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SEA FENNEL (*Crithmum maritimum* L.): SUSTAINABLE SOURCE OF OMEGA-3 FATTY ACIDS OR FUNCTIONAL INGREDIENT FOR THE DESIGN OF ALGINATE-BASED CAPSULES

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“If we knew what we were doing, it would not be called research, would it?”

- Albert Einstein

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ACRONYMS AND ABBREVIATIONS

ACRONYM	DEFINITION
ALA	α-linolenic acid
BP	By product
CAL-F	Calabria flower
CAL-L	Calabria leave
CON-L	Conero leave
COR-F	Corsica flower
COR-L	Corsica leave
COR-S	Corsica seed
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
GRAS	Generally Recognized As Safe
LA	Linoleic acid
LDL	Low density lipoprotein
LIG-F	Liguria flower
LIG-L	Liguria leave
MAR-F	Marche flower
MAR-L	Marche leave
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
PUG-F	Puglia flower
PUG-L	Puglia leave
RIN-L-AD	Rinci leave air-dried
RIN-L-FD	Rinci leave freeze-dried
SAR-F	Sardinia flower
SAR-L	Sardinia leave
SD	Standard deviation
SF	Sea fennel
SFA	Saturated fatty acid
SIC-F	Sicily flower
SIC-L	Sicily leave
TOS-F	Tuscany flower
TOS-L	Tuscany leave

SUMMARY

Sea fennel (*Crithmum maritimum* L.), also known as rock samphire, is a halophyte plant. In the Mediterranean countries, *C. maritimum* L. aerial parts are used in cuisine and folk medicine. *C. maritimum* is considered a good source of dietary fiber, proteins, polyunsaturated fatty acids, minerals, vitamins, polyphenols and essential oils. Limited information is available regarding fatty acid composition particularly in aerial parts of (leaves and flowers) sea fennel, and several recent studies have reported substantial differences in fatty acid composition between wild and cultivated plant of the same species. Therefore, this study aims to: i) characterize the fatty acid composition of leaves, flowers, seeds and by-product of wild and cultivated *C. maritimum* and ii) to test the dropping encapsulation method on sea fennel extracts, not yet tested on such plant extract, for the research and development of new sea fennel-based functional foods.

Fatty acid methyl esters (FAME) were analyzed using a GC-FID system (gas chromatography coupled with flame ionization detector). The results of fatty acid composition herein collected suggest that different parts of the sea fennel are far from each other in terms of fat composition. The amount of omega-3 in 100 g of sea fennel was calculated to determine whether a nutrition or health claim can be used under the Regulation (EC) No 1924/2006 and the highest amount was found in the leaves sample from Puglia. Alginate-based spherification was achieved using an ethanolic extract of sea fennel especially with the sample E75 which has turned out to be the best formulation for stable and appropriate capsules.

The results of this thesis will help researchers to set new experimental design to valorize sea fennel in the food industry.

RIASSUNTO

Il finocchio marino (*Crithmum maritimum* L.), noto anche come spaccasassi, è una pianta alofita. Nei Paesi del Mediterraneo le parti aeree di *C. maritimum* L. sono utilizzate in cucina e nella medicina popolare. Il *C. maritimum* è considerato una buona fonte di fibre alimentari, proteine, acidi grassi polinsaturi, minerali, vitamine, polifenoli e oli essenziali. Tuttavia, le informazioni sulla composizione degli acidi grassi sono limitate, in particolare delle parti aeree (foglie e fiori) di finocchio marino. Diversi studi recenti hanno riportato differenze sostanziali nella composizione degli acidi grassi tra piante selvatiche e coltivate della stessa specie. Pertanto, questo studio si propone di: i) caratterizzare la composizione degli acidi grassi delle foglie, fiori, semi e sottoprodotti di *C. maritimum* da popolazione selvatiche e coltivate e ii) testare il metodo di incapsulamento detto "dropping method" su estratti di finocchio marino, non ancora testato su tale estratto vegetale, per la ricerca e lo sviluppo di nuovi alimenti funzionali a base di finocchio marino.

Gli esteri metilici degli acidi grassi (FAME) sono stati analizzati con un sistema GC-FID (gascromatografia accoppiata a rivelatore a ionizzazione di fiamma). I risultati della composizione degli acidi grassi qui raccolti suggeriscono che le diverse parti del finocchio marino sono molto distanti tra loro in termini di composizione degli acidi grassi. La quantità di omega-3 in 100 g di finocchio marino è stata calcolata per determinare la possibilità di utilizzare un'indicazione nutrizionale o salutistica ai sensi del regolamento (CE) n. 1924/2006 e la quantità più elevata è stata trovata nel campione di foglie proveniente dalla Puglia. La sferificazione a base di alginato è stata ottenuta utilizzando un estratto etanolico di finocchio marino, in particolare con il campione E75, che si è rivelato la migliore formulazione per capsule edibili e stabili.

I risultati di questa tesi aiuteranno i ricercatori a impostare nuovi disegni sperimentali per valorizzare il finocchio marino nell'industria alimentare.

Keywords: halophyte, chlorogenic acid, fatty acids, nutraceuticals, encapsulation.

1. INTRODUCTION

1.1. General characteristics of C. maritimum or sea fennel

Sea fennel (*Crithmum maritimum* L.), also known as rock samphire the only species of the genus *Crithmum* within the *Apiaceae* family. It is a halophyte plant, meaning that it is among the few species capable of surviving in saline environments [1]. It grows on maritime cliffs and sand in the European Atlantic coasts, Azores, Madeira, Canarias Islands, Mediterranean and Black Sea coasts, north-west Africa, and western Asia [3]. *C.maritimum* L. is a highly branched plant up to 30–60 cm tall, the root of which is strong, thick and knotty, and the leaves are fleshy and succulent. The leaves (Fig.2) are 2 to 5 cm long and 0.6 cm wide. This plant blooms from June to September, and the fruits begin to ripen in November-December [2]. Pale yellow, five-petaled flowers (Fig.2) with 10–30 rays are grouped in umbellets and encircled by bracts. The fruits that develop from these flowers ripen between November and December. The flowering season typically lasts from June to September [4]. The endospermic seed (Fig.4) of *C. maritimum* L. is highly nutrient-rich, measuring around 3.5 mm in length and 1 mm in width [28]. Nowadays, sea fennel is protected in some regions, such as England and the Conero Natural Park (Marche region, central Italy), as indiscriminate collection of sea fennel in the wild has caused this specie to disappear from some European habitats and it is now forbidden to collect sea fennel in the wild [4].

Figure 2. Sea fennel leaves

Figure 1. Sea fennel flowers

Figure 3. Sea fennel by-product

Figure 4. Sea fennel seeds

1.2. Application of sea fennel in food

In the Mediterranean countries, *C.maritimum* L. aerial parts are used in cuisine and folk medicine for their aromatic, antiscorbutic, diuretic, digestive, and carminative properties. Traditionally, succulent, and crunchy leaves are consumed as a condiment in salads, sauces, soups, pickled in vinegar or oil and as dried herb (Fig. 5) [4, 1]. They can also be consumed as an appetizer with a variety of meals, such as bread and olive oil or even cooked with capers, in several parts of the Mediterranean basin [4]. Also, a traditional meal from the British Isles, "Rock Samphire Hash" is made with pickled cucumbers, capers, and sea fennel leaves [1]. Renna and Gonnella [5] reported the use of the sea fennel as a new spice-colorant useful in several culinary preparations. Additionally, sea fennel is a perfect component for creating industrial-scale prototypes of two brand-new, shelf-stable green sauces [4]. Fresh-cut sea fennel is currently available in a niche market for a variety of culinary applications. Sea fennel is an aromatic plant that can be used both dried and in fresh products [1]. Recently, in order to investigate the functional properties of sea fennel in different physical states, innovative foods have been developed, such as fermented preserves, co-fermented preserves with other vegetables, artificially acidified preserves, powders, and functional beverages (Fig. 6) [6].

Figure 5. Application of sea fennel in soups and salads

Figure 6. Application of sea fennel in fermented preserves, green sauces and pasta

1.3.Sustainability of sea fennel

Environmental stress factors such as drought, salinity, freezing and high temperatures have become a hot topic due to concerns about the impact of climate change on plant resources, biodiversity and global food security as they affect plant growth and pose a growing threat to sustainable agriculture [15]. Heat stress is often followed by water scarcity and drought by salinization. Soil salinization is a serious soil degradation that can be caused by both natural causes and anthropogenic activities, such as deforestation or irrigation with poor quality water, and is found in many countries around the world, especially in arid and semi-arid zones [16]. Halophytes have evolved several adaptive traits expressed at various levels of organization that allow them to germinate, grow, and achieve their complete cycle of development under such conditions [17]. Sea fennel is a promising vegetable ingredient, and further studies are recommended to deepen the knowledge of this emerging crop and to encourage its large-scale diffusion through an increase in both consumer and farmer awareness about its highly valuable nutritional and functional traits. In this regard, a project titled "Innovative Sustainable Organic Sea Fennel (*Crithmum maritimum L.*) based Cropping Systems to Boost Agrobiodiversity, Profitability, Circularity, and Resilience to Climate Changes in Mediterranean Small Farms" [18] aims to introduce new sustainable organic sea fennel-based cropping systems that can cope with limited resources (freshwater and fertile soils), environmental constraints (biodiversity loss and chemical pollution), and climate-related risks (soil salinization and water drought) to enhance food production stability over time [4].

1.4. General composition and fatty acids

Depending on the genotype, environment, and vegetative stage, the composition of this halophyte varies [4]. Table 1 indicates that water (87.60%), carbohydrates (7.33%), and dietary fibre (3.74%) make up the bulk of this plant. Ashes makes up 2.78%, while proteins takes 1.57% of the total weight. Lastly, total lipids and total sugar make up roughly 0.73% and 0.65%, respectively.

Table 1. Proximate composition of C.maritimum L.

Crop	Water	Total lipid	Protein	Total Carbohydrate	Total Sugar	Total Dietary Fiber	Ashes
	g 100 g^{-1} FW						
Sea fennel	87.60	0.73	1.57	7.33	0.65	3.74	2.78

Data retrieved from Renna [1].

C.maritimum is considered a good source of dietary fiber, proteins, polyunsaturated fatty acids (e.g., linoleic and linolenic acids), minerals, vitamins (C, A, E), bioactive compounds such as polyphenols (hydroxycinnamic acids and flavonoids), and essential oils [6]. Fatty acids are organic acids with at least one carboxyl group and a long carbon chain, that are distributed to cells where they serve as fuel for muscular contraction and general metabolism in human body. According to the presence and number of double bonds FA can be classified as saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). As biological compounds, FAs play crucial roles in human metabolism, health, and disease. Epidemiological and clinical trials showed that fatty acids are associated with cardiovascular diseases, neurological diseases, non-alcoholic fatty liver disease, allergic diseases, and so on [10]. The human body cannot synthesize PUFAs with the first double bond on C3 and C6 from the methyl-end that are essential for health and must be obtained from a diet [9]. Leaves of wild edible plants used for culinary purposes produce low oil yields, but it has been indicated that lipids from the leaves of *C.maritimum* are a rich source of essential fatty acids such as linoleic acid (LA, C18:2 ω3) and alfa-linolenic acid (ALA C18:3 ω3) [12]. Many scientific studies have documented importance of essential fatty acids in many biochemical pathways, leading to cardioprotective effects due to their significant antiatherogenic, antithrombotic, anti-inflammatory, antiarrhythmic, hypolipidemic effects, due to the potential to reduce the risk of serious diseases, especially cardiovascular diseases, cancer, osteoporosis, diabetes and other health promotion activities [11]. Increased dietary intake of LA leads to oxidation of LDL, platelet aggregation, and interference with the incorporation of EPA and DHA into cell membrane phospholipids [25].

For instance, according to Liu [14], lipids in Antarctic krill are among the richest sources of n-3 polyunsaturated fatty acids (PUFAs), which contain abundant eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Numerous studies

have shown that n-3 PUFAs have positive effects on improving physical health and reducing the risk of diseases. For example, they could reduce the risk factors for cardiovascular diseases, decreasing inflammatory diseases, improving immune function, and decreasing tumor cell growth and survival [6–8]. Therefore, krill oil can be considered a potential source of lipids with unique biological efficacy [14]. Krill oil production has recently become one of the most attractive activities in the food industry. As a novel food ingredient, krill oil has great potential in food, nutraceutical and pharmaceutical applications due to its diverse health benefits. Krill oil products are commonly available as dietary supplements in the form of soft gels, capsules, gummies, and tablets popular in the European and American health markets [13]. Furthermore, more research is required in this area because there is a growing market need for sustainable sources of polyunsaturated fatty acids, specifically omega-3 and omega-6. In this case, the focus of the research is on sea fennel as a source of these essential fatty acids.

Sea fennel is a promising vegetable crop due to its composition. Moreover, limited information is available regarding fatty acid composition particularly in aerial parts of (leaves and flowers) sea fennel, and several recent studies have reported substantial differences in fatty acid composition between wild and cultivated plant of the same species. Therefore, the presence of this fatty acid in edible plant tissues will be of great importance in human nutrition [12].

1.5. Fatty acids health claims in the Regulation 1924/2006

Within the European Union Permitted health claims the list of health claims which may be made on foods, is set out in Annex to the Regulation (EC) No 1924/2006, and contain following nutrition and health claims related to unsaturated fatty acids:

1. “Replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels [MUFA and PUFA are unsaturated fats].”

2. “Essential fatty acids are needed for normal growth and development of children. Information to the consumer that the beneficial effect is obtained with a daily intake of 2 g of α-linolenic acid (ALA) and a daily intake of 10 g of linoleic acid (LA).”

3. “ALA contributes to the maintenance of normal blood cholesterol levels. The claim may be used only for food which is at least a source of ALA as referred to in the claim SOURCE OF OMEGA 3 FATTY ACIDS as listed in the Annex to Regulation (EC) No 1924/2006. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 2 g of ALA.”

4. “Linoleic acid contributes to the maintenance of normal blood cholesterol levels. The claim may be used only for a food which provides at least 1,5 g of linoleic acid (LA) per 100 g and per 100 kcal. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 10 g of LA.”

5. “SOURCE OF OMEGA-3 FATTY ACIDS” A claim that a food is a source of omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0,3 g alpha-linolenic acid per 100g and per 100kcal, or at least 40mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100g and per 100kcal.”

6. “HIGH OMEGA-3 FATTY ACIDS” A claim that a food is high in omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0,6 g alpha-linolenic acid per 100 g and per 100 kcal, or at least 80 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal.”

7. “HIGH POLYUNSATURATED FAT” A claim that a food is high in polyunsaturated fat, and any claim likely to have the same meaning for the consumer, may only be made where at least 45% of the fatty acids present in the product derive from polyunsaturated fat under the condition that polyunsaturated fat provides more than 20% of energy of the product.

An important determinant in decreasing the risk of coronary heart disease, both in primary and secondary prevention, is the balance of omega-6/omega-3 fatty acids. Diets with a high omega-6/omega-3 ratio may increase the risk of depression and inflammatory diseases [25]. A higher intake of ALA with a ratio of 5/1 for LA/ALA could possibly constitute a nutritional answer to the main cause of morbidity and mortality due to cardiovascular diseases in industrialized countries [26].

