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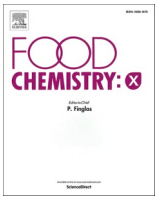

# The alphabet of sea fennel: Comprehensive phytochemical characterisation of Croatian populations of *Crithmum maritimum* L.

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ABSTRACT

Extreme environmental conditions affect the synthesis and accumulation of bioactive metabolites in halophytic plants. The aim of this study was to investigate the presence and quantity of key health-promoting phytochemicals in Croatian sea fennel, one of the most popular Mediterranean halophytes with a wide range of uses. The EOs were characterised by a high content of limonene (up to 93%), while the fatty acid profile shows a low content of oleic acid and the presence of valuable linoleic acid (ω-6) and linolenic acid (ω-3) in high percentages. The dominances of lutein and α-tocopherol were also confirmed in all samples. The results confirm the great variability in the chemistry of sea fennel populations in the Mediterranean region, with significant differences in the composition of the Croatian samples compared to the others, as well as the presence and high concentrations of the analysed bioactive compounds that contribute to the plant's health-promoting attributes.

## 1. Introduction

Halophytic species represent only 1% of all plants (around 6000 species), but their importance has been growing exponentially in the last two decades due to the scarcity of fresh water resources, salinization of the soil derived from climate change, soil erosion and intensive agriculture, as well as due to limited crop production conditions in arid and semi-arid climatic zones (Amoruso et al., 2022; Sánchez-Faure et al., 2020). During their growing cycle, halophytes are exposed to extreme environmental conditions like drought, high temperatures, nutrient limitations in addition to high salinity conditions, etc. Due to different mechanisms of adaptation against these stressful environmental conditions, these plants represent an exceptional opportunity for sustainable saline agriculture (Castillo et al., 2022;).

Rock samphire, marine samphire, crest marine, sea fennel, or marine fennel (*Crithmum maritimum* L.) is one of the most widespread Mediterranean perennial, facultative halophyte with a great ability of surviving in saline environments (Kraouia et al., 2023b). It belongs to the Apiaceae plant family, but is the only species of the *Crithmum* genus (Kovačić et al., 2008). It grows along the Adriatic coast in an environment characterised by saline conditions and abundant sunlight so it is a valuable example of halophytes and heliophytes. Sea fennel is a plant with well-developed mechanisms of adaptation to the Mediterranean climate and resilience to climate changes what makes it an ideal candidate for the promotion of halophyte agriculture. Therefore, it is not surprising that it has been recognised as a "cash" crop for saline agriculture (Atia, Barhoumi, Mokded, Abdelly, & Smaoui, 2011; Kraouia et al., 2023b; Renna, 2018).

Croatian sea fennel grows mainly in large populations inhabiting scrublands, on rocks and in coastal areas with rugged terrain (Kovačić et al., 2008). The occurrence of sea fennel in these habitats meets the criteria established in the Manual for categorizing terrestrial ecosystems

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in Croatia under the European Union directive, as outlined by Topić and Vukelić (2009). In particular, sea fennel thrives on rocks and cliffs along the Mediterranean and Black Sea coasts and on the South Atlantic coast of the Iberian Peninsula. This ecological niche corresponds to the halophytic vegetation community of *Crithmo-Limoniete*. Halophytic communities of ridge species have developed in the cracks of coastal reefs in the zone of salt spray and splash water from sea waves. In this habitat, plants are exposed to strong mechanical impacts of waves and wind, large temperature fluctuations, lack of soil and high salt content. The floristic composition includes the species *Crithmum maritimum* L. and several endemic species of the genus *Limonium*. Within this group, there are three types of communities in which sea fennel occurs on the Croatian coast: 1) *Plantagini-Limonietum cancellati* Horvatić (1934) 1939 - in the northern to central part of the eastern Adriatic coast, an exceptionally open halophytic community; 2) *Limonietum anfracti* Ilijanić & S. Hećimović 1982 - a rare, endemic and halophytic community in the coastal areas of southern Dalmatia; 3) *Crithmo-Limonietum vestiti* Trinajstić in Zi. Pavletić 1989 - endemic reef communities located on the coastal ridges of volcanic islands such as Jabuka and Brusnik, characterised by open, species-poor vegetation. Another habitat type where we sampled sea fennel is the delta of the Neretva River, where sea fennel grows in close proximity to the dominant reed species.

Confrontation with various abiotic stressors leads to changes in the structure, morphology and biochemistry of plants (Munns & Millar, 2023; Saharan et al., 2022). To counteract this, they activate molecular and physiological responses, adapt metabolism and produce specific antioxidant secondary metabolites such as phenolics and terpenes, which are crucial for defense (Salam et al., 2023; Qaderi, Martel, & Strugnell, 2023). In addition to their protective function, these compounds also offer a pleasant taste and health benefits. Several studies in scientific journals reviewed nutritional composition and functional traits of sea fennel, as well as its consumption potential due to its richness in terms of health-promoting compounds, especially those with notable antioxidant activity (Atia et al., 2011; Kraouia et al., 2023b; Renna, 2018). Sea fennel leaves and stems, which are mainly harvested from spring until early autumn, are widely used in traditional Mediterranean cuisine and in the food industry, due to their distinctive sensory attributes in terms of taste, scent and colour. They are used raw, cooked or preserved as pickles, in salads, as appetizers, for the preparation of condiments, dried spices, sauces, soups, herbal liqueurs, etc. (Atia et al., 2011; Kraouia et al., 2023b; Renna, 2018; Renna, Gonnella, Caretto, Mita, & Serio, 2017). In addition, sea fennel is also used for medicinal purposes, as numerous nutritive and health-beneficial components have been detected and identified in all parts of the sea fennel, such as ascorbic acid (Franke, 1982; Maoloni et al., 2021), minerals (Amoruso et al., 2022; Martins-Noguerol et al., 2022; Meot-Duros & Magné, 2009; Nabet et al., 2017; Pedreiro et al., 2023; Sánchez-Faure et al., 2020), carotenoids (Guil-Guerrero & Rodríguez-García, 1999; Nabet et al., 2017; Sarrou et al., 2019; Sousa, Alves, Neves, Tecelão, & Ferreira-Dias, 2022), organic acids (Maoloni et al., 2021; Meot-Duros & Magné, 2009; Sánchez-Faure et al., 2020), fatty acids (Ben Hamed, Ben Youssef, Ranieri, Zarrouk, & Abdelly, 2005; Castillo et al., 2022; Guil-Guerrero & Rodríguez-García, 1999; Labiad et al., 2021; Maoloni et al., 2021; Martins-Noguerol et al., 2022; Martins-Noguerol, Matías, et al., 2022; Sánchez-Faure et al., 2020), phenolic compounds (Maoloni, Pirker, Pferschy-Wenzig, Aquilanti, & Bauer, 2023; Martins-Noguerol, Matías, et al., 2022; Martins-Noguerol, Pérez-Ramos, et al., 2022; Pedreiro et al., 2023; Sánchez-Faure et al., 2020; Sarrou et al., 2019; Veršić Bratinčević, Kovačić, Popović, Radman, & Generalić Mekinić, 2023), volatiles (Nabet et al., 2017; Özcan, Akgül, Bascr, Özck, & Tabanca, 2001; Pateira et al., 1999; Pedreiro et al., 2023; Sarrou et al., 2019).

When comparing the results of different studies investigating the chemical composition of sea fennel, differences were found in the profiles and concentrations of the bioactive metabolites detected depending on the to the main parameters such as genotype (Kadoglidou et al., 2022; Pateira et al., 1999), plant part studied (Generalić Mekinić et al., 2016; Pavela et al., 2017), habitat/sampling location (Martins-Noguerol, Matías, et al., 2022; Martins-Noguerol, Pérez-Ramos, et al., 2022; Meot-Duros & Magné, 2009; Özcan et al., 2001; Pavela et al., 2017), growing conditions (Castillo et al., 2022; Martins-Noguerol, Pérez-Ramos, et al., 2022; Sarrou et al., 2019) and plant vegetative stage/harvest period (Generalić Mekinić et al., 2018).

There are several publications on the chemical composition of Croatian sea fennel genotypes, but they mainly focus on the main antioxidant secondary metabolites; plant phenolic profile (Generalić Mekinić et al., 2016, 2018; Maleš, Žuntar, Nigović, Plazibat, & Vundać, 2003; Politeo et al., 2023; Politeo et al., 2023; Veršić Bratinčević et al., 2023) and essential oil components (terpenes) (Generalić Mekinić et al., 2016; Kulisic-Bilusic, Blažević, Dejanović, Miloš, & Pifat, 2010; Politeo, Popović, Veršić Bratinčević, Koceić, et al., 2023; Politeo, Popović, Veršić Bratinčević, Kovačević, et al., 2023) while other compounds of interest are unattended. Following the results of previous studies, our hypothesis assumes that the Croatian sea fennel population has different profiles for each examined phytochemical group, with different proportions of individual components within these phytochemical groups. Therefore, this study aims to investigate five major groups of phytochemicals with health-beneficial properties in ten wild sea fennel populations along the Croatian Adriatic coast. The targeted phytochemical groups include essential oil (EO) components, fatty acids, tocopherols, carotenoids, and phenolics. The use of statistical tools is proposed to compare and differentiate these phytochemicals among the sea fennel populations. Additionally, the study aims to identify the phytochemical factors responsible for variations observed between the Croatian populations.

