The PRIMA programme is an Art. 185 initiative supported and funded under Horizon 2020, the European Union's Framework Programme for Research and Innovation'

Deliverable title	D6.5 Report with elaborated data overall collected within WP6
Deliverable Lead:	UNIBRE
Related Work Package:	WP6
Related Task:	Task 6.1 (R&D). Preparation and analysis of extracts from see fennel crop by-products Task 6.2 (R&D) Exploitation of the extracts as functional food ingredients and nutraceuticals Task 6.3 (R&D) Composting of residual sea fennel biomass Task 6.4 (R&D) Preparation and analysis of essential oils from sea fennel crop by-products
Author(s)	Dr Christian Magné (UNIBRE)
Dissemination level	PU
Due Submission Date:	MONTH 35 (30.03.2025)
Actual submission:	30.03.2025
Start date of project	30.05.2022
Duration	36 MONTHS
Summary of Deliverable D6.5 – Integrated Results from WP6	The work spanned multiple countries and research teams, combining chemical analyses, technological innovations, and biological testing. On the extracts side, both aqueous and hydroethanolic formulations were produced and characterized. They proved rich in chlorogenic acid, rutin, and flavonoids, with strong antioxidant, anti-diabetic, anti-obesity, and antimicrobial activities. Italy and Croatia optimized extraction methods - microwave-assisted and accelerated solvent extraction - to maximize yields, while Tunisia confirmed additional anti-inflammatory and antifungal potential. France contributed advanced NMR profiling, ensuring precise chemical fingerprints. Encapsulation trials using sodium alginate showed that extracts could be stabilized into edible capsules, extending shelf life and consumer appeal. Tunisia further tested by-products as animal feed supplements. Preliminary trials with rabbits showed good palatability and consistent growth at moderate inclusion levels, opening pathways for functional feed. In parallel, nutraceutical formulations (spray-dried powders, capsules) demonstrated high encapsulation efficiency and stability, pointing to opportunities in dietary supplements. Another major outcome was the valorization of essential oils. Croatia showed their potential in preserving vegetable oils from oxidation and enhancing flavor, while Tunisia successfully

	integrated them into confectionery prototypes like jelly candies, illustrating cross-sectoral applications. Finally, composting studies in Croatia confirmed that sea fennel residues can be turned into soil amendments with good water retention properties, usable in blends to reduce reliance on peat. Complementary compost teas provided nutrient release suitable for horticultural systems. Altogether, WP6 demonstrates that sea fennel is not only a promising crop for novel foods but also a multi-purpose resource supporting nutraceuticals, animal nutrition, natural preservatives, cosmetics, and sustainable farming. The results strongly position sea fennel within a circular bioeconomy framework, where no part of the plant is wasted and every fraction contributes to sustainability and innovation.

Versioning and Contribution History

Version	Date	Modified by	Modification reason
v1.0	20/08/2023	Christian Magné	First version
V2.0	30/03/2025	Christian Magné	Comments after peer reviewing process

Table of Contents

Report with elaborated data overall collected within WP6 (detailed for each Task and Partner)

1 Preparation and analysis of extracts from sea fennel crop by-products and exploitation of extracts as functional food ingredients and nutraceuticals

1.1 UNIVPM - Italy

1.1.1 Preparation and analysis of extracts from sea fennel crop by-products

Material and Methods

Sea fennel by-product supply

C. maritimum by-product was kindly provided by a local company Rinci s.r.l. (Castelfidardo, Italy; 43°26'51"N 13°33'21"E). The by-product consisted of heterogeneous materials such as wood parts, stems, old leaves, and flowers. After a visual inspection to ensure the absence of mold, the biomass was stabilized by air-drying in a De Cloet Dryer at 40 °C until it reached a relative humidity (RH) of less than 15% at the company facilities. The dried by-product was sealed in hermetic bags, transferred to the laboratory and stored at room temperature in the darkness. The hardest inedible parts were manually removed and then, the by-product was ground with a cutter mill using a 6 mm grid to obtain a fine powder (BP-RAW) prior to extract preparation.

Preparation of aqueous and hydroethanolic extracts

The aqueous extract (BP-W) was obtained using BP-RAW and deionized water at a ratio of 1:20 w/v, as reported by Alemán et al. (2019). The extraction was carried out at room temperature in the darkness, under agitation for 18 h, then centrifuged (3500 rpm, 5 min), filtered through a regenerated cellulose filter (0.45 µm), freeze-dried, and stored at -20 °C for further analysis.

On the other hand, the ethanolic extract (BP-ET) was obtained from BP-RAW using ethanol: water (80:20, v/v) as solvent at a ratio of 1:20 w/v. Subsequently, the mixture was subjected to ultrasound-assisted extraction for 30 min.

The extract was then centrifuged (3500 rpm, 5 min), filtered through a regenerated cellulose filter (0.45 µm), and the ethanol was evaporated using a vacuum rotary evaporator (40 °C), while the water was removed by freeze-drying. The extract was stored at -20 °C before further analysis. Figure 1 illustrates sea fennel BP-RAW, BP-W and BP-ET preparation.
The following formula was used to determine the extraction yield of BP-ET and BP-W extracts:
Extraction yield (%) = $\frac{W_1}{W_2} \times 100$;
where W1 is the mass of the final dried extract and W2 is the mass of the initial sample prior to extraction.

Results

The aqueous extract of by-product displayed:

- 296.37 ± 5.44 IC50 (µg/mL) for DPPH test
- 5.15 ± 0.16 IC50 (µg/mL) for ABTS test
- 65.14 ± 2.44 µM Fe (II)/g for FRAP test.
- 40.45 mg GAE/g DW (TPC)
- 18.78 mg QE/g DW (TFC)
- 0.4 mg βC/g DW (TCC)
- 166.75 ± 3.21 Lipase inhibitory activity of C. maritimum extracts [IC50 (µg/mL)]
- 673.96 ± 8.85 α-amylase inhibitory activity of C. maritimum extracts [IC50 (µg/mL)]
- 203.12 ± 3.08 α-glucosidase inhibitory activity of C. maritimum extracts [IC50 (µg/mL)]
- No antimicrobial activity towards the 8 strains of pathogens tested, belonging to the species Staphylococcus aureus, Escherichia coli and Listeria innocua.
- This extract could be used to increase antioxidant properties of nutraceuticals. http://dx.doi.org/10.1016/j.fbio.2023.103417

The ethanolic extract of by-product displayed:

- 354.55 ± 10.31b IC50 (µg/mL) for DPPH test
- 121.82 ± 9.04a IC50 (µg/mL) for ABTS test
- 60.20 ± 3.98a µM Fe (II)/g for FRAP test.
- 3.38 ± 0.35a b-Carotene bleaching test (30 min incubation)
- 3.27 ± 0.29a b-Carotene bleaching test (60 min incubation)
- 39.2 ± 1.5 mg GAE/g DW (TPC)
- 21.6 ± 0.9 mg QE/g DW (TFC)
- Antimicrobial acvitity: the ethanol extract demonstrated notable antimicrobial activity, with the largest inhibition growth zone observed for Staphylococcus aureus DSM 20231 (4.50 ± 0.00 mm). Additionally, the ethanol extract exhibited comparable inhibition zones against other S. aureus strains, including S. aureus ATCC 29213 (3.31 ± 0.09 mm) and S. aureus ATCC 25923 (3.13 ± 0.00 mm). For the three strains of L. innocua, the inhibition zones were also significant, with values of 3.00 ± 0.18 mm for Li 1, 3.13 ± 0.00 mm for Li 2, and 3.19 ± 0.27 mm for Li 3. These findings highlight the effectiveness of the ethanol extract in inhibiting the growth of a range of bacterial strains, particularly S. aureus and L. innocua, suggesting its potential as a natural antimicrobial agent. The ethanolic extract exhibited strain-dependent growth inhibition zones against S. aureus and L. innocua, but not against E. coli. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the ethanolic extract against S. aureus were 2.5 MIC and 10.0 MBC mg/mL, respectively. Ethanolic extracts could potentially be used in food formulations to enhance lipid peroxidation resistance and antimicrobial capacity for food preservation purposes.