1.6. Valorization of *C. maritimum* through encapsulation

A part fatty acids composition, sea fennel contains also other bioactive compounds such as essential oils and polyphenols. The bioactive pool of sea fennel can also deliver several functional (e.g., anti-inflammatory, antioxidant, and antiatherogenic) properties as a nutraceutical or as a functional food ingredient. To date, the addition of sea fennel extracts to foods as powders or encapsulated forms with liposomes has been explored, however alginate-based encapsulation has not been yet tested on sea fennel extracts. Sea fennel polar extracts are now being studied for their beneficial effect in the Seafennel4Med research activities, but one the antioxidant and microbial effect will be validated, and encapsulation step would be needed to stabilize the extracts and protect them from oxidation and degradation [4]. Encapsulation is an alternative to physical or chemical instability of compounds [22]. Encapsulation is a process in which a core material is coated in a wall material that protects it by creating capsules that are effective against chemical and environmental interactions, allowing the controlled release of active matter in a certain environment [23]. In this process, many polymers such as chitosan, gums, maltodextrin, pectin, starch, whey protein, sodium alginate, cellulose, carboxymethylcellulose, zein, pullulan, galactomannan, and sodium caseinate are used as wall materials to protect the core, which is generally formed by bioactive compounds [22]. Significant attention in the field of material science is focused on alginate-based materials, which are natural polymers [24]. Alginate is linear naturally occurring polysaccharide, extracted especially from brown marine algae [21], consisted of D-mannuronic (M) and L-guluronic (G) acids [19]. Because of their highly porous and hydrated structure, alginate gels are appealing for use in a range of biological applications, such as wound healing, tissue engineering, and drug delivery [24]. This anionic polymer is extensively investigated and used for many biomedical applications, as it is Generally Recognized As Safe (GRAS), nontoxic, nonantigenic, satisfactorily biocompatible, sufficiently biodegradable [21], and due to its biocompatibility and relatively low cost [20]. The capacity of alginate to form gels in the presence of divalent cations like Ca2+ and Ba2+ is one of its special qualities [24]. By using the ionotropic gelation process, which involves dropping alginate and the material to be encapsulated into a multivalent ion solution, beads can be prepared using alginate's capacity to form gel in the presence of multivalent ions. When droplets come into contact with multivalent ions, gel spheres with material

evenly distributed throughout the crosslinked alginate matrix are instantly formed (Fig.7).

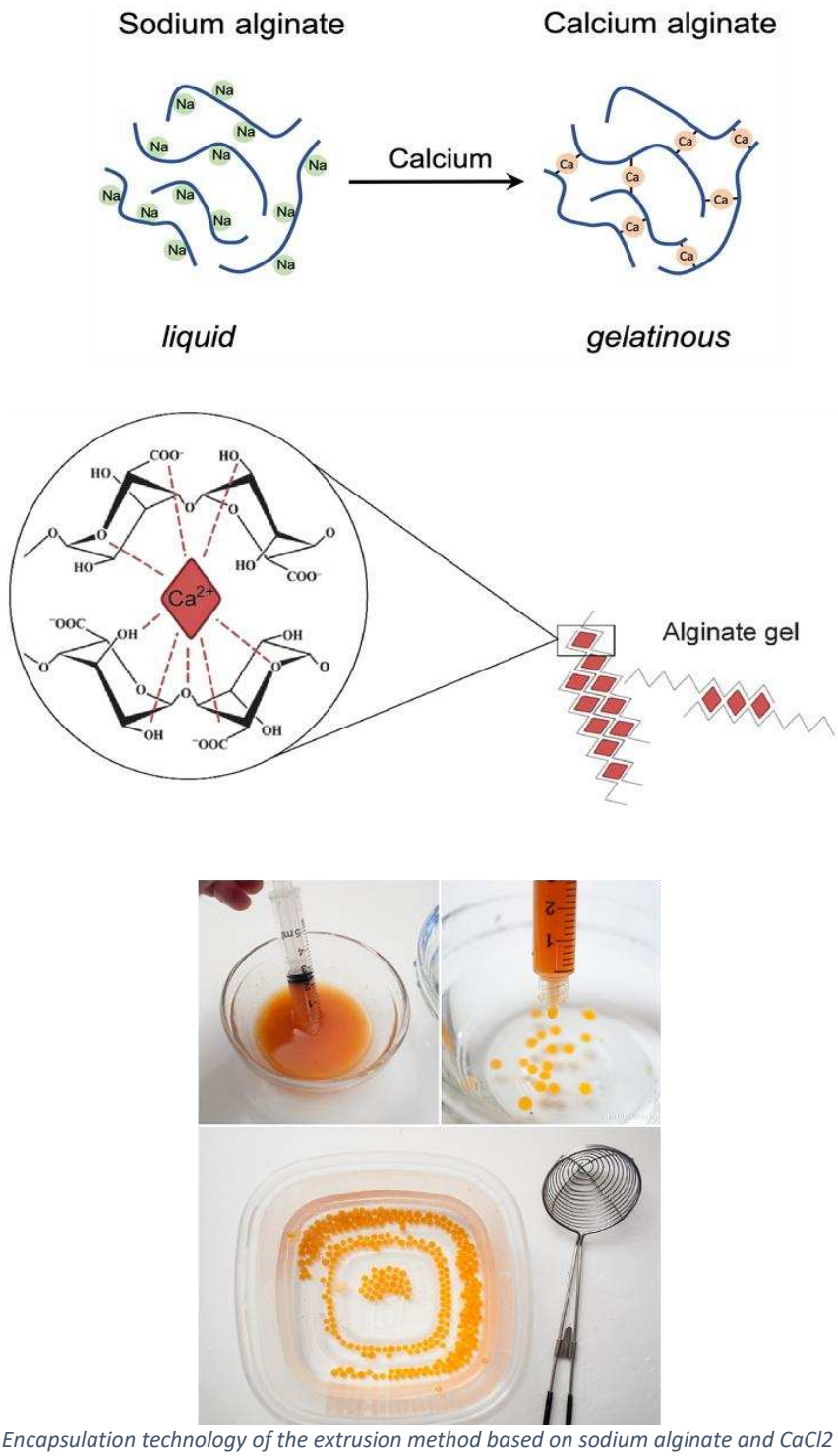


Figure 7. Encapsulation technology of the extrusion method based on sodium alginate and CaCl2

The diameter of the nozzle and the viscosity of the polymer dispersion have an impact on the size of the droplet of polymer dispersion, which determines the size of the wet beads [19].

2. AIM OF THE THESIS

Within this framework, the first aim of this study is to characterize and compare the fatty acid composition between leaves, flowers, seeds and by-product of wild and cultivated *C.maritimum*. Sea fennel is a promising vegetable ingredient and is considered a good source of essential fatty acids, which play crucial roles in human metabolism, health, and disease. Moreover, limited information is available regarding fatty acid composition particularly in aerial parts of (leaves and flowers) of sea fennel. Moreover, to valorize sea fennel in the commercial framework, the meeting criteria to nutritional or health claims conditions regarding omega-3 and omega-6 were verified. The second goal of this thesis is was to test the encapsulation of sea fennel water and ethanol extract using sodium alginate on a lab scale to explore the possibility of a new method of encapsulation, not yet tested on such plant extract, for the research and development of new sea fennel-based functional foods.

3. MATERIALS AND METHODS

3.1. Sampling of wild and cultivated C.maritimum populations

3.1.1. Cultivated C. maritimum leaves

A sample of organic C. maritimum crop produced in an open field in the Marche region (Central Italy) was collected to compare wild (WT) and cultivated (C) C.maritimum populations in terms of fatty acid profile. Leaves from organic sea fennel crop were manually harvested, air-dried in a De Cloet Dryer at <40 ◦C till reaching RH% <15, milled to obtain a powder, and stored at 20 ◦C (Fig.8).

Figure 8. Cultivated C. maritimum leaves

3.1.2. By-product of cultivated C. maritimum

By-product (BP) of the Italian sea fennel crop produced in open field in the Marche region (Central Italy), consisting of woody stems, old leaves, and flowers (Fig.3, Fig.9) was manually harvested from the field and air-dried in a De Cloet Dryer at T<40°C till reaching RH%<15.

Figure 9. C.maritimum by-product

3.1.3. Wild C.maritimum populations

Leaves (L; n =8) and flowers (F; n =7) (Fig. 10) were collected at the plant flowering period in August/September 2023 from wild sea fennel populations growing spontaneously in Italian coastal areas: Calabria (CAL); Marche, Conero Regional Park (CON); Marche, Porto Potenza Picena (MAR); Apulia (APU); Sardinia (SAR); Sicily (SIC); Tuscany (TUS) and COR (Corsica). C. maritimum plant species were identified by the botanists of the Department of Agricultural, Food, and Environmental Science, Università Politecnica delle Marche, using morphological traits and geographical distribution of C. maritimum. About 500 g of fresh tender leaves were sampled by mixing leaves/sprouts from different individuals of the same population; about 500 g of flowers (umbels) from each population were also sampled. Samples of leaves and flowers were stored separately in sterile plastic bags, transported to the laboratory under refrigerated conditions, and kept at − 20 ◦C till the freeze-drying stabilization for long-term storage until further analysis.

Figure 10. Leaves, flowers and freeze-dried powder of wild C.maritimum populations

3.1.4. Seeds of wild Sicilian C. maritimum population

Fruits of C. maritimum (Fig. 11) were collected during the ripening period in October 2023 from wild sea fennel populations (n =1) growing spontaneously in Sicilian coastal areas. About 1000 fruits were sampled by mixing leaves/sprouts from different individuals of the same population; stored in sterile plastic bags, transported to the laboratory under refrigerated conditions, and kept at room temperature till the mechanical extraction of seeds (Fig. 11). Each fruit contains a single dry seed from 4 to 10 mm long [29]. Seeds were ground manually using a mortar and pestle, just before the Soxhlet oil extraction.

Figure 11. Fruits (left) and seeds (right) of C. maritimum (Renna, 2018)

3.2. Oil extraction

The oil extraction of *C. maritimum* vegetable material was carried out using a Soxhlet extractor (Fig. 12). An aliquot of 20 g of *C. maritimum* powders (leaves, flowers and by-product) or 5 g in the case of seed sample was extracted with 100 mL of n-hexane for 5 h. The extract was then dried over anhydrous sodium sulphate and n-hexane was evaporated by means of a vacuum rotary evaporator for further UHPLC analysis [30].

Figure 12. Buchi B-811 Extraction System, Soxhlet

3.3. Fatty acids composition determination

Fatty acid methyl esters (FAME) were obtained from total lipids through alkaline methylation. Briefly, 20 mg of oil were added of n-hexane (0.5 mL), KOH 2M in methanol solution (0.5 mL) and vortexed. An aliquot (100 µl) of the organic phase was diluted 10 times and then inject in the capillary gas chromatographer. Samples were analyzed in triplicate.

The qualitative analysis of FAMEs (weight% of total fatty acids) was performed by means of gas chromatography using Thermo Scientific TRACE 1300 apparatus (Massachusetts, USA) equipped with a flame ionization detector set at 270°C (FID) and an RT-2560 fused silica capillary column (100 m 0.25 mm i.d., film thickness 0.2 µm; Restek, USA). The carrier gas was helium at a flow rate of 1.6 mL/min. The oven temperature program was: 5 min at 140°C, raised to 240°C at a rate of 4°C/min, then held for 15 min. The injector temperature was 250°C. The identification was performed using F.A.M.E mix C4-C24 (Sigma-Aldrich, St. Louis, USA). The processing software of data was Chromeleon 7.

Experimental results of the performed analysis were processed by the MetaboAnalyst 5.0 online platform. A normalized and scaled dataset was used for total visualization. Principle component analysis (PCA), heatmaps, and variable importance in projection (VIP) were applied to monitor the variations in the fatty acids profile of leaves, flowers, by-product and seeds of SF.

3.4. Preparation of aqueous and ethanolic extracts from sea fennel by-product

For the water extract, an aliquot (0.5 g) of dried powder from sea fennel by-product was extracted with 10 mL of deionized water, following the ratio 1:20 w/v, as reported by Alemán et al. (2019), at room temperature in dark conditions, under agitation for 18 h, centrifuged (3500 rpm, 5 min), and filtered on regenerated cellulose filter (0.45 µm) and kept at -20 °C for further analysis (Fig.13, Table 2).

For the ethanolic extract, an aliquot (0.5 g) of dried powder from sea fennel by-product was extracted with 10 mL of ethanol/water solution (80:20) following the ratio 1:20 w/v, at room temperature in dark conditions, with ultrasound assisted extraction for 30 min, centrifuged (3500 rpm, 5 min), and filtered on regenerated cellulose filter (0.45 µm). Ethanol was evaporated with vacuum rotavapor (40°C), while water was evaporated with freeze-drying process. The extract was kept at -20 °C before further analysis (Fig.13, Table 2).

Table 2. Preparation of by-product polar extracts: BP-W (by-product water extract) and BP-E (by-product ethanolic extract)

	BP-W	BP-E
Solvent	distilled water	ethanol 80: distilled water 20
Ratio	1:20	1:20
T	room	room
Time	18 h	30 min
Assist extraction	agitation	ultrasound
Drying	freeze-drying	rotavapor 40°C + freeze-drying

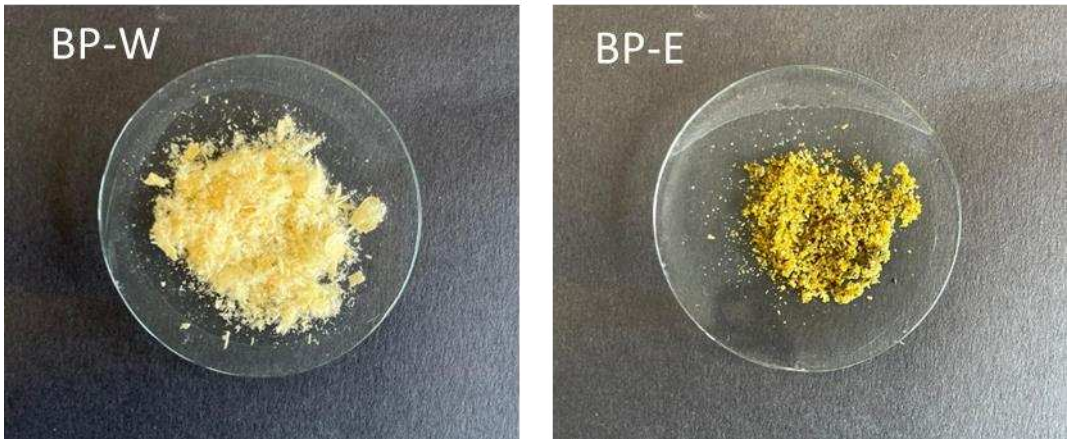


Figure 13. BP-W (by-product water extract) and BP-E (by-product ethanolic extract)

3.5. Alginate-based capsules

Alginate-based capsules were produced by the dropping method following the ionotropic gelation technique (Fig.14). Briefly, encapsulant solutions, alginate (1,34 and 2% w/v) in combination with pectin (0.66 and 1% w/v), enriched with 1,34 and 2% (w/v) of BP-W or BP-E, were prepared using an Ultra-Turax (Ika- Werke GmbH & Co, Germany) until complete dissolution and stored 8 hours at 4°C to allow both hydration and deaeration [31]. Preliminary formulations were experimented before selecting 4 formulations (Table 3) to allow the formation of capsules. A chloride calcium salt solution was prepared at 0,05M in water. Alginate-based capsules were produced by dropping the enriched alginate solution in the calcium chloride bath using a manual syringe (plastic, 5 mL). Capsules were left in the calcium bath for 5 min to allow complete gelation, then a sieve was used to recover them. A washing step with mineral water was applied to capsules to remove the bitter taste of calcium chloride. Capsules were moved to jars and stored under refrigeration before the physicochemical analysis.