## 2. Materials and methods

### 2.1. General

All chemicals, reagents and solvents used were of adequate analytical grade, and were purchased from Sigma-Aldrich (St. Louis, MO, USA), VWR (Radnor, PA, USA) and Honeywell Fluka (Charlotte, NC, USA). For the identification of the essential oil components and fatty acids series of *n*-hydrocarbons (C8-C40, Supelco Inc., Sigma Aldrich) and fatty acid methyl esters (FAMEs) set of standards (Supelco 37 Component FAME Mix, Sigma-Aldrich) were used. Carotenoid standards were acquired as follows: neoxantin, violaxanthin and lutein were purchased from Sigma-Aldrich (St. Louis, MO, USA), while $\beta$-carotene was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Phenolic compounds (gallic acid, protocatechuic acid, neochlorogenic acid, *p*-hydroxybenzoic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, ferulic acid, sinapic acid and rutin) were purchased from Sigma-Aldrich. Ultrapure water was obtained from Milli-Q System (Millipore, Bedford, MA).

### 2.2. Plant material and sampling

Sea fennel grows along the sea coast and on the islands of Croatia, following a northwest-southeast trajectory. In order to comprehensively assess its distribution range, we strategically identified and selected ten sampling locations. The selected sites covered the entire coastal area, including the coast and islands, from Krk, the northernmost site, to Cavtat, the southernmost site, with distances between sampling sites $<$100 km. Also, sampling was performed on the largest southern island, Korčula, as well as the firth of the Neretva River, which is characterised by brackish water (Fig. 1).

The geographical coordinates of each sampling site are given in Table 1, while the specific ecological habitats where the sea fennel specimens were collected are shown in Fig. 1 and Supplementary 1 Sea fennel leaf samples were harvested from a total of 20 individual bushes at each of the ten collecting sites. All leaves from each plant was used, while damaged, yellow and dry leaves were discard from samples for


**Fig. 1.** Sea fennel sampling sites in Croatia.

further analyses. Sampling was carried out during the peak flowering period, which takes place in the first half of August (2022). The precise species identification was carried out by Ph. D. Tonka Ninčević Runjić (Department of Plant Sciences, Institute for Adriatic Crops, Split, Croatia). Subsequently, all herbarium samples were carefully preserved and stored at the Faculty of Chemistry and Technology (University of Split, Split, Croatia).

The main objectives of these sampling sites were to investigate the chemotypic variations, assess the biochemical characteristics and evaluate the nutritional properties of sea fennel. The samples intended for the determination of essential oils were air-dried (about 500 g of plant material for about two weeks in dark at room temperature), while the samples (again around 500 g) selected for other chemical analytical procedures were freeze-dried (FreeZone 2.5, Labconco, Kansas City, MO, USA) to preserve their composition.

### 2.3. Essential oils

The dry sea fennel leaves were hydrodistilled in a Clevenger apparatus for 3 h according to Bektašević et al. (2023). The isolated essential oils were dissolved in pentane/diethyl ether (1:1), dried using anhydrous sodium sulphate and stored at −4 °C until analysis.

The separation and analysis of the sea fennel essential oils constituents was performed by gas chromatography coupled with mass spectrometry (GC–MS; Agilent Inc., Santa Clara, CA, USA), using a gas chromatograph (model 8890 equipped with an automatic liquid injector model 7693 A) equipped with a non-polar column HP-5MS (5% phenyl methylpolysiloxane, 30 m × 0.25 mm, 0.25 μm, Agilent Inc.), and a tandem mass spectrometer (model 7000D GC/TQ). Helium was used as the carrier gas at a flow rate of 1 mL/min. The column temperature programme was used as follows: 3 min at 60 °C, ramped to 246 °C at 3 °C/min, and kept isothermal for 25 min. The inlet temperature was 250 °C, the sample injection volume was 1 μL, and the split ratio was 1:50. The following conditions were used for the MS: Ion source temperature 200 °C, ionization energy 70 eV, full-scan range (33–350 *m/z*).

Individual peaks were identified by comparison of their retention indices with the series of *n*-hydrocarbons and by computer matching of the mass spectra with commercial databases (Wiley 7 MS library, Wiley, NY, USA) and NIST02 (Gaithersburg, MD, USA) and by comparison of their mass spectra and retention indices with literature data (Adams,

**Table 1**
Geographical coordinates of the sampling sites.

| Location | Krk | Senj | Pag | Šibenik | Split | Drašnice | Korčula | Pelješac | Neretva | Cavtat |
|---|---|---|---|---|---|---|---|---|---|---|
| Geographic latitude | 45°9′3.87″N | 45°3′20.96″N | 44°29′49.74″N | 43°43′42.09″N | 43°30′2.62″N | 43°13′25.06″N | 42°57′7.80″N | 42°49′0.93″N | 43° 1′13.47″N | 42°35′13.29″N |
| Geographic longitude | 14°31′59.04″E | 14°52′12.82″E | 14°54′58.19″E | 15°50′6.09″E | 16°29′21.63″E | 17°6′28.94″E | 17°8′21.84″E | 17°40′33.40″E | 17°27′50.37″E | 18°12′41.90″E |

2007)(Supplementary 2 A). All analyses were performed in triplicate. The percentages of the identified compounds were calculated as mean ± standard deviation.

### 2.4. Fatty acids

The analysis of fatty acids in sea fennel samples was performed through the analysis of FAMEs prepared by methylation. The lipids were extracted from dry sea fennel samples (1 g) using 2-propanol (4 mL) and by heating (at 80 °C for 15 min) the suspensions. Then, hexane (6 mL) and sodium sulphate (6.7%, *w/v*, 5 mL) were added and the suspension was shaken vigorously. After centrifugation (3000 rpm, 3 min), and the upper phase was transferred to a clean tube. The aqueous phase was extracted again with a hexane: 2-propanol mixture (7:2 *v*/v, 7.5 mL), and the upper phase was combined with the previous one. After removal of the solvent, the fatty acids methylation was performed by adding methanol: toluene: sulphuric acid (88:10:2 v/v/v, 3 mL) and sample heating (at 80 °C during 1 h). The FAMEs were extracted from the cooled samples with heptane (2 × 1 mL) and analysed by GC-FID.

The prepared FAMEs were analysed using a gas chromatograph (model 3900; Varian Inc., Lake Forest, CA, USA) equipped with a flame ionization detector and an RTX 2330 capillary column (30 m, 0.25 mm, 0.2 μm; Restek Corp., Bellefonte, PA, USA) by injecting 1 μL of the sample. The split ratio was 1:50 and helium (flow rate 2 mL/min) was used as the carrier gas. The oven temperature was 140 °C, held for 4 min, raised to 210 °C at the rate 4 °C/min and held at 210 °C for 11 min. The total run time was 32.5 min. The injector and detector temperature was 250 °C. A set of FAME standards was used for the identification of the compounds (Supplementary 2B). Analyses were performed in duplicate, and the results are expressed as the mean of percentage of fatty acid (calculated as the ratio between the FAME peak area and the total peaks area) ± standard deviation.

### 2.5. Carotenoids

The extraction of carotenoids from sea fennel was performed according to Nartea et al. (2021) with slight modifications. Briefly, 100 mg of the sample was extracted in 5 mL of acetone, stored at 4 °C for 15 min and centrifuged at 1370 rpm for 10 min; the whole procedure was repeated once more. Afterwards, the sample was filtered through a 0.45 μm PVDF filter, and the solvent was evaporated under the stream of nitrogen. Prior to analysis, the dried residue was re-suspended in 0.5 mL acetone for the determination of β-carotene and in 80% methanol for the determination of neoxanthine, violaxantine and lutein.

The analysis was performed by ultra-high performance liquid chromatography (UHPLC) coupled with diode array detector (DAD) (Ultimate 3000RS, Thermo Fisher Scientific, Waltham, Massachusetts, SAD), on a reversed phase column (Halo C30; 150 × 3 mm, 2.7 μm; Advanced Materials Technology, DE, USA). Carotenoids were separated by gradient elution with solvent A (1% acetic acid in water), solvent B (MeOH) and solvent C (ACN) as follows: 70% C isocratic for 0.2 min; 0.2–8 min to 100% C; 8–9 min to 30% B and 70% C; 9–17 min 30% B and 70% C. The total analysis time was 17 min, including 0.1 min for start conditions and 4.9 min for re-equilibration. The carotenoids in the sample extracts were monitored at 415, 425 and 450 nm, the flow rate was set to 1 mL/min and 10 μL of the sample was injected into the system. The peaks were identified by comparing their retention times with those of authentic standards and spiked samples using standard solutions (Supplementary 2C). Calibration curves for neoxanthine, violaxantine and lutein were constructed using 6 calibration points in the concentration range of 0.05–20 μg/mL from standard solutions using methanol/water (80:20, *v*/v), and for *β*-carotene from 0.05 to 20 μg/mL by diluting the standard in acetone. Cromeleon software was used to collect, record, process and integrate the data. Analyses were performed in triplicate, and the results, expressed as mg of compound tested per gram of dry extract (mg/g), are expressed as mean ± standard deviation.

### 2.6. Tocopherols

Tocopherols were isolated by direct acetone extraction and saponification as reported by Knecht, Sandfuchs, Kulling, and Bunzel (2015). Ascorbic acid (1 g), sodium sulphate (0.1 g), ethanol (20 mL), and potassium hydroxide solution 60% (4 mL) were added to sea fennel leaf powder (0.4 g). The suspension was saponified in a water bath (at 85 °C for 30 min), shaken from time to time, and cooled to room temperature. After water was added (12 mL) and a triple extraction with *n*-hexane (10 mL) was performed. The organic phases were pooled, washed four times with water (10 mL), dried in a rotary evaporator (Laborota 4000 efficient G3, Heildoph GmbH, Schwabach, Germany) at 35 °C, and finally dissolved in *n*-hexane.