1.1.2 Exploitation of the extracts as functional food ingredients

Material and Methods

Preparation of capsules

Method Agar Agar

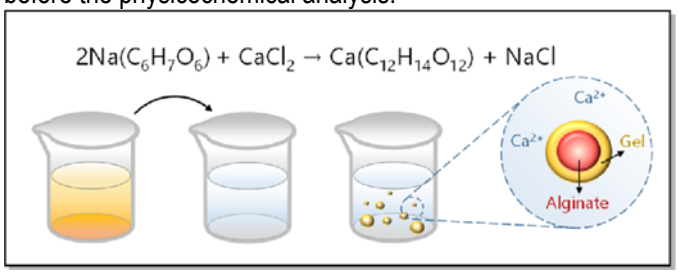
The spherification process to produce SEASONIG EDIBLE CAPSULS FROM AQUEOUS EXTRACTS was tested and preliminary results are provided. The optimization of the sphericationis under evaluation. The spherification was achieved using agar ingredient. 100 g of extract was prepared using 10 g of dried sea fennel by-product and 100 mL of water. After extraction, it was filtered. The formulation was designed as:

- 100 g of extract
- 1 g of agar
- 1 g of citric acid

The mixture was heated at 100°C for 30 seconds and then cooled down to 60°C. The mixture was then moved into a syringe and the capsules were formed by dropping the drops in 400g of sunflower oil at 1°C. Finally, the capsules were washed with cold water 10°C and kept at °4C for further analysis.

Method Sodium Alginate

Alginate-based capsules were produced by the dropping method following the ionotropic gelation technique. 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. Preliminary formulations were experimented with before selecting 4 formulations 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.



Alginate-base gelation technique

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%

pH and a_w

The pH of the samples was measured using a pH meter equipped with a HI2031 solid-state electrode (Hanna Instruments, Padova, Italy). Water activity (aw) was determined using an AW LabMaster instrument (Novasina AG, Lachen, Switzerland).

Color

The color measurements 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).

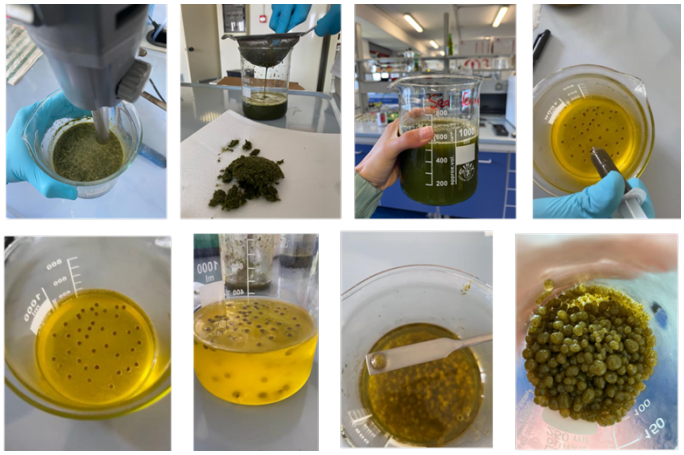
Texture parameters

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). The probe compressed the sample to a depth of 2 mm at a speed of 15 mm/s. Measurements were performed at room temperature using 10 g of sample placed in containers with capsules of 10 mm thickness. Three independent measurements were carried out, for each sample.

Results

Method Agar Agar

The spherification was possible but the capsules were not stable at 4°C, they melted, so the method with sodium alginate was performed.

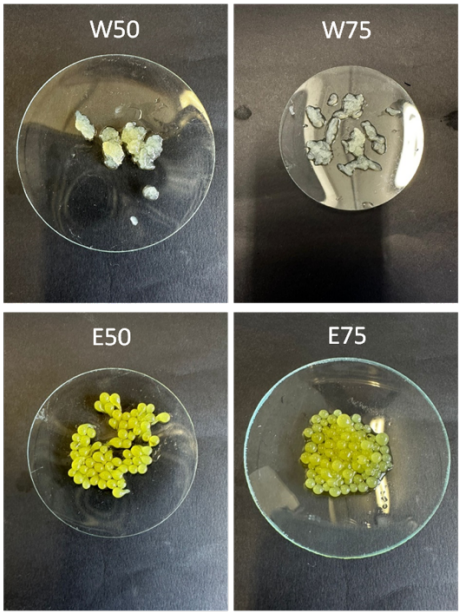


Preparation of capsules by agar-agar method

Method Sodium Alginate

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.
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%). 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.



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

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

Conclusion

For the encapsulation of sea fennel ethanolic extract the dropping method with sodium alginate was used in laboratory environment. 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. The encapsulation of water extract is not performing well, thus only ethanolic extract will be optimized as ingredient for laboratory scale production of stable capsules with defined shelf life.

References

Flamminii, F., Paciulli, M., Di Michele, A., Littardi, P., Carini, E., Chiavaro, E., Pittia, P., & Di Mattia, C. D. (2020). Alginate-based microparticles structured with different biopolymers and enriched with a phenolic-rich olive leaves extract: A physico-chemical characterization. Current Research in Food Science, 4, 698-706. https://doi.org/10.1016/j.crfs.2021.10.001

1.1.2.1 Optimization of Sodium alginate encapsulation of ethanolic extract

Material and Methods

Preparation of capsules by Sodium Alginate method

Alginate-based capsules were produced by the dropping method following the ionotropic gelation technique. Briefly, encapsulant solutions, alginate (1.5% w/v) was mixed with 1.7% (w/v) of BP-E (ethanolic extract powder), homogenized using an Ultra-Turax (Ika- Werke GmbH & Co, Germany) until complete dissolution. pH of the mixture was adjusted in the range of 4.2 – 4.6 using lactic acid solution. Ulrasound-assisted extraction was applied for 5 mins to release entrapped air and the mixture was stored 2 hours at room temperature in the dark to allow better hydration. A chloride calcium salt solution was prepared at 1% 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 15 min to allow complete gelation, then a sieve was used to recover them. A washing step with distilled 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.

Preservation Medium for Encapsulated Samples

The brine used in the study was kindly provided by the local company Rinci s.r.l. (Castelfidardo, Italy). Its composition included 3% salt, 25% wine vinegar, and 72% water, with a measured pH of 3.32. Prior to use, the brine was pasteurized at 80 °C for 15 minutes, cooled to room temperature, and subsequently stored at 4 °C.

pH and a_w

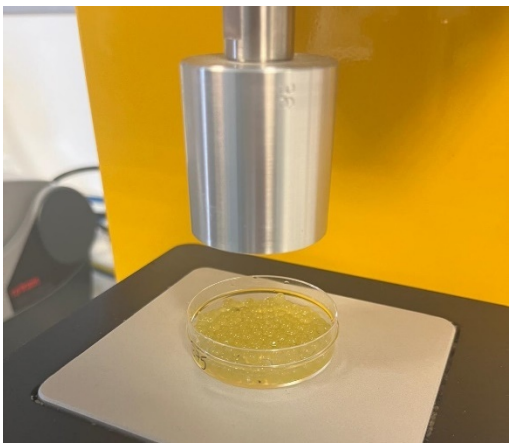
The pH of the samples was measured using a pH meter equipped with a HI2031 solid-state electrode (Hanna Instruments, Padova, Italy). Water activity (aw) was determined using an AW LabMaster instrument (Novasina AG, Lachen, Switzerland).

