences. Values are the mean ± SE (^x $n = 9$, ^y $n = 6$).

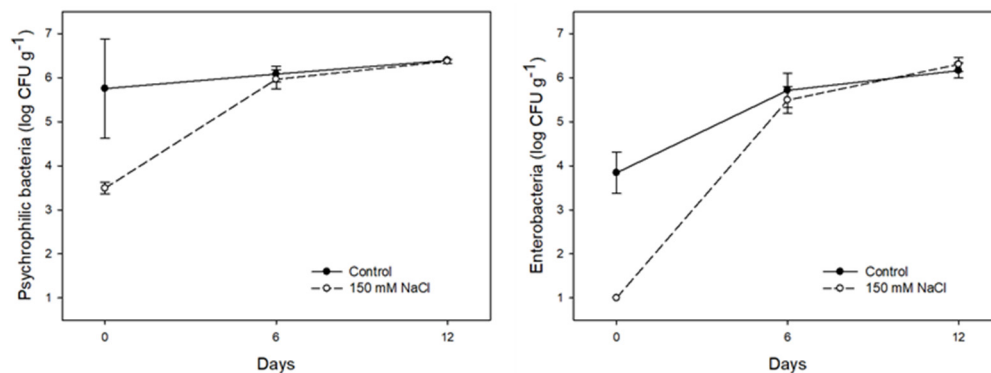


Figure 2. Psychrophilic bacteria and enterobacteria (log CFU g⁻¹) of *C. maritimum* leaves with different salinity treatment (control and 150 mM NaCl) after storage at 5 °C for 0, 6 and 12 days. Values are the mean ± SE ($n = 3$).

Table 8. Effect of 150 mM NaCl addition in the nutrient solution on sensory quality of *C. maritimum* leaves at 0, 6 and 12 days of storage at 5 °C.

	Sensorial Quality					
	Day 0		Day 6		Day 12	
	Control	150 mM NaCl	Control	150 mM NaCl	Control	150 mM NaCl
Acceptance						
Visual appearance	5	4	4.5	4	4	3.5
Colour	4	4	4	4	4	4
Texture (Crispness)	5	5	4	3.5	3	3
Flavour (Freshness)	5	4	4	4	3	3
Aroma	5	5	5	5	5	5
Global acceptance	5	4.5	4	3.5	3.5	3
Alterations						
Off-odours	5	5	5	5	5	5
Mechanical damage	4	4	4	4	3.5	3.5

4. Discussion

In this study the impact of salinity (150 mM NaCl) on plant growth, quality and self-life of *C. maritimum* was analysed. The salinity had no effect on the biomass and

growth traits measured, indicating that sea fennel is a facultative halophyte with moderate tolerance to salinity, which does not require salt for maximal growth [29,30]. Similarly, Jiménez-Becker et al. [14] did not find differences in this species with respect to biomass growth when NaCl concentrations of 100 mM, 200 mM, and 300 mM were used. Nevertheless, the response to the salinity of *C. maritimum* is population-dependent, being this trait often correlated with the growth of the plants in their natural habitat [29]. The leaf area was reduced under 150 mM NaCl, in agreement with previous results of Hamed et al. [29], provoking a reduction of the specific leaf area and consequently an increase in leaf thickness and succulence (measured as leaf FW:leaf area ratio [31], the latter being one of the major factors involved in plant salt tolerance, the main quality trait for stimulated growth in halophytes [32]. However, Jiménez-Becker et al. [14] detected a decrease in leaves FW:DW ratio (leaves succulence) together with a decrease in plant water content in the plants grown with 300 mM with respect to that of those grown with 100 mM NaCl; therefore, the salt concentration in the nutrient solution is one of the main factors to consider in leaf succulence of halophytes plants. It is important to bear in mind that succulence together with firmness and juiciness procure leaf texture, which is an important sensory attribute for determining the post-harvest quality and consumers' acceptance [33]. Consequently, acquiring leaf succulence in the crop cycle and keeping it during post-harvest through adequate technology could be a useful strategy for guaranteeing the quality and shelf-life of sea fennel.

Sea fennel can be also a good source of daily minerals required in a healthy diet. The increase in Na^+ and Cl^- as the result of NaCl salinity was a common and expected response that was previously reported in sea fennel [32,34] since these elements are compartmentalized in vacuoles to avoid causing cytotoxicity [35]. However, differences between Na^+ and Cl^- accumulation in the aerial part were observed. It was postulated that differences in ion charge are responsible for the more expensive energetically sequestration of Na^+ compared to the sequestration of Cl^- , as the potential inside the vacuole is positive relative to the cytoplasm [36]. This would explain that the Cl^- content in the aerial part was found to be systematically higher than Na^+ , under control and saline conditions. In this study, the K^+/Na^+ ratio dropped dramatically with salinity treatment as it was reported in Tunisian [29,37] and Argelian *C. maritimum* populations [34], when increasing salinity concentrations were applied, although the degree of resilience was population dependent. Maintaining a high K^+/Na^+ ratio is likely to be important to avoid the effects of ion toxicity under salt stress [38]. In our study, the accumulation of Na^+ in the control plant leaves was lower than in other halophytes [39]. In addition, K^+ was accumulated 4.68-fold higher than Na^+ , which could mean that sea fennel grown in a floating system could be suitable to cover part of the amount of K^+ required daily. However, due to the high Na^+ concentration, it would be better to use it as a meal accompaniment or as a condiment [7,39], instead of as a main fresh vegetable dish. In control plants, Ca^{2+} concentration was 2-fold than the Ca^{2+} accumulation found by Sánchez-Faure et al. [40] in sea fennel plants grown in their natural habitat. Despite saline treatment reducing the available Ca^{2+} , its content in sea fennel leaves remained relatively high (532.05 mg kg^{-1} FW), with the potential benefit of preventing salt-induced oxidative damages, due to the protecting function of Ca^{2+} when plants face extreme heat, dry, or saline conditions [41,42]. The above-mentioned Ca^{2+} reduction with the salinity treatment could be due not only to the Na^+ accumulation but also to its reduce mobility and transport to the shoot under salinity stress [43,44]. An adequate Ca^{2+} intake for adults of 750 mg per day was marked by EFSA. Therefore, 100 g of fresh sea fennel grown in our conditions may represent 15.8% (control plants) and 7.6% (plant grown with 150 mM NaCl) of the daily recommended doses.