Samples were injected (20 μL) into the HPLC system (Series 200, Perkin Elmer, Walthamn, Massachusetts, USA) equipped with a fluorimetric detector (FLD Series 200, Perkin Elmer) and an Ultra-silica column (250 × 4.6 mm, 5 μm, Restek Corporation, USA). Linear elution was performed with solvent A (hexane) and solvent B (isopropanol) in a 96:4 ratio at a flow rate of 0.8 mL/min. The temperature of the column was maintained at 25 °C. Detection was performed using a fluorescence detector (excitation 290 nm and emission 330 nm). The α-, β-, and γ-tocopherols were identified based on the retention times of the standards (Supplementary 2D). The calibration curve was used for α-tocopherol, while the concentrations of β-, and γ-tocopherol were reported relative to its concentration. The results obtained are expressed as mg of compound detected per kg of dry plant material (mg/kg). All analyses were performed in triplicate and final results are expressed as mean ± standard deviation.

### 2.7. Phenolic compounds

The homogenised, dry sea fennel samples (0.5 g) were extracted with methanol (80:20 v/v, 5 mL) by sonication (15 min at room temperature) followed by mixing in an orbital shaker (3 h at room temperature). The samples were left overnight at +4 °C in the dark, filtered (Chromafil™ Xtra PTFE Syringe filters, 0.45 μm, Macherey-Nagel, Düren, Germany) and further used for the detection of the individual phenolic compounds.

High-performance liquid chromatographic (HPLC) analyses of the individual phenolic compounds were performed on the Shimadzu Nexera HPLC system LC-40 (Shimadzu, Kyoto, Japan) equipped with a UV/VIS detector using a Phenomenex C18 (250 mm × 4.6 mm, 5 μm; Torrance, CA, USA) reverse-phase column. The mobile phase flow rate was 1.0 mL/min and the temperature was maintained at 35 °C. The mobile phase (A) was 0.2% phosphoric acid in water, while the mobile phase (B) was methanol-acetonitrile (1:1, *v/v*). Elution started isocratically with 4% B, and then the gradient program was set as follows: 0–16 min (linear gradient up to 15% B), 16–50 min (linear gradient up to 35% B), 50–62 min (linear gradient up to 4% B), and 62–65 min (4% B). The initial conditions were established in 2 min and maintained for 10 min to equilibrate the column. Six-point standard curves were prepared by diluting the working standard solution with methanol/water (80:20, v/v). Calibration curves for neochlorogenic and criptochlorogenic acids were prepared in a concentration range of 0.1–50 μg/mL, for chlorogenic acid from 5 to 500 μg/mL and for all other phenolics tested in a range of 0.1 to 10 μg/mL. The phenolic compounds were identified by comparing the retention times of the sample peaks and their absorbance spectra (at 220 and 320 nm) with those obtained for the standard compounds tested under the same conditions, while quantification was performed using an external standard calibration curve (Supplementary 2E). The presented results are obtained from two injections and are expressed as milligrams of compound per gram of dry plant material (mg/g) (as mean value ± standard deviation).

### 2.8. Statistical analysis

The statistical difference between samples in terms of significant

differences in fatty acid shares, phenolic concentrations, essential oil composition and pigment concentration between sampling areas was determined using the analyses of variance (one-way ANOVA, followed by Fisher's least significant difference test). Analyses were performed using software Statgraphics Centurion-Ver.16.1.11 (StatPoint Technologies, Inc., Warrenton, VA, USA). Additionally, the relationship between the dominant fatty acids (>15%, $n = 4$), phenolics (chlorogenic acid, $n = 3$), essential oil components (>5%, $n = 8$), tocopherols ($\alpha$- and $\beta$- form, form, $n = 2$) and pigments ($n = 3$) in relation to the sampling areas was determined by principal component analysis (PCA) using the software STATISTICA® (version 13, StatSoft Inc., Tulsa, OK, USA). Before analyses the data were log-transformed.

## 3. Results and discussion

The number of studies on halophytic species is exponentially increasing, especially since 20% of all irrigated land is affected by salinization, and projections indicate that 50% of all cropland will be affected by it by 2050 (Grigore & Vicente, 2023). Halophytes are wild plants native to saline habitats, where they are able to survive and complete their life cycle due to their unique adaptive mechanisms to the harsh conditions they are exposed to. One way halophytes respond and tolerate salt stress is by synthesising secondary metabolites with biological activity (Grigore & Vicente, 2023; Lombardi et al., 2022). Apart from the biological function of these secondary plant compounds in the mechanisms of stress tolerance, their bioactivity and health-promoting properties increase the interest in the use of these species for various purposes, especially culinary.

Among the numerous halophytes in the Mediterranean region, sea fennel is one of the most widespread and best studied species, mainly because of its essential oil and phenolic compounds. The reports of various studies led to the hypothesis that several different chemotypes of sea fennel grow in the Mediterranean region (Pateira et al., 1999; Renna et al., 2017) and that Croatian sea fennel may be a special chemotype (Generalić Mekinić et al., 2016, 2018; Politeo, Popović, Veršić Bratinčević, Koceić, et al., 2023; Politeo, Popović, Veršić Bratinčević, Kovačević, et al., 2023). However, the studies on Croatian sea fennel focused only on its essential oil (Generalić Mekinić et al., 2016; Politeo, Popović, Veršić Bratinčević, Koceić, et al., 2023; Politeo, Popović, Veršić Bratinčević, Kovačević, et al., 2023) or phenolic compounds (Generalić Mekinić et al., 2016, 2018; Maleš et al., 2003; Politeo, Popović, Veršić Bratinčević, Koceić, et al., 2023; Politeo, Popović, Veršić Bratinčević, Kovačević, et al., 2023; Veršić Bratinčević et al., 2023), while there are no previous reports on the presence and profile of other phytochemicals in it. Therefore, this study was conducted to obtain a comprehensive phytochemical characterisation of Croatian wild populations of sea fennel, such as essential oil components, fatty acids, carotenoids, tocopherols, and phenolic compounds.

For a long time, sea fennel was mainly known in coastal and island regions, where it was traditionally preserved and used seasonally. Historically, it served as a vital resource in times of scarcity, especially during the two world wars when food was in short supply. Today, sea fennel has become a popular gastronomic delicacy. This research will therefore comprehensively investigate the biochemical and nutritional properties of sea fennel to demonstrate its potential for wider use compared to its historical use.

### 3.1. Essential oils

The volatile metabolites of sea fennel, particularly the EOs, are of great industrial interest due to their pleasant sensory properties, especially their aroma and flavour, commonly described as citrus peel-like. Due to their positive biological properties and health-promoting effects, sea fennel and its isolates are therefore widely used in the food industry and for culinary purposes, in cosmetics and perfumery, but also in traditional medicine and the pharmaceutical industry (Kraouia et al., 2023a; Renna, 2018).

A total of 35 constituents were found during the chemical analysis of the chemical composition of the sea fennel leaves EOs collected from various locations along the Adriatic coast. The oil content also varied between the samples, ranging from 0.04% in the sample from Cavtat to 0.88% in the sample from Senj. The most abundant EOs compounds belong to the group of monoterpenes and monoterpenoids (Table 2). Sesquiterpenes and phenolic compounds are present in lower amounts. Limonene was identified as the most abundant constituent of the leaf oil (24.36 to 93.20%). The highest concentration of limonene was found in the essential oils of samples collected on the southern and central Adriatic coast, in Neretva (93.20%), Split (79.13%), Korčula (79.44%) and Senj (71.07%), and the lowest in the southernmost region, in sample from Cavtat (24.36%). A high percentage of sabinene (0.80–31.18%) and a slightly lower percentage of $\gamma$-terpinene (0.38–8.74%) and terpinen-4-ol (1.55–18.96%) were also found. All investigated essential oils belong to chemotype II, as no dillapiole was detected in their chemical composition (Pateira et al., 1999), while according to Renna et al. (2017), they belong to the monoterpene hydrocarbon essential oil chemotype. A previous analysis of the EOs from dried leaves of Croatian sea fennel (collected in Central Dalmatia- Split region) (Generalić Mekinić et al., 2016; Politeo, Popović, Veršić Bratinčević, Koceić, et al., 2023; Politeo, Popović, Veršić Bratinčević, Kovačević, et al., 2023), showed that the most abundant components were limonene (36.3–74.2%), terpinen-4-ol (1.3–10.4%), $\gamma$-terpinene (2.8–7.1%), and sabinene (8.1–51.5%).

The main compounds characterizing Greek cultivated sea fennel in the study by Sarrou et al. (2019) were also limonene (10.6–36.4%), $\gamma$-terpinene (8.2–31.5%) and sabinene (5.6–26.9%), while Pedreiro et al. (2023) found monoterpene hydrocarbons, namely $\gamma$-terpinene (37.2%) and sabinene (21.2%) were present in the highest concentrations in the essential oil of sea fennel from Portugal. The essential oil of the dried leaves of sea fennel collected in Sicily (Italy) contained thymol methyl ether (47.4%), $\gamma$-terpinene (38.9%), and *p*-cymene (10.1%) (Salinelle, Palermo), and thymol methyl ether (42.9%) and $\gamma$-terpinene (36.3%) (Acquacalda, Lipari) (Pavela et al., 2017). The essential oil from the fresh leaves of the plant from Tunisia contains dillapiole as the main constituent (83.3–94.6%) (Mustapha et al., 2020), while the most abundant constituents of the essential oil of the leaves of cultivated sea fennel were dillapiole (41.4%), thymol methyl ether (27.8%) and $\gamma$-terpinene (22.5%) (Houta, Akrout, Najja, Neffati, & Amri, 2015). Previous analyses have shown that dillapiole is the major constituent of the essential oil of sea fennel from France (fresh plant material), followed by thymol methyl ether, *p*-cymene and $\gamma$-terpinene (Pavela et al., 2017). The most abundant constituents of the essential oil isolated from the dry leaves of sea fennel collected in Cyprus are $\gamma$-terpinene (39.3%), $\beta$-phellandrene (22.6%), carvacrol methyl ether (10.4%), (*Z*)-$\beta$-ocimene (8.2%), and *p*-cymene (6.4%) (Polatoğlu et al., 2016), while in the Algerian samples the most abundant components were $\gamma$-terpinene (50.5%), thymol methyl ether (33.6%) and *p*-cymene (12.6%) (Nabet et al., 2017).