Color

The color measurements 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).

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). The probe compressed the sample to a depth of 3 mm, with 10g trigger and at a speed of 1.4 mm/s. Measurements were performed at room temperature using 10 g of sample placed in containers with capsules of 10 mm thickness. Three independent measurements were carried out, for each sample.



Texture analysis of capsules

Total phenolic content

The mixture to be encapsulated and capsules were analyzed for total phenolic content (TPC). The extraction was carried out according to Fanesi et al. (2023) with minor modifications. Briefly, 0.25 g of dry sample was extracted with 3.75 ml ethanol:water (50:50, v/v) at 40 °C for 1 h under agitation, then centrifuged (3500 rpm, 10 min at 4°C). TPC were determined according to the Folin–Ciocalteu method. Therefore, 20 µL of the supernatant was added to 1.58 mL water and 100 µL of Folin reagent, left for 7 min in the darkness, then 300 µL of sodium carbonate solution was added. After 30 min, the absorbance was measured at λ = 750 nm using an UV-Vis spectrophotometer (Onda, UV-31 SCAN, Beijing, China) and results were expressed as mg gallic acid equivalents (GAE) per g of dry sample.

The Encapsulation efficiency

The Encapsulation efficiency (EE %) of Total Phenolic Content was calculated by the following formula:

$$EE\ \% = (Wt/Wi) \times 100\%$$

where Wt is the TPC (mg GAE/ g) of the mixture to be encapsulated and Wi is the TPC (mg GAE/ g) of the capsules.

Microbiological enumeration

Microbiological analyses were performed by aliquoting 10g of each replicate and adding 90mL sterile 0,1% (w v-1) peptone water, The suspension was then homogenized for 5min at 230rpm in a stomacher machine (400 Circulator, International PBI, Milan, Italy). Enumeration was performed by preparing tenfold serial dilutions for: (i) mesophilic aerobic bacteria on Plate Count Agar (PCA) (VWR International Srl, Milan, Italy), by incubating at 30°C for 48 h; (ii) yeasts on Rose Bengal chloramphenicol Agar (RB) (VWR), by incubating at 25°C for 5 days; (iii) Enterobacteriaceae on Violet Red Bile Agar (VRBGA) (VWR), by incubating at 37°C for 24 h, (iv) spore-forming bacteria were first heat-treated at 80 °C for 12 minutes, cooled, and then plated on PCA (VWR), incubated at 37 °C for 24 h. The results of viable counting were expressed as the mean Log colony forming units (CFU) g−1 of three replicates ± standard deviation.

Additionally, Aliquots of sea fennel capsules were aseptically collected from each replicate of each sample on day 0, and were subjected to the enumeration of: (i) *Escherichia coli* on count coliform agar (CCA) (VWR) at 37°C for 24 h, (ii) *Listeria monocytogenes* on Agar Listeria Ottaviani and Agosti (ALOA) (BIOLIFE Italiana Srl, Milan, Italy) supplemented with Listeria Enrichment Supplement Ottaviani and Agosti (VWR)
at 37°C for 24 h, (iii) *Clostridium perfringens* on Tryptose Sulfite Neomycin agar (TSN) (VWR) at 37°C for 24 h.

Accelerated shelf-life test (ASLT)

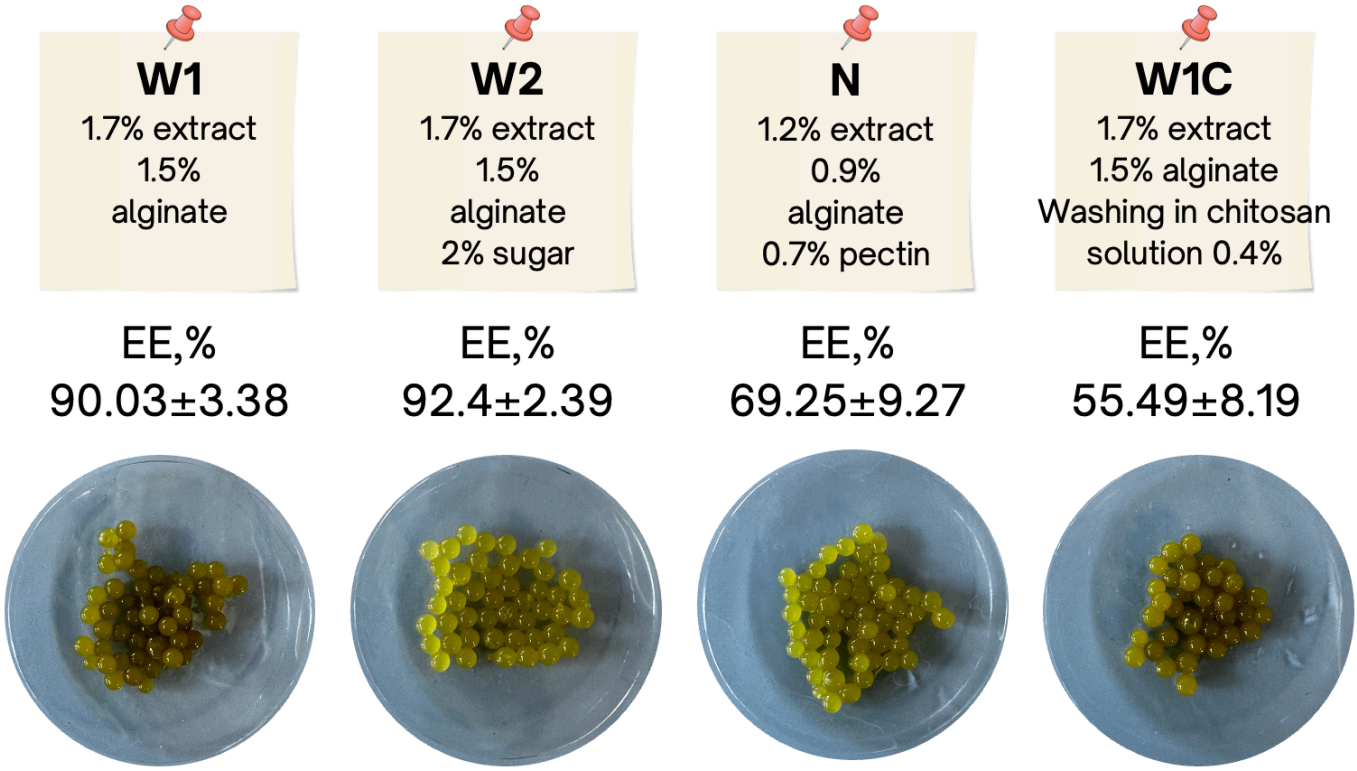
The effectiveness of the storage using brine or lactic acid preservation was evaluated through accelerated shelf-life test. Aliquots (10 g) of the brine preserved capsules and lactic acid preserved capsules (control) were aseptically collected prior to incubation (t_0) and after 5, 15, and 30 days of incubation at 37 °C and were subjected to microbiological analyses as described in the previous section.

Sensory Analysis

Sea fennel hydro-ethanolic extract-containing alginate-based capsules were produced on the same day as the sensory panel and served to panelists in two forms: freshly prepared capsules without preservation and capsules preserved in brine. The sensory evaluation followed the method described by Maoloni et al. (2022), with minor modifications. Samples were coded with random numbers and presented to 18 non-smoking panelists, including 8 men and 10 women, aged between 18 and 53 years. Participants were asked to evaluate the samples based on appearance, color, aroma, texture acceptability—covering both hardness and springiness—and overall acceptability, using a 5-point hedonic scale, where 5 indicated "extremely like" and 1 indicated "extremely dislike."

