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Differences in nutrient composition of sea fennel (*Crithmum maritimum*) grown in different habitats and optimally controlled growing conditions

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ABSTRACT

Crithmum maritimum L. is an edible halophyte with large potential in human nutrition field. However, it is unclear whether its nutritional value is maintained throughout the contrasting habitats where it commonly grows (cliffs, sandy and rocky beaches) and the nutritional profile of cultivated plants still remains uncertain. In this work, we provided for the first time a comparison of the nutritional profile of *C. maritimum* across its different type of habitats in the south of Spain and between wild plants and plant material under optimal growing conditions. The protein, amino acids, lipids, fatty acids, minerals composition and phenolic content of plants were analysed. Plants under field conditions exhibited a nutritionally balanced composition (3.8-6.2 g protein/100 g DW, 4.9-7.5 mg lipids/g WW, 3.9-5.0 g Na/100 g DW), with high phenolic content (30.2-48.0 mg/g DW) regardless of the variability of the contrasting habitats. In contrast, under optimal conditions, *C. maritimum* showed a greater protein and lipid content (10.2 g/100g DW and 9.6 mg/g WW, respectively), and lower sodium accumulation (1.2 g/100 g DW), allowing a greater consumption of this halophyte without exceeding the daily intake recommendations. Conversely, phenolics were strongly decreased in these plants (6.1 mg/g DW) likely due to the absence of stress factors.

Keywords: halophyte, sea fennel, food analysis, food composition, plant valorization.

Abbreviation list: DW, dry weight; EC, electrical conductivity; GAE, gallic acid equivalent; PUFA, polyunsaturated fatty acid; SD, standard deviation; SFA, saturated fatty acid; TFC, total flavonoid content; THC, total hydroxycinnamic content; TPC, total phenolic content, WW, wet weight.

1. Introduction

Halophytes represent approximately 1% of all worldwide land plants, including nearly 6000 species. They are commonly found in coastlines worldwide where they are subjected to several abiotic stresses, including exposure to fluctuating soil salinity or temporal droughts. In a global scenario where, agricultural land is increasingly limited due to salinization and desertification processes, together with shortage of freshwater, exploitation of halophytes has been highlighted as an interesting crop in saline or salinized soils where other species are not able to grow (Li et al., 2020). Most conventional crops are glycophytes to which salt excess impairs their growth by affecting nutrient and water uptake (Talbi Zribi et al., 2020). By contrast, halophytes have developed morphological, physiological and biochemical adaptations to tolerate excess salt and reproduce under high saline conditions of at least 200 mM NaCl (Petropoulos, Karkanis, Martins & Ferreira, 2018).

Halophytes are commonly used for the production of food, fertilizers, phyto-fuels, as well as for processes of phytoremediation and desalination (Shaer & Attia-Ismail, 2015). Furthermore, halophytes have been consumed by local populations and used in traditional medicine due to their nutritional and therapeutic properties for centuries (Panta et al., 2014). These plants are considered a good source of protein, fiber and fatty acids (Ventura & Sagi, 2013; Castañeda-Loaiza et al., 2020) and vitamins A, C or B6 and tocopherols providing antioxidant properties (Lima et al., 2020; Castañeda-Loaiza et al., 2020). In addition, they are good sources of minerals, such as calcium, magnesium and potassium (Agudelo et al., 2021). Additionally, they synthetize secondary metabolites such as phenolic compounds as a response to salt stress-induced oxidative damage, with known antioxidant properties highly appreciated for human consumption (Ventura &

Sagi, 2013). This nutritional and antioxidant profile makes halophytes an interesting food supply with functional potential (Romojaro et al., 2013), providing chemical compounds with biological properties. Recently, some halophytes such as *Salicornia spp. and Sarcocornia* spp. have gained increasing interest in gourmet cuisine (Barreira et al., 2017; Maciel, Domingues, Domingues, Calado & Lillebø, 2020) and other species such as *Halimione portulacoides*, *Atriplex halimus* and *Cakile maritima* have been proposed as potential crops with high economic interest in the human nutrition field (Maciel et al., 2018; Martins-Noguerol et al., 2021). Nonetheless, halophytes still constitute an underexploited resource with great potential for the food industry (Nikalje et al., 2018). Current knowledge of the nutritional profiles of halophytes is still scarce, and it has been proved that environmental conditions including edaphic variables such as soil texture, electrical conductivity or pH considerably affect the plant elemental composition (Jan et al., 2018). Furthermore, limited information is available regarding nutritional composition of cultivated halophytes, and several recent studies have reported substantial differences in nutritional composition between wild and cultivated plants of the same species (e.g. see Castañeda-Loaiza et al., 2020).

Sea fennel (*Crithmum maritimum* L., Apiaceae), also known as rock samphire, is an herbaceous and edible halophyte in coastal habitats throughout Western Europe. It is consumed in Spain, Greece and Italy as an ingredient in salads, sauces, soups, pickled in vinegar or as condiments (Meot-Duros & Magne, 2009). Its aerial parts have considerable nutritional and functional value since they are rich in phenolic compounds and mineral elements (Nabet et al., 2017), and it has recently received special interest in modern and innovative cuisine due to its sensorial properties (Romojaro et al., 2013). In an ecological context, recent studies reported the ability to grow this species by watering with brine

withouth influence negatively the plant development (Gómez-Bellot et al., 2021), which highlights its potential in saline agriculture.

To date, research on nutritional profile of *C. maritimum* has been mostly focused on plant material collected only from a narrow range of local wild genotypes (Meot-Duros & Magne, 2009; Sánchez-Faure et al., 2020) and a considerable variation has been identified in the nutrient and antioxidant profiles depending on its geographic origin. Moreover, seasonal variations were reported within phenolics in this species (Barroso et al., 1992). It is well known that *C. maritimum* can thrive in a wide range of habitats (including cliffs, sandy and rocky beaches), growing in soils with highly variable physicochemical properties and subjected to highly contrasted environmental conditions. Given the recent interest in the explotaition of this halophyte for human consumption and as a source of bioactive compounds in nutraceutical industry, it is increasingly necessary to test whether its phytochemical composition remains unchanged under the different soil physicochemical properties of contrasting habitats. Furthermore, it is not clear if cultivated plants would maintain the attractive nutritional profile showed by wild plants.

The aim of this study is to analyse whether the nutritional composition and phenolic content of wild *C. maritimum* plants (in terms of proteins, aminoacids, lipid composition, mineral elements and phenolic compounds) varies depending on the type of habitat and to evaluate whether the nutritional profile is modified when plants grow under optimal controlled conditions. Solving these questions would provide substantial information in order to develop agrotechnical practices aimed at improving the quality of vegetable products derived from this halophyte.

2. Material and methods

2.1. Field sampling and plant material

Four wild populations of *C. maritimum* were selected along the southern coast of Spain to reflect the variety of ecosystems where the species grows. The selected populations included at least 30 adult plants with at least one flowering stem each. The sampling habitats presented different topographies and soil properties which are representative of main types of habitat for the study species: El Toyo (sandy beach; Retamar, Almería), Los Muertos (rocky beach; Carboneras, Almería), Calblanque (cliffs; Cartagena, Murcia) and Roche (sandy beach; Conil de la Frontera, Cádiz). The populations showed average distances to the high tide line of 20.6 ± 3.2 meters (El Toyo), 44.5 ± 3.5 m (Los Muertos), 16.9 ± 2.1 m (Calblanque) and 47.4 ± 2.8 m (Roche). In mid-September 2019, twelve adult plants were selected at each population, with an average plant height of 39.6 ± 14.3 cm. Plants were separated by at least 4 m each other. For each plant, we randomly collected 35-40 fully expanded leaves for protein, amino acids, lipids, fatty acids, phenolic compounds, and mineral nutrients analyses.