Although, according to Pateira et al. (1999) there are two sea fennel chemotypes with respect to the presence and concentration of dillapiole in EOs, the results of this and previous studies confirm some other differences between Croatian sea fennel and sea fennel from other Mediterranean countries. In our samples, limonene was identified as the most abundant constituent (up to 93%). This monoterpene compound is the main component of various citrus essential oils and is responsible for the characteristic odour and, of course, citrus notes of Croatian sea fennel. In other studies, its presence in appreciable amounts was confirmed only in cultivated samples from Greece, with a content of 36% (Sarrou et al., 2019), and these samples also showed high levels of the other two dominant compounds, $\gamma$-terpinene and sabinene. In addition to the absence of dillapiole, thymol methyl ether, which dominates in EOs from Italy (Pavela et al., 2017), Tunisia (Houta et al., 2015), Algeria (Nabet et al., 2017) and France (Houta et al., 2015) was also not

**Table 2**
EO profiles of Croatian sea fennel leaves from different locations along the Adriatic coast.

| Compound (%) | RI | Krk | Senj | Pag | Šibenik | Split | Drašnice | Korčula | Pelješac | Neretva | Cavtat | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *α*-thujene | 926 | 0.19 ± 0.00 c | nd | 0.29 ± 0.06 c | 0.43 ± 0.03 b | nd | nd | nd | 0.54 ± 0.01 a | nd | nd | 0.0004 |
| *α*-pinene | 932 | 0.88 ± 0.12 | 0.51 ± 0.073 | 7.60 ± 7.09 | 0.47 ± 0.04 | nd | nd | nd | 2.84 ± 0.07 | 0.49 ± 0.00 | 0.47 ± 0.02 | 0.4720 |
| sabinene | 970 | 18.66 ± 1.09 c | 16.48 ± 1.85 c | 23.74 ± 1.13 b | 31.67 ± 1.63 a | 0.80 ± 0.06 d | 18.21 ± 0.87 c | 3.07 ± 0.48 d | 15.89 ± 1.21 c | 3.39 ± 0.94 d | 1.24 ± 0.08 d | <0.0001 |
| *β*-pinene | 974 | nd | 0.16 ± 0.16 | nd | 0.12 ± 0.07 | nd | nd | nd | nd | nd | nd | 0.3626 |
| octanal | 999 | nd | nd | 0.29 ± 0.01 d | 0.11 ± 0.06 e | 0.69 ± 0.01 b | 0.32 ± 0.01 d | nd | 0.99 ± 0.06 a | nd | 0.49 ± 0.04 c | <0.0001 |
| *α*-terpinene | 1015 | 1.20 ± 0.19 bc | 0.87 ± 0.18 cd | 1.45 ± 0.06 b | 2.01 ± 0.27 a | nd | 0.56 ± 0.01 d | nd | 1.67 ± 0.08 ab | nd | nd | 0.0004 |
| *p*-cymene | 1022 | 1.34 ± 0.33 b | 0.10 ± 0.10 b | 1.40 ± 0.16 b | 0.86 ± 0.17 b | nd | 3.76 ± 0.03 a | 5.00 ± 1.55 a | 4.3 ± 0.14 a | nd | 0.15 ± 0.02 b | <0.0001 |
| limonene | 1026 | 63.10 ± 5.15 c | 71.07 ± 6.31 bc | 39.71 ± 3.67 d | 35.29 ± 2.78 d | 79.13 ± 1.21 b | 44.81 ± 1.12 d | 79.44 ± 5.28 b | 45.32 ± 2.96 d | 93.20 ± 1.57 a | 24.36 ± 1.356 e | <0.0001 |
| *(Z)*-*β*-ocimene | 1035 | 1.13 ± 0.21 b | 3.32 ± 1.04 a | 0.30 ± 0.02 b | 0.12 ± 0.07 b | 0.42 ± 0.05 b | nd | 0.79 ± 0.182b | 0.34 ± 0.03 b | 0.47 ± 0.27 b | nd | 0.0007 |
| Benzeneacetaldehyde | 1039 | nd | nd | 0.10 ± 0.06 d | 0.13 ± 0.08 d | nd | 0.63 ± 0.08 b | nd | 0.39 ± 0.01 c | nd | 1.58 ± 0.123a | <0.0001 |
| *γ*-terpinene | 1057 | 5.41 ± 0.82 b | 1.94 ± 1.21 c | 6.64 ± 0.65 ab | 6.00 ± 0.57 b | 0.55 ± 0.06 c | 5.43 ± 0.34 b | 8.74 ± 1.86 a | 7.80 ± 0.81ab | 0.89 ± 0.14 c | 0.38 ± 0.03 c | <0.0001 |
| *cis*-sabinene hydrate | 1065 | 0.19 ± 0.11 c | nd | 0.87 ± 0.12 a | 0.78 ± 0.14 ab | nd | nd | nd | 0.23 ± 0.01 c | nd | 0.56 ± 0.034 b | 0.0016 |
| terpinolene | 1088 | 0.48 ± 0.05 bc | 0.13 ± 0.13 d | 0.65 ± 0.02 ab | 0.73 ± 0.09a | nd | 0.29 ± 0.01 cd | nd | 0.60 ± 0.01 ab | nd | nd | 0.0004 |
| linalool | 1097 | 0.19 ± 0.11 c | nd | 0.99 ± 0.17 a | 0.83 ± 0.18 a | nd | nd | nd | 0.37 ± 0.00 bc | nd | 0.68 ± 0.023 ab | 0.0053 |
| *cis*-*p*-mentha-2,8-dien-1-ol | 1133 | nd | nd | 0.22 ± 0.01 c | 0.10 ± 0.06 d | 0.53 ± 0.04 a | nd | nd | 0.22 ± 0.02 c | nd | 0.33 ± 0.01 b | <0.0001 |
| *trans*-*p*-menth-2-en-1-ol | 1136 | 0.29 ± 0.17 b | nd | 0.60 ± 0.22 b | 0.84 ± 0.17 b | 1.00 ± 0.23 b | 1.3 ± 0.11 b | 0.21 ± 0.12 b | 0.75 ± 0.14 b | nd | 10.32 ± 1.20 a | <0.0001 |
| *cis*-verbenol | 1137 | nd | nd | nd | 0.09 ± 0.05 d | 0.90 ± 0.12 b | 0.51 ± 0.01 c | nd | 0.23 ± 0.01 cd | nd | 6.29 ± 0.18 a | <0.0001 |
| *trans*-verbenol | 1142 | 0.22 ± 0.13 d | nd | 0.63 ± 0.08 bcd | 0.50 ± 0.13 cd | 1.04 ± 0.02 b | 0.82 ± 0.04 bc | 0.22 ± 0.18 d | 0.88 ± 0.05 bc | nd | 2.12 ± 0.32 a | <0.0001 |
| pinocarvone | 1160 | nd | nd | 0.09 ± 0.06 | 0.09 ± 0.052 | nd | nd | nd | nd | nd | nd | 0.9669 |
| terpinen-4-ol | 1177 | 6.14 ± 1.50 c | 0.52 ± 0.33 d | 19.20 ± 2.68 a | 17.07 ± 1.38 a | 1.55 ± 0.03 d | 15.69 ± 0.98 ab | 2.30 ± 0.80 d | 13.19 ± 1.16 b | 1.56 ± 3.18 d | 2.35 ± 0.09 d | <0.0001 |
| isocarveol | 1187 | nd | nd | nd | 0.08 ± 0.05 b | 1.14 ± 0.04 b | 0.45 ± 0.02 b | nd | nd | nd | 6.44 ± 1.03 a | <0.0001 |
| *α*-terpineol | 1189 | 0.22 ± 0.13 e | nd | 0.69 ± 0.13 de | 0.47 ± 0.10 de | 1.63 ± 0.02 b | 1.33 ± 0.39 bc | 0.21 ± 0.12 e | 0.96 ± 0.04 cd | nd | 3.64 ± 0.11 a | <0.0001 |
| myrtenol | 1197 | nd | nd | 0.13 ± 0.08 d | 0.20 ± 0.12 d | 0.89 ± 0.023 b | 0.55 ± 0.01 c | nd | 0.23 ± 0.01 d | nd | 2.69 ± 0.15 a | <0.0001 |
| verbenone | 1207 | nd | nd | 0.12 ± 0.07 c | 0.11 ± 0.07 c | 1.57 ± 0.12 b | 0.64 ± 0.02 bc | nd | 0.25 ± 0.02 bc | nd | 12.01 ± 1.10 a | <0.0001 |
| *trans*-carveol | 1218 | nd | nd | 0.10 ± 0.06 d | 0.10 ± 0.06 d | 1.82 ± 0.06 b | 0.94 ± 0.03 c | nd | 0.33 ± 0.01 cd | nd | 7.82 ± 0.57 a | <0.0001 |
| *cis*-carveol | 1229 | nd | nd | nd | 0.11 ± 0.06 b | nd | 0.36 ± 0.02 a | nd | nd | nd | nd | 0.0191 |
| *trans*-chrysanthenyl acetate | 1239 | nd | nd | 0.20 ± 0.16 | 0.16 ± 0.09 | nd | 0.44 ± 0.04 | nd | nd | nd | nd | 0.1296 |
| carvone | 1242 | nd | nd | nd | nd | 0.47 ± 0.01 b | nd | nd | nd | nd | 2.08 ± 0.12 a | 0.0002 |
| carvacrol | 1301 | nd | nd | nd | nd | nd | 0.41 ± 0.01 | nd | nd | nd | nd | |
| myrtenyl acetate | 1328 | nd | nd | 0.11 ± 0.06 c | 0.10 ± 0.06 c | 0.64 ± 0.01 a | 0.37 ± 0.03 b | nd | 0.04 ± 0.02 d | nd | nd | <0.0001 |
| 10-(acetylmethyl)-3-carene | 1380 | nd | nd | 0.11 ± 0.06 e | 0.09 ± 0.05 f | 0.91 ± 0.02 b | 0.61 ± 0.02 c | nd | 0.38 ± 0.01 d | nd | 1.82 ± 0.21 a | <0.0001 |
| *β*-longipinene | 1405 | nd | nd | nd | 0.09 ± 0.06 e | 0.82 ± 0.01 b | 0.52 ± 0.02 c | nd | 0.30 ± 0.00 d | nd | 1.05 ± 0.10 a | <0.0001 |
| cuparene | 1508 | nd | nd | nd | nd | 0.66 ± 0.01 b | nd | nd | nd | nd | 1.72 ± 0.09 a | 0.0003 |
| *β*-vatirenene | 1538 | nd | nd | nd | nd | 0.61 ± 0.01 | nd | nd | nd | nd | nd | |
| spathulenol | 1582 | nd | nd | 0.08 ± 0.05 c | nd | nd | 0.55 ± 0.04 b | nd | 0.49 ± 0.03 b | nd | 1.63 ± 0.23 a | 0.0001 |
| EO Yield (5) | | 0.23 | 0.88 | 0.14 | 0.24 | 0.05 | 0.05 | 0.17 | 0.09 | 0.19 | 0.04 | |