Results

- The viscosity of the mixture to be encapsulated increases with increasing concentration of extract and alginate, as the result drop shape capsules were obtained.
- Sea fennel is a rich source of calcium, which causes premature thickening when sodium alginate is added and as a result limits the extent to which the extract can be increased. Distilled water was used instead of drinking water to minimize the calcium concentration in the mixture.
- When the concentration of the extract increases and the concentration of alginate decreases, unstable capsules are obtained.
- Adjusting acidity and pectin concentration improves EE.
- An increase in the concentration of the extract should be accompanied by an increase in the concentration of alginate or pectin to maintain the loading efficiency of at least 50%.



- 2 formulations (W1, W2) were selected according to texture parameters and TPC encapsulation efficiency and therefore storage and stability study will be performed on them.

Samples	W1	W2
Water	50 mL	50 mL
Sodium Alginate	0.75 g	0.75 g
Extract	0.85 g	0.85 g
Sugar	0 g	1 g
Sodium Alginate %	1.5%	1.5%
Extract %	1.7%	1.7%
Sugar %	-	2%

Formulation of alginate-based capsules

- Due to its appealing color appearance, formulation W2 was selected for upscale production, sensory testing and further microbiological analyses including shelf-life.
- To evaluate capsule stability during shelf life, two storage conditions were compared: (L) capsules without any preservation (W2 formulation), and (B) capsules preserved in brine (W2 formulation in brine). Sensory analysis, microbiological and accelerated shelf life experiments were conducted on both formulations.

pH, aw, color and texture parameters

Samples	L	B

Day	0	30	0	30
pH	4.6	3.48	4,6	3.99
aw	0.96	0.93	0.94	0.92
L*	33.07 ± 3.06	31.32 ± 0.16	34.93 ± 1.74	36.21 ± 1.12
a*	2.39 ± 0.23	1.72 ± 0.12	2.62 ± 0.48	0.69 ± 0.12
b*	24.64 ± 1.07	22.24 ± 0.51	25.75 ± 1.33	14.61 ± 0.37
Hardness, g	374.94 ± 83.30	1602.63 ± 512.4	934.93 ± 283.92	453.5 ± 306.32
Cohesiveness	0.62 ± 0,13	0.64 ± 0.06	0.85 ± 0.19	0.80 ± 0.11
Springiness, mm	1.81 ± 0.34	2.43 ± 0.46	7.46 ± 3.09	2.68 ± 0.29
Adhesion, mJ	0.34 ± 0.34	0.53 ± 0.22	0.51 ± 0.19	0.44 ± 0.17

Edible capsules: physico-chemical, color and texture parameters. Data are reported as means of three replicas ± standard deviation
L - capsules without preservation (W2 formulation)
B - capsules preserved in brine (W2 formulation in brine)

The table above presents the pH, water activity (aw), color, and texture parameters of edible capsules formulated using the W2 matrix, with and without preservation in brine, measured at day 0 and day 30 of storage.

Water activity (aw) slightly decreased over time in both formulations, from 0.96 to 0.93 in non-brined capsules and from 0.94 to 0.92 in brined ones, indicating a slight reduction in available moisture.

Color parameters revealed changes over the storage period. Lightness (L*) decreased slightly in the non-preserved capsules (from 33.07 ± 3.06 to 31.32 ± 0.16), while it increased in brined samples (from 34.93 ± 1.74 to 36.21 ± 1.12), suggesting brining may have helped preserve or enhance brightness. The a* (red-green) and b* (yellow-blue) values declined in both groups, indicating a general fading of color over time. Notably, the decrease in b* values was more pronounced in the brined capsules (from 25.75 ± 1.33 to 14.61 ± 0.37), suggesting a loss of yellow hues during storage.

Texture profile analysis showed significant changes. The hardness of non-preserved capsules increased markedly from 374.94 ± 83.30 g to 1602.63 ± 512.4 g by day 30, indicating a firming effect over time. In contrast, the hardness of brined capsules decreased from 934.93 ± 283.92 g to 453.5 ± 306.32 g, likely due to softening effects of brine. Cohesiveness values remained relatively stable across both formulations, while springiness increased in the non-preserved capsules (from 1.81 ± 0.34 mm to 2.43 ± 0.46 mm) but decreased sharply in brined ones (from 7.46 ± 3.09 mm to 2.68 ± 0.29 mm). Adhesion values fluctuated slightly but without a consistent trend. Overall, storage and preservation method significantly influenced the physical and textural quality of the edible capsules.

Total Phenolic Content

Sample	**Mix**	**L**		**B**	
Day	0	0	30	0	30
TPC (mg GAE/g dw)	1.58±0.11	0.98±0.02	1.11±0.25	1.00±0.06	1.37±0.08

EE (%)	-	61.80	70.25	63.21	86.70

Total phenolic content of: Mix – mixture to be encapsulated, L - capsules without preservation, B - capsules preserved in brine Data are expressed as mean ± standard deviation (n= 3).

The data presented in the table above refer to the total phenolic content in the mixture prior to encapsulation, as well as in the edible capsules, both with and without preservation in brine. All samples were stored under dark and refrigerated conditions. Interestingly, the encapsulation efficiency of phenolic compounds in the capsules was found to be higher on day 30 compared to day 0, indicating improved retention or potential stabilization of phenolics over time.

Microbiological analyses results

Microbiological monitoring of sea fennel extract capsules stored under 4°over 30 days are presented in the table below. The results revealed distinct differences in microbial stability between samples preserved with sea fennel brine and those treated with lactic acid. The brine-preserved capsules (B) consistently exhibited no detectable growth of mesophilic aerobic bacteria, yeasts, spore-forming bacteria or Enterobacteriaceae across all time points, indicating strong antimicrobial efficacy.
Conversely, the lactic acid-treated samples (L) demonstrated progressive microbial proliferation, particularly from day 15 onward. Mesophilic aerobic counts increased steadily from 2.83 log CFU g^{-1} at day 15 to 6.49 log CFU g^{-1} by day 30, and yeasts were already present on day 5, reaching levels above 6 log CFU g^{-1} by day 25. Spore-forming bacteria appeared later reaching 2.24 log CFU g^{-1} by day 30. However, Enterobacteriaceae remained undetected throughout.
The results of enumeration of *Escherichia coli, Listeria monocytogenes* and *Clostridium perfringens* at day 0 *showed* counts below detection limit in all samples.
These findings suggest that the brine provided a more robust antimicrobial barrier than lactic acid, potentially due to its intrinsic bioactive compounds and saline properties.

Viable counts of control and brine preserved capsules.