To evaluate the nutritional value of *C. maritimum* under optimal greenhouse conditions, in January 2019 root cuttings (c. 2 cm long) were collected from 20 individuals at the Roche wild population. Root cuttings were planted at the greenhouse facilities of the University of Seville in wet perlite during one month until they developed roots and sprouts. Experimental plants (n=10) were then potted in individual plastic pots (13.5 cm diameter x 18 cm height) with bottom drainage holes using commercial washed sand (0.5-1 mm size particle) as substrate. To achieve optimal growing conditions, plants were grown under non-limiting nutrient supply by irrigation with 20% Hoagland´s solution (Hoagland & Arnon, 1938) supplemented with 50 mM NaCl. During the experiment, the pH of the irrigation solution was maintained between 8.19-8.45. At the beginning of the experiment, a 3 L volume of the solution was placed in each of the trays, to a depth of 1 cm. To maintain 50 mM NaCl concentration during the experiment, solution levels in the

trays were monitored and topped up to the marked level with 20% Hoagland's solution (without additional NaCl) whenever necessary. The average frequency of top-up of the solution was every 3 days with approximately 400 mL of non-NaCl containing solution. The entire solution (including 50 mM NaCl) was changed every two weeks. Greenhouse conditions were maintained under natural daylight (200 μmol m^{-2} s^{-1} as the minimum and 1000 μmol m^{-2} s^{-1} as the maximum light flux), temperature between 23-25 °C and 40-60% relative humidity. After 60 days of plant growth, 20-25 randomly selected and fully developed leaves from each plant were collected, and samples were pooled to generate three replicates for protein, amino acids, phenolic compounds, lipids and mineral nutrients analyses.

2.2. Soil characterisation

In each wild population above described, we collected top soil samples (0-30 cm depth) adjacent to sampled plants for electrical conductivity, pH, organic matter content and texture analyses (n=12). The electrical conductivity (EC) was determined in a 1:5 (w/v) soil:water suspension using a conductivity meter (Crison-522, Barcelona, Spain). Soil pH was potentiometrically determined in a 1:2.5 (w/v) soil:water suspension using a digital meter (Crison pH-25, Barcelona, Spain). The organic matter content was estimated by using a muffle furnace calcination (muffle HD-230, Hobersal S.L., Barcelona, Spain) at 450 °C for 4 h (Steubing et al., 2002). For soil texture analysis, coarse elements were removed (> 2 mm) by sieving and the percentage of gravel was estimated. The proportions of coarse and fine sand were determined by sieving in the 2-0.5 mm fraction. Then, the proportions of fine sand, silt and clay were determined in the < 0.5 mm fraction according to the Bouyoucos hydrometer method (Bouyoucos, 1962).

2.3. Protein and amino acid composition

Leaves samples were washed with water diluted HCl (1%) and deionized water. Then samples were dried during 48 h at 70 °C. Dried samples were ground using a plant grinder. The total nitrogen content was determined by the N- Kjeldahl method (Kjeldahl, 1883). Samples were digested with concentrated H_2SO_4 in the presence of a catalyst (Se and K_2SO_4 mixture) during 2 h at 380 °C. Ammoniacal nitrogen assay was carried out by an indophenol method. Nitrogen content was expressed in % on dry weight. Total protein content was calculated by multiplying the total nitrogen content of leaves by a factor of 4.43 according to Yeoh & Wee (1994) for angiosperms.

Amino acids contents were determined in lyophilised leaf material Alaiz et al. (1992). Fresh frozen samples were milled using a knife mill Grindomix GM 200 (Retsch GmbH, Haan, Germany). Samples (4-6 mg of proteins) were hydrolyzed with 4 mL of HCl 6N for 24 h at 110 °C in sealed tubes under nitrogen atmosphere. Later, samples were dried using a rotary evaporator and then resuspended in 10 mL of sodium borate 1M pH 9.0. Next, derivatization process was performed using diethyl ethoxymethylenemalonate (Sigma Chemical Co., Missouri, USA) at 50 °C for 50 minutes. Separation of amino acids was developed by UPLC using a reverse phase column (XSelect HSS T3 2.5 µm of 3.0 × 150 mm, Waters, Massachusetts, USA) in a binary gradient system with 25 mM sodium acetate 0.02% (w/v) sodium azide pH 6.0 (Buffer A) and acetonitrile (Buffer B) as solvents. The elution was developed at 25 °C with a elution flow of 0.8 mL/min with the following gradient: time 0-1 min, elution with A:B 92:8; time 1-4.33 min, linear gradient from A:B 92:8 to A:B 86:14; time 4.33-7.32 min, elution with A:B 86:14; time 7.32-11.65 min, linear gradient from A:B 86:14 to A:B 72:28; time 11.65-13.31, linear gradient from A:B 72:28 to A:B 65:35; time 13.31-15.64, linear gradient from A:B 72:28 to A:B 92:8. D,L-α-aminobutyric (Sigma Chemical Co., Missouri, USA) was used as an internal

204 standard to calculate the content of each amino acid using calibration lines obtained for
205 each one. The amino acids used for obtaining the calibration lines were submitted to the
206 same analytical conditions of the samples to avoid the mistakes made for the modification
207 or loss of amino acids during acid hydrolysis. Results are expressed in percentage (g
208 amino acid/ 100 g amino acids) as mean ± SD of three-twelve independent replicates. To
209 determinate the tryptophan content, samples of 20 mg of proteins were hydrolyzed with
210 3 mL of NaOH 4N at 110 °C for 4 h in sealed tubes under inert nitrogen atmosphere
211 according to Yust et al. (2004). Subsequently, samples were neutralized with HCl and
212 completed with 1 M sodium borate buffer pH 9.0 (up to 10 mL). Quantification of
213 tryptophan was developed by UPLC using a reverse phase column (XSelect HSS T3 2.5
214 µm of 3.0 × 150 mm, Waters, Massachusetts, USA) using as elution solvents the buffers
215 A:B (91:9) in a elution flow of 0.8 mL/min and 25 °C of analytical temperature. Results
216 are expressed in percentage (g amino acid/ 100 g amino acids) as mean ± SD of three-
217 twelve independent replicates.

218

219 ***2.4. Lipid extraction and fatty acid composition***

220 Samples (approximately 1 g of fresh leaves) were kept in tubes with 4 mL of 2-propanol
221 and they were transported to the lab for lipid analysis. Total lipids were extracted
222 according to Hara & Radin (1978). Plant material was ground in a glass homogenizer
223 with 4 mL of 2-propanol and some sea sand. Then, the mixtures were heated at 80 °C
224 during 15 min to inactivate phospholipases and increase the yield extraction.
225 Accordingly, 6 mL hexane were added to the samples and shaken vigorously, and then 5
226 mL sodium sulphate 6.7% (w/v) were also added and mixed again. The mixture was
227 centrifugated and the upper hexane-rich phase containing lipids was transferred to clean

tube. The aqueous phase was extracted again with 7.5 mL of hexane:2-propanol (7:2, v/v), and the upper phase was extracted and combined with the previously obtained.

Fatty acids methylation was performed by adding 3 mL methanol:toluene:sulphuric acid (88:10:2, v/v/v) to the lipid samples and the mixtures were heated at 80 °C during 1 h (Garcés & Mancha, 1993). Fatty acid methyl esters (FAMEs) were extracted twice with 1 mL heptane and analysed by GLC using a Perkin-Elmer Clarus500 GC gas chromatograph and a Supelco SP-2380 capillary column (60 m length, 0.25 mm i.d., 0.2 µm film thickness; Supelco, Bellefonte, PA, USA). Hydrogen was the carrier gas at 20 cm/s, with 220 °C temperature of flame ionization detector and injector, 185 °C for the oven temperature being the split ratio 100:1. As internal standard for lipid and fatty acid quantification heptadecanoic acid (17:0, Sigma-Aldrich, Missouri, USA) was used. A combination of standards was used for identification of the different methyl esters. The area of the peaks were determined as final step of the peak integration using ChemStation V.B04 software (Agilent, Santa Clara, USA). The % values reported were determined as % of each peak respect to total area detected.