RI- Retention Index. nd- not detected. Different letters (a-f) in the same row denote statistically significant difference ($p < 0.05$).

detected in Croatian samples.

### 3.2. Fatty acids

Fatty acids play various key roles in all organisms as essential components of lipids and cell membranes, where they influence their fluidity, serve as energy substrates, regulators of stress signalling, precursors of other biologically active compounds or due to their protective function against various biotic and abiotic stress factors. Unsaturated fatty acids are of particular importance due to their positive effects on human health, especially essential fatty acids such as linoleic acid (ω-6) and linolenic acid (ω-3) (He & Ding, 2020; Tvrzicka, Kremmyda, Stankova, & Zak, 2011).

The fatty acid profile of the investigated Croatian sea fennel samples is shown in Table 3. A total of 21 fatty acids were identified in different proportions; among them two polyunsaturated fatty acids (PUFAs), namely linoleic (C18:2n6) and linolenic acid (C18:3n3) and six monounsaturated fatty acids (MUFAs), namely pentadecenoic (C15:1n10), palmitoleic acid (C16:1n7), *cis*-7-hexadecenoic acid (C16:1n9), heptadecenoic acid (C17:1), oleic acid (C18:1), and eicosenoic acid (C20:1). All other detected compounds were saturated fatty acids (SFAs).

From all detected compounds, only four of them were detected in high amount. The dominant SFAs in the samples were palmitic acid (C16:0) with the amounts ranging from 19.0% in the Senj sample to 22.4% in the Pag sample, and stearic acid (C18:0) with concentrations ranging from 15.8% in the Neretva sample to 26.6% in Split sample. The content of linoleic acid ranged from 19.6 to 24.6%, with the highest found in the Korčula sample, while the content of linolenic acid ranged from 17.4 to 23.0%, with the highest content again found in the Korčula sample.

Total SFAs of the samples ranged from 47.6 to 56.4%, MUFAs from 5.2 to 6.6% and PUFAs from 37.7 to 47.6%.

Researchers (Ben Hamed et al., 2005; Castillo et al., 2022; Guil-Guerrero & Rodríguez-García, 1999; Labiad et al., 2021; Martins-Noguerol, Matías, et al., 2022; Oliveira-Alves et al., 2023; Sánchez-Faure et al., 2020) reported the dominance of PUFAs, namely linolenic and linoleic acids, in sea fennel samples, and among MUFAs, oleic acid was the most abundant. Among SFAs, palmitic acid dominated, while significantly lower amounts of stearic acid were found. Also, significantly lower content of stearic acid (from 3 to 8.8%) have been reported (Ben Hamed et al., 2005; Castillo et al., 2022; Labiad et al., 2021; Oliveira-Alves et al., 2023; Sánchez-Faure et al., 2020) while our results are closer to those of Martins-Noguerol et al. (Martins-Noguerol, Matías, et al., 2022) where significantly higher amounts were found (from 14.8 to 21.6%). However, the wild-collected sea fennel samples in the studies by Martins-Noguerol, Pérez-Ramos, et al. (2022) and Ben Hamed et al. (2005) had higher content of PUFAs than those found in present study, up to 27.3% for linoleic and 31% for linolenic acid, and up to 33.3% for linoleic and 29% for linolenic acid, respectively. Guil-Guerrero and Rodríguez-García (1999) also found significantly higher amounts of

**Table 3**
Fatty acids profiles (%) of Croatian sea fennel from different locations along the Adriatic coast.

| Compound (%) | Krk | Senj | Pag | Šibenik | Split | Drašnice | Korčula | Pelješac | Neretva | Cavtat | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C8:0 | 0.1 ± 0.0 cd | 0.0 ± 0.0 f | 0.0 ± 0.0 e | 0.0 ± 0.0 ef | 0.1 ± 0.0 c | 0.1 ± 0.0 b | 0.0 ± 0.0 e | 0.2 ± 0.0 a | 0.0 ± 0.0 d | 0.1 ± 0.0 b | <0.0001 |
| C10:0 | 0.1 ± 0.0 ef | 0.7 ± 0.0 a | 0.4 ± 0.0 b | 0.3 ± 0.0 cd | 0.1 ± 0.0 ef | 0.1 ± 0.0 f | 0.0 ± 0.0 f | 0.3 ± 0.0 bc | 0.1 ± 0.0 ef | 0.2 ± 0.1 de | <0.0001 |
| C11:0 | 0.2 ± 0.0 a | 0.0 ± 0.0 ef | 0.1 ± 0.0 d | 0.0 ± 0.0 e | 0.1 ± 0.0 bc | 0.2 ± 0.0 a | 0.0 ± 0.0 f | 0.1 ± 0.0 b | 0.1 ± 0.0 bc | 0.1 ± 0.0 cd | <0.0001 |
| C12:0 | 0.7 ± 0.0 h | 1.6 ± 0.0 b | 1.4 ± 0.0 d | 2.5 ± 0.0 a | 0.9 ± 0.0 g | 1.7 ± 0.0 b | 1.0 ± 0.0 f | 2.4 ± 0.0 a | 1.3 ± 0.0 e | 1.4 ± 0.0 c | <0.0001 |
| C13:0 | 0.7 ± 0.0 a | 0.6 ± 0.0 d | 0.5 ± 0.0 f | 0.6 ± 0.0 c | 0.7 ± 0.0 b | 0.6 ± 0.0 c | 0.5 ± 0.0 e | 0.7 ± 0.0 b | 0.5 ± 0.0 de | 0.6 ± 0.0 d | <0.0001 |
| C14:0 | 1.2 ± 0.0 g | 3.6 ± 0.0 e | 3.5 ± 0.0 e | 4.0 ± 0.0 d | 4.1 ± 0.1 d | 5.4 ± 0.0 a | 3.1 ± 0.0 f | 5.3 ± 0.0 ab | 5.1 ± 0.1 bc | 5.0 ± 0.0 c | <0.0001 |
| C15:0 | 0.2 ± 0.0 de | 0.2 ± 0.0 de | 0.2 ± 0.0 g | 0.2 ± 0.0 cd | 0.4 ± 0.0 a | 0.3 ± 0.0 b | 0.2 ± 0.0 fg | 0.3 ± 0.0 bc | 0.3 ± 0.0 ab | 0.2 ± 0.0 ef | <0.0001 |
| C15:1 (10) | 0.8 ± 0.0 h | 0.8 ± 0.0 g | 1.4 ± 0.0 c | 1.4 ± 0.0 b | 1.3 ± 0.0 d | 1.5 ± 0.0 a | 0.9 ± 0.0 f | 1.0 ± 0.0 e | 0.7 ± 0.0 i | 0.8 ± 0.0 g | <0.0001 |
| C16:0 | 22.1 ± 0.0 c | 20.3 ± 0.0 g | 22.4 ± 0.0 a | 21.0 ± 0.0 e | 21.2 ± 0.0 d | 22.3 ± 0.0 ab | 20.6 ± 0.0 f | 19.0 ± 0.1 h | 22.1 ± 0.0 bc | 20.9 ± 0.0 e | <0.0001 |
| C16:1 *ω*-7 | 1.9 ± 0.0 a | 1.1 ± 0.0 f | 1.4 ± 0.0 b | 1.4 ± 0.0 bc | 1.2 ± 0.0 e | 1.4 ± 0.0 cd | 1.4 ± 0.0 b | 1.0 ± 0.0 g | 1.3 ± 0.0 d | 1.2 ± 0.0 e | <0.0001 |
| C16:1 *ω*-9 | 0.1 ± 0.0 a | 0.0 ± 0.0 b | 0.0 ± 0.0 b | 0.0 ± 0.0 b | 0.0 ± 0.0 b | 0.0 ± 0.0 b | 0.0 ± 0.0 b | 0.2 ± 0.1 a | 0.0 ± 0.0 b | 0.0 ± 0.0 b | 0.0035 |
| C17:0 | 0.9 ± 0.0 b | 0.4 ± 0.0 f | 0.6 ± 0.0 d | 0.6 ± 0.0 de | 0.4 ± 0.0 g | 0.7 ± 0.0 c | 0.9 ± 0.0 b | 0.5 ± 0.0 e | 0.4 ± 0.0 f | 1.8 ± 0.0 a | <0.0001 |
| C17:1 | 0.3 ± 0.0 a | 0.2 ± 0.0 bc | 0.0 ± 0.0 f | 0.1 ± 0.0 def | 0.2 ± 0.0 ab | 0.1 ± 0.0 cd | 0.1 ± 0.0 ef | 0.1 ± 0.0 ef | 0.1 ± 0.0 de | 0.1 ± 0.0 ef | <0.0001 |
| C18:0 | 20.8 ± 0.0 e | 20.0 ± 0.0 f | 19.0 ± 0.0 h | 16.4 ± 0.0 i | 26.6 ± 0.0 a | 23.8 ± 0.0 c | 19.4 ± 0.0 g | 25.5 ± 0.0 b | 15.8 ± 0.0 j | 22.2 ± 0.0 d | <0.0001 |
| C18:1 | 2.1 ± 0.0 h | 4.0 ± 0.0 a | 2.8 ± 0.0 c | 2.7 ± 0.0 d | 1.9 ± 0.0 i | 2.3 ± 0.0 g | 2.4 ± 0.0 f | 2.8 ± 0.0 c | 3.0 ± 0.0 b | 2.5 ± 0.0 e | <0.0001 |
| C18:2 | 24.2 ± 0.0 b | 23.7 ± 0.0 cd | 23.9 ± 0.0 c | 23.4 ± 0.0 d | 19.6 ± 0.0 h | 20.2 ± 0.1 f | 24.6 ± 0.0 a | 19.9 ± 0.0 g | 23.5 ± 0.0 d | 22.1 ± 0.0 e | <0.0001 |
| C20:0 | 0.0 ± 0.0 bc | 0.0 ± 0.0 bc | 0.1 ± 0.0 a | 0.1 ± 0.0 a | 0.0 ± 0.0 b | 0.0 ± 0.0 b | 0.1 ± 0.0 a | 0.0 ± 0.0 bc | 0.0 ± 0.0 bc | 0.0 ± 0.0 c | 0.0004 |
| C18:3 | 22.1 ± 0.0 c | 20.6 ± 0.0 d | 20.3 ± 0.0 e | 22.7 ± 0.0 b | 18.7 ± 0.0 f | 17.4 ± 0.0 i | 22.9 ± 0.0 a | 18.3 ± 0.0 h | 23.0 ± 0.0 a | 18.5 ± 0.0 g | <0.0001 |
| C20:1 | 0.1 ± 0.0 f | 0.3 ± 0.0 bc | 0.2 ± 0.0 de | 0.3 ± 0.0 bc | 0.2 ± 0.0 cd | 0.3 ± 0.0 ab | 0.2 ± 0.0 e | 0.3 ± 0.0 bc | 0.2 ± 0.0 c | 0.3 ± 0.0 a | <0.0001 |
| C22:0 | 0.0 ± 0.0 f | 0.2 ± 0.0 c | 0.3 ± 0.0 b | 0.2 ± 0.0 d | 0.1 ± 0.0 e | 0.0 ± 0.0 f | 0.2 ± 0.0 d | 0.2 ± 0.0 d | 0.3 ± 0.0 a | 0.2 ± 0.0 d | <0.0001 |
| C24:0 | 0.5 ± 0.0 | 0.7 ± 0.0 | 0.6 ± 0.0 | 1.1 ± 0.0 | 1.2 ± 0.0 | 0.7 ± 0.0 | 0.6 ± 0.0 | 0.9 ± 0.0 | 0.9 ± 0.0 | 0.9 ± 0.0 | <0.0001 |