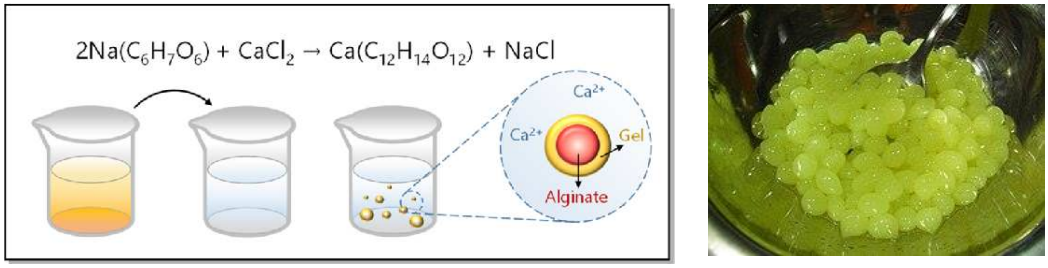


Figure 14. Alginate-base gelation technique

Table 3. Formulation of alginate-based capsules

Samples	W50	W75	E50	E75
Water	50 mL	75 mL	50 mL	75 mL
Sodium Alginate	1 g	1 g	1 g	1 g
Extract	1 g	2 g	3 g	4 g
Pectin	0,5 g	0,5 g	0,5 g	0,5 g
Sodium Alginate %	2%	1,34%	2%	1,34%
Extract %	2%	1,34%	2%	1,34%
Pectin %	1%	0,66%	1%	0,66%

3.6. Physicochemical parameters of alginate-based capsules

3.6.1 pH and aw

The pH of the samples was determined using a pH meter equipped with a HI2031 solid electrode (Hanna Instruments, Padova, Italy). aw was measured with the instrument AW LabMaster.

3.6.2 Texture parameters

The texture properties of the capsules were measured with a Texture Analyzer (model CT3-4500, Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) using a 36 mm diameter cylindrical probe (mod. TA-AACC36). A 2.0 g load cell was used. The probe compressed the the sample till 2 mm compression depth at a speed of 15 mm s−1. The measurements were performed at room temperature in the middle of the container with capsules (10 mm thickness) (Fig.15). Three independent measurements were carried out, for each sample.

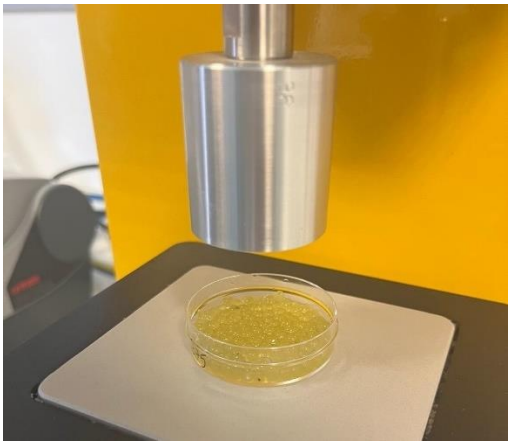


Figure 15. Hardness measurement

3.6.3 Color

The color measurements (Fig.16) were performed using a Chroma Meter CR-200 (Minolta,Osaka, Japan) with a D65 illuminant. Color parameters were determined according to the CIE L*a*b* system (L*, brightness; a*, redness/greenness; b*, blueness/yellowness) as reported by [32].

Figure 16. Color measurement

4. RESULTS

4.1. Fatty acid profile analysis

4.1.1. PCA: effect of plant part and geographic origin

Limited information is available regarding fatty acid composition, particularly in the aerial parts (leaves and flowers) of sea fennel, and several recent studies have reported substantial differences in fatty acid composition between wild and cultivated plants of the same species. Therefore, analyzing and comparing the different plant parts of sea fennel makes it possible to study this halophyte more closely and see the whole picture. Leaves (L; n =8) and flowers (F; n =7) were collected at the plant flowering period in August/September 2023 from wild sea fennel populations (n =8) growing spontaneously in Italian coastal areas: Calabria (CAL); Marche, Conero Regional Park (CON); Marche, Porto Potenza Picena (MAR); Apulia (APU); Sardinia (SAR); Sicily (SIC); Tuscany (TUS); plus one French population, COR (Corsica).

Based on the location of the clusters in the Scores plot in Figure 17, it is clear that the different vegetable parts (leaves, flowers, seeds and by-products) of the sea fennel are far from each other in terms of fat composition. It should also be noted that the site of collection, different Italian regions, of the sea fennel samples did not play a significant role in the arrangement of the clusters, only the effect of plant part outstands. Interestingly seeds and flowers from Corsica site are very close in their quadrant of the score plot. The similarity in

fatty acid composition between seeds and flowers is because the collection of flower occurred in October during the ripening of fruits, thus their FA composition is more similar to a seed.

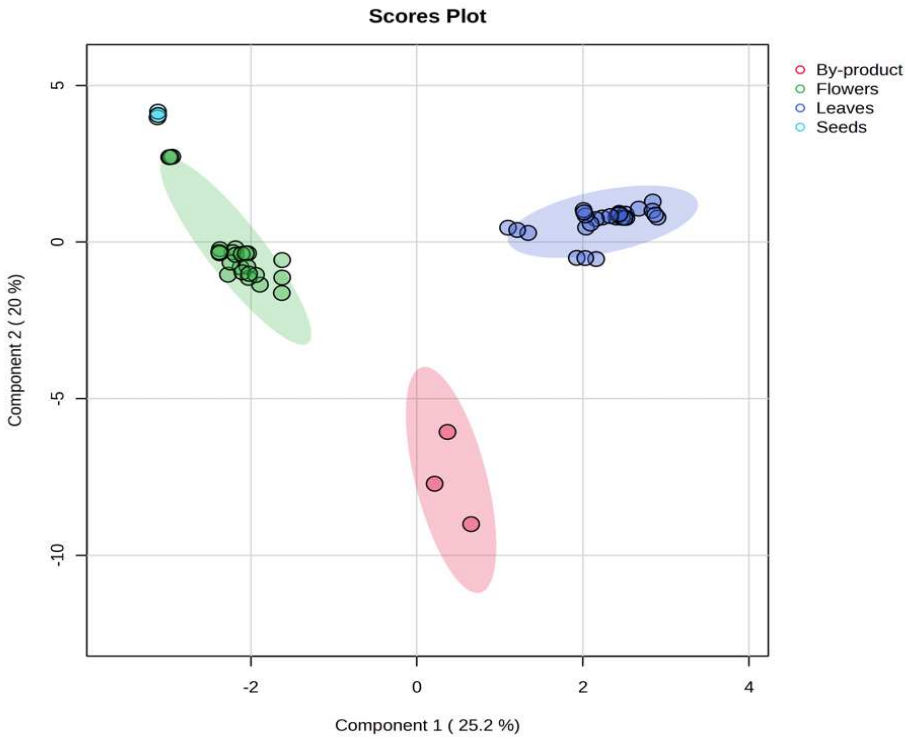


Figure 17. Scores plot of the principal component analysis (PCA) of sea fennel by-product, flowers, leaves and seeds

The fatty acid composition of each part of sea fennel can be described generally as follows (Fig. 18): polyunsaturated fatty acids predominate in the flowers, particularly omega 6, while high levels of omega 3 are present in the leaves. The by-product contains medium levels of omega 3 and omega 6 and a high level of saturated fatty acids. The seeds, in turn, show a high content of monounsaturated fatty acids, while in flowers this indicator is average, and in leaves and by-products it is at a low level.

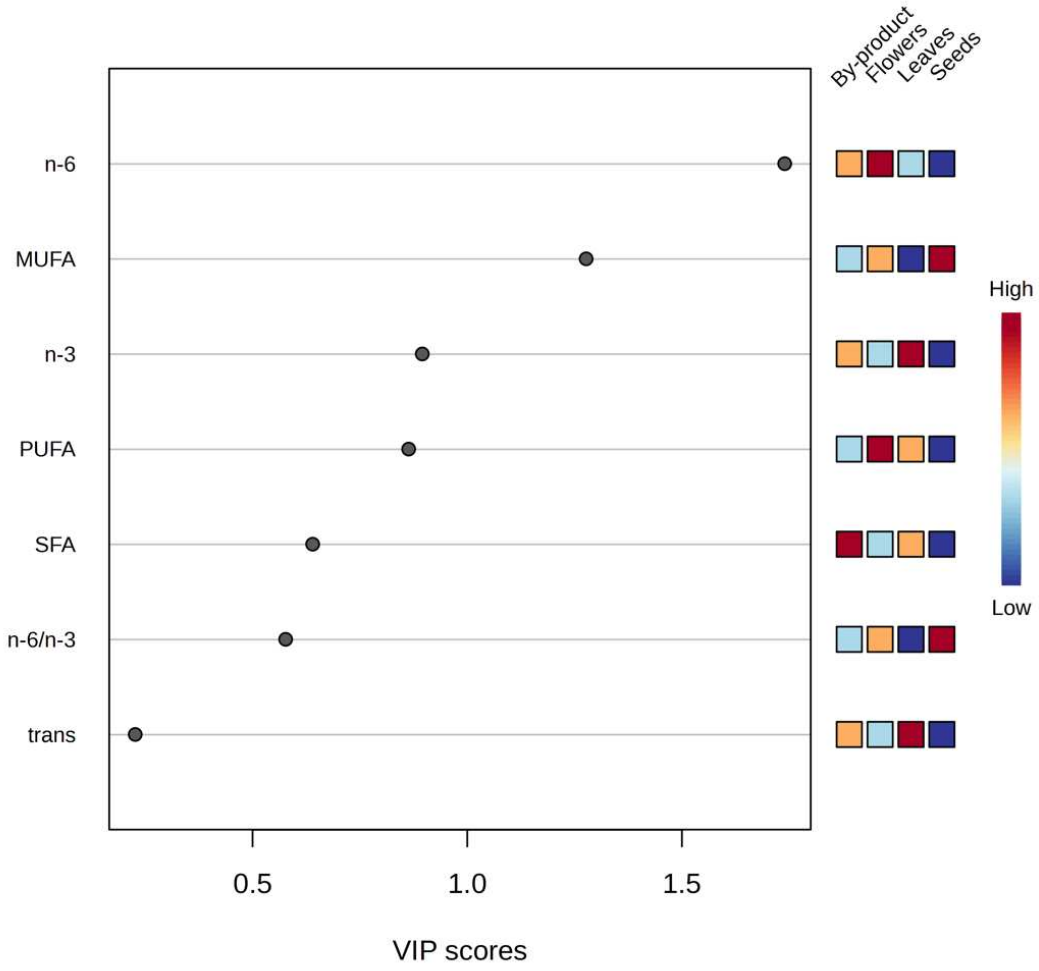


Figure 18. VIP scores of Sea fennel by-product, flowers, leaves and seeds

Figure 19 shows the difference in fatty acid content between wild and cultivated sea fennel populations. RIN-L-FD and RIN-L-AD samples were cultivated in the Rinci field, air (AD) and freeze dried (FD), while other samples are wild populations. Samples have significant differences in levels of saturated, monounsaturated and polyunsaturated fatty acids, which may indicate different growing, processing and storage conditions, as well as differences between leaves, flowers and plant by-products. SFA (saturated fatty acids) values vary from 9.83% to 44.70%, the lowest value is for sample SAR-S, the highest is for BP. MUFA (monounsaturated fatty acids) values vary from 2.30% to 77.17%, the lowest value is for sample RIN-L-FD, the highest is for SAR-S. PUFA (polyunsaturated fatty acids) values vary from 12.95% to 69.81%, the lowest value is for the SAR-S sample, the highest is for the RIN-L-FD. The values of trans fats range from 0.04% to 8.80%, the lowest value is for sample SAR-S, the highest is for RIN-L-FD. n-6 (omega-6) values vary from 12.57% to 49.54%, the lowest value is for the SAR-S

sample, the highest for the MAR-F sample. n-3 (omega-3) values vary from 0.35% to 31.92%, the lowest value is for sample SAR-S, the highest is for RIN-L-AD. Ratio of omega-6 to omega-3 vary from 1.18 to 36.39, the lowest value is for sample RIN-L-AD, the highest is for SAR-S. The highest content of polyunsaturated fatty acids, including omega-3, among the selected samples was shown by the sample RIN-L-FD. While the sample MAR-F showed the next highest result in polyunsaturated fatty acid content and the closest value to the recommended ratio of omega-6 to omega-3.