Microbial group	**Sampling time**	**Prototypes**	
Mesophilic aerobic bacteria (Log CFU g^{-1})		B	L
	t_0	< 1.0	< 1.0
	t_5	< 1.0	< 1.0
	t_{10}	< 1.0	< 1.0
	t_{15}	< 1.0	2.83 ± 0.69^c
	t_{20}	< 1.0	5.05 ± 0.12^b
	t_{25}	< 1.0	6.40 ± 0.06^a
	t_{30}	< 1.0	6.49 ± 0.07^a
spore-forming bacteria (Log CFU g^{-1})			
	t_0	< 1.0	< 1.0
	t_5	< 1.0	< 1.0
	t_{10}	< 1.0	< 1.0
	t_{15}	< 1.0	< 1.0
	t_{20}	< 1.0	< 1.0
	t_{25}	< 1.0	< 1.0
	t_{30}	< 1.0	2.24 ± 2.00^a

Yeasts (Log CFU g^{-1})			
	t_0	< 1.0	< 1.0
	t_5	< 1.0	2.38 ± 0.14^{cd}
	t_{10}	< 1.0	1.69 ± 0.06^{d}
	t_{15}	< 1.0	2.87 ± 0.01^{c}
	t_{20}	< 1.0	4.94 ± 0.03^{b}
	t_{25}	< 1.0	6.19 ± 0.11^{a}
	t_{30}	< 1.0	5.71 ± 0.11^{ab}
Enterobacteriaceae (Log CFU g^{-1})			
	t_0	< 1.0	< 1.0
	t_5	< 1.0	< 1.0
	t_{10}	< 1.0	< 1.0
	t_{15}	< 1.0	< 1.0
	t_{20}	< 1.0	< 1.0
	t_{25}	< 1.0	< 1.0
	t_{30}	< 1.0	< 1.0

The results were expressed as the mean of three replicates ± standard deviation.
B: sea fennel capsules with brine; L: sea fennel capsules with lactic acid.
For each microbial group and sample, overall means with different small superscript letters in the same column are significantly different ($p < 0.05$).

Accelerated shelf-life test

The results of microbial counting determined during the ASLT test are presented in the table below.
The accelerated shelf-life test confirms the efficiency of sea fennel brine under thermal stress.
Throughout the 30-day incubation at 37 °C, the brine-treated capsules (B) remained microbiologically stable, showing no detectable growth of mesophilic aerobes, yeasts, spores, or Enterobacteriaceae.
In contrast, lactic acid-treated samples (L) were more susceptible to microbial growth. A notable peak in mesophilic aerobes was recorded on day 15 (7,50 log CFU g^{-1}), Yeasts and Enterobacteriaceae were transiently present at day 5, with yeast levels at 2,80 log CFU g^{-1} and Enterobacteriaceae increasing to 7,39 log CFU g^{-1} by day 15. Spore-forming bacteria were not detected during the storage period.
The consistent absence of microbial growth in B highlights the potential of sea fennel brine as a natural preservative, outperforming lactic acid in both static and stressed storage conditions.

Viable counts results during accelerated shelf-life test ASLT of control and brine preserved capsules.

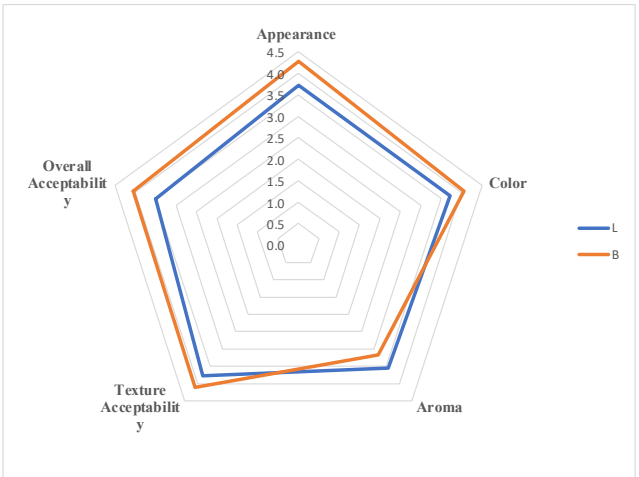
Microbial group	**Sampling time**	**Prototypes**	
Mesophilic aerobic bacteria (Log CFU g^{-1})		B	L
	t_0	< 1.0	< 1.0
	t_5	< 1.0	< 1.0
	t_{15}	< 1.0	7.50 ± 0.38^{a}
	t_{30}	< 1.0	$6.79 \pm 0.05^{a,b}$
spore-forming bacteria (Log CFU g^{-1})			

	t_0	< 1.0	< 1.0
	t_5	< 1.0	< 1.0
	t_{15}	< 1.0	< 1.0
	t_{30}	< 1.0	< 1.0
Yeasts (Log CFU g^{-1})			
	t_0	< 1.0	< 1.0
	t_5	< 1.0	2.80 ± 0.73^{a}
	t_{15}	< 1.0	< 1.0
	t_{30}	< 1.0	< 1.0
Enterobacteriaceae (Log CFU g^{-1})			
	t_0	< 1.0	< 1.0
	t_5	< 1.0	3.92 ± 0.88^{c}
	t_{15}	< 1.0	7.39 ± 0.52^{a}
	t_{30}	< 1.0	6.77 ± 0.05^{ab}

The results were expressed as the mean of three replicates ± standard deviation.
B: brine preserved capsules
L: lactic acid preserved capsules (control)
For each microbial group and sample, overall means with different small superscript letters in the same column are significantly different ($p < 0.05$).

Sensory Evaluation

The sensory evaluation results of the freshly prepared capsules (without preservation) and those preserved in brine are presented in the spider plot below. The assessed parameters included appearance, aroma, texture, color, and overall acceptability. The sensory evaluation revealed that the capsules preserved in brine were generally preferred over the freshly prepared capsules in most attributes. Specifically, brine-preserved capsules received higher scores for appearance (4.3 vs. 3.7), color (4.1 vs. 3.7), texture acceptability (4.1 vs. 3.8), and overall acceptability (4.1 vs. 3.5). However, the freshly prepared capsules scored slightly higher in aroma (3.6 vs. 3.2), suggesting that preservation in brine may slightly affect aromatic perception.



Sensory evaluation (scale 1-5) for edible capsule with sea fennel extract (1.7%)
L - capsules without preservation (W2 formulation)

B - capsules preserved in brine (W2 formulation in brine)

1.1.3 Exploitation of the extracts as nutraceuticals

UNIVPM (P1) CREA-AN (P2) AND RINCI (P3)

Material and methods

Spray-drying of aqueous extract

Aqueous extract from by-product of sea fennel was produced under agitation for 18 h and/or with ultrasonic bath (30 min) using 10g of by-product sample and 500 mL distilled water. Each 10 mL of extract 1 g of maltodextrin was added as carrier of the spray-drying process.

A MiniSpray-Dryer S-300 apparatus at pilot scale was used for the operation of spray-drying and the parameters of spray-drying were studied.

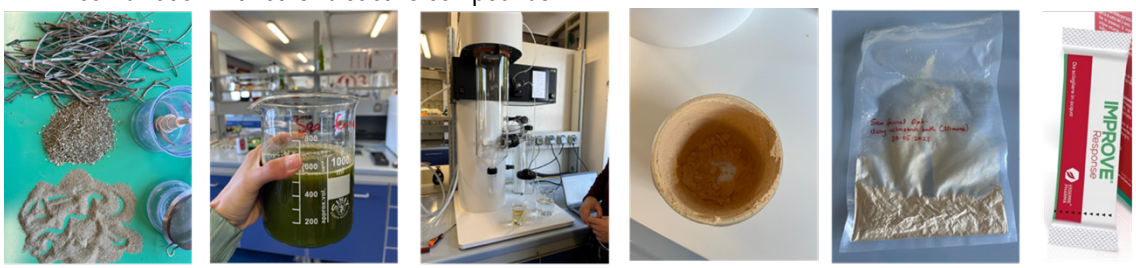
MiniSpray-Dryer S-300 apparatus

Results

The optimal parameters for the sea fennel aqueous extract were found as follows:

- Ratio 1:20 w/v (ex 10 g + 200 mL of water)
- Room temperature
- Ultrasound assisted extraction 30 min
- Filtration
- Add 1 g of maltodextrin (MD) each 10 mL extract (ratio 1:10)
- Spray-dry MiniSpray-Dryer S-300 apparatus
- Drying gas = 32 mL/h
- Inlet T = 160°C
- Spray Gas = 1000 L/h
- Feeding solution rate = 10 mL/min

- Encapsulation efficiency of 85% of aqueous extract thus this spray-dry powder could be potentially used in the formulation of nutraceuticals to prevent diseases associated with oxidative stress and hyperglycemic conditions in combination with other bioactive compounds.