Different letters (a-j) in the same row denote statistically significant difference ($p < 0.05$).

linoleic acid (48.4%) in their study, as did Labiad et al. (2021) in cultivated sea fennel from Spain (49.5%).

It is estimated that the most abundant fatty acids in plant tissues contain 16 and 18 carbon atoms (Tvrzicka et al., 2011) and that palmitic, linoleic and linolenic acids are the dominant fatty acids in halophyte plants (Oliveira-Alves et al., 2023) which is also confirmed by this study on sea fennel. Studies have also found an increase in the content of fatty acids such as palmitic, palmitoleic and oleic acids in nutrient-deficient plants under high salinity (Castillo et al., 2022), which could also be the reason for the variability in the results of this study. Compared to previous investigations on the fatty acid profile of sea fennel from Tunis (Ben Hamed et al., 2005), Italy (Maoloni et al., 2021), Portugal (Castillo et al., 2022; Oliveira-Alves et al., 2023), Spain (Castillo et al., 2022; Guil-Guerrero & Rodríguez-García, 1999; Labiad et al., 2021; Martins-Noguerol, Matías, et al., 2022), the Croatian samples contained a significantly higher content of SFAs, a lower content of PUFAs and an almost negligible content of oleic acid (1.9–3%), again indicating the difference in chemical composition between Croatian sea fennel and plants from other Mediterranean countries.

### 3.3. Carotenoids

Determination of four carotenoids (neoxanthin, violaxanthin, lutein and $\beta$-carotene) in sea fennel leaves was performed by UHPLC-DAD, and the results are shown in Table 4.

The most abundant compounds in all tested samples, collected from all locations was the xanthophyll lutein (107.1–435.4 mg/kg), followed by $\beta$-carotene (16.8–80.2 mg/kg) and neoxanthin (0.5–8.4 mg/kg). Sea fennel samples from south locations showed higher abundance of all quantified carotenoids: the sample from peninsula Pelješac had the highest amount of lutein and neoxanthin, while $\beta$-carotene was most abundant in this sample and in the sample from the southernmost location of Cavtat. Sea fennel samples from Split generally had the lowest carotenoid content among all tested locations, with the absence of neoxanthin.

Most of the researches on sea fennel carotenoids have so far been performed on wild populations with spectrophotometric determination of total carotenoids expressed as $\beta$-carotene (62.6 mg/kg according to Nabet et al. (2017) and 410 mg/kg according to Sousa et al. (2022). However, Sarrou et al. (2019) investigated carotenoids from two harvest seasons of the commercially grown Greek genotype of sea fennel. In the first season the dominant compound was lutein with concentrations of 545.5 and 741.5 μg/g, while in the second season it was neoxanthin (935.0–1459.4 μg/g). The presence of violaxanthin was detected in both seasons and in all samples, but at significantly lower concentrations. In this study, violaxanthin, the precursor of neoxanthin, was not detected in Croatian sea fennel populations, in contrast to the research of Sarrou et al. (2019).

Carotenoids, the most abundant photosynthetic pigments in plants, are known for their functions in plants during photosynthesis and/or the reproductive cycle, but also for their role as powerful antioxidants, antiumor, neuroprotective, anti-inflammatory and chemoprotective agents, immunomodulators, etc. However, carotenoids with an unsubstituted $\beta$-terminal group (such as $\alpha$-carotene, $\beta$-carotene, and $\beta$-cryptoxanthin) are also important and recognised for their provitamin A activity. Carotenoids can be classified as carotenes (hydrocarbons, e.g. $\beta$-carotene, lycopene) and xanthophylls (oxygenated carotene derivatives, e.g. lutein, violaxanthin, zeaxanthin) (Crupi et al., 2023). Lutein, one of the most potent xanthophyll antioxidants, plays an important role in various pathological conditions (Fuad et al., 2020), It was the most abundant xanthophyll in Croatian sea fennel populations (up to 435.4 mg/kg), while in second place was $\beta$-carotene (up to 80.2 mg/kg), which is the only carotenoid that can be enzymatically cleaved into two molecules of vitamin A (Biesalski, Chichili, Frank, von Lintig, & Nohr, 2007). Neoxanthin was found in the lowest amounts (up to 8.4 mg/kg) only in samples from several locations, mostly the southern ones. It can be noted that neoxanthin, which is found to have a specific role in protecting against oxidative stress (Dall'Osto, Cazzaniga, North, Marion-Poll, & Bassi, 2007), was not found in northern populations.

### 3.4. Tocopherols

Tocopherols, composed of a polar chromanol group and a unipolar saturated isoprenoid side chain, are commonly known as vitamin E and can occur as four isomers depending on the nature of the R1 and R2 radicals on the chromanol group: $\alpha$-, $\beta$-, $\gamma$-, and $\delta$-isomer (Niki & Abe, 2019). Analysis of sea fennel samples from different locations along the Croatian Adriatic coast identified $\alpha$-, $\beta$- and $\gamma$-tocopherol isomers, the profiles of which are shown in Table 5.

$\alpha$-Tocopherol was predominant in all samples except the sample from Šibenik, which is consistent with previous studies on halophyte species (Ksouri et al., 2012). Maatallah Zaier et al. (2020) examined the tocopherol profile in young shoots of four Tunisian *Amaranthaceae* halophytes, and of the four tocopherol isomers detected ($\alpha$-, $\beta$-, $\gamma$-, and $\delta$-), α-tocopherol was the most abundant (1.95–11.42 mg/100 g fresh weight). $\alpha$- and $\beta$-tocopherols were detected in two Tunisian populations of *Cakile maritima* fruit oil (Stambouli-Essassi et al., 2020), with the α-isomer being predominant (28.29–31.13 mg/kg oil). Barreira et al. (2017) studied the nutritional profiles of four halophytes: *Sarcocornia perennis* subsp. *perennis*, *S. perennis* subsp. *alpini* and *Salicornia ramosissima* and *Arthrocnemum macrostachyum* and among the tocopherols, the α-isomer was predominant in all species and the highest in *A. macrostachyum* (8.74 mg/100 g dw). El-Shami and El-Negoumy (1993) detected four tocopherol isomers in *Salicornia* halophyte oil from ground seeds and found that $\gamma$-tocopherol was the most abundant (58.7%), followed by $\alpha$- (38.2%) and $\delta$- (2.1%), and finally $\beta$- (1.0%). In this study, the content of $\alpha$-tocopherol was lowest in the sample from Šibenik (9.68 mg/kg) and highest in the sample from Pelješac (53.91 mg/kg). The content of the $\beta$-isomer ranged from 2.22 mg/kg (Pelješac) to 10.11 mg/kg (Šibenik) and that of the $\gamma$-isomer from 1.19 mg/kg (Šibenik) to 2.43 mg/kg (Neretva). It should be noted that the sea fennel from Šibenik had the lowest content of $\alpha$-tocopherol, while the other two isomers were present in the highest amounts in this sample.