On the other hand, nitrate, bromide, and sulphate were reduced in the leaves of plants treated with NaCl, confirming a reduction in the absorption capacity of nutrients by the roots under salt stress [45]. The difference in nitrate accumulation in response to salinity is generally linked with the inhibition of NO_3^- uptake by Cl^- [46], which could happen by the interaction between these ions at the site of entry and for ion transport [47,48]. The

nitrate content in the plants studied was generally quite low, and lower than the maximum legislated in the EU (Commission Regulation (EC) No 1258/2011) for other leafy vegetables such as spinach, lettuce, or rocket plant (2000–7000 mg kg⁻¹).

Salinity increased the content of total flavonoids but decreased phenolic content, while total antioxidant capacity was unaffected. Plants vary widely in their phenolic composition and content also accordingly to genetics and environmental conditions [49]. Our results agree with those of Labiad et al. [50], who demonstrated an increase in flavonoids content in NaCl treated sea fennel plants. Similarly, Yuan et al. [51] demonstrated on radish sprouts that moderate concentration of NaCl (100–150 mM) reduced total phenolic content while total antioxidant capacity remained unchanged. More recently, Emami Bistgani et al. [52] observed an increase in total phenolic content by around 20% after saline irrigation (60 mM NaCl) was applied to *Thymus vulgaris* and *Thymus daenensis*, compared with control plants. Additionally, an increase in leaf flavonoid content by 38.6% and 36.6% was observed in plants grown under salt stress conditions after the application of 60 and 90 mM NaCl. Plants cope with salinity-induced stress by altering metabolic processes and stimulating antioxidant activity to scavenge free radicals and ions chelators. Therefore, salt tolerance seems to be favoured by increased antioxidative compounds against oxidative stress induced by a toxic ion action [53]. Flavonoids are frequently induced by abiotic stress and promote roles in plant protection [54] as happened in our study. Hence, based on previous evidence and current data, it is possible to affirm that salt stress (150 mM NaCl) could be a feasible approach to keep, or even increase, the content of health-promoting compounds in *C. maritimum*.

Few studies have examined the storage conditions for edible halophytes leaves, with a clear lack of knowledge on *C. maritimum* shelf-life. The storage period of halophytes is usually limited to around a week, so high-tech storage and shipping conditions are required for longer periods [55]. The results presented here show that crop cultivation in controlled soilless conditions, even when salty, can yield production of high quality and good storability. The leaves kept their marketability until 12 days at 5 °C. Concerning the colour, NaCl produced clearer leaves, probably due to the presence of salt crystals. To corroborate this, a detailed microscopy study would be needed. These results are in agreement with D'Imperio et al. [56] who found similar colour parameters on wild sea fennel collected along sea shoreline, which is the natural habitat of this species. Colour is among the first quality parameters catching the attention of consumers with a strong influence on consumers' choice and opinion about the food quality [1,7]. Changes of colour observed in our study were subtle and undetected by the trained sensory panel.

Modified atmosphere packaging is commonly used for fresh produce quality maintenance, prolonging shelf-life, and decreasing the microbial growth on perishable commodities [57]. The atmosphere reached in our experiments seems to be adequate, since no off-odours related to anaerobic metabolism were detected. The high relative humidity inside the packages made the weight loss almost negligible, indicating that the modified atmosphere is convenient for retaining succulence and firmness. The relatively lower firmness of leaves obtained from salinity did not affect the shelf-life.

The reduced microbial load at harvest for leaves grown with 150 mM NaCl would be related to the fact that they had less aerial biomass, so microorganisms appeared later and/or in fewer number than in the control samples. However, at the end of storage, that difference was negligible. Abadias et al. [58] obtained similar values (10⁶ to 10⁷ CFU g⁻¹) of yeast and moulds in fresh-cut lettuce grown under salty conditions. Enterobacteriaceae, a common species in raw vegetables, even when reduced with NaCl, was still present, being an indicator of contamination, that should be carefully avoided in a floating system. The absence of Enterobacteriaceae is an ideal starting point prior to storage and commercialisation.

5. Conclusions

A saline-nutrient solution may be used successfully in hydroponic-grown sea fennel plants to enhance raw product quality and post-harvest shelf-life. The product presents a high concentration of flavonoids, a good sensory quality, and a reduction of microbial load. Consequently, it could be said that the saline treatment can be useful for the hydroponic culture of sea fennel obtaining a product with good marketability as an emerging crop for fresh consumption.

Nonetheless, variability in yields and chemical composition with geographical origins, harvesting and post-harvesting conditions needs to be explored and better understood prior to large-scale commercialisation for both farmers and consumers.

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