2.5. Mineral composition in plant leaves

Samples were washed with water diluted HCl (1%) and deionized water. Then samples were oven-dried during 48 h at 70 °C and ground using a plant grinder. Samples of approximately 0.5 g of dried material were weighed directly into Teflon vessels. Accordingly, 4 mL NHO_3 suprapur (TracepureTM 140 HNO_3; Merck, New Jersey, USA) were added to the samples and they were shaken gently. Samples were then subjected to microwave digestion (START D Microwave Digestion System, Milestone, Sorisole, Italy). After cooling, the digests were diluted with ultrapure water (<18 MΩ/cm) up to 50 mL and they were passed through nylon filters (0.45 µm). The extracts were cold stored

until further use. The foliar concentrations of mineral elements were analysed by inductively coupled plasma optical emission spectroscopy, ICP-OES, with a Varian ICP 720-ES (Agilent Technologies, Inc., Santa Clara, CA, USA). The operating conditions for ICP-OES were as follows: power: 1.30 kW; plasma gas flow: 16.5 L/min; auxiliary gas flow: 1.50 L/min; spray chamber type: glass cyclonic; Torch: standard axial torch; Nebulizer type: seaspray; Nebulizer gas flow: 200 kPa; Replicated read time: 10 s; Number of replicates: 3; Sample delay time: 40 s; Stabilization time: 15 s; Rinse time: 10 s; Fast pump: On; Background correction: fitted. Y 1000 mg/L (Merck, New Jersey, USA) was used as internal standard. The accuracy and precision of method were confirmed by standard reference material (*Brassica oleracea* sample from Plant-analytical Exchange (IPE) international program, Wageningen Evaluating Programmes for Analytical Laboratories, WEPAL). Calibration curves were performed in HNO_3 8% with the multi-elemental standars Certipur multi-elemental standard solution (Merck, New Jersey, USA) and Spectrascan certified reference solution (LGC Standards GmbH, Wesel, Germany) and the phosphorus mono-elemental standard for its calibration curve. The LOD and LOQ, recovery test and RSD% values are provided in Supplementary Table 1. The elements sodium (Na), calcium (Ca), potassium (K), magnesium (Mg) and phosphorous (P) were expressed in percentage (g/100 g dry weight, DW) and the elements copper (Cu), iron (Fe), manganese (Mn), chromium (Cr) and zinc (Zn) as well as the toxic metals lead (Pb) and cadmium (Cd) were expressed in mg/kg DW.

2.6. Identification and quantification of phenolic compounds

Phenolic compounds were extracted from 20 mg of dried leaf material with 0.25 mL of 70% methanol in an ultrasonic bath for 15 min, followed centrifugation, the extract was filtered through a 0.20-µm micropore PTFE membrane and placed in vials for

chromatographic analysis (Moreira et al., 2021). Chemical identification of the polyphenol composition was performed using an ultra-performance liquid chromatography coupled with electrospray ionization quadrupole (Thermo Dionex Ultimate 3000 LC) time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS) (Compact™) (Bruker Daltonics GmbH, Bremen, Germany). Chromatographic separation was developed in a Kinetex™ 2.6 μm C18 82-102 Å, LC Column 100 × 4.6 mm column with a binary gradient solvent mode consisting of 0.05% formic acid in water (solvent A) and acetonitrile (solvent B). The gradient used was the following: from 10% to 30% B (0–5 min), from 30 to 50% B (5-10 min), from 50 to 100% B (10-12 min), hold 100% B until 14 min, from 100% to 10% B (14-15 min), hold 10% B until 17 min. The injection volume was 3 μL, the flow rate was established at 0.4 mL/min and column temperature was controlled at 35 °C. MS analysis was operated in a spectra acquisition range from 50 to 1200 *m/z*. Negative (-) ESI modes were used under the following specific conditions: gas flow 8 l/min, nebulizer pressure 38 psi, dry gas 7 l/min, and dry temperature 220 °C. Capillary and end plate offset were set to 4500 and 500 V, respectively. MS/MS analysis was performed based on the previously determined accurate mass and RT and fragmented by using different collision energy ramps to cover a range from 15 to 50 eV. Individual compounds were identified on the basis of the data obtained from the standard substances or published literature, including RT, λmax, ($[M–H]^-$), and major fragment ions.

For the quantitative analysis of phenolic compounds, 10 μL of each sample was then analysed using the same column and conditions described previously, in an UHPLC (Nexera LC-30AD; Shimadzu, Tokio, Japan) with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS photodiode array detector (Shimadzu, Tokio, Japan); see Moreira et al. (2021) for more details of the chromatographic analyses. Chromatograms were recorded at 330 nm. The flavonoids were quantified as rutin equivalents and

hydroxycinnamic acids as chlorogenic acid equivalents. We achieved the quantification of these phenolic compounds by external calibration using calibration curves at least with six data points, from 0.01 to 1mM. Caffeoyl quinic acis and p-coumparoyl quinic acids derivatives were quantified as chlorogenic acid (hydroxycinnamic acids) (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), flavonoids were quantified as rutin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). The limits of detection and quantification for the compounds were in the range of 0.3 and 1 ng for chlorogenic acid and 0.6 and 1 ng for rutin. The recoveries of compounds were calculated in three different concentrations in the range of 93.7–104.1%. Total phenolic content was calculated as the sum concentration of each individual compound.. Phenolic compound concentrations were expressed in mg/ g tissue on a dry weight (DW) basis as mean ± SD of three-twelve independent assays.

2.7. Statistical analyses

All experiments were performed at least in triplicate and the results expressed as mean ± standard deviation of the mean. Statistical analyses were performed using IBM SPSS v. 24.0 software (IBM Corp., New York, USA). Data were analysed by one-way analysis of variance (ANOVA) and significant differences were determined by Tukey test. First, data were tested for normality with Kolmogorov-Smirnov test and for homogeneity of variance with Levene test. For data not normally distributed, the non-parametric Kruskal-Wallis test followed by Mann-Whitney U test was employed.

3. Results

3.1. Soil characteristics

The physicochemical properties of soils samples collected at the different habitats of *C. maritimum* were analysed (Table I). Roche presented signifficantly lower organic matter content than the other sites, while pH was strongly alkaline and constant across sites. Concerning the electrical conductivity (EC), Calblanque and Roche displayed the lowest EC values whereas it was higher in Los Muertos and El Toyo (Table 1), although not statistically significant in the latter. Concerning soil physical analysis, the highest gravel percentage appeared in Los Muertos and the lowest in Roche; fine sand content was highest at El Toyo and lowest at Roche, whereas all habitats were similar in terms of coarse sand, slit and clay contents (Table 1).

3.2. Nutritional profile

3.2.1. Total protein, lipid and phenolic content

Total protein, lipid and phenolic compounds content of *C. maritimum* leaves are shown in Fig. 1. Crude protein content of plants under field conditions ranged from 3.8% (DW) in Roche to 6.2% (DW) in El Toyo (Fig. 1A). Lipid content ranged from 4.9 mg/g (wet weight, WW) in Calblanque to 7.5 mg/g (WW) in Los Muertos (Fig. 1B). Concerning total phenolic content (TPC), *C. maritimum* plants under field conditions showed between 30.3-48.0 mg/g DW, showing plants from Calblanque cliffs values significantly higher than other wild populations (Fig. 1C).