Production steps of possible nutraceutical from sea fennel by-product

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Maoloni A, Milanović V, Osimani A, Cardinali F, Garofalo C, Belleggia L, Foligni R, Mannozzi C, Mozzon M, Cirlini M, et al., 2021. Exploitation of sea fennel (Crithmum maritimum L.) for manufacturing of novel high-value fermented preserves. Food Bioprod Process. 127:174–197
Fanesi, B.; Ismaiel, L.; Nartea, A.; Orhotohwo, O.L.; Kuhalskaya, A.; Pacetti, D.; Lucci, P.; Falcone, P.M. Bioactives and Technological Quality of Functional Biscuits Containing Flour and Liquid Extracts from Broccoli By-Products. Antioxidants 2023, 12, 2115, doi:10.3390/ANTIOX12122115/S1.

1.2 UNIST - Croatia

1.2.1 Preparation and analysis of extracts from sea fennel crop by-products

1.2.1.1 Novel vs. Conventional extraction methods, solvent choice

Materials and Methods

Extraction

The aerial parts of sea fennel plants (Crithmum maritimum L.) collected in May 2022 were used as plant material. Young and healthy green leaves and shoots, about one kilogram of fresh material from the same population, were selected for the study, while the woody parts of the plant were discarded. The plant material was frozen and dried by lyophilization, and the dry plant material was homogenized in a hand mill and sieved through a 1 mm diameter sieve for 20 min.

To prepare the extracts, 10 g of the powdered plant material was mixed with 100 mL of solvent. Different water–ethanol mixtures were used for the preliminary experiments: 0%, 25%, 50%, and 75% aqueous ethanol (v/v). To compare different extraction methods, the CSE, MAE, and UAE methods were performed. MAE was carried out using ETHOS X (Milestone Srl, Bergamo, Italy), while for the UAE, a Transsonic 310/H ultrasonic bath (Elma, Singen, Germany) was used. Nine samples were extracted with water–ethanol at a 1:1 ratio (v/v) for 30 min. For each extraction method, three samples were extracted under different conditions (Table 1). After each extraction, the suspensions were filtered through folded filter paper, and the resulting filtrates were stored at a temperature of +4◦C for 24 h, then filtered again and used for further analyses.

Table 1. The parameters of the applied microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and conventional solvent extraction (CSE) of the samples.

Sample	Extraction Method	Time	Conditions	
A	MAE	30 min	Power	300 W
B				500 W
C				700 W
D	UAE		Temperature	RT
E				40 °C
F				60 °C
G	CSE		Temperature	RT
H				40 °C
I				60 °C

RT—room temperature.

Total phenolic content and HPLC analysis of individual phenols

The highest TPC was obtained with MAE (from 25.91 mg GAE/g at 700 W to 28.80 mg GAE/g at 300 W), while UAE appeared to be the extraction method that achieved the lowest content of extracted phenols (from 18.46 mg GAE/g at 40 °C to 19.97 mg GAE/g at 60 °C). The CSE method resulted in a TPC of 20.61 mg GAE/g at RT and 23.41 mg GAE/g at 60 °C. Similar to the TPC, the highest TFC was obtained using MAE (from 89.61 mg RE/g d.p.m. at 700 W to 102.95 mg RE/g d.p.m. at 300 W). The other two extraction methods, UAE and CSE, showed similar results, with slightly higher values observed for CSE. The amount of total flavonoids extracted ranged from 77.69 mg RE/g d.p.m. at 60 °C to 81.75 mg RE/g d.p.m. at RT when UAE was used, and from 76.56 mg RE/g d.p.m. at RT to 85.20 mg RE/g d.p.m. at 40 °C when the CSE method was used. The TTC was pproximately equal for MAE at 700 W (5.63 mg CE/g d.p.m.) and UAE at RT (5.67 mg CE/g d.p.m.)

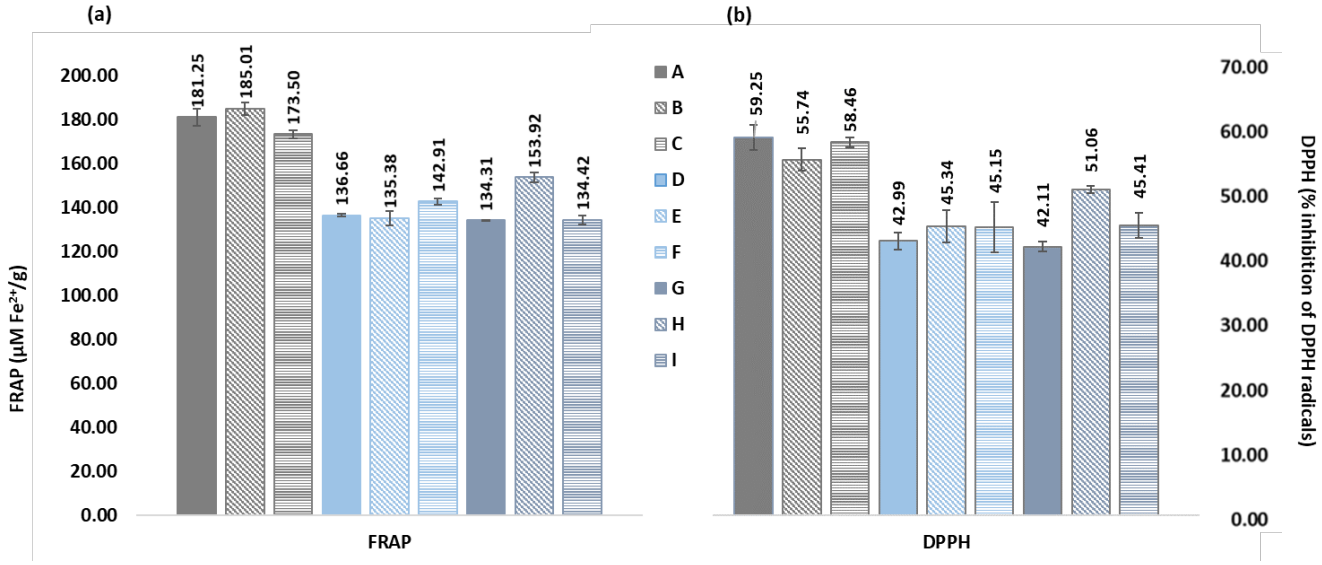
Total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC), expressed as equivalent (mg eq./g d.p.m.), in sea fennel extracts obtained by different extraction methods.

Type of extraction	Condition	TPC (mg GAE/g d.p.m.)	TFC (mg RE/g d.p.m.)	TTC (mg CE/g d.p.m.)
MAE	300 W	28.80 ± 0.88	102.95 ± 1.01	3.68 ± 0.10
	500 W	27.17 ± 0.42	97.83 ± 1.29	3.14 ± 0.08
	700 W	25.91 ± 0.45	89.61 ± 1.04	5.63 ± 0.06
UAE	RT	19.96 ± 0.52	81.75 ± 2.62	5.67 ± 0.09
	40 °C	18.46 ± 0.83	80.53 ± 0.35	3.87 ± 0.09
	60 °C	19.97 ± 0.58	77.69 ± 0.56	3.34 ± 0.02
CSE	RT	20.61 ± 077	79.56 ± 0.84	4.17 ± 0.13
	40 °C	23.41 ± 0.57	85.20 ± 0.76	3.35 ± 0.14
	60 °C	20.87 ± 0.53	82.96 ± 2.05	3.40 ± 0.09

GAE - galic acid equivalents, RE - rutin equivalents, CE - catechin equivalents, d.p.m. - dry plant material, RT - room temperature, MAE -microwave-assisted extraction, UAE - ultrasound-assisted extraction, CSE - conventional solvent extraction.