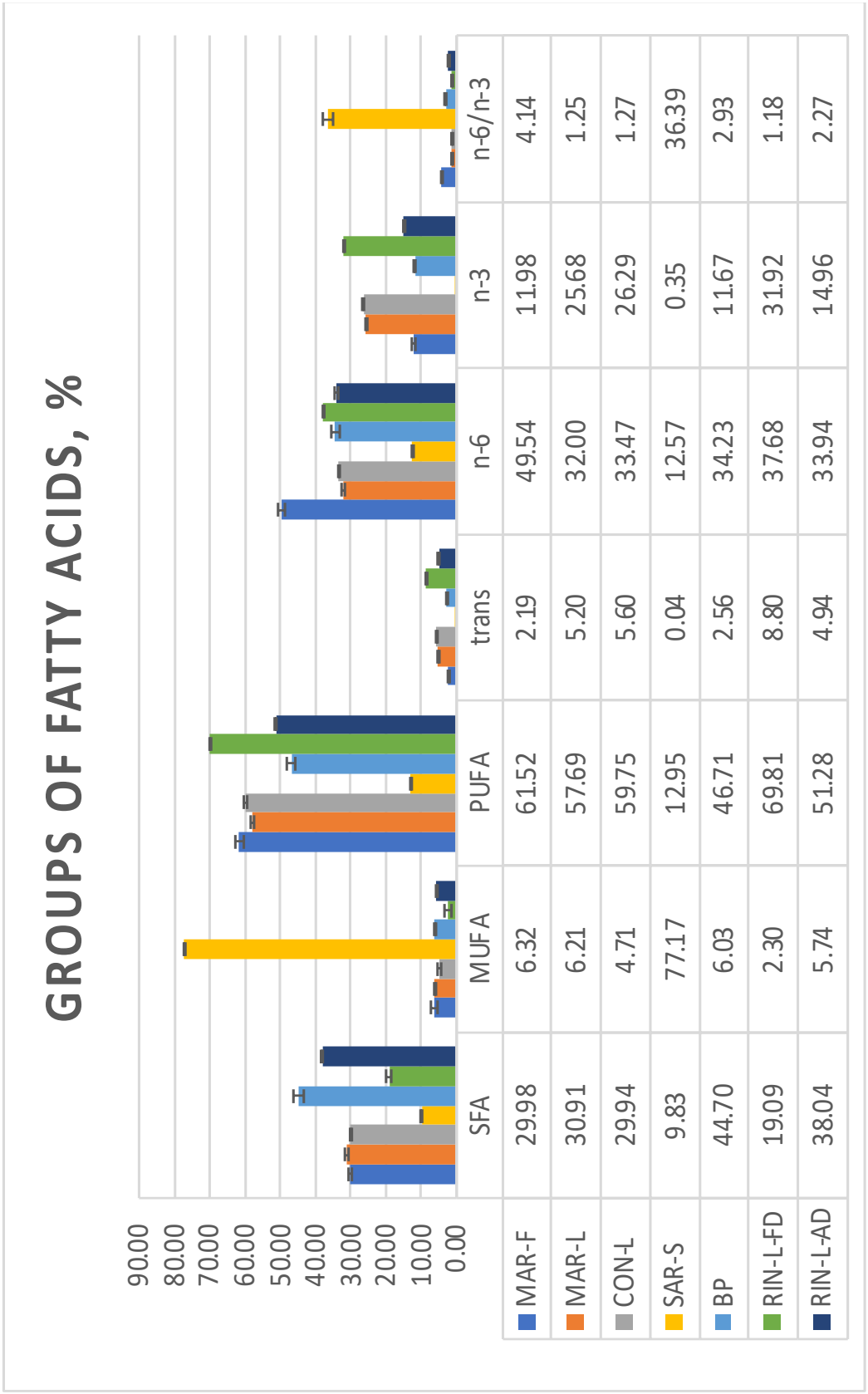


Figure 19. Levels of fatty acids groups and types in wild and cultivated sea fennel leaves and flowers. Data are presented as means of three replicas ± standard deviation

4.1.2. Fatty acid composition of SF flowers

Table 4. Fatty acid composition of sea fennel flowers from different Italian regions. The fatty acids contents are expressed as weight percentages of total FAME. Data are presented as means of three replicas ± standard deviation

FAs, %	LIG-F			TOS-F			COR-F			MAR-F			CAL-F			SIC-F			SAR-F			PUG-F		
C4:0	0.17	±	0.18	1.04	±	0.11	0.12	±	0.08	0.65	±	0.05	0.15	±	0.01	4.05	±	0.01	0.58	±	0.12	0.57	±	0.02
C6:0	2.52	±	1.90	0.44	±	0.00	0.38	±	0.26	1.69	±	0.75	0.70	±	0.05	1.50	±	0.20	0.00	±	0.00	1.48	±	1.17
C10:0	0.04	±	0.04	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.16	±	0.00	1.07	±	0.47	0.18	±	0.00	0.00	±	0.00
C11:0	0.19	±	0.17	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.77	±	0.02	0.66	±	0.07	0.00	±	0.00
C12:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.11	±	0.01	0.15	±	0.00	0.00	±	0.00	0.00	±	0.00
C13:0	0.07	±	0.07	0.00	±	0.00	0.00	±	0.00	0.15	±	0.05	0.00	±	0.00	0.14	±	0.04	0.00	±	0.00	0.00	±	0.00
C14:0	0.43	±	0.15	0.45	±	0.05	0.28	±	0.01	0.50	±	0.04	0.35	±	0.00	0.40	±	0.02	0.28	±	0.01	0.33	±	0.01
C15:0	0.24	±	0.03	0.00	±	0.00	0.00	±	0.00	0.23	±	0.02	0.21	±	0.00	0.22	±	0.01	0.24	±	0.02	0.20	±	0.00
C16:0	18.51	±	0.55	19.59	±	0.56	9.65	±	0.07	19.13	±	0.06	19.38	±	0.09	16.76	±	0.17	18.67	±	0.28	17.81	±	0.36
C16:1	0.15	±	0.06	0.00	±	0.00	0.00	±	0.00	0.29	±	0.01	0.30	±	0.06	0.18	±	0.02	0.19	±	0.06	0.29	±	0.05
C17:0	0.22	±	0.05	0.00	±	0.00	0.00	±	0.00	0.27	±	0.02	0.22	±	0.02	0.22	±	0.01	0.29	±	0.03	0.22	±	0.01
C17:1	0.22	±	0.03	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.24	±	0.00	0.30	±	0.01	0.28	±	0.03	0.14	±	0.01
C18:0	3.54	±	0.22	3.31	±	0.00	1.96	±	0.03	3.29	±	0.08	3.34	±	0.03	3.73	±	0.06	3.77	±	0.07	3.33	±	0.00
C18:1n9T	1.23	±	0.06	2.08	±	0.09	0.63	±	0.01	1.91	±	0.02	0.85	±	0.01	0.83	±	0.02	1.65	±	0.00	1.41	±	0.04
C18:1c	6.24	±	0.23	5.21	±	0.34	56.15	±	0.37	5.14	±	0.05	7.86	±	0.02	6.14	±	0.23	4.87	±	0.12	6.11	±	0.38
C18:2 t9,12	0.08	±	0.08	0.00	±	0.00	0.00	±	0.00	0.28	±	0.06	0.14	±	0.01	0.00	±	0.00	0.12	±	0.12	0.32	±	0.02
LA	50.78	±	0.39	50.87	±	0.36	24.24	±	0.16	48.43	±	0.70	52.91	±	0.03	48.25	±	0.19	50.23	±	0.42	51.65	±	0.81
C20:0	0.76	±	0.19	0.70	±	0.02	0.37	±	0.02	0.59	±	0.12	0.62	±	0.07	0.81	±	0.02	0.65	±	0.09	0.64	±	0.10
ALA	9.90	±	0.24	11.89	±	0.10	4.60	±	0.16	11.98	±	0.37	9.04	±	0.05	8.92	±	0.06	12.31	±	0.08	10.70	±	0.13
C20:1 c	0.18	±	0.13	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.26	±	0.01	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C18:3 c6,9,12	1.15	±	0.34	1.52	±	0.25	0.18	±	0.01	1.11	±	0.09	0.77	±	0.01	0.75	±	0.01	1.13	±	0.03	1.44	±	0.00
C21:0	0.19	±	0.06	0.00	±	0.00	0.15	±	0.03	0.29	±	0.03	0.19	±	0.01	0.63	±	0.01	0.57	±	0.13	0.27	±	0.01
C20:2 c	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C22:0	1.09	±	0.20	1.14	±	0.22	0.42	±	0.02	1.11	±	0.22	0.95	±	0.01	0.96	±	0.00	0.92	±	0.07	1.14	±	0.04
C20:3 c8,11,14	0.15	±	0.13	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.26	±	0.04	0.21	±	0.01	0.25	±	0.00	0.00	±	0.00
C22:1 c	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C22:2 c	0.00	±	0.00	0.00	±	0.00	0.24	±	0.05	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C24:0	1.56	±	0.38	1.76	±	0.17	0.64	±	0.01	2.08	±	0.17	0.95	±	0.03	1.40	±	0.00	1.13	±	0.05	1.22	±	0.02
C24:1	0.37	±	0.62	0.00	±	0.00	0.00	±	0.00	0.89	±	0.84	0.03	±	0.01	1.58	±	0.01	1.01	±	0.07	0.72	±	0.70
SFA	**29.54**	**±**	**0.74**	**28.42**	**±**	**0.30**	**13.96**	**±**	**0.34**	**29.98**	**±**	**0.35**	**27.33**	**±**	**0.01**	**32.82**	**±**	**0.49**	**27.95**	**±**	**0.09**	**27.21**	**±**	**0.69**
MUFA	**7.17**	**±**	**0.39**	**5.21**	**±**	**0.34**	**56.15**	**±**	**0.37**	**6.32**	**±**	**0.89**	**8.70**	**±**	**0.05**	**8.21**	**±**	**0.23**	**6.35**	**±**	**0.28**	**7.27**	**±**	**0.26**
PUFA	**61.98**	**±**	**0.58**	**64.29**	**±**	**0.72**	**29.26**	**±**	**0.04**	**61.52**	**±**	**1.16**	**62.97**	**±**	**0.05**	**58.14**	**±**	**0.28**	**63.93**	**±**	**0.31**	**63.79**	**±**	**0.93**
trans	**1.32**	**±**	**0.14**	**2.08**	**±**	**0.09**	**0.63**	**±**	**0.01**	**2.19**	**±**	**0.08**	**0.99**	**±**	**0.02**	**0.83**	**±**	**0.02**	**1.78**	**±**	**0.12**	**1.73**	**±**	**0.02**
n-6	**51.93**	**±**	**0.71**	**52.40**	**±**	**0.62**	**24.41**	**±**	**0.16**	**49.54**	**±**	**0.79**	**53.68**	**±**	**0.04**	**49.01**	**±**	**0.20**	**51.36**	**±**	**0.39**	**53.09**	**±**	**0.80**
n-3	**9.90**	**±**	**0.24**	**11.89**	**±**	**0.10**	**4.60**	**±**	**0.16**	**11.98**	**±**	**0.37**	**9.04**	**±**	**0.05**	**8.92**	**±**	**0.06**	**12.31**	**±**	**0.08**	**10.70**	**±**	**0.13**
n-6/n-3	**5.25**	**±**	**0.16**	**4.41**	**±**	**0.01**	**5.32**	**±**	**0.22**	**4.14**	**±**	**0.06**	**5.94**	**±**	**0.04**	**5.50**	**±**	**0.02**	**4.17**	**±**	**0.06**	**4.96**	**±**	**0.01**

Fatty acids (FA), expressed in % to flowers (F) weight of sea fennel wild populations sampled in different Italian regions and one French region. LIG = Liguria; TOS = Tuscany; COR = Corsica; MAR =Marche; CAL = Calabria; SIC = Sicily; SAR = Sardinia, PUG = Puglia.

Table 4 allows for a comparative analysis of the fatty acid profile of sea fennel from different regions, which is important for identifying regional differences in chemical composition and, possibly, plant adaptation to different environmental conditions. SFA content varies from 13.96% to 32.82%, lowest in COR-F and highest in SIC-F region. MUFA

range from 5.21% to 56.15%, lowest in the TOS-F region and highest in COR-F region. PUFA predominant in all regions, highest content in TOS-F region (64.29%) and lowest in COR-F region (29.26%).

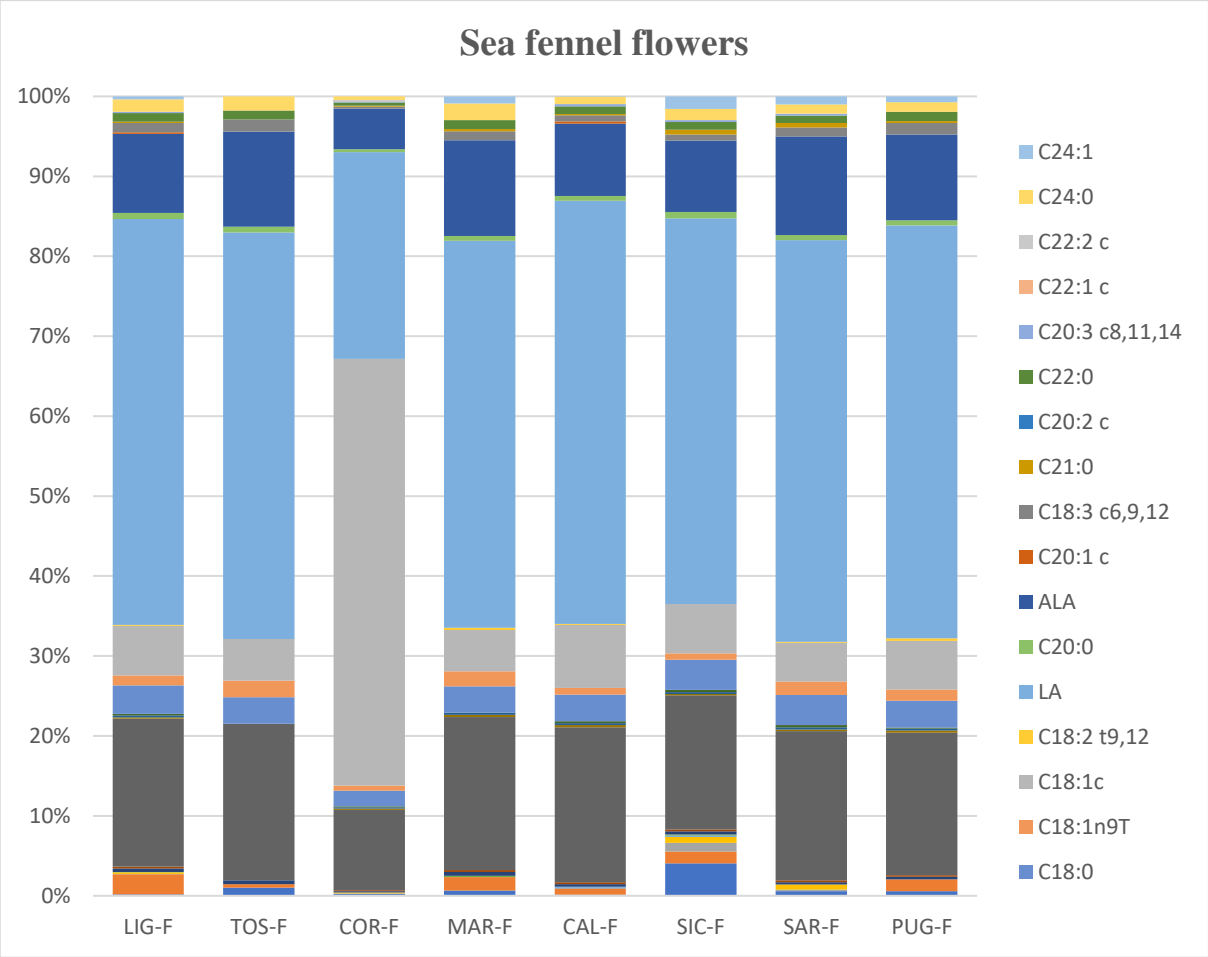


Figure 20. Fatty acid content in flowers of sea fennel from different regions

The major proportion of fatty acids in sea fennel flowers from all regions are fatty acids designated as C16:0 (palmitic acid), LA and C18:1c (oleic acid). Their content varies but remains high in most samples (Fig.20). ALA was also present in all samples and the percentage (Table 4) varies from 4.60% (COR-F) to 12.31% (SAR-F), although the amount was significantly lower compared to C16:0 and C18:1c. The samples generally have a similar percentage of fatty acids, also because in all samples the C16:0 fatty acid dominates, while the sample COR-F differs sharply from the other samples because C18:1c was the dominant fatty acid (56.15%). The oleic acid varies from 4.87% to 7.86% in from 4.87% to 7.86% in the other

samples. This graph clearly demonstrates how the ripening can influence the chemical composition of plants, in this case the fatty acid content of sea fennel flowers since the flowers of Corsica where collected during the period of the ripening of fruits while the rest of samples were collected at the flowering period.