Total protein and lipid contents were significantly higher ($p<0.05$) in plants under optimal greenhouse conditions in comparison to those from the same genotype under field conditions. Crude protein increased more than two-fold, reaching 10.2% (DW) (Fig. 1A) and total lipids increased by 25% displaying a value of 9.6 mg/g WW (Fig. 1B). However,

TPC drastically decreased by 80% in plants under optimal conditions in comparison with the same genotype under field conditions ($p<0.05$) (Fig. 1C).

3.2.2. Amino acid composition

The essential amino acid profile of *C. maritimum* plants analysed is listed in Table 2. The most abundant amino acids detected in *C. maritimum* plants under field conditions were Leu, Lys, Val, Phe and Thr. Otherwise, the sulphur amino acids (Met + Cys) and Trp were detected in the lowest proportion. In these plants, the total essential amino acids percentage registered values between 41.2% and 42.7%.

In plants under optimal growing conditions, the amino acids Phe, His and Ile significantly increased in comparison with values registered in field plant material ($p<0.05$). This increase was reflected in total essential amino acid percentage, which also rised significantly in plants under optimal controlled conditions (45.6%) ($p<0.05$).

3.2.3. Fatty acid profile

The lipid fraction of plants under field conditions was dominated by unsaturated fatty acids, particularly by polyunsaturated fatty acids (PUFA) (Table 3), ranging from 46.4% in Roche to 64.0% in Los Muertos. PUFA linoleic ($18:2\Delta^{9,12}$) and α-linolenic ($18:3\Delta^{9,12,15}$) acids displayed the most remarkable levels. The monounsaturated fatty acids (MUFA) ranged from 4.3% (Los Muertos) to 25.4% (Roche). Within MUFA, petroselinic acid ($18:1\Delta^{6}$) showed the most variable levels depending on the type of habitat, displaying the highest value in Roche (18.1%) and being practically undetectable in Los Muertos. No significant differences were observed in the polyunsaturated to saturated fatty acid

ratio (PUFA/SFA) among contrasting habitats, which displayed values in the range of 1.7 to 2.0.

The lipid profile of plants under optimal controlled conditions in greenhouse was also dominated by PUFA, which increased significantly by 11% ($p<0.05$) in comparison with those values obtained in leaf material from the same genotype under field conditions. Otherwise, no significant differences were observed in MUFA or saturated fatty acids (SFA) contents between material under field and optimal controlled conditions. Accordingly, the PUFA/SFA ratio was also significantly increased (2.4) in plant material under optimal conditions ($p<0.05$). Concerning the fatty acid species mostly represented, α-linolenic acid increased significantly ($p<0.05$) whereas no significant differences were observed in linoleic and petroselinic acids regarding the same genotype under field conditions.

3.2.4. Phenolic compounds

The foliar concentration of phenolic compounds identified in *C. maritimum* plants is listed in Table 4. We detected phenolic compounds from two groups: hydroxycinnamic acids and flavonoids. In *C. maritimum* plants under field conditions the phenolic profile was mostly represented by flavonoids (in the range of 25.1-41.0 mg/g DW, showing Calblanque cliffs significantly higher values ($p<0.05$)), and being rutin the dominant compound. The total hydroxycinnamic acids content displayed values in the range of 4.1-7.0 mg/g DW across the wild studied populations.

Although no differences were detected in the total hydroxycinnamic acids content in *C. maritimum* plants under controlled conditions compared with the same genotype under field conditions, total flavonoid content was significantly decreased by 89%

397 ($p<0.05$). Furthermore, kaempferol 3-glucoside-7-rhamnoside was detected only in plants
398 under optimal greenhouse conditions whereas ferulic acid, quercetin-O-hexoside and
399 quercetin-7-xyloside were only detected in samples from field conditions.
400
401 ***3.2.5. Mineral composition***

402 Na was the most abundant mineral element in plant material under field conditions
403 (Table 5), without significant differences among the studied populations. The following
404 most abundant elements were Ca>K>Mg>P. However, significant differences were
405 observed among different populations regarding the content of these elements (Table 5).

406 Other minerals were detected in a lower proportion in all samples as following:
407 Fe>Mn>Zn>Cu>Cr. However, significant differences were observed in the content of
408 some of these elements regarding the contrasting habitats (Table 5). Fe content was the
409 most variable, showing Roche and El Toyo the highest levels whereas the lowest content
410 was detected at Los Muertos. El Toyo showed the significantly higher values of Mn, Zn
411 and Cu ($p<0.05$), together with Los Muertos for the latter element. The toxic metals Cd
412 and lead Pb showed values between 0-0.6 mg/kg DW and <0.1-0.9 mg/kg DW,
413 respectively.

414 *C. maritimum* plants under optimal greenhouse conditions significantly increased
415 the K, P, and Cr contents in comparison to the levels from the same genotype under field
416 conditions ($p<0.05$). In contrast, Na and Fe significantly decreased in plants under
417 optimal controlled conditions ($p<0.05$), respectively.
418
419 **4. Discussion**
420

4.1. Protein and amino acids

The protein content we reported in this study for *C. maritimum* leaves from contrasting habitats was lower than previously described by Sánchez-Faure et al. (2020), who reported 11% (DW) for plants growing in north coast of Galicia (northwest of Spain). Nevertheless, despite the diversity of constrasting habitats, the protein contents of the sampled plants were within the range of other green leaves and vegetables (0.2-3.9%, WW) (Slavin & Lloyd, 2012) and it was close to values recorded for other edible halophytes such as *Sarcocornia perennis* (6.9 g/100 g DW) and *Salicornia ramosissima* (5.5 g/100 g DW) (Barreira et al., 2017). Under optimal growing conditions *C. maritimum* plants exceeded these values. Previously, Castañeda-Loaiza et al. (2020) described an increase in protein content of cultivated halophytes when comparing with the same species growing wild in their natural habitats. Here, the increase that we observed in *C. maritimum* plants protein content is particularly remarkable since it reaches values close to other cultivated halophytes highly appreciated in gourmet cuisine such as *Sarcocornia fruticosa* (12.6 g/100 g DW) (Castañeda-Loaiza et al., 2020).

The nutritional value of food protein not only depends on the quantity but also on their amino acid composition. Concerning the amino acid profile of *C. maritimum* plants under field conditions, all the values met the recommended dietary allowance (RDA) according to FAO (2002), with the exception of sulphur amino acids for the sandy beach El Toyo and rocky beach Los Muertos. Within essential amino acids, the high proportion of Lys was remarkable since it is limiting in cereal grains (together with Trp), which represents one of the main sources for human food. Moreover, Lys is also involved in protein synthesis and degradation, and it plays a crucial role in metabolism, brain development, electrophysiology and neurotransmitter regulation in humans (Tomé & Bos, 2007; Hallen et al., 2013). The high levels of branched amino acids (Leu, Val, Ile)

were also noteworthy, since they are involved in protein synthesis and glucose and energy metabolism in humans (Monirujjaman & Ferdouse, 2014). The greater content of essential amino acids His, Ile and Phe in plants under optimal growing conditions increased total essential amino acids percentage up to a higher value than those previously reported for *C. maritimum* (37%) (Sánchez-Faure et al., 2020).

4.2. Total lipids and fatty acids

The total lipid content of *C. maritimum* plants from the different sampling habitats was in agreement with those previously reported for this species (0.4-0.7 g/100 g WW) (Sánchez-Faure et al., 2020) and they were within the range of common leafy vegetables (0.2-1.4 g/100 g WW) (Slavin & Lloyd, 2012). Moreover, it was higher than those previously reported in other halophytes with food potential such as *Mesembryanthemum crystallinum* (0.1 g/100 g WW) and *Triglochin maritima* (0.2% g/100 g WW) (Sánchez-Faure et al., 2020).