HPLC-UV/VIS analysis was performed for the qualitative and quantitative detection of phenolic compounds. Of the ten compounds detected, nine were non-flavonoids (phenolic acids), and only one flavonoid compound was detected (rutin). CGA was the dominant phenolic constituent in all samples, with the highest content achieved when using MAE (from 10.10 mg/g at 700 W to 10.67 mg/g at 500 W). CSE, as the second most effective extraction method, achieved rates from 81% at 60 °C to 92% at 40 °C, and UAE demonstrated rates from 78% at 60 °C to 81% at RT, the most effective temperature for MAE.
Rutin (R), the only flavonoid detected and the second most abundant phenolic compound, was maximally extracted using MAE (0.42 mg/g at 700 W to 0.45 mg/g at 500 W). As for CGA, the CSE method was more effective than the UAE method, with a yield of 88% at 40 °C, and the content achieved by MAE was the highest. The two phenolic acids, cryptochlorogenic acid (cCGA) and neochlorogenic acid (nCGA), were extracted most effectively by the MAE method. The concentration values for cCGA ranged from 0.37 mg/g at 300 W to 0.39 mg/g at 700 W, and those for nCGA ranged from 0.16 mg/g at 300 W to 0.17 mg/g at 700 W. The second-best conditions for the extraction of cCGA and nCGA were CSE at 40 °C.

Antioxidant activity:
As expected, the highest antioxidant activity obtained by both the FRAP (173.50–185.01 µmol Fe^{2+}/g) and DPPH assays (55.74–58.46% inhibition of DPPH radicals) was observed in the samples extracted by the MAE method. The second-best antioxidant potential was observed for samples extracted by the CSE method and mixed at 40 °C, with 153.92 µmol Fe^{2+}/g (FRAP) and 51.06% inhibition of DPPH radicals (DPPH). This was consistent with the analysis of the phenolic compounds, as studies have confirmed that phenolic compounds have important antioxidant properties in plants, especially chlorogenic acid and its derivatives.



The free-radical scavenging activity of extracted samples obtained using (a) ferric reduction antioxidant power (FRAP) and (b) 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (mean ± SD, n = 3). Extraction conditions: MAE (A – 300 W; B – 500 W; C – 700 W), UAE (D – RT; E – 40 °C; F – 60 °C), CSE (G – RT; H – 40 °C; I – 60 °C).

Main conclusions:
In this study, the effects of different extraction methods on the extraction of phenolic compounds from sea fennel were investigated.

- the maximum amount of extracted phenolic compounds was obtained with a mixture of water and ethanol at a ratio of 1:1 (v/v),
- microwave-assisted extraction showed the highest efficiency in phenolic extraction,
- a higher microwave power resulted in a lower phenolic content in extracts
- temperature effect was observed for conventional solvent extraction, and the highest content of phenolics was obtained in the extract prepared at 40 °C,

- high-performance liquid chromatography revealed chlorogenic acid as the dominant phenolic compound in all samples, followed by rutin a
- the highest antioxidant potential was detected for the samples extracted by microwave-assisted extraction.

1.2.1.2 Accelerated Solvent Extraction, solvent choice, temperature effect

Material and methods

Extraction

Sea fennel was harvested in June 2023 on the island of Čiovo, Croatia (43°30'N 16°17'E). The fresh plant material was cleaned and washed with tap water to remove dirt. The leaves were then manually removed and stored at -18°C. The frozen leaves were freeze-dried (FreeZone 2.5 L, - 50 °C, Labconco, Kanzas City, MO, USA) and ground to a powder using a commercial (coffee) grinder (Delimano, Croatia). Pressurized liquid extraction (accelerated solvent extraction, ASE) was performed using a Dionex™ ASE™ 350 extractor (Thermo Fisher Scientific Inc., Sunnyvale, CA, USA). In this experiment, the solvent (ethanol concentration) and extraction temperature were optimized as shown in Table.

Pressurized liquid extraction parameters

Eluent content (%)	Extraction temperature (°C)					
	20	40	60	80	100	120
20	1A/A*	2A/A*	3A/A*	4A/A*	5A/A*	6A/A*
40	1B/B*	2B/B*	3B/B*	4B/B*	5B/B*	6B/B*
60	1C/C*	2C/C*	3C/C*	4C/C*	5C/C*	6C/C*
80	1D/D*	2D/D*	3D/D*	4D/D*	5D/D*	6D/D*

For extraction, the 22 mL stainless steel cells (Dionex™, Thermo Fisher Scientific Inc., Sunnyvale, CA, USA) were fitted with cellulose filters (Dionex™, Thermo Fisher Scientific Inc., Sunnyvale, CA, USA) to prevent clogging of the metal frit and to prevent the sample particles from entering the extracts. Samples were prepared by mixing 1 ± 0.01 g of plant material with 2 g of diatomaceous earth (Thermo Fisher Scientific Inc., Sunnyvale, CA, USA) and covered with an additional 1 g of diatomaceous earth to fill the cells. The first step of the extraction procedure was to fill the extraction cell with the selected solvent until a pressure of 1500 psi (10.34 MPa) was reached. Then the instrumentally determined heating time was applied depending on the extraction temperature (i.e. 5 minutes for temperatures up to 100 °C and 6 minutes for 100 and 120 °C). Static extraction was performed for 5 minutes for all samples. During heating and static extraction, the static valve opened at 11.72 MPa (1700 psi), releasing a small amount of solvent each time. After static extraction, the cells were flushed with a fresh volume of solvent equal to 60% of the cell volume. Finally, the cells and lines were purged with nitrogen gas for 90 seconds. After each extraction, the system was thoroughly flushed to avoid contamination between experiments. Extractions were performed in duplicate and extracts were collected in 60 mL glass vials with Teflon-lined septa and stored at +4 °C for 24 hours to precipitate tannins. The ethanol from the combined extracts was evaporated using a rotary evaporator (Heidolph, Schwabach, Germany) and the extracts were then freeze-dried. For the analyses, the extracts were diluted with 50% ethanol solution at various concentrations between 0.01 and 10 mg/mL depending on the sensitivity of the method.

Total phenolic content and HPLC analysis of individual phenols

Total phenolics were determined using the Folin-Ciocalteau method as previously described, same as individual phenolics by HPLC method. The total phenolic content (TPC) in the samples was calculated using a gallic acid standard calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry plant material (mg GAE/g DM) and individual compounds are expressed in milligrams of compound per gram of dry extract (mg/g).

Antioxidant activity

DPPH and FRAP method procedures are described.