4.1.3. Fatty acid composition of SF leaves

In the case of sea fennel leaves (Fig. 21), all samples are similar in the percentage of fatty acids and there are no distinctive cases as in the case of flowers. SFA varies from 27.69% to 35.32%, lowest in COR-L and highest in TOS-L region (Table 5). MUFA ranges from 3.31% to 11.42%, lowest in the COR-L region and highest in SIC-L region. PUFA, also as in case of flowers, are predominant in all regions, highest content in COR-L region (61.32%) and lowest in SIC-L region (54.34%). The dominant fatty acids in the leaves are palmitic acid, linoleic acid and alpha linoleic acid.

Table 5. Fatty acid composition of sea fennel leaves from different Italian regions. The fatty acids contents are expressed as weight percentages of total FAME. Data are presented as means of three replicas ± standard deviation

FAs, %	MAR-L			CAL-L			CON-L			SIC-L			PUG-L			COR-L			SAR-L			TOS-L			LIG-L		
C4:0	0.31	±	0.08	0.23	±	0.10	0.00	±	0.00	0.36	±	0.02	0.11	±	0.03	0.33	±	0.07	0.71	±	0.11	3.51	±	0.08	0.53	±	0.10
C6:0	1.22	±	0.18	1.16	±	0.75	2.58	±	0.07	0.96	±	0.38	1.53	±	0.34	1.32	±	0.30	1.29	±	0.11	2.75	±	0.59	1.42	±	0.16
C10:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.56	±	0.05	0.16	±	0.10	0.00	±	0.00	0.25	±	0.01	0.42	±	0.08	0.00	±	0.00
C11:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.28	±	0.07	0.00	±	0.00	0.20	±	0.01	0.00	±	0.00	0.52	±	0.02	0.00	±	0.00
C12:0	0.65	±	0.02	2.02	±	0.10	1.06	±	0.04	1.10	±	0.03	1.04	±	0.00	0.48	±	0.01	0.73	±	0.02	0.89	±	0.07	0.68	±	0.01
C13:0	0.00	±	0.00	0.00	±	0.00	0.35	±	0.01	0.35	±	0.02	0.14	±	0.01	0.25	±	0.07	0.21	±	0.03	0.39	±	0.12	0.14	±	0.04
C14:0	3.08	±	0.03	2.33	±	0.11	2.34	±	0.07	3.43	±	0.03	3.55	±	0.10	2.26	±	0.05	2.20	±	0.02	3.11	±	0.08	3.20	±	0.00
C15:0	0.00	±	0.00	0.21	±	0.01	0.00	±	0.00	0.24	±	0.03	0.53	±	0.43	0.18	±	0.01	0.17	±	0.01	0.21	±	0.04	0.23	±	0.02
C16:0	20.61	±	0.13	19.26	±	0.28	18.09	±	0.24	18.10	±	0.35	18.91	±	0.23	17.58	±	0.25	18.36	±	0.22	17.59	±	0.42	19.37	±	0.09
C16:1	1.70	±	0.01	1.63	±	0.05	1.32	±	0.02	0.47	±	0.01	1.05	±	0.01	0.97	±	0.03	0.99	±	0.01	0.94	±	0.22	0.95	±	0.01
C17:0	0.33	±	0.02	0.46	±	0.01	0.39	±	0.01	0.44	±	0.02	0.47	±	0.01	0.35	±	0.00	0.41	±	0.02	0.29	±	0.00	0.41	±	0.00
C17:1	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.16	±	0.01	0.06	±	0.03	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C18:0	2.96	±	0.17	3.27	±	0.19	3.24	±	0.03	2.93	±	0.11	2.78	±	0.04	2.43	±	0.03	2.70	±	0.04	3.09	±	0.06	2.73	±	0.00
C18:1n9T	5.20	±	0.04	4.15	±	0.11	5.60	±	0.20	3.23	±	0.05	5.52	±	0.09	7.68	±	0.15	6.02	±	0.13	5.94	±	0.16	5.40	±	0.00
C18:1c	4.10	±	0.13	4.59	±	0.06	2.69	±	0.60	10.79	±	0.47	4.25	±	0.03	2.06	±	0.01	4.48	±	0.13	3.29	±	0.49	4.94	±	0.23
C18:2 t9,12	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
LA	31.33	±	0.32	32.37	±	0.42	32.82	±	0.24	35.12	±	1.01	27.96	±	0.18	31.29	±	1.04	29.11	±	0.05	27.88	±	0.79	30.17	±	0.17
C20:0	0.43	±	0.05	0.40	±	0.13	0.32	±	0.07	0.93	±	0.08	0.71	±	0.15	0.70	±	0.11	0.75	±	0.02	0.68	±	0.01	1.00	±	0.02
ALA	25.68	±	0.14	26.54	±	0.25	26.29	±	0.21	18.03	±	0.41	28.96	±	0.26	29.19	±	0.32	28.76	±	0.01	25.06	±	0.49	26.27	±	0.04
C20:1 c	0.00	±	0.00	0.00	±	0.00	0.34	±	0.01	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C18:3 c6,9,12	0.68	±	0.06	0.46	±	0.02	0.64	±	0.03	1.19	±	0.00	0.70	±	0.02	0.85	±	0.02	1.06	±	0.10	1.05	±	0.03	0.87	±	0.10
C21:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.09	±	0.00	0.00	±	0.00	0.00	±	0.00
C20:2 c	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.87	±	0.01
C22:0	0.75	±	0.24	0.61	±	0.09	1.00	±	0.06	0.86	±	0.03	0.86	±	0.01	0.95	±	0.02	0.76	±	0.03	0.91	±	0.08	0.00	±	0.00
C20:3 c8,11,14	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.52	±	0.01	0.00	±	0.00
C22:1 c	0.41	±	0.03	0.00	±	0.00	0.36	±	0.07	0.00	±	0.00	0.41	±	0.02	0.28	±	0.01	0.20	±	0.00	0.00	±	0.00	0.00	±	0.00
C22:2 c	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C24:0	0.58	±	0.03	0.31	±	0.03	0.57	±	0.02	0.50	±	0.03	0.30	±	0.04	0.64	±	0.05	0.75	±	0.08	0.96	±	0.00	0.81	±	0.08
C24:1	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
SFA	**30.91**	**±**	**0.47**	**30.26**	**±**	**0.78**	**29.94**	**±**	**0.33**	**31.02**	**±**	**1.00**	**31.09**	**±**	**0.22**	**27.69**	**±**	**0.64**	**29.39**	**±**	**0.05**	**35.32**	**±**	**0.09**	**30.53**	**±**	**0.25**
MUFA	**6.21**	**±**	**0.09**	**6.22**	**±**	**0.01**	**4.71**	**±**	**0.54**	**11.42**	**±**	**0.45**	**5.77**	**±**	**0.03**	**3.31**	**±**	**0.05**	**5.67**	**±**	**0.12**	**4.23**	**±**	**0.27**	**5.89**	**±**	**0.24**
PUFA	**57.69**	**±**	**0.52**	**59.37**	**±**	**0.69**	**59.75**	**±**	**0.42**	**54.34**	**±**	**0.60**	**57.62**	**±**	**0.10**	**61.32**	**±**	**0.74**	**58.92**	**±**	**0.06**	**54.51**	**±**	**0.34**	**58.18**	**±**	**0.01**
trans	**5.20**	**±**	**0.04**	**4.15**	**±**	**0.11**	**5.60**	**±**	**0.20**	**3.23**	**±**	**0.05**	**5.52**	**±**	**0.09**	**7.68**	**±**	**0.15**	**6.02**	**±**	**0.13**	**5.94**	**±**	**0.16**	**5.40**	**±**	**0.00**
n-6	**32.00**	**±**	**0.38**	**32.83**	**±**	**0.44**	**33.47**	**±**	**0.21**	**36.31**	**±**	**1.01**	**28.67**	**±**	**0.16**	**32.14**	**±**	**1.06**	**30.16**	**±**	**0.05**	**28.93**	**±**	**0.82**	**31.04**	**±**	**0.06**
n-3	**25.68**	**±**	**0.14**	**26.54**	**±**	**0.25**	**26.29**	**±**	**0.21**	**18.03**	**±**	**0.41**	**28.96**	**±**	**0.26**	**29.19**	**±**	**0.32**	**28.76**	**±**	**0.01**	**25.06**	**±**	**0.49**	**26.27**	**±**	**0.04**
n-6/n-3	**1.25**	**±**	**0.01**	**1.24**	**±**	**0.00**	**1.27**	**±**	**0.00**	**2.02**	**±**	**0.10**	**0.99**	**±**	**0.01**	**1.10**	**±**	**0.05**	**1.05**	**±**	**0.00**	**1.16**	**±**	**0.06**	**1.18**	**±**	**0.00**

Fatty acids (FA), expressed in % to leaves (L) weight of sea fennel wild populations sampled in different Italian regions and one French region. MAR =Marche; CAL =Calabria; CON =Marche, Conero Regional Park; SIC =Sicily; PUG = Puglia; COR = Corsica; SAR = Sardinia; TOS = Tuscany; LIG = Liguria.

The minimum values of C16:0 fatty acid are recorded in the samples from COR-L region (17.58%). The minimum value of linoleic acid was found in TOS-L sample (27.88%) and of alpha linoleic acid was found in SIC-L sample (18.03%). The maximum values of these dominant fatty acids are as follows: C16:0 - MAR - L (20.61%), linoleic acid - SIC-L (35.12%), alpha linoleic acid - COR-L (29.19%) (Table 5).

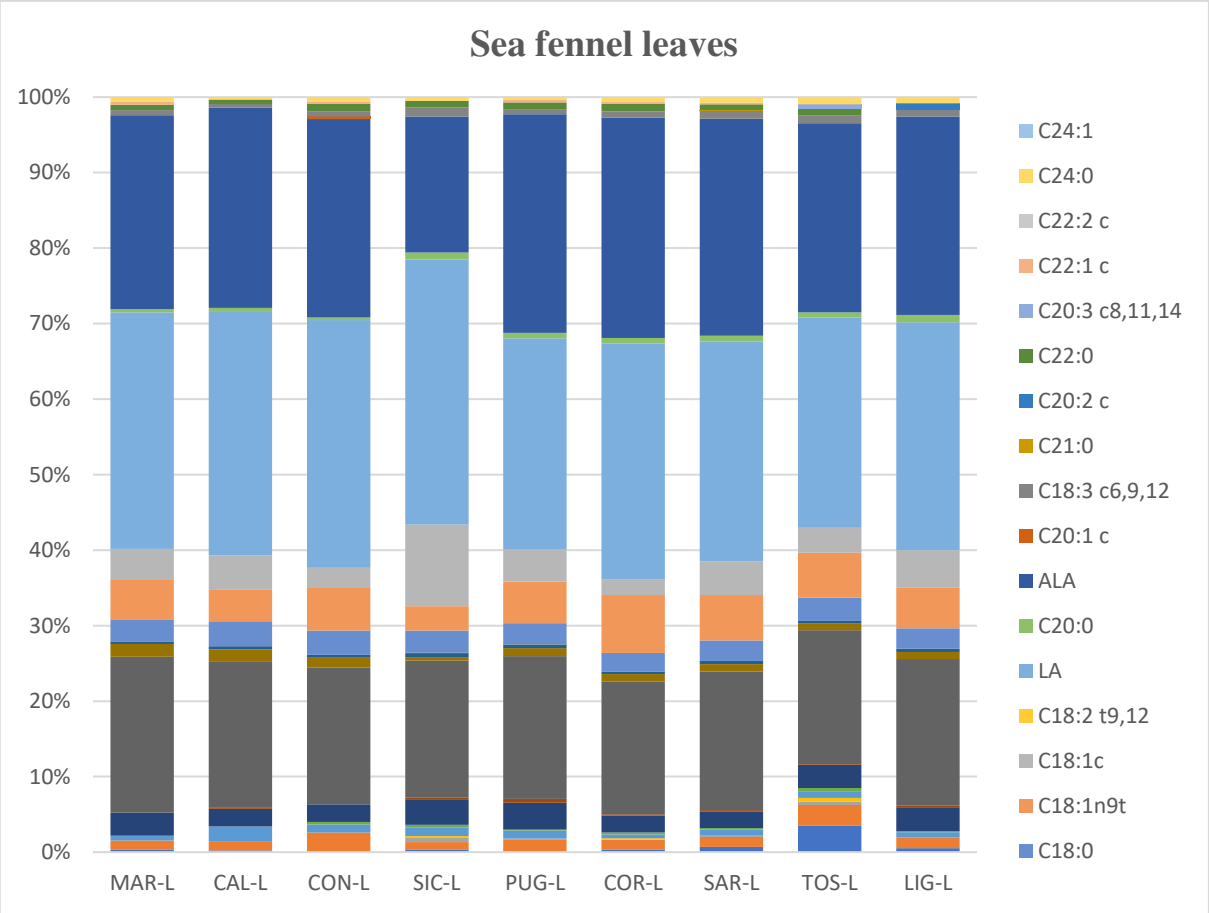
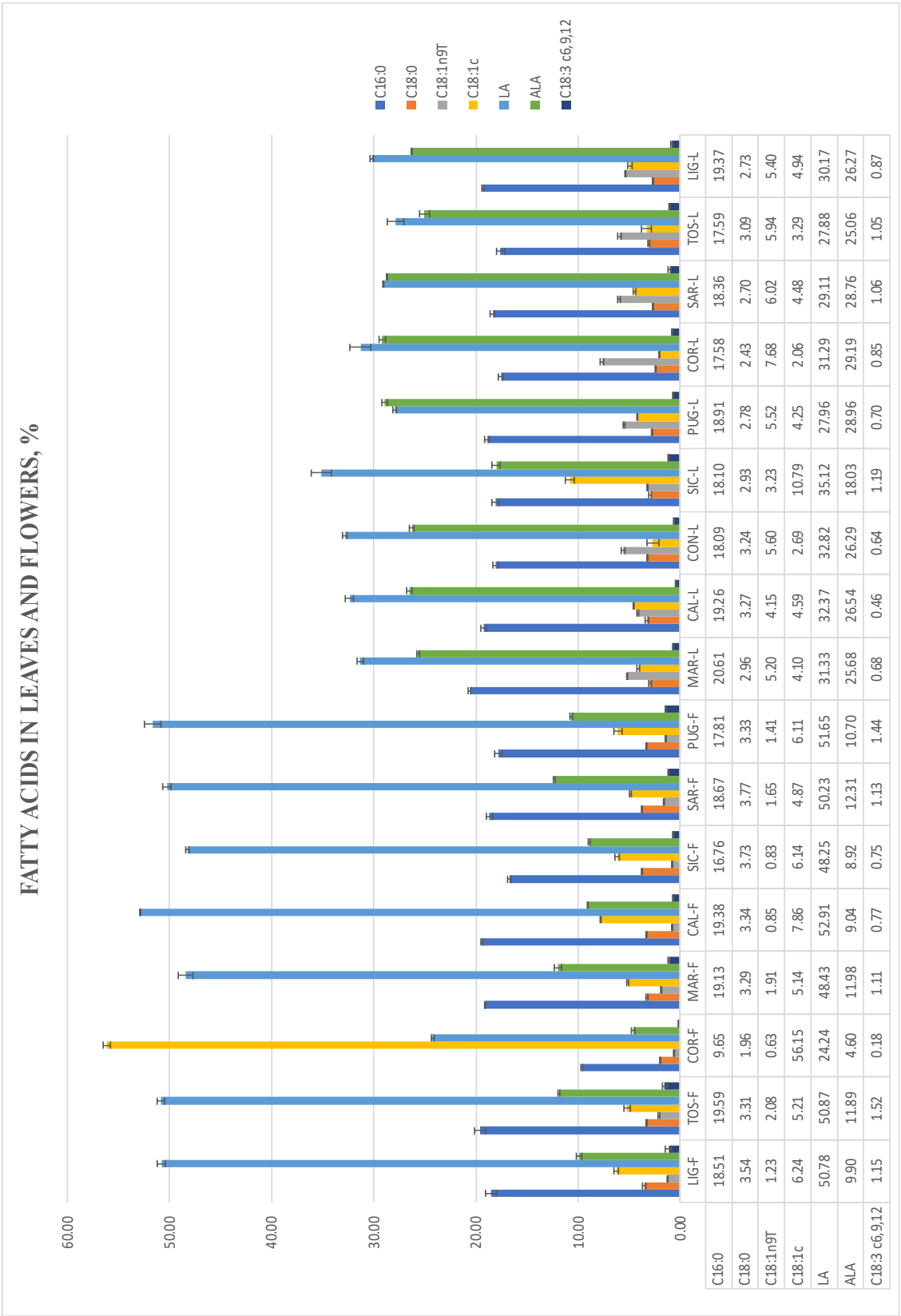