Fatty acids are bioactive molecules present in vegetables, and some of them, such as essential fatty acids linoleic and α-linolenic acids, must be acquired through the diet since humans cannot synthesize them (Loconsole et al., 2019). The fatty acid profile of all plants under field conditions was dominated by PUFA and characterized by a relative abundance of linoleic and α-linolenic acids. PUFA are bioactive compounds with antifungal properties, and additionally they inhibit carcinogenesis and the progression of atherosclerosis (Margină et al., 2020). Halophytes are considered a good source of α-linolenic acid comparing with other green leafy vegetables as lettuce, red leaf lettuce, spinach or mustard, which have less than 0.9 mg/g WW (Simopoulos, 2004). α-linolenic is a precursor of several ω-3 fatty acids and shows antinflammatory and anti-thrombotic activities, being the consumption of ω-3 rich foods recommended to prevent

cardiovascular diseases (Marangoni et al., 2020). In this sense, *C. maritimum* leaves showed relatively high amounts of α-linolenic regardless of the type of habitat, in a range of ~1.5 to 2.3 mg/g WW, supporting the potential of this halophyte as a healthy food. Moreover, the PUFA/SFA ratio observed in the studied populations is in agreement with nutritional guidelines that recommend a minimum ratio of PUFA/SFA of 0.4-0.5 (WHO/FAO, 2003).

While unexpected, considerable values of petroselinic acid were detected in *C. maritimum* leaves and its level was significantly variable depending on the sampled population. Petroselinic acid is a less-common monounsaturated isomer of oleic acid with dietary benefits that is present in high quantities in plant seed oils belonging to the Apiaceae family. This fatty acid has many applications in functional food and for pharmaceutical and nutraceutical industries (Delbeke et al., 2016), thus representing an added value for the full exploitation of *C. maritimum*. To our knowledge, we reported for the first time noticeable levels of petroselinic acid in *C. maritimum* leaves. Based on our results, petroselinic acid production appears to be specific on sampled population, since it was the fatty acid with the most variable levels among the different wild populations and no significant differences were detected when compared material from field and under optimal controlled conditions in greenhouse. In *C. maritimum* plants growing under optimal conditions, the fatty acid profile was similar to that of the same genotype under field conditions except that it showed higher levels of PUFA, showing α-linolenic the highest increase. In addition to α-linolenic, other unsaturated fatty acids increased their content in these plants, whereas some saturated fatty acids decreased, and that was reflected in a higher PUFA/SFA, which is a more favorable trait from a health perspective (Chen & Liu, 2020). These findings, together with the higher lipid accumulation under

controlles conditions, suggests that specific cultivation conditions could produce plants with higher bioactive profile for functional food or nutraceutical industries.

4.3. Mineral composition

Concerning the mineral composition, Na was the most abundant element in plants collected from the contrasting habitats under field conditions. Halophytes usually accumulate Na in their tissues mainly due to the natural abundance of this element in soils where they commonly grow. Although Na is an essential nutrient in the human diet, its excess intake is associated with the increase in blood pressure, which represents a risk factor for cardiovascular diseases (Mozaffarian et al., 2014). Consequently, a maximum intake of 2 g of Na per day is recommended (WHO, 2012). Accordingly, the consumption of some gourmet halophytes is recommended only as a condiment or salt substitute in order to not exceed the maximum daily intake recommended (Castañeda-Loaiza et al., 2020). Likewise, high Na content was previously reported in wild *C. maritimum* (14.7 g/kg WW) (Sánchez-Faure et al., 2020). However, we reported lower Na levels in *C. maritimum* plants from contrasting habitats. Assuming 88% leaf moisture content (mean value registered in field plant material -data not shown-) still a meal containing 100 g of these fresh plants will not exceed the maximum recommended per day (0.46-0.60 g). Concerning the elements K, Ca, Mg, and P the daily reference intake are 2000, 800, 375, and 700 mg, respectively (Regulation (EU) N° 1169/2011). Considering this information, the consumption of a serving of 100 g of fresh plant material collected from the populations analysed in our study would represent 10-14% for K, 34-56% for Ca, 12-19% for Mg, and 2-3% for P of the daily intake recommended of these elements. Among the mineral elements, Ca and Mg are particularly important in human nutrition due to their critical role in in cellular metabolism and bone structure and development. *C. maritimum*

was reported to present high Ca content (Gómez-Bellot et al., 2021), even higher than broccoli, which is one of the best vegetable sources of Ca in the human diet (Romojaro et al., 2013). Our results support this observation and indicate Ca content remains high in *C. maritimum* leaves across the contrasting habitats.

Within the elements Fe, Cu, Mn, Zn and Cr, the nutrient reference value for human consumption are 14, 1, 2, 10 mg, and 40 µg, respectively (EU N ° 1169/2011). Based in our results of plant material under field conditions, a meal containing 100 g of fresh plants could supply 22-48% for Mn or 16-41% for Cr whereas Fe, Zn and Cr supply would reach 5-18%, 3-5% and 16-41%, respectively. Our results indicate that the wide range of mineral accumulation in these plants depends on the habitat type. Further studies including a large array of sites and edaphic conditions should be conducted to test whether the variation of specific soil properties contributes to variable leaf mineral compositions. In a health and safety perspective, Cd and Pb toxic metals were practically undetected in all samples, below the maximum permissible threshold in leafy vegetables according the Codex Alimentarius Commission of the Food of FAO and WHO (Codex Alimentarius-1995).

It is interesting to remark that *C. maritimum* plants under optimal greenhouse conditions showed the lowest Na content (143 mg Na per 100 g serving). Considering that these experimental plants were grown under moderate salt levels (50 mM NaCl), this reduced salinity in leaves would allow a greater consumption of this halophyte, thus avoiding the high salt intake commonly associated with the consumption of this type of plants. This finding highlights the potential of *C. maritimum* for human consumption in comparison with other halophytes exhibiting high Na levels even when they are cultivated with frequent irrigation (Castañeda-Loaiza et al., 2020). *C. maritimum* is considered as a salt-includer halophyte that accumulates Na^+ and Cl^- toxic ions into vacuoles without

compromising their water status, and being able to accumulate salt in roots, shoots and leaves (Hamdani et al., 2017). However, the existence of different ecotypes regarding the response to salinity has been suggested (Ventura et al., 2014). More recently, Jiménez-Becker et al. (2019) described that the salt tolerance of *C. maritimum* is conferred by the ability to restrict the entry of saline ions through the root limiting the transport of Cl^- to the aerial parts, salt excretion and accumulation of proline and soluble sugars.

The levels of the other minerals detected in higher proportion were unaffected or even increased in *C. maritimum* plants under optimal conditions in comparison to field plant material. Indeed, we detected significantly higher levels of K under optimal controlled conditions. Wild *C. maritimum* plants are usually more exposed to Na^+ and Ca^{2+} ions than K^+ ions, so the inhibition of K uptake could be produced due to the high concentration of Na in natural environments (Gupta & Huang, 2014). Higher levels of K than Na were also observed in *C. maritimum* plants irrigated with wastewater or brine (Gómez-Bellot et al., 2021). Accordingly, the consumption of plant material grown under optimal growing conditions would supply a more balanced mineral elements intake (36% for K, 44% for Ca, 15% for Mg, and 10% for P of the aforementioned daily references intake). An increase in Cr content in plants under optimal conditions in relation to field plant material was observed, representing this value a contribution of 80% of the recommended daily intake of this mineral per 100 g serving. In this sense, it can be considered an excellent Cr source, being able to supply the daily recommended intake without surpassing the toxicity threshold.