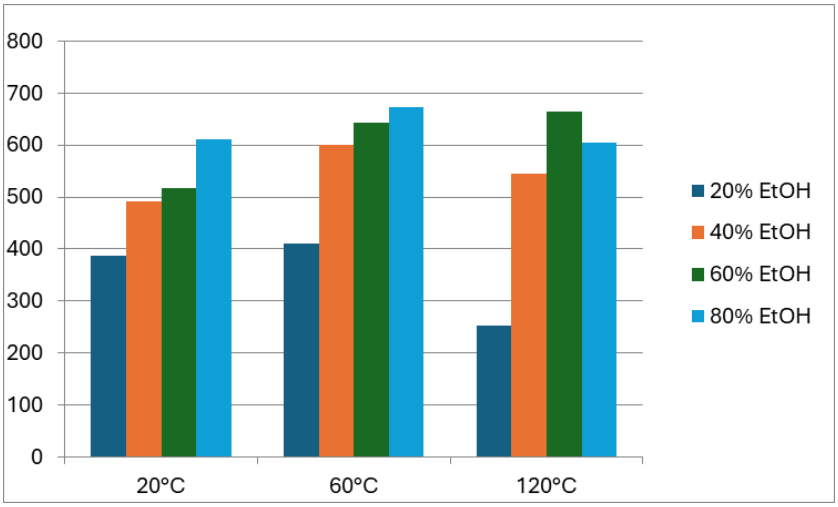
The ORAC (Oxygen Radical Absorbance Capacity) method is used to measure the ability of antioxidants to protect the fluorescent probe (fluorescein) from free radical damage, particularly peroxyl radicals produced by the spontaneous decomposition of AAPH [2,2'-azobis(2-amidino-propane) dihydrochloride] (AOAC, 2013). Fluorescence measurements were performed using a Synergy HTX Multimode Reader spectrofluorimeter (BioTek, Charlotte, VT, USA); excitation was measured at 495 nm and emission at 520 nm. Prior to the experiment, the spectrofluorimeter was heated to 37 °C so that the rate of radical generation was constant. For the measurements, which were performed in sextuplicates, 150 µL of fluorescein solution and 25 µL of the samples were mixed in a black microplate well and thermostated at 37 °C. After 30 minutes, 25 µL of AAPH solution was added and the fluorescence intensity was measured every minute for 80 minutes.

Antimicrobial activity

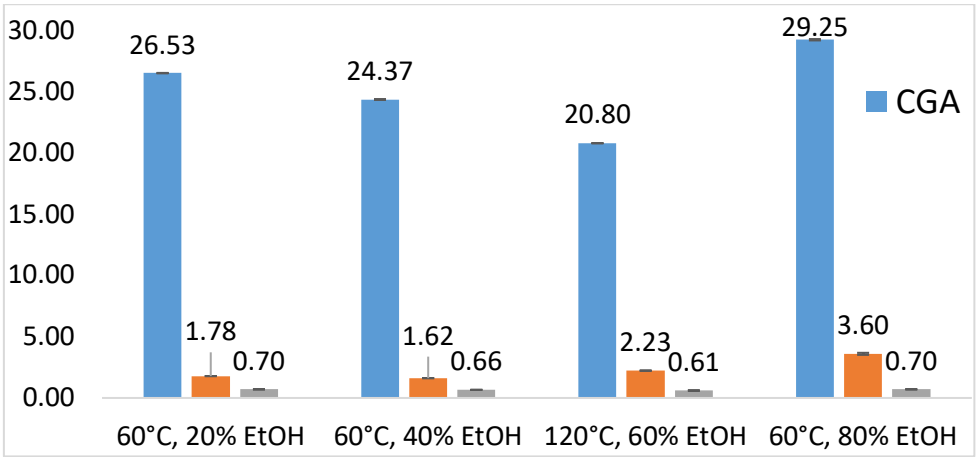
The bacterial strains Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Listeria monocytogenes ATCC 7644, Bacillus cereus ATCC 14579 and Pseudomonas aeuriginosa ATCC 27853 were used for antibacterial testing in this study. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the microdilution method for sea fennel extracts dissolved in 50% EtOH at a concentration of 10 mg/mL. The MBC method was performed according to the method previously described by Skroza et al. In brief, 100 µL of the diluted extracts (mixture of Mueller–Hinton broth (MHB) and extracts in a 1:1 ratio) were added to the first wells of the 96-well microtiter plate and diluted twofold in the next wells. After addition of the 50 µL inoculum (1×106 colony forming units (CFU)/mL), each plate was shaken for 1 minute on a microtiter plate shaker at 600 rpm (plate shaker thermostat PST-60 HL, Biosan, Riga, Latvia). The positive control (50 µL inoculum and 50 µL broth), the negative control (50 µL broth and 50 µL extract), the blank sample (100 µL broth) and 50% EtOH were tested. After 24 hours at 37 °C, 20 µL of the bacterial metabolic activity indicator, 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (2 mg/mL), was added. The MIC values were visually determined as the lowest concentration of the extract at which no suppression of bacterial growth was detected by the reduction of INT to red formazan. The MBC was determined as the lowest concentration at which no microbial growth was detected in the wells where the MIC was determined and in the wells with higher concentrations of the extract. All measurements were repeated in triplicate and the mean values are given in the tables/figures.

Results

TPC increases evenly at all ethanol content at 60 °C, reaching a maximum of 673.16 mg/L in 80% ethanol, but the results become less persistent at 120 °C.



Graphical representation of the TPC change at different conditions (temperatures and pressure combinations).



Content of CGA (chlorogenic acid), cCGA (criptochlorogenic acid) and nCGA (neochlorogenic acid) in ASE samples

Sea fennel is known for its richness in hydroxycinnamic acids (ref), and chlorogenic acid (CGA) is the most prevalent among the secondary metabolites. Of the analysed samples, CGA had concentrations from 7.87 mg/g in sample 6A (120°C, 20% ethanol) to 29.25 mg/g in sample 3D (60°C, 80% ethanol). This indicates that moderate thermal conditions at 60 °C coupled with a high ethanol concentration of 80% are optimal for the extraction of CGA, whereas higher temperatures (120 °C) may reduce extraction efficiency due to likely thermal degradation.

Antioxidant activity of sea fennel extracts

Extraction conditions	DPPH (%)	FRAP (mM Fe^{2+})	ORAC (0.01mg)	NO (%)
60°C, 20% EtOH	13.79 ± 0.6	0.154 ± 0.003	38.41 ± 2.63	3.838 ± 0.604
60°C, 40% EtOH	9.78 ± 0.25	0.261 ± 0.003	51.91 ± 5.08	6.371 ± 1.083
120°C, 60% EtOH	16.56 ± 0.54	0.282 ± 0.005	50.30 ± 2.48	N/A
60°C, 80% EtOH	19.25 ± 0.33	0.283 ± 0.005	43.64 ± 1.92	N/A

The antioxidant activity of extracts varied based on ethanol concentration and extraction temperature. The highest DPPH radical scavenging activity was observed at 60°C with 80% EtOH (19.25%), followed by 120°C with 60% EtOH (16.56%). The lowest activity was recorded at 60°C with 40% EtOH (9.78%). The reducing power, measured through the FRAP assay, increased with ethanol concentration, reaching the highest value (0.283 mM Fe^{2+}) at 60°C with 80% EtOH. The ORAC assay showed the highest oxygen radical absorbance capacity at 60°C with 40% EtOH (51.91), while the lowest was observed at 60°C with 20% EtOH (38.41). The NO scavenging assay revealed the highest nitric oxide scavenging effect at 60°C with 40% EtOH (6.371%), while the lowest was at 60°C with 20% EtOH (3.838%). Data for higher ethanol concentrations were not available.

Antimicrobial activity of sea fennel extract described as MIC and MBC values (mg/mL).

	E. faecalic	*B. cereus*	*L. monocytogenes*	*E. coli*	*P. aeruginosa*
3A	nd/nd	nd/nd	1.25/2.5	nd/nd	2.5/nd
3B	nd/nd	1.25/2.5	1.25/2.5	2.5/nd	1.25/2.5

6C	1.25/1.25	1.25/2.5	0.62/0.62	2.5/nd	1.25/2.5
3D	1.25/1.25	2.5/2.5	0.62/0.62	nd/nd	1.25/2.5

nd—not determined, >2.5 mg/mL.

Among the Gram-positive bacteria, *L. monocytogenes* appeared to be one of the most susceptible species, as samples 6C