Figure 21. Fatty acid content in leaves of sea fennel from different regions

4.1.4. Comparing FA composition in leaves and flowers

For comparison, in particular, the content of LA and ALA in the leaves and flowers of sea fennel (Fig.22), it can be noted that in percentage terms of the total amount of FAME in the flowers of sea fennel, LA predominates, while in the leaves the percentage of ALA from the total amount of FAME is twice as high as in the flowers and the ratio of these two essential fatty acids is almost 1:1. The only sample that differs in the predominant content of monounsaturated fatty acid (C18:1c), instead of polyunsaturated fatty acid LA, is COR-F. The percentage content of LA and ALA in this sample is also 2-fold less in comparison with samples from other regions of sea fennel flowers.



	LIG-F	TOS-F	COR-F	MAR-F	CAL-F	SIC-F	SAR-F	PUG-F	MAR-L	CAL-L	CON-L	SIC-L	PUG-L	COR-L	SAR-L	TOS-L	LIG-L
C16:0	18.51	19.59	9.65	19.13	19.38	16.76	18.67	17.81	20.61	19.26	18.09	18.10	18.91	17.58	18.36	17.59	19.37
C18:0	3.54	3.31	1.96	3.29	3.34	3.73	3.77	3.33	2.96	3.27	3.24	2.93	2.78	2.43	2.70	3.09	2.73
C18:1n9T	1.23	2.08	0.63	1.91	0.85	0.83	1.65	1.41	5.20	4.15	5.60	3.23	5.52	7.68	6.02	5.94	5.40
C18:1c	6.24	5.21	56.15	5.14	7.86	6.14	4.87	6.11	4.10	4.59	2.69	10.79	4.25	2.06	4.48	3.29	4.94
LA	50.78	50.87	24.24	48.43	52.91	48.25	50.23	51.65	31.33	32.37	32.82	35.12	27.96	31.29	29.11	27.88	30.17
ALA	9.90	11.89	4.60	11.98	9.04	8.92	12.31	10.70	25.68	26.54	26.29	18.03	28.96	29.19	28.76	25.06	26.27
C18:3 c6,9,12	1.15	1.52	0.18	1.11	0.77	0.75	1.13	1.44	0.68	0.46	0.64	1.19	0.70	0.85	1.06	1.05	0.87

Figure 22. Main fatty acids in leaves and flowers from different Italian regions. The fatty acids contents are expressed as weight percentages of total FAME. Data are presented as means of three replicas ± standard deviation

4.1.5. Comparing FA composition in seeds and by-product

Table 6. Fatty acid composition of sea fennel seed. The fatty acids contents are expressed as weight percentages of total FAME. Data are presented as means of three replicas ± standard deviation

FAs, %	**COR-S**		
C4:0	3.60	±	0.21
C6:0	0.24	±	0.09
C10:0	0.00	±	0.00
C11:0	0.00	±	0.00
C12:0	0.00	±	0.00
C13:0	0.00	±	0.00
C14:0	0.02	±	0.00
C15:0	0.00	±	0.00
C16:0	4.61	±	0.03
C16:1	0.10	±	0.01
C17:0	0.04	±	0.00
C17:1	0.00	±	0.00
C18:0	1.14	±	0.10
C18:1n9T	0.00	±	0.00
C18:1c	77.08	±	0.11
C18:2 t9,12	0.04	±	0.01
LA	12.57	±	0.02
C20:0	0.16	±	0.07
ALA	0.35	±	0.01
C20:1 c	0.00	±	0.00
C18:3 c6,9,12	0.00	±	0.00
C21:0	0.00	±	0.00
C20:2 c	0.03	±	0.00
C22:0	0.00	±	0.00
C20:3 c8,11,14	0.00	±	0.00
C22:1 c	0.00	±	0.00
C22:2 c	0.00	±	0.00
C24:0	0.03	±	0.00
C24:1	0.00	±	0.00
SFA	**9.83**	**±**	**0.10**
MUFA	**77.17**	**±**	**0.10**
PUFA	**12.95**	**±**	**0.00**
trans	**0.04**	**±**	**0.01**
n-6	**12.57**	**±**	**0.02**
n-3	**0.35**	**±**	**0.01**
n-6/n-3	**36.39**	**±**	**1.58**

Fatty acids (FA), expressed in % to seeds (S) weight of sea fennel wild population sampled in Corsica region.

Table 7. Fatty acid composition of sea fennel by-product. The fatty acids contents are expressed as weight percentages of total FAME. Data are presented as means of three replicas ± standard deviation

FAs,%	**BP**		
C4:0	2.18	±	2.87
C6:0	1.76	±	0.71
C10:0	0.00	±	0.00
C11:0	0.00	±	0.00
C12:0	1.64	±	0.15
C13:0	0.00	±	0.00
C14:0	3.84	±	1.24
C15:0	0.26	±	0.10
C16:0	21.99	±	0.62
C16:1	0.41	±	0.36
C17:0	0.23	±	0.20
C17:1	0.00	±	0.00
C18:0	6.63	±	2.10
C18:1n9T	2.12	±	0.14
C18:1c	5.62	±	0.27
C18:2 t9,12	0.44	±	0.03
LA	30.31	±	0.79
C20:0	3.00	±	1.80
ALA	11.67	±	0.15
C20:1 c	0.00	±	0.00
C18:3 c6,9,12	3.92	±	0.43
C21:0	0.00	±	0.00
C20:2 c	0.00	±	0.00
C22:0	1.36	±	0.52
C20:3 c8,11,14	0.81	±	0.16
C22:1 c	0.00	±	0.00
C22:2 c	0.00	±	0.00
C24:0	1.82	±	0.61
C24:1	0.00	±	0.00
SFA	**44.70**	**±**	**1.35**
MUFA	**6.03**	**±**	**0.13**
PUFA	**46.71**	**±**	**1.25**
trans	**2.56**	**±**	**0.17**
n-6	**34.23**	**±**	**1.21**
n-3	**11.67**	**±**	**0.15**
n-6/n-3	**2.93**	**±**	**0.14**

Fatty acids (FA), expressed in % to by-product (BP) weight of sea fennel wild population.

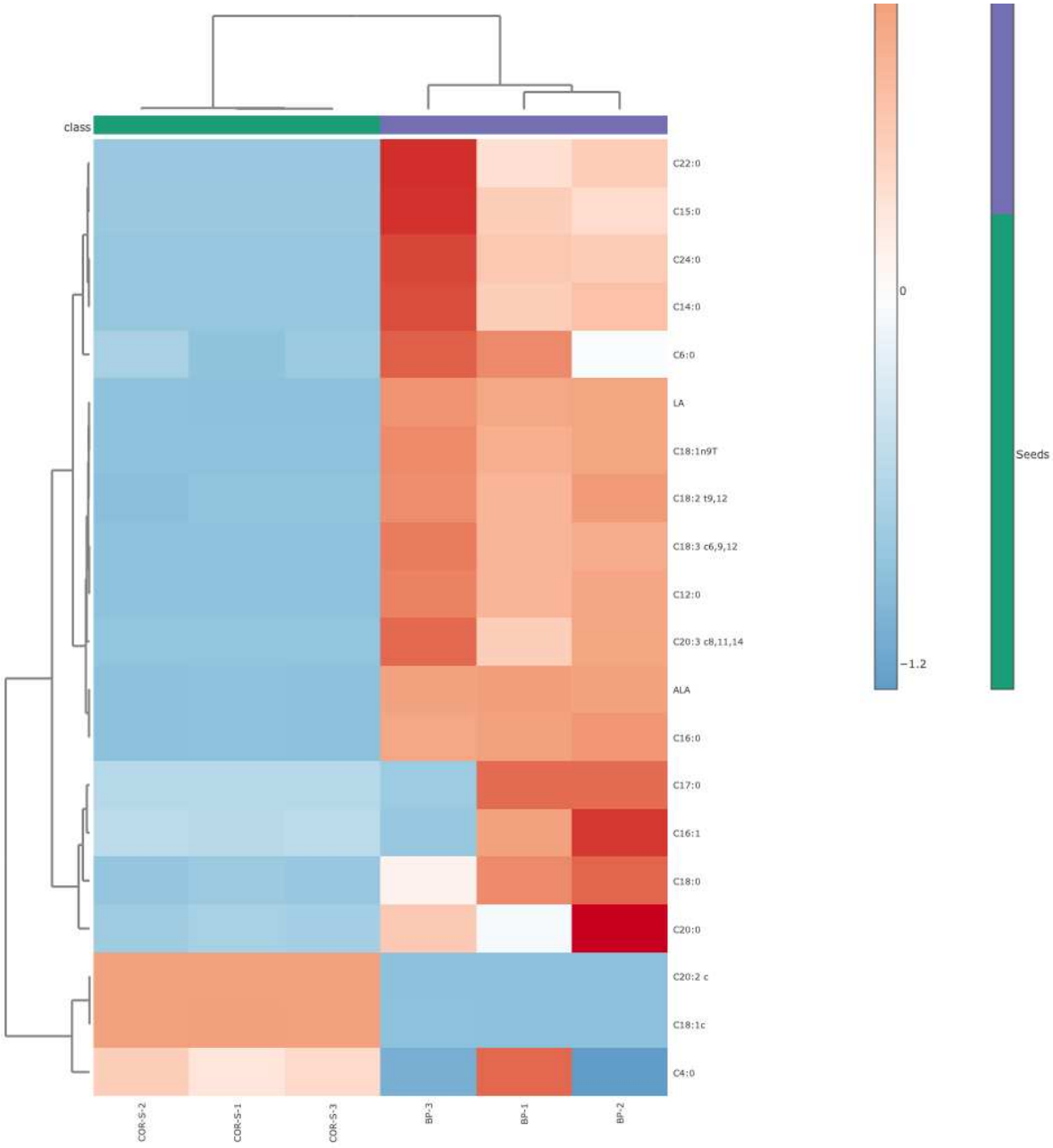
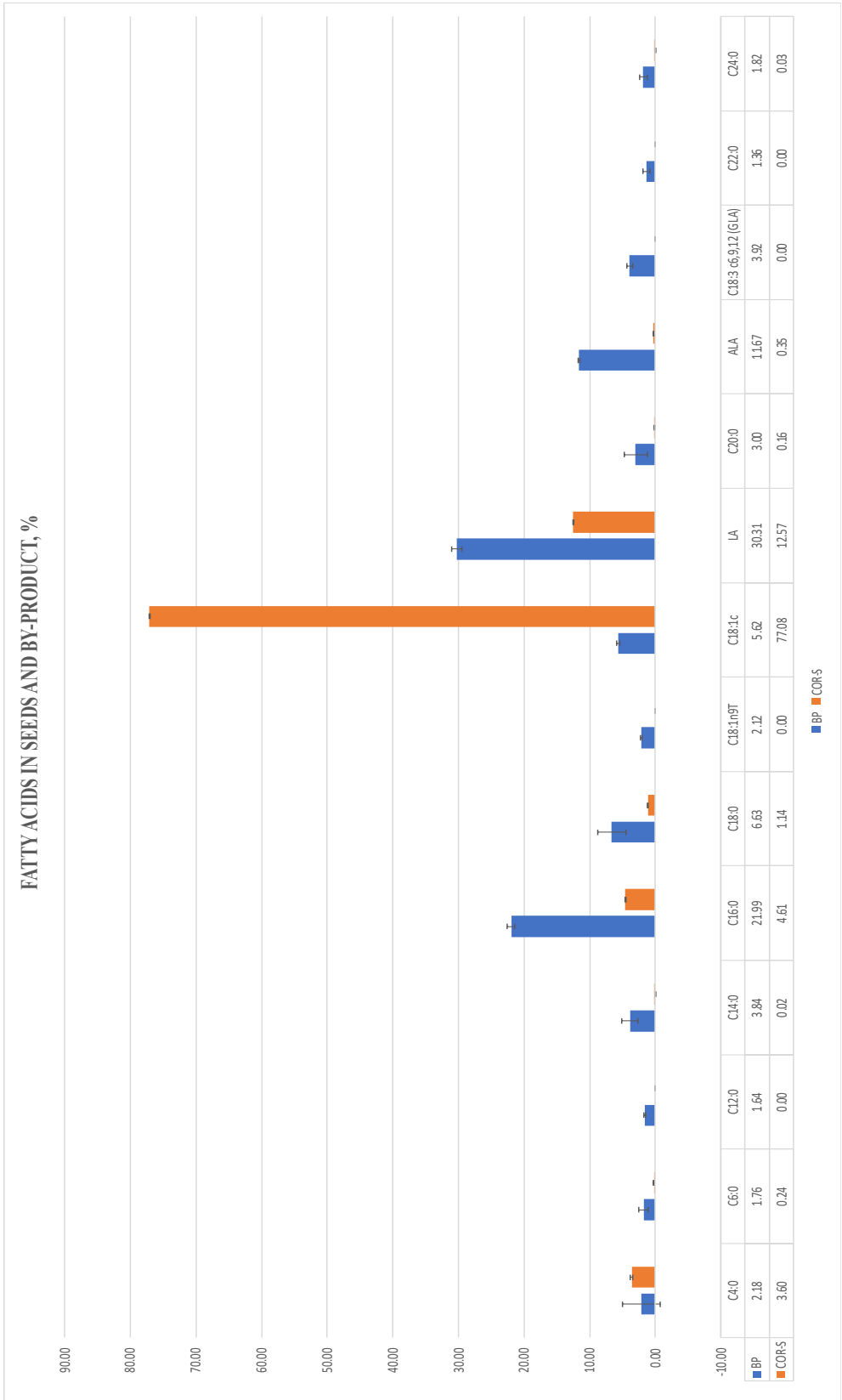


Figure 23. Heatmap of by-product and seeds samples

The Fig. 23, Tables 6 and 7 can be used to show differences between studied samples and relationships between variables, allowing the results of a study to be visualized and analyzed. Along the vertical axis, fatty acids are located in clusters which are displayed by a dendrogram to the left of the heat map. Along the horizontal axis are samples of sea fennel by-product and

seeds are located. To the right of the heat map there is a color scale that indicates the degree of deviation of the values for each variable. The scale ranges from blue (low values) to red (high values), allowing quickly the assessment of the major impact of variables have high or low values in different samples. For example, seeds from the Corsica region are indicated in orange only in the case of three fatty acids: C4:0, C18:1c and C20:2c, and in other cases are indicated in blue, which means that the content of other fatty acids is either in very small quantities or absent at all. While the by-product is indicated in the color palette from light beige to red in all other fatty acids except for C4:0, C18:1c and C20:2c.