4.4. Phenolic compounds

Phenolic compounds are known as powerful antioxidants and they play important roles in human health, since their intake is associated with the prevention of adverse

effects caused by oxidative stress (Lu & Yen, 2015). In this study, TPC in *C. maritimum* leaves collected from different natural habitats was considerably higher than levels previously reported for other vegetables commonly consumed like spinach (13 mg of gallic acid equivalent, GAE/g DW) or broccoli (10.6 mg GAE/g DW) (Chu et al., 2002). In addition, these values were similar or even higher than those in halophytes such as *Salicornia ramosissima* and *Sarcocornia perennis* which are highly appreciated as gourmet food (33.0 mg GAE/g DW and 20.5 mg GAE/g DW, respectively) (Barreira et al., 2017). Indeed, our results showed that plants growing in cliffs displayed the highest TPC values. Rocky cliffs are harsh environments where plants are commonly exposed to several sources of stress, like mechanical effects of wind, salt-spray and nutrient scarcity. Considering that polyphenols accumulation in plants is strongly influenced by abiotic stress, the higher TPC content recorded in plants collected from cliffs was probably related to the specific environmental conditions of this kind of ecosystems. Recently, Gil et al. (2019) detected that *C. maritimum* accumulates more polyphenols in habitats close to the coastline than inland due to the different exposure to salt. Furthermore, variable levels of phenolics have been reported for *C. maritimum* regarding the season and site collection (10-30 mg GAE/g DW) (Barroso et al., 1992; Meot-Duros & Magne, 2009).

TPC was drastically diminished when *C. maritimum* plants were grown under optimal growing conditions, likely because plants were less stressed, down-regulating the antioxidant defense system including phenolics. Notwithstanding this reduction, leaf-TPC in *C. maritimum* plants under optimal conditions still showed similar values than other wild edible halophytes like *Mesembryanthemum nodiflorum* or *Sarcocornia fruticosa* and even higher than both *M. nodiflorum* and *S. fruticosa* cultivated material (Castañeda-Loaiza et al., 2020). Increasing saline concentration in soil substrate has been proposed to be an interesting strategy to get plants with more antioxidant capacity. Further

595 future studies should be performed to find a salt concentration at which the yield for these
596 valuable metabolites is higher than the drawback of reduced growth mediated by salt
597 stress.

598 Phenolic profile has been suggested to be species-specific in some halophytes, not
599 influenced by either cultivation method or collection site (Castañeda-Loaiza et al., 2020).
600 However, our study did not support this hypothesis in *C. maritimum*, since we found
601 considerable variation in the phenolic profile of the species in studied populations in
602 comparison with those reported in previous studies. Although some differences in
603 phenolic profile could be attributed to the physiological stage and the extraction method
604 (Jallali et al., 2012), our results showed that the most accumulated compounds were
605 flavonoids, with rutin as the most represented, whereas previous works reported phenolic
606 profiles mostly represented by hydroxycinnamic acids in *C. maritimum* plants collected
607 from coasts of western France and northern Spain (Meot-Duros & Magne, 2009; Sánchez-
608 Faure et al., 2020). Rutin, also known as vitamin P, is a flavonoid with neuroprotective
609 effects (Hao et al., 2016) that is widely present in a variety of fruits and vegetables (Marín
610 et al., 2002). Flavonols containing more hydroxyl groups, such as rutin, exhibit a strong
611 capacity for scavenging of free radicals and are well-known potent antioxidants (Cai et
612 al., 2006). The high content of rutin in *C. maritimum* leaves detected in our study gives
613 *C. maritimum* great potential for functional food applications. Otherwise, within the
614 hydroxycinnamic acids, the chlorogenic acid isomers (namely caffeoylquinic, di-
615 caffeoylquinic and feruloylquinic acids) are phytochemicals highly appreciated as
616 utraceutical and food additive attending to their multifunctional properties (Santana-
617 Gàlvez et al., 2017). Besides, chlorogenic acid has several biological activities including
618 antimicrobial, antioxidant and anti-carcinogenic properties (Onakpoya et al., 2015;
619 Santana-Gàlvez et al. 2017).

In addition, focusing in the comparison of field plant material and plants under optimal conditions from the same genotype, it is interesting to remark that some phenolic species only appeared in plants uder field conditions (ferulic acid, quercetin-O-hexoside and quercetin-7-xyloside), whereas other was only detected in plants under optimal growing conditions (kaempferol 3-glucoside-7-rhamnoside). These variations appear to be related to phenotypic plasticity of *C. maritimum* regarding phenolics biosynthesis, both qualitatively and quantitatively, depending on the environmental conditions. In practice, these findings suggest that different cultivation conditions could lead to produce plant products with different phenolic profile. Additional studies should be performed to fully elucidate the phenolic synthesis mechanisms underlying adaptation to different environmental conditions in this species.

5. Conclusions

In this work, plant material of *C. maritimum* from field conditions exhibited a nutritionally balanced composition with high phenolic content regardless of the variability of the environmental conditions in the studied populations. These findings demonstrate the potential of this species regarding its cultivation in poor-nutrient and underutilized saline soils although more studies with higher number of populations should be performed. Furthermore, under optimal growing conditions, *C. maritimum* plants improved its nutritional profile by increasing protein and lipid content and decreasing sodium accumulation, but conversely phenolics were drastically decreased, likely due to the absence of stressors. Our findings provide for the first time a comparison of the nutritional profile of the edible halophyte *C. maritimum* across its different type of habitats. Moreover, this work compares the nutrient composition between wild plants and

plant material under optimal growing conditions, which provides a basic knowledge leading to to optimize cultivation of this edible halophyte.

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Tables

Table 1. Physicochemical properties of the soil in the different studied populations. Data represent mean ± SD of twelve independent replicates. Different letters indicate significant differences among different populations ($p<0.05$).

	Type of habitat	Geographical coordinates	Organic matter (mg C/g dry weight)	pH	Conductivity ($\mu S\ cm^{-1}$)	Gravel (%)	Texture			
							Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)
El Toyo	Sandy beach	36.835718/ -2.325802	23.8 ± 15.3**a**	9.5 ± 0.4**a**	525.5 ± 523.0**ab**	13.5 ± 14.7**b**	83.8 ± 20.6**a**	4.7 ± 4.7**b**	7.7 ± 10.1**a**	3.7 ± 6.5**a**
Los Muertos	Rocky beach	36.956220/ -1.899545	31.4 ± 25.9**a**	9.8 ± 0.3**a**	466.4 ± 249.8**b**	75.9 ± 24.6**c**	88.8 ± 7.8**a**	3.0 ± 3.2**ab**	6.8 ± 4.2**a**	1.4 ± 1.1**a**
Calblanque	Cliffs	37.602117/ -0.731187	51.2 ± 29.2**b**	9.5 ± 0.5**a**	160.0 ± 78.5**a**	2.6 ± 4.1**ab**	93.01 ± 3.8**a**	2.1 ± 2.2**ab**	3.4 ± 1.7**a**	1.4 ± 1.3**a**
Roche	Sandy beach	36.314138/ -6.153952	20.2 ± 9.8**a**	9.4 ± 0.3**a**	168.8 ± 116.8**a**	0.3 ± 0.5**a**	94.8 ± 2.9**a**	1.1 ± 1.0**a**	2.8 ± 1.5**a**	1.3 ± 0.9**a**

Table 2. Essential amino acid composition (g amino acid/ 100 g amino acids) in leaves of *C. maritimum*. Data represent mean ± SD of twelve independent replicates for field samples and three replicates for plants under optimal greenhouse conditions. Different letters indicate significant differences among different populations ($p<0.05$).