Tocopherols as non-polar compounds, especially α-tocopherol, have a protective role in membranes against oxidative stress (Havaux, Eymery, Porfirova, Rey, & Dörmann, 2005). This role has been applied to the prevention of some chronic diseases and cancers (Ortiz et al., 2006). Horvath et al. (2006) found that $\alpha$-tocopherol is most abundant in

**Table 4**
Carotenoids (neoxantin, lutein, $\beta$-carotene) profiles (mg/kg) of Croatian sea fennel from different locations along the Adriatic coast.

| Compound (mg/kg) | Krk | Senj | Pag | Šibenik | Split | Drašnice | Korčula | Pelješac | Neretva | Cavtat | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| neoxantin | nd | nd | nd | 0.77 ± 0.01 c | nd | 0.68 ± 0.01 c | 3.96 ± 0.27 b | 8.40 ± 0.42 a | nd | 0.54 ± 0.02 c | <0.0001 |
| lutein | 250.00 ± 0.10 c | 127.70 ± 0.10 i | 147.30 ± 1.60 h | 244.80 ± 0.50 d | 107.10 ± 1.10 j | 178.40 ± 0.20 f | 273.90 ± 1.00 b | 435.40 ± 0.10 a | 151.40 ± 0.10 g | 236.30 ± 0.30 e | <0.0001 |
| $\beta$-carotene | 51.00 ± 0.01 d | 23.40 ± 0.03 f | 23.50 ± 0.12 f | 54.20 ± 0.37 c | 16.80 ± 0.02 h | 35.40 ± 0.43 e | 74.60 ± 0.19 b | 79.60 ± 0.33 a | 21.00 ± 0.10 g | 80.20 ± 0.15 a | <0.0001 |

nd- not detected. Different letters (a-j) in the same row denote statistically significant difference ($p < 0.05$).

**Table 5**
Tocopherols ($\alpha$-, $\beta$- and $\gamma$-) profiles (mg/kg) of Croatian sea fennel from different locations along the Adriatic coast.

| Compound (mg/kg) | Krk | Senj | Pag | Šibenik | Split | Drašnice | Korčula | Pelješac | Neretva | Cavtat | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $\alpha$-tocopherol | 38.25 ± 0.26 b | 14.98 ± 0.19 f | 13.31 ± 0.07 g | 9.68 ± 0.1 h | 18.66 ± 0.46 e | 23.29 ± 0.58 d | 37.43 ± 0.24 b | 53.91 ± 0.57 a | 22.76 ± 0.04 d | 27.26 ± 0.15 c | <0.0001 |
| $\beta$-tocopherol | 5.18 ± 0.01 d | 4.7 ± 0.07 e | 3.95 ± 0.04 f | 10.11 ± 0.13 a | 5.15 ± 0.26 d | 5.56 ± 0.28 d | 6.4 ± 0.09 c | 2.22 ± 0.02 g | 9.17 ± 0.11 b | 4.44 ± 0.02 e | <0.0001 |
| $\gamma$-tocopherol | 1.92 ± 0.15 b | 1.33 ± 0.01 de | 1.34 ± 0.01 de | 1.19 ± 0.01 e | 1.71 ± 0.02 bc | 1.54 ± 0.01 cd | 1.34 ± 0.13 de | 1.65 ± 0.05 c | 2.43 ± 0.12 a | 1.27 ± 0.05 e | <0.0001 |

Different letters (a-g) in the same row denote statistically significant difference ($p < 0.05$).

leaves, which are classified as photosynthetic tissues, while $\gamma$-tocopherol is most abundant in the non-photosynthetic tissues of the plant such as seeds, cotyledons, roots, stems, flowers, fruits, tubers and hypocotyls.

### 3.5. Phenolic compounds

According to the results presented in Table 6, chlorogenic acid (3-*O*-caffeoylquinic acid) and its derivatives, cryptochlorogenic acid (4-*O*-caffeoylquinic acid) and neochlorogenic acid (5-*O*-caffeoylquinic acid), were the predominant phenolic compounds in the sea fennel samples studied, which is consistent with our previous reports on Croatian sea fennel samples (Generalić Mekinić et al., 2016, 2018; Politeo, Popović, Veršić Bratinčević, Koceić, et al., 2023; Politeo, Popović, Veršić Bratinčević, Kovačević, et al., 2023; Veršić Bratinčević et al., 2023), as well as with studies by other authors on samples from other Mediterranean countries (Alemán, Marín, Taladrid, Montero, & Carmen Gómez-Guillén, 2019; Alves-Silva et al., 2020; Kadoglidou et al., 2022; Maoloni et al., 2023; Martins-Noguerol, Matías, et al., 2022; Meot-Duros & Magné, 2009; Nabet et al., 2017; Pedreiro et al., 2023; Pereira et al., 2017; Sánchez-Faure et al., 2020).

The highest content of chlorogenic acid was determined in the Korčula sample (248.44 mg/g), followed by the Neretva sample (154.65 mg/g). High concentrations (over 80 mg/g) were also found in the samples collected from Pelješac and Cavtat, so it can be concluded that all samples from the southern areas were extremely rich in this compound. All other samples contained significantly lower amounts of chlorogenic acid (between 24.65 and 48.38 mg/g), with the exception of the Krk sample, where the presence of a higher amount of chlorogenic acid was detected (81.15 mg/g).

Meot-Duros and Magné (2009) studied the antioxidant activity and phenolic content of *Crithmum maritimum* (L.) aerial parts collected along the shoreline in France during one year, and proved that a higher concentration of chlorogenic acid was detected in samples collected in a stressful sand hill environment, which corresponds to our samples collected in the Neretva. A similar trend can be observed for neochlorogenic acid, the content of which was highest in the sample from Korčula (30.05 mg/g), while in the other samples it ranged from 2.63 mg/g (Drašnice) to 9.71 mg/g (Pelješac). The content of criptochlorogenic acid ranged from 4.35 mg/g in the sample from Drašnice to 15.93 mg/g in the sample from Neretva. Several in vitro and in vivo studies have highlighted the great potential of chlorogenic acid and its derivatives to prevent and/or reverse neurodegenerative events (Amato, Terzo, & Mulè, 2019).

The presence of cinnamic acids derived from esterification with quinic acid was reported by Sánchez-Faure et al. (2020). The detected acids were also found in previous reports (Kadoglidou et al., 2022; Özcan et al., 2001; Pereira et al., 2017; Sarrou et al., 2019). High concentrations of ferulic acid and *p*-hydroxybenzoic acid were determined in the Korčula sample (1.42 and 3.36 mg/g, respectively), while the sea fennel from Pelješac was rich in caffeic acid (0.73 mg/g).

Researchers (Alemán et al., 2019; Kadoglidou et al., 2022; Martins-Noguerol, Matías, et al., 2022; Piatti et al., 2023; Sarrou et al., 2019) also reported the presence of significant amounts of rutin in sea fennel samples, and the highest concentration of this flavonoid in our study was found in the sample from Senj (9.04 mg/g), followed by Cavtat (6.78 mg/g) and Korčula (3.65 mg/g). Phenolic compounds are among the most important phytochemicals that play a key role in plant survival under various abiotic stress factors due to their increased synthesis and accumulation (He & Ding, 2020). In addition to defense against various abiotic stresses, they also serve as pathogen growth inhibitors (Reginato et al., 2014). However, the concentrations of these compounds differed considerably between samples, and are most likely the result of