	C4:0	C6:0	C12:0	C14:0	C16:0	C18:0	C18:1n9T	C18:1c	LA	C20:0	ALA	C18:3 c6,9,12 (GLA)	C22:0	C24:0
BP	2.18	1.76	1.64	3.84	21.99	6.63	2.12	5.62	30.31	3.00	1.167	3.92	1.36	1.82
COR-S	3.60	0.24	0.00	0.02	4.61	1.14	0.00	77.08	12.57	0.16	0.35	0.00	0.00	0.03

Figure 24. Fatty acid content in seed and by-product of sea fennel. Data are presented as means of three replicas ± standard deviation

Figure 24 shows the content of individual fatty acids in sea fennel seeds (S) from Corsica and by-product (BP). Short and medium chain fatty acids (from C4:0 to C14:0) are present in minor quantities. Levels of these fatty acids did not exceed 1% in both samples. C16:0 is present in both samples with the highest value in BP (about 8%). C18:0 content in both samples is low, about 2%. There is a strong difference in C18:1c content between the samples: in COR-S it reaches almost 80%, while in BP-L its amount is minimal. LA is present in both samples at a level of about 10%. Long chain fatty acids (C20:0, C20:1, C22:0, etc.) are present in low quantities in both samples, rarely exceeding 1%.

Notably, the seeds contains a very high oleic acid % compared to by-products. This information can be used to further investigate the functional properties of sea fennel depending on its components.

4.2. Claims

4.2.1. Verification of conditions of making any nutritional or health claims

The amount of omega-3 and omega-6 in 100 g of sea fennel was calculated based on the amount of fat obtained using Soxhlet extraction and the percentage of fatty acids obtained using a gas chromatograph. Based on the calculations presented in Table 8, each samples except sample COR-S, satisfy the criteria to be assigned the subsequent nutrition claim set out in Annex to the Regulation (EC) No 1924/2006 : “SOURCE OF OMEGA-3 FATTY ACIDS” A claim that a food is a source of omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0,3 g alpha-linolenic acid per 100g and per 100kcal, or at least 40mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100g and per 100kcal.”

Table 8. Calculation of the percentage of polyunsaturated fatty acids omega-3 and omega-6 in different parts of dry sea fennel wild and cultivated populations

#	Name	g, Sample weight	g, Extracted oil	%, g oil/ g sample weight	%, ALA	g ALA / 100g dry SF	%, LA	g LA / 100g dry SF
1	CAL-L	5.001	0.240	4.800	26.540	1.274	32.370	1.554
2	CAL-F	5.077	0.220	4.333	9.040	0.392	52.910	2.293
3	MAR-L	1.000	0.140	13.994	25.680	3.594	31.330	4.384
4	MAR-F	5.055	0.270	5.341	11.980	0.640	48.430	2.587
5	SAR-L	2.002	0.267	13.315	28.760	3.829	29.110	3.876
6	SAR-F	5.003	0.240	4.797	12.310	0.591	50.230	2.410
7	SIC-L	5.003	0.280	5.596	18.030	1.009	35.120	1.965
8	SIC-F	5.033	0.280	5.563	8.920	0.496	48.250	2.684
9	PUG-L	2.006	0.270	13.460	28.960	3.898	27.960	3.763
10	PUG-F	5.000	0.170	3.400	10.700	0.364	51.650	1.756
11	TOS-L	5.000	0.430	8.599	27.880	2.397	25.060	2.155
12	TOS-F	2.009	0.110	5.476	11.890	0.651	50.870	2.785
13	CON-L	2.002	0.120	5.993	26.290	1.576	32.820	1.967
14	LIG-L	2.005	0.156	7.800	26.270	2.049	30.170	2.353
15	LIG-F	5.000	0.185	3.706	9.900	0.367	50.780	1.882
16	COR-L	2.005	0.239	11.925	29.190	3.481	31.290	3.731
17	COR-F	2.008	0.172	8.572	4.600	0.394	24.240	2.078
18	RIN-L-FD	2.001	0.144	7.175	31.920	2.290	36.340	2.607
19	RIN-L-AD	5.000	0.460	9.200	14.960	1.376	31.020	2.854
20	BP	2.001	0.088	4.413	11.670	0.515	30.310	1.337
21	COR-S	6.000	1.511	25.187	0.350	0.088	12.570	3.166

Furthermore, samples from MAR-L, SAR-L, PUG-L, TOS-L, LIG-L, COR-L and RIN-L-FD satisfy the criteria to be assigned the subsequent nutrition claim “ALA contributes to the maintenance of normal blood cholesterol levels. The claim may be used only for food which is at least a source of ALA as referred to in the claim SOURCE OF OMEGA 3 FATTY ACIDS

as listed in the Annex to Regulation (EC) No 1924/2006. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 2 g of ALA." if consider the amount of daily intake of sea fennel as 100g.

It should be noted that the samples from the regions CAL-L, MAR-L, MAR-F, SAR-L, SIC-L, PUG-L, TOS-L, TOS-F, CON-L, LIG-L, COR-L, RIN-L-AD and RIN-L-FD can be covered under the claim "HIGH OMEGA-3 FATTY ACIDS" A claim that a food is high in omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0,6 g alpha-linolenic acid per 100 g and per 100 kcal, or at least 80 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal."

Moreover, sea fennel may be covered under the health claim "Replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels". However, all those claims could be used only during the commercialization of dried plant parts, except for the seed and by-product. The percentage of water in flowers and leaves varied from 80% and 87%. The following conclusion can be reached by examining the calculation that was performed with the presumption that sea fennel leaves and flowers had an average water content of 84% (Table 9): highest amount of omega-3 fatty acid was presented in the sample PUG-L (3.274 g) and highest amount of omega-6 was noticed in sample MAR-L (3.683 g). The samples from the regions MAR-L, SAR-L, PUG-L, TOS-L and COR-L can be covered under the claim "ALA contributes to the maintenance of normal blood cholesterol levels. The claim may be used only for food which is at least a source of ALA as referred to in the claim SOURCE OF OMEGA 3 FATTY ACIDS as listed in the Annex to Regulation (EC) No 1924/2006. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 2 g of ALA." As we do not take into account seeds in fresh state as for consuming, each samples satisfy the criteria to be assigned the subsequent nutrition claim "SOURCE OF OMEGA-3 FATTY ACIDS" A claim that a food is a source of omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0,3 g alpha-linolenic acid per 100g and per 100kcal, or at least 40mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100g and per 100kcal." And lastly, samples from the regions CAL-L, MAR-L, SAR-L, SIC-L, PUG-L, TOS-L, CON-L, LIG-L, COR-L, RIN-L-AD and RIN-L-FD can be covered under the claim "HIGH OMEGA-3 FATTY ACIDS" A claim that a food is high in omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0,6 g alpha-linolenic acid per 100 g and per 100 kcal, or at least 80

mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal. In addition to this, the health claim "Linoleic acid contributes to the maintenance of normal blood cholesterol levels. The claim may be used only for a food which provides at least 1,5 g of linoleic acid (LA) per 100 g and per 100 kcal. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 10 g of LA." can be applied to dry sea fennel for all samples and in case of the fresh state it can be applied to all samples except CAL-L and PUG-F; however, 400–500g is needed to fulfil the daily requirement of 10g.

Table 9. Calculation of the percentage of polyunsaturated fatty acids omega-3 and omega-6 in fresh flowers and leaves of wild and cultivated sea fennel populations

#	Name	g ALA / 100g dry SF	g ALA / 100g fresh SF	g LA / 100g dry SF	g LA / 100g fresh SF
1	**CAL-L**	1.274	1.070	1.554	1.305
2	**CAL-F**	0.392	0.329	2.293	1.926
3	**MAR-L**	3.594	3.019	4.384	3.683
4	**MAR-F**	0.640	0.538	2.587	2.173
5	**SAR-L**	3.829	3.217	3.876	3.256
6	**SAR-F**	0.591	0.496	2.410	2.024
7	**SIC-L**	1.009	0.848	1.965	1.651
8	**SIC-F**	0.496	0.417	2.684	2.255
9	**PUG-L**	3.898	3.274	3.763	3.161
10	**PUG-F**	0.364	0.306	1.756	1.475
11	**TOS-L**	2.397	2.014	2.155	1.810
12	**TOS-F**	0.651	0.547	2.785	2.340
13	**CON-L**	1.576	1.324	1.967	1.652
14	**LIG-L**	2.049	1.721	2.353	1.977
15	**LIG-F**	0.367	0.308	1.882	1.581
16	**COR-L**	3.481	2.924	3.731	3.134
17	**COR-F**	0.394	0.331	2.078	1.745
18	**RIN-L-FD**	2.290	1.924	2.607	2.190
19	**RIN-L-AD**	1.376	1.156	2.854	2.397

Aside from this, it would be more accurate to compare cultivated and wild sea fennel samples from the same geographical region. Table 9 in this instance leads to the following conclusion regarding dry sea fennel: The wild sample from the region of Marche MAR-L contains the third highest amount of omega-3 polyunsaturated fatty acid (3.594 g in 100 g of dry extract SF) when compared to other wild samples. The cultivated samples from the same region, RIN-L-FD and RIN-L-AD, this value is lower (alternately 2.290 g and 1.376 g), but comparatively higher than other wild samples from other regions. Regarding omega-6, among all the samples, the wild sample MAR-L has the highest level of this polyunsaturated fatty acid, whereas the cultivated samples RIN-L-FD and RIN-L-AD display an average value (2.607 g and 2.854 g, respectively).

4.2.2. Laboratory scale encapsulation

Field by-product (BP) was subjected to green extraction technologies (ultrasound,) using food grade hydro-alcoholic (water/ethanol) solvent mixtures. The choice of these solvents fully complies with the European legislation (Directive 2009/32/EC), also considering the effectiveness towards the extraction of bioactive substances and the toxicity and environmental impact. The crude extracts (= 2, water W and ethanolic E) were tested for the alginate-based encapsulation for the first time.

The sea fennel extract encapsulation process using sodium alginate was performed using the dropping method. Freeze-dried sea fennel extracts were and mixed with sodium alginate powder and water. Sodium alginate can be used as a thickener, suspending agent, stabilizer, and gel, so when a suspension of extract in sodium alginate was added into a solution of calcium chloride, an exchange between the calcium ions with sodium ions occurs and form a gel. In the encapsulation procedure, the hydrogel was allowed to react for 15 min in the calcium chloride solution so that all the sodium alginate reacted with calcium ions (Fig. 25, Fig. 26).

Figure 26. Sea fennel extract encapsulation in calcium bath

Figure 25. Encapsulated sea fennel extract

Different formulations were tested in order to obtain stable capsules using two extracts: water (W) and ethanolic one (E). Two percentage of sodium alginate (2% and 1,34%) were tested and two percentage of the sea fennel extracts were tested (2% and 1,34%). Form figure 27 it is possible to notice that the spherification was achieved using the ethanolic extract especially with the sample E75. With the water extract it was not possible to obtain spherical capsules because the pH was to high and it does not allow the gelation of the alginate. To confirm those results the physico-chemical and the texture parameters were measured as reported in Table 10. Formulation E50 produced hard and springy capsules, while E75 produced soft and cohesive capsules. This experiment was a preliminary trial with the aim to optimize the encapsulation of sea fennel extract with alginate polysaccharides.

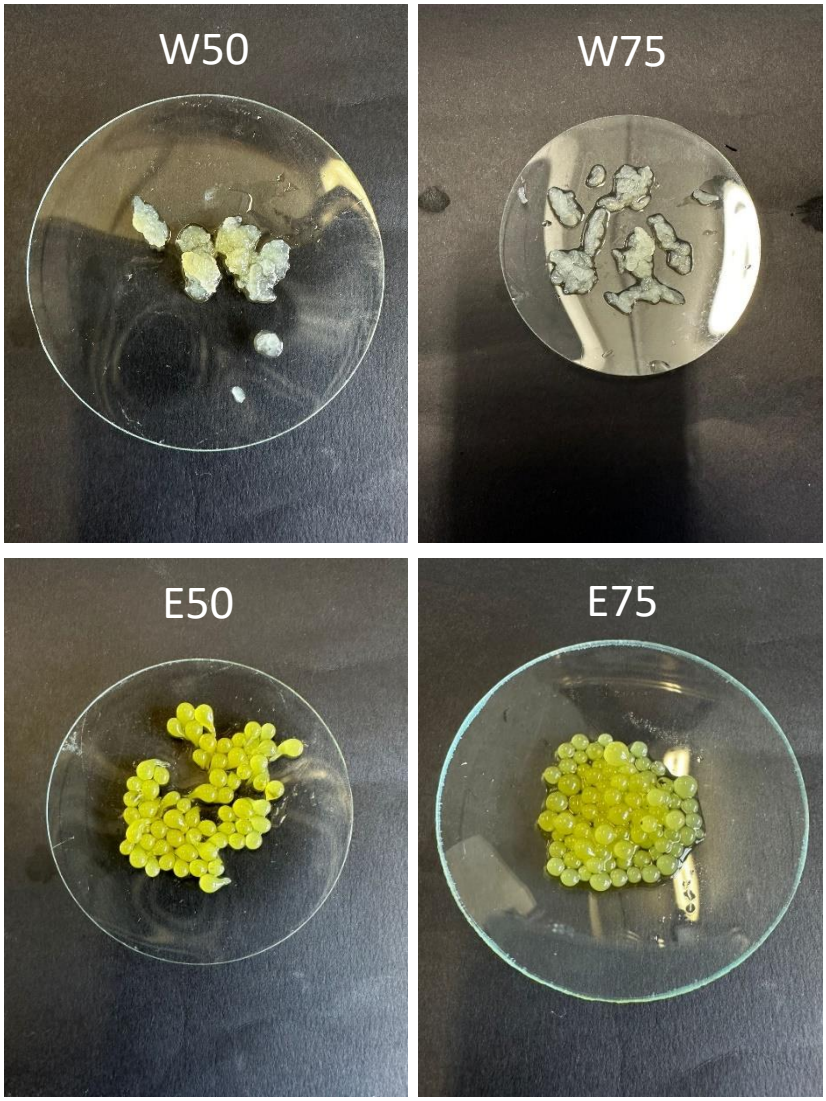


Figure 27. Alginate-based capsule testing 4 formulation. W= water extract and E (ethanolic extract).