	El Toyo	**Los Muertos**	**Calblanque**	**Roche (field conditions)**	**Roche (optimal conditions)**	**RDA[1]**
Histidine	2.0 ± 0.1**a**	2.0 ± 0.1**a**	2.2 ± 0.1**b**	2.1 ± 0.2**ab**	3.2 ± 0.2**c**	1.5
Threonine	5.3 ± 0.2**a**	5.3 ± 0.2**a**	5.4 ± 0.2**a**	5.7 ± 0.3**a**	5.5 ± 0.0**a**	2.3
Tyrosine	3.7 ± 0.2**a**	3.9 ± 0.2**ab**	4.1 ± 0.1**c**	4.0 ± 0.2**bc**	3.8 ± 0.2**ab**	3.8*****
Valine	7.3 ± 3.7**a**	5.8 ± 0.6**a**	5.3 ± 0.4**a**	5.5 ± 1.0**a**	6.2 ± 0.1**a**	3.9
Methionine	0.8 ± 0.4**a**	1.1 ± 0.3**a**	1.5 ± 0.3**b**	1.6 ± 0.3**b**	1.5 ± 0.2**b**	2.2******
Cysteine	0.5 ± 0.1**a**	0.5 ± 0.1**a**	1.0 ± 0.1**b**	0.6 ± 0.0**c**	0.6 ± 0.2**ac**	2.2******
Isoleucine	4.1 ± 0.5**a**	4.4 ± 0.7**ab**	4.5 ± 0.4**ab**	4.2 ± 0.8**a**	5.2 ± 0.1**b**	3.0
Tryptophan	0.8 ± 0.2**a**	1.0 ± 0.3**ab**	0.9 ± 0.2**ab**	1.3 ± 0.4**b**	0.9 ± 0.0**ab**	0.6
Leucine	9.1 ± 0.4**a**	9.3 ± 0.4**a**	9.5 ± 0.2**a**	9.5 ± 0.4**a**	9.7 ± 0.1**a**	5.9
Phenylalanine	5.5 ± 0.3**a**	5.7 ± 0.3**a**	5.6 ± 0.2**a**	5.7 ± 0.2**a**	6.2 ± 0.3**b**	3.8*****
Lysine	6.6 ± 0.4**a**	6.6 ± 0.4**a**	7.1 ± 0.2**b**	7.1 ± 0.3**ab**	7.1 ± 0.1**b**	4.5
Essential amino acids (%)	41.5 ± 3.9**ab**	41.2 ± 2.1**b**	42.1 ± 1.3**b**	42.7 ± 1.8**b**	45.6 ± 0.2**a**	

*Phe + Tyr; **: Met + Cys;

[1] Recommended Dietary Allowance. Reference values from FAO (2002). Data are expressed in mg amino acids/100 mg protein.

Table 3. Fatty acid species (mol%) detected in *C. maritimum* leaves. Data represent mean ± SD of twelve independent replicates for field samples and three replicates for plants under optimal greenhouse conditions. Different letters indicate significant differences among different populations ($p<0.05$). MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, SFA, saturated fatty acids; PUFA/SFA, polyunsaturated to saturated ratio.

	El Toyo	**Los Muertos**	**Calblanque**	**Roche (field conditions)**	**Roche (optimal conditions)**
14:0	3.2 ± 1.0**a**	3.4 ± 01.1**a**	3.7 ± 1.4**a**	2.8 ± 1.2**a**	4.4 ± 0.1**a**
16:0	20.9 ± 1.9**a**	21.6 ± 0.8**a**	19.9 ± 2.6**ab**	17.9 ± 2.0**b**	14.8 ± 0.8**c**
16:1$^{\Delta 9}$	1.6 ± 0.4**ac**	1.6 ± 0.5**ac**	1.1 ± 0.3**b**	1.2 ± 0.3**ab**	1.9 ± 0.1**c**
16:3$\Delta^{7,10,13}$	6.0 ± 1.1**ab**	7.1 ± 1.4**ac**	5.6 ± 1.1**ab**	5.2 ± 1.0**b**	7.9 ± 0.6**c**
18:0	5.2 ± 0.8**a**	5.3 ± 0.7**a**	6.1 ± 1.3**a**	6.0 ± 1.4**a**	3.5 ± 0.5**b**
18:1Δ^{9}	1.5 ± 0.5**a**	2.3 ± 2.0**a**	2.0 ± 1.2**a**	6.0 ± 8.1**a**	2.1 ± 0.5**a**
18:1$^{\Delta 6}$	6.0 ± 5.4**a**	0.3 ± 1.0**b**	11.5 ± 8.4**ac**	18.1 ± 4.8**d**	14.9 ± 0.9**cd**
18:2$\Delta^{9,12}$	27.3 ± 3.0**a**	26.0 ± 2.7**a**	25.2 ± 4.2**ab**	19.3 ± 2.0**c**	21.2 ± 0.5**bc**
18:3$\Delta^{9,12,15}$	27.5 ± 2.4**ab**	31.0 ± 3.4**a**	24.0 ± 3.7**bc**	21.9 ± 4.3**c**	28.0 ± 1.4**ab**
20:0	0.8 ± 0.4**a**	1.4 ± 0.5**ab**	0.9 ± 0.3**ab**	1.5 ± 0.4**b**	1.3 ± 0.1**ab**
MUFA	9.1 ± 5.2**a**	4.3 ± 1.8**c**	14.6 ± 8.1**ab**	25.4 ± 7.0**d**	18.9 ± 1.0**bd**
PUFA	60.8 ± 3.3**ab**	64.0 ± 2.7**a**	54.8 ± 4.7**b**	46.4 ± 6.1**c**	57.01 ± 1.5**b**
SFA	30.1 ± 3.4**ab**	31.7 ± 2.0**a**	30.7 ± 5.1**a**	28.2 ± 3.1**bc**	24.0 ± 1.2**c**
PUFA/SFA	2.0 ± 0.3**ab**	2.0 ± 0.2**ab**	1.8 ± 0.3**a**	1.7 ± 0.3**a**	2.4 ± 0.2**b**

Table 4. Profile of phenolic compounds from the *C. maritimum* leaves expressed in mg/g DW. Data represent mean ± SD of twelve independent replicates for field samples and three replicates for plants under optimal greenhouse conditions. Different letters indicate significant differences among different populations ($p<0.05$). TFC, total flavonoid content; THC, total hydroxycinnamic content; nd, non detected.