**Table 6**
Phenolic profiles (mg/g) of Croatian sea fennel from different locations along the Adriatic coast.

| Compound (mg/g) | Krk | Senj | Pag | Šibenik | Split | Drašnice | Korčula | Pelješac | Neretva | Cavtat | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| gallic acid | 0.02 ± 0.00 e | 0.05 ± 0.00 b | 0.03 ± 0.00 c | 0.09 ± 0.00 a | 0.01 ± 0.00 f | 0.03 ± 0.00 cd | 0.03 ± 0.00 d | 0.02 ± 0.00 e | 0.03 ± 0.00 cd | 0.01 ± 0.00 f | <0.0001 |
| protocatechuic acid | 0.16 ± 0.00 bc | 0.23 ± 0.01 b | 0.04 ± 0.00 d | 0.37 ± 0.11 a | 0.17 ± 0.00 bc | 0.18 ± 0.02 cd | 0.23 ± 0.00 b | 0.10 ± 0.00 cd | 0.14 ± 0.01 bcd | 0.09 ± 0.00 cd | <0.0001 |
| neochlorogenic acid | 7.47 ± 0.17 d | 4.26 ± 0.00 f | 2.96 ± 0.00 h | 3.85 ± 0.01 g | 4.27 ± 0.00 f | 2.63 ± 0.05 i | 30.05 ± 0.010 a | 9.71 ± 0.04 b | 9.54 ± 0.00 c | 5.56 ± 0.02 e | <0.0001 |
| *p*-hydroxybenzoic acid | 1.30 ± 0.08 d | 0.89 ± 0.01 ef | 0.79 ± 0.00 f | 1.56 ± 0.00 c | 1.81 ± 0.00 b | 1.40 ± 0.08 d | 3.36 ± 0.00 a | 1.55 ± 0.01 c | 0.91 ± 0.00 e | 0.59 ± 0.01 g | <0.0001 |
| chlorogenic acid | 81.15 ± 0.16 e | 48.38 ± 0.06 f | 32.40 ± 0.01 h | 43.93 ± 0.00 g | 27.04 ± 0.00 i | 24.65 ± 0.00 j | 248.44 ± 0.03 a | 97.08 ± 0.40 c | 154.65 ± 0.00 b | 82.04 ± 0.02 d | <0.0001 |
| cryptochlorogenic acid | 10.43 ± 0.08 d | 6.73 ± 0.01 f | 4.70 ± 0.00 i | 6.05 ± 0.00 g | 5.94 ± 0.08 h | 4.35 ± 0.00 j | 42.06 ± 0.01 a | 13.67 ± 0.06 c | 15.93 ± 0.01 b | 8.92 ± 0.04 e | <0.0001 |
| caffeic acid | 0.09 ± 0.00 ef | 0.37 ± 0.17 bc | 0.20 ± 0.00 de | 0.06 ± 0.00 ef | 0.02 ± 0.00 f | 0.06 ± 0.00 ef | 0.09 ± 0.01 ef | 0.73 ± 0.01 a | 0.27 ± 0.00 cd | 0.50 ± 0.02 b | <0.0001 |
| ferulic acid | 0.57 ± 0.00 c | 0.47 ± 0.02 cde | 0.25 ± 0.00 g | 0.46 ± 0.00 de | 0.27 ± 0.00 fg | 0.38 ± 0.06 ef | 1.42 ± 0.08 a | 0.72 ± 0.06 b | 0.46 ± 0.04 de | 0.53 ± 0.00 cd | <0.0001 |
| sinapic acid | 0.09 ± 0.00 e | 0.22 ± 0.02 c | 0.10 ± 0.00 de | 0.37 ± 0.00 b | 0.12 ± 0.00 cde | 0.20 ± 0.06 cd | 0.58 ± 0.08 a | 0.35 ± 0.03 b | 0.22 ± 0.04 c | 0.05 ± 0.00 e | <0.0001 |
| rutin | 2.11 ± 0.04 g | 9.04 ± 0.01 a | 2.04 ± 0.02 g | 2.69 ± 0.02 e | 1.49 ± 0.02 h | 2.35 ± 0.02 f | 3.65 ± 0.03 c | 2.34 ± 0.06 f | 3.12 ± 0.02 d | 6.78 ± 0.04 b | <0.0001 |

Different letters (a-j) in the same row denote statistically significant difference ($p < 0.05$).

divergent phylogenetic evolutionary pathways, different responses to stressors and morphological traits (Lopes, Sanches-Silva, Castilho, Cavaleiro, & Ramos, 2023), while variations in the concentration of chlorogenic acid between samples can be explained by the influence of different environmental conditions, including salt stress and nutrient deficiency as one of the most important parameters (Wang, Ge, Tian, & Mai, 2022).

### 3.6. Principal component analysis

Principal component analysis (PCA) was used to describe the variations between the dominant fatty acids (palmitic, stearic, linoleic and linolenic acids), phenolics (chlorogenic acid, neochlorogenic acid and criptochlorogenic acid), essential oil components (limonene, sabinene, γ-terpinene, terpinen-4-ol, verbenone, *cis*-verbenol, *trans*-*p*-menth-2-en-1-ol, trans-carveol), tocopherols (α-tocopherol and β-tocopherol) and pigments (lutein, β-carotene, neoxanthin) in relation to the sampling areas. The results of PCA, the correlation plot (a) and the scoreplot (b) of the dominant are shown in Fig. 2. The first two PCs described 60.22% of the initial data variability. The highest variable contribution to Factor 1 was observed for *trans*-*p*-menth-2-en-1-ol, *cis*-verbenol, *trans*-carveol, verbenone, neoxantin, lutein and β-carotene, while phenolic acids (neochlorogenic, chlorogenic and cryptochlorogenic) and α-tocopherol contributed to Factor 2.

A clear separation was observed along PC1 between the samples from Cavtat and other locations. It was positioned on the left part of the multivariate space, followed by Split and Drašnice samples, while remaining samples were positioned in the right part of the score plot

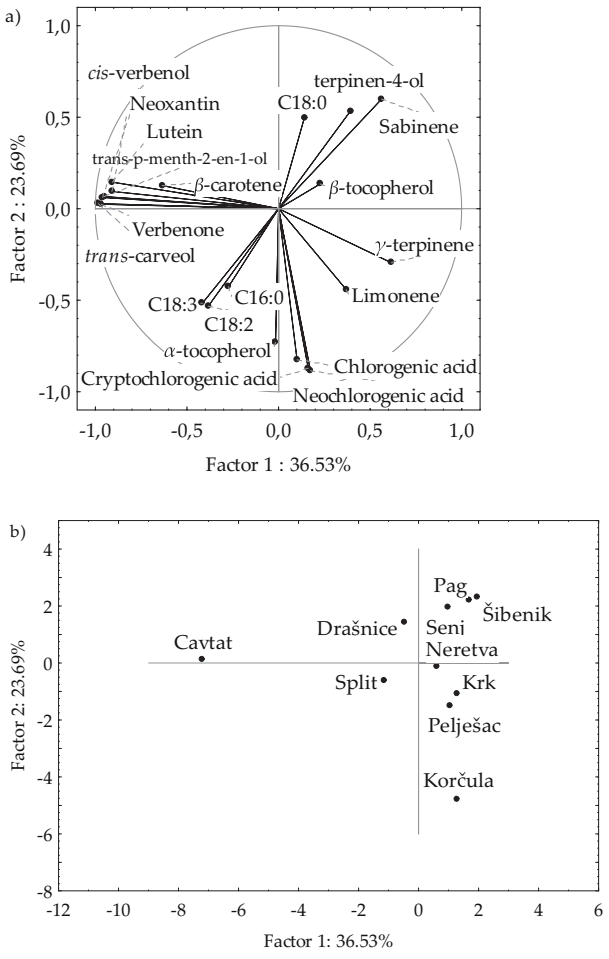


**Fig. 2.** Correlation loadings (**a**) and score plots (**b**) of the dominant fatty acids, phenolics, essential oils and pigments in relation to sampling area.

(Fig. 2b). The main difference between the sample from Cavtat and the other samples is the content of essential oil components. This sample contained the lowest amount of limonene (only 24%), while components that were present only in small amounts or not at all in other samples were present in the sea fennel from Cavtat in amounts of over 5% (such as *trans*-*p*-menth-2-en-ol, *cis*-verbenol, *trans*-carveol, isocarveol, verbenone). Split sample was also characterised by the lowest content of sabinene, low content of terpinen-4-ol and high content of limonene.

On the contrary, the samples from Split and Drašnice exhibited the lowest levels of chlorogenic acid, as well as relatively low levels of linolenic acid and elevated levels of stearic acid. These variations in composition and the positioning of the samples along principal components may also be influenced by the environmental (climatic/weather) conditions to which the plants were exposed prior to harvest. Data provided by the Croatian Meteorological and Hydrological Service (https://meteo.hr/) indicates that the August 2022 harvest period experienced slightly higher temperatures compared to the preceding 30-year period across all locations. Significantly warmer measurements were observed in the southernmost (Cavtat, Neretva, Pelješac, Korčula) and northernmost (Pag, Senj, Krk) regions. Cavtat, in particular, experienced prevailing drought conditions, while an even greater absence of rainfall was recorded in the most northern collection sites. These meteorological parameters could potentially influence the chemical composition of the plants and their ability to combat stressors and could also be a reason for the variation observed in the Cavtat sample in Fig. 2. However, to draw definitive and comprehensive conclusions, a more exhaustive study would be required. Interestingly, the distribution along PC2 showed a clear separation of the Korčula sample (highest case contribution) in the lower right area and Pag and Šibenik in the upper right area. The Korčula sample is characterised by an extremely high content of chlorogenic acid and its two derivatives (for example, a concentration >10 times higher than in the Drašnice sample). The highest content of lutein was also found in the sea fennel from Korčula, while the samples from Pag and Šibenik also had a low content of chlorogenic acid, but also a low content of α-tocopherol and limonene, but a high content of sabinene.

The results obtained from all locations for each group of phytochemicals tested show agreement with the expected result and thus confirm the accuracy of the hypothesis formulated.

## 4. Conclusions

Salinity is one of the biggest environmental problems in the world, with solutions being found in the cultivation of salt-tolerant crops. Therefore, it is not surprising that due to rapid climate change and population growth, twenty-first century is expected to be the century of halophyte agriculture expansion. This study was conducted to provide a comprehensive phytochemical characterisation of antioxidant and nutritive valuable metabolites from wild Croatian sea fennel populations, one of the most representative halophyte species in the Mediterranean region The results confirmed the valuable chemical composition of the plant, with a high content of nutritive and bioactive antioxidant compounds, especially PUFAs (linoleic and linolenic acids), monoterpenes and monoterpenoids (limonene, sabinene γ-terpinene, and terpinen-4-ol), carotenoids (lutein), tocopherols (α-tocopherol), and phenolic compounds (chlorogenic acid and its derivatives). Differences in chemistry have been found between Croatian populations and those from other Mediterranean regions, which could indicate the existence of a special chemotype of the plant. Due to the richness of sea fennel samples in valuable compounds, the authors believe it is justified to include the adjective “functional” in the list of descriptions of this valuable plant species.

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## CRediT authorship contribution statement

**Ivana Generalić Mekinić:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Olivera Politeo:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Ivica Ljubenkov:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Linda Mastelić:** Writing – review & editing, Formal analysis. **Marijana Popović:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Maja Veršić Bratinčević:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Vida Šimat:** Writing – review & editing, Visualization, Validation, Investigation, Data curation. **Sanja Radman:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Danijela Skroza:** Writing – review & editing, Investigation. **Tonka Ninčević Runjić:** Writing – review & editing, Visualization, Methodology, Investigation. **Marko Runjić:** Writing – review & editing, Visualization, Methodology, Investigation. **Gvozden Dumičić:** Writing – review & editing, Visualization, Methodology, Investigation. **Branimir Urlić:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

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