Table 10. Edible capsules: physico-chemical, color and texture parameters. Data are reported as means of three replicas ± standard deviation

Samples	W50		E50		E75	
pH	5,05		4,21		4,12	
aw	0,95		0,95		0,96	
L*	45,55	± 0,60	37,54	± 2,01	32,68	± 1,50
a*	3,82	± 0,09	1,77	± 1,07	3,03	± 1,03
b*	15,30	± 0,33	22,00	± 1,05	26,81	± 2,03
Hardness g	225,0	± 5,3	1434,0	± 9,5	29,0	± 3,2
Cohesiveness	0,84	± 0,20	0,84	± 0,34	0,74	± 0,02
Springiness mm	1,60	± 0,23	1,80	± 1,32	0,30	± 0,07
Adhesion mJ	0,53	± 0,05	0,73	± 0,01	0,79	± 0,06

5. DISCUSSION

The data obtained from this thesis were compared with data from other scientific articles, and some small discrepancies in the percentages of individual fatty acids were noted.

Table 11. Fatty acid profile of leaves, flowers, and fruits/schizocarps of sea fennel, C. maritimum.

	Leaves		Flowers		Schizocarps	
	% Total FA	µg/mg dw	% Total FA	µg/mg dw	% Total FA	µg/mg dw
SFA						
C12:0	0.45 ± 0.29	0.077 ± 0.064	0.066 ± 0.03	0.011 ± 0.005	0.02 ± 0.01	0.013 ± 0.008
C14:0	2.49 ± 0.09	0.429 ± 0.019	0.547 ± 0.003	0.142 ± 0.008	0.27 ± 0.01	0.131 ± 0.042
C15:0	0.22 ± 0.06	0.030 ± 0.004	0.18 ± 0.01	0.048 ± 0.005	0.11 ± 0.00	0.055 ± 0.019
C16:0	18.53 ± 0.18	3.210 ± 0.130	17.57 ± 0.10	4.570 ± 0.270	8.89 ± 0.12	4.359 ± 1.440
C17:0	0.25 ± 0.01	0.043 ± 0.001	0.225 ± 0.003	0.059 ± 0.004	0.12 ± 0.00	0.058 ± 0.020
C18:0	7.97 ± 0.08	1.377 ± 0.062	3.54 ± 0.02	0.918 ± 0.051	0.42 ± 0.03	0.200 ± 0.080
C20:0	1.43 ± 0.02	0.246 ± 0.011	0.72 ± 0.01	0.186 ± 0.014	0.28 ± 0.01	0.134 ± 0.044
C24:0	2.12 ± 0.07	0.364 ± 0.006	2.64 ± 0.02	0.684 ± 0.038	1.00 ± 0.02	0.49 ± 0.16
MUFA						
C15:1	0.32 ± 0.05	0.059 ± 0.006	0.17 ± 0.02	0.043 ± 0.008	0.07 ± 0.01	0.035 ± 0.016
C16:1	1.43 ± 0.26	0.220 ± 0.018	0.27 ± 0.01	0.067 ± 0.004	0.16 ± 0.01	0.076 ± 0.026
C17:1	0.22 ± 0.01	0.039 ± 0.002	0.53 ± 0.01	0.138 ± 0.010	0.07 ± 0.02	0.040 ± 0.012
C18:1 n-9	2.03 ± 0.07	0.355 ± 0.013	3.43 ± 0.03	0.889 ± 0.043	50.1 ± 1.00	29.7 ± 2.00
C18:1 n-7	0.44 ± 0.05	0.072 ± 0.008	0.52 ± 0.01	0.134 ± 0.007	0.48 ± 0.06	0.24 ± 0.06
C22:1 n-9	6.62 ± 0.07	1.139 ± 0.052	10.04 ± 0.17	2.610 ± 0.160	7.05 ± 0.30	3.43 ± 1.05
C24:1 n-9	2.63 ± 0.07	0.456 ± 0.015	4.12 ± 0.06	1.074 ± 0.076	3.07 ± 0.03	1.51 ± 0.51
PUFA						
C16:3 n-4	0.33 ± 0.01	0.057 ± 0.002	0.07 ± 0.01	0.015 ± 0.003	0.07 ± 0.00	0.033 ± 0.009
C18:2 n-6	29.47 ± 0.39	5.11 ± 0.33	45.25 ± 0.24	11.77 ± 0.64	23.70 ± 0.50	11.6 ± 3.8
C18:3 n-3 (ALA)	22.50 ± 0.36	3.90 ± 0.24	8.54 ± 0.04	2.22 ± 0.12	2.67 ± 0.10	1.30 ± 0.41
C20:5 n-3 (EPA)	0.56 ± 0.04	0.091 ± 0.001	1.58 ± 0.02	0.406 ± 0.025	1.51 ± 0.02	0.74 ± 0.25
SFA	33.45 ± 0.44		25.48 ± 0.11		11.10 ± 0.14	
MUFA	13.69 ± 0.30		19.08 ± 0.20		60.95 ± 0.76	
PUFA	52.86 ± 0.63		55.44 ± 0.27		27.95 ± 0.62	
n-3	23.07 ± 0.35		10.12 ± 0.04		4.18 ± 0.12	
n-6	29.47 ± 0.39		45.25 ± 0.24		23.70 ± 0.50	
n-3/n-6	0.78 ± 0.01		0.22 ± 0.00		0.176 ± 0.001	
H/H	2.57 ± 0.06		3.16 ± 0.03		8.35 ± 0.16	
AI	1.247 ± 0.020		0.992 ± 0.007		0.405 ± 0.006	
TI	0.317 ± 0.005		0.345 ± 0.002		0.174 ± 0.002	

Values in mean ± SD of triplicates of three independent samples. FA, fatty acid. SFA, saturated fatty acid. MUFA, monounsaturated fatty acid. PUFA, polyunsaturated fatty acid. dw, dry weight.

Data retrieved from Correia [27].

After examining the table in the scientific publication from Correia [27], it is evident that the fatty acid listing in that piece starts with C12:0, but in this paper, fatty acids C4:0, C6:0, C10:0, and C11:0 were mentioned in trace amounts (Table 4,5). Overall, the discrepancy

between the results of the two works does not exceed ± 4%, as well as the results of n6/n3 ratio. Some fatty acids were also absent from Table 11, including C13:0, C21:0, C22:0, C20:1, C20:2, C20:3, and C22:2. This is most likely because these peaks are hard to see due to the low percentage content (up to 1%).

Among the wild populations of sea fennel, a geographic effect on the fatty acid composition was not find. Lipids may be considered primary or secondary metabolites because they can regulate several biological functions within plant cells. Temperature and other environmental factors might influence their biosynthesis, however in this study no difference due to geographic origin was find, but only due to plant part. In accordance with Sánchez–Faure et al. [33] who analyzed the composition of lipid fractions in lyophilized stems, the sea fennel fatty acid profile was PUFAs > SFAs > MUFAs, while in seeds, the distribution of the fractions was MUFAs > PUFAs > SFAs.

Table 12. Fatty acid composition of seed oils of some halophytes

Species	C16:0	C18:0	C16:1	C18:1	C18:2	C18:3
Crithmum maritimum L.	4.8	0.7	0.2	78.6	15.4	0.3
Arthrocnemum macrostachyum	26.93	3.17	0.9	-	63.02	-
Batis maritima	5.5	1.2	1.1	17.8	73.0	1.4
Alhagi maurorum	29.38	11.01	0.23	-	53.28	-
Kosteletzkya virginica	27.31	2.51	0.46	23.62	37.53	5.53
Salicornia bigelovii	trace	13.6	trace	67.8	3.6	1.8

Data retrieved from Atia [28].

Sea fennel seeds were one of the six species of halophytic plants whose fatty acid composition was examined in the scientific paper by Atia. It can be immediately noted that the order starts with the fatty acid C16:0, while in this work, the peak of C4:0 was noted with a percentage content of 3.60%. The variation between the comparative statistics of fatty acids C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3 in both tables (Table 4 and Table 5) is only ±3%.

Regarding the sea fennel by-product, no comparable research studies have been found to compare the outcomes. The by-product has a high quantity of saturated and polyunsaturated fatty acids; in particular, the amount of alpha-linoleic acid (±10%) is comparable to the amount of ALA found in sea fennel flowers. The n6/n3 ratio likewise revealed outcomes that were nearly in line with the ratio (5/1) suggested for lowering the incidence of cardiovascular illnesses (Table 7).

In addition to polyunsaturated fatty acids, sea fennel is thought to be a good source of minerals, proteins, dietary fibre, vitamins C, A, and E, and bioactive substances such flavonoids, polyphenols (hydroxycinnamic acids), and essential oils. Sea fennel's bioactive

compounds are what give it its useful properties, which include anti-inflammatory, antioxidant, antibacterial, and anti-proliferative properties. These compounds have enormous potential in the pharmaceutical and nutraceutical industries [6]. Polyphenols are a widespread and large group of plant metabolites that perform key functions throughout the life cycle. They are considered useful for the prevention of diabetes, cancer, cardiovascular disease and neurological diseases, as they exhibit important physiological activities in humans, mainly counteracting oxidative stress. Previous studies have identified significant amounts of phenolic acids in sea fennel, mainly chlorogenic acids [7] which has been extensively studied since it is widely distributed in plants, is one of the main polyphenols in the human diet, and it possesses many health-promoting properties. Furthermore, it has been found that chlorogenic acid has antioxidant, anti-inflammatory, anticancer, antilipidemic, antidiabetic, antihypertensive, and anti-neurodegenerative activities [8]. In agreement with previous studies, leaves of sea fennel wild populations contained a higher amount of chlorogenic acid than flowers, furthermore in leaves, chlorogenic acid was the most abundant compound [6]. Moreover, most research regarding the health benefits of chlorogenic acid has been done on disorders related to metabolic syndrome, which is defined as a group of interconnected physiological, biochemical, clinical, and metabolic factors that increase the risk of cardiovascular diseases, type 2 diabetes mellitus, and all-cause mortality. It has been estimated that 25% of the world's adult population has this syndrome. In addition, the syndrome is considered a worldwide epidemic with high socioeconomic cost and increasing prevalence in both childhood and young adulthood [8]. This gives rise to the notion of conducting more research on the encapsulated extract of this halophyte in a complex in order to determine the proportion of these chemical components in the final product in addition to the data that has previously been supplied regarding the presence of polyunsaturated fatty acids. And hence, it is possible to attribute additional nutrition and health claims.

6. CONCLUSION

Salinity is one of the world's biggest environmental problems, which pushes us to learn and study salt-tolerant crops for cultivation. It is therefore not surprising that with rapid climate change and population growth, the twenty-first century is expected to be the century of expansion of halophytic agriculture. Sea fennel is a promising vegetable ingredient and is considered a good source of essential fatty acids, which play crucial roles in human metabolism, health, and disease. Analyzing and comparing the different parts of sea fennel makes it possible to study this halophyte more closely and see the whole picture. The results herein collected suggest that different parts of the sea fennel are far from each other in terms of fat composition. The fatty acid composition of each part of sea fennel can be described generally as follows: polyunsaturated fatty acids predominate in the flowers, particularly omega-6, while high levels of omega-3 are present in the leaves. The by-product contains medium levels of omega-3 and omega-6 and a high level of saturated fatty acids. The seeds, in turn, show a high content of monounsaturated fatty acids, while in flowers this indicator is average, and in leaves and by-products it is at a low level.

The amount of omega-3 and omega-6 in 100 g of sea fennel was calculated based on the amount of fat obtained using Soxhlet extraction and the percentage of fatty acids obtained using a gas chromatograph. Based on the calculations presented in Table 8, each samples except sample COR-S, satisfy the criteria to be assigned the subsequent nutrition claim set out in Annex to the Regulation (EC) No 1924/2006 : “SOURCE OF OMEGA-3 FATTY ACIDS.” Furthermore, samples from MAR-L, SAR-L, PUG-L, TOS-L, LIG-L, COR-L and RIN-L-FD satisfy the criteria to be assigned the subsequent nutrition claim “SOURCE OF OMEGA 3 FATTY ACIDS”. It should be noted that the samples from the regions CAL-L, MAR-L, MAR-F, SAR-L, SIC-L, PUG-L, TOS-L, TOS-F, CON-L, LIG-L, COR-L, RIN-L-AD and RIN-L-FD can be covered under the claim “HIGH OMEGA-3 FATTY ACIDS”, however, all those claims could be used only during the commercialization of dried plant parts, except for the seed and by-product. For this reason, taking into account water content in leaves and flowers of SF, the following conclusion can be reached (Table 9): the samples from the regions MAR-L, SAR-L, PUG-L, TOS-L and COR-L can be covered under the claim “ALA contributes to

the maintenance of normal blood cholesterol levels. The claim may be used only for food which is at least a source of ALA as referred to in the claim SOURCE OF OMEGA 3 FATTY ACIDS as listed in the Annex to Regulation (EC) No 1924/2006. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 2 g of ALA." As we do not take into account seeds in fresh state as for consuming, each samples satisfy the criteria to be assigned the subsequent nutrition claim "SOURCE OF OMEGA-3 FATTY ACIDS." And lastly, samples from the regions CAL-L, MAR-L, SAR-L, SIC-L, PUG-L, TOS-L, CON-L, LIG-L, COR-L, RIN-L-AD and RIN-L-FD can be covered under the claim "HIGH OMEGA-3 FATTY ACIDS." In addition to this, the health claim "Linoleic acid contributes to the maintenance of normal blood cholesterol levels." can be applied to dry extract of sea fennel for all samples and in case of the fresh state it can be applied to all samples except CAL-L and PUG-F; however, 400–500g is needed to fulfil the daily requirement of ALA.

Moreover, sea fennel may be covered under the health claim "Replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels".

For the encapsulation of sea fennel ethanolic extract the dropping method with sodium alginate was used in laboratory environment. Form figure 27 it is possible to notice that the spherification was achieved using the ethanolic extract especially with the sample E75 which has turned out to be the best formulation for stable and appropriate capsules. With the water extract it was not possible to obtain spherical capsules because the pH was to high and it does not allow the gelation of the alginate. To confirm those results the physico-chemical and the texture parameters were measured as reported in Table 10.

In the next future, author believes that research on encapsulated sea fennel extract will continue in a complex with other bioactive compounds of this halophyte in addition to the data that has been supplied during this research.

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