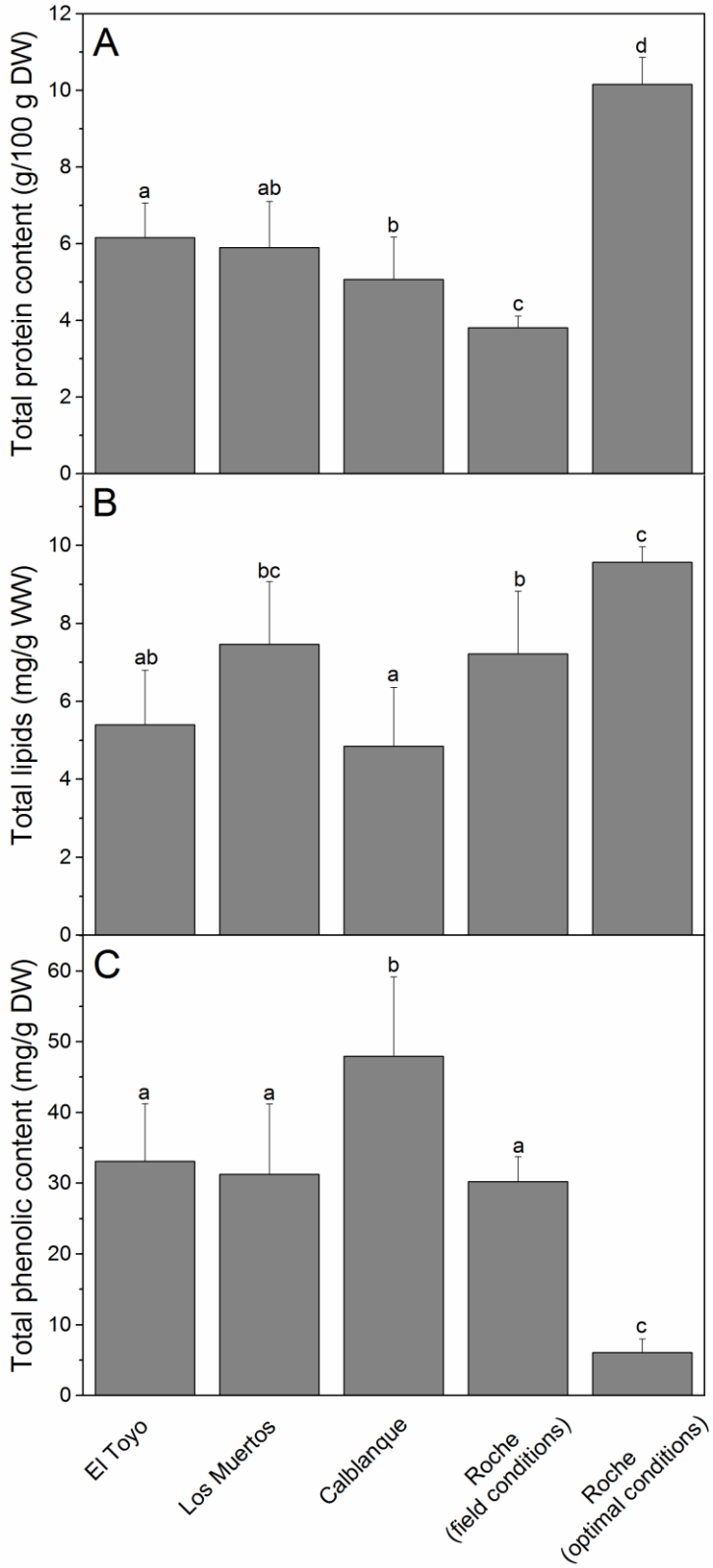
	El Toyo	**Los Muertos**	**Calblanque**	**Roche (field conditions)**	**Roche (optimal conditions)**
Hydroxycinnamic acids					
3-caffeoyl quinic acid	0.3 ± 0.1**ab**	0.3 ± 0.1**ab**	0.4 ± 0.2**b**	0.3 ± 0.1**a**	0.1 ± 0.0**c**
5-caffeoyl quinic acid	4.4 ± 1.2**ab**	4.3 ± 1.5**ab**	4.8 ± 1.2**a**	2.8 ± 0.6**bc**	1.4 ± 0.7**c**
p-coumaroyl quinic acid	0.5 ± 0.2**a**	0.4 ± 0.2**a**	0.3 ± 0.2**a**	0.3 ± 0.1**a**	0.2 ± 0.1**a**
Feruloyl quinic acid	0.2 ± 0.1**abc**	0.2 ± 0.1**a**	0.2 ± 0.1**bc**	0.1 ± 0.1**b**	0.2 ± 0.0**ac**
Ferulic acid	0.2 ± 0.1**a**	0.2 ± 0.1**a**	0.4 ± 0.1**a**	0.3 ± 0.1**a**	nd
3,5-Di-Caffeoyl quinic acid	0.5 ± 0.2**a**	0.3 ± 0.2**ab**	0.5 ± 02**a**	0.3 ± 0.1**ab**	0.1 ± 0.1**b**
4,5-Di-Caffeoyl quinic acid	0.3 ± 0.1**ac**	0.2 ± 0.1**b**	0.3 ± 0.1**c**	0.1 ± 0.0**d**	0.7 ± 0.5**a**
Flavonoids					
Quercetin-O-hexoside	1.0 ± 1.6**ab**	1.2 ± 2.3**ab**	0.2 ± 0.6**a**	2.5 ± 2.3**b**	nd
Quercetin-7-xyloside	3.1 ± 1.2**ab**	2.3 ± 2.1**b**	11.7 ± 8.3**a**	3.9 ± 0.9**c**	nd
Rutin	22.1 ± 6.0**ab**	20.0 ± 6.4**a**	27.4 ± 7.5**b**	19.7 ± 2.9**a**	1.7 ± 0.4**c**
Kaempferol 3-glucoside-7-rhamnoside	nd	nd	nd	nd	1.6 ± 0.1
THC	6.4 ±1.6**a**	6.2 ±2.0**ab**	7.0 ± 1.5**a**	4.1 ± 0.9**bc**	3.3 ± 0.5**c**
TFC	26.7 ± 7.4**a**	25.1 ± 8.2**a**	41.0 ± 10.5**b**	26.1 ± 3.1**a**	2.8 ± 1.4**c**

Table 5. Total concentration of Ca, K, Mg, Na, P (expressed in percentage, g/100 g DW), Fe, Mn, Zn, Cu and Cr (expressed in mg/kg DW) in *C. maritimum* leaves. Data represent mean ± SD of twelve independent replicates for field samples and three replicates for plants under optimal greenhouse conditions. Different letters indicate significant differences among different populations ($p<0.05$).

	El Toyo	**Los Muertos**	**Calblanque**	**Roche (field conditions)**	**Roche (optimal conditions)**
Mineral elements					
Ca	2.6 ± 0.4**a**	2.2 ± 0.3**b**	2.9 ± 0.4**c**	3.7 ± 1.0**c**	2.9 ± 0.2**ac**
K	1.8 ± 0.4**a**	2.2 ± 0.5**a**	2.0 ± 0.9**a**	2.4 ± 0.5**a**	6.0 ± 0.2**b**
Mg	0.5 ± 0.1**ab**	0.4 ± 0.1**b**	0.6 ± 0.1**a**	0.5 ± 0.1**a**	0.5 ± 0.0**ab**
Na	4.0 ± 1.6**a**	3.9 ± 0.9**a**	4.1 ± 1.4**a**	5.0 ± 1.5**a**	1.2 ± 0.0**b**
P	0.2 ± 0.0**a**	0.2 ± 0.0**a**	0.1 ± 0.0**b**	0.1 ± 0.0**ab**	0.6 ± 0.1**c**
Fe	150.6 ± 35.2**a**	58.4 ± 17.4**b**	77.8 ± 16.9**c**	191.7 ± 75.4**a**	68.9 ± 14.0**bc**
Mn	80.3 ± 23.6**a**	43.3 ± 12.5**b**	60.1 ± 28.1**ab**	37.2 ± 18.8**b**	41.4 ± 18.2**b**
Zn	41.3 ± 9.0**a**	31.2 ± 7.0**b**	25.5 ± 19.7**bc**	26.4 ± 10.2**bd**	23.5 ± 1.1**cd**
Cu	7.3 ± 2.0**a**	6.6 ± 1.7**a**	4.5 ± 1.9**b**	4.3 ± 0.9**b**	2.8 ± 0.4**b**
Cr	1.0 ± 0.5**ac**	0.5 ± 0.3**b**	0.7 ± 0.2**ab**	1.4 ± 0.6**c**	2.7 ± 1.1**d**
Toxic metals					
Pb	<0.4	<0.1	<0.9	<0.1	1.4 ± 1.2
Cd	<0.1	<0.2	<0.1	<0.6	0.1 ± 0.0

Figure legends

Figure 1. (A) Total protein (in percentage, g/100 g dry weight), **(B)** total lipid (mg/g wet weight) and **(C)** total phenolic content (mg/g dry weight) of *C. maritimum* leaves collected from different contrasting habitats (El Toyo, sandy beach; Los Muertos, rocky beach; Calblanque, cliffs; Roche field conditions, sandy beach) and plants collected from Roche under optimal greenhouse conditions (Roche optimal conditions). Data represent mean and standard deviation of twelve independent replicates for field samples and three replicates for plants under optimal greenhouse conditions. Different letters indicate significant differences ($p<0.05$).


A
Total protein content (g/100 g DW)
0
2
4
6
8
10
12
a
ab
b
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d
B
Total lipids (mg/g WW)
0
2
4
6
8
10
ab
bc
a
b
c
C
Total phenolic content (mg/g DW)
0
10
20
30
40
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60
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El Toyo
Los Muertos
Calblanque
Roche (field conditions)
Roche (optimal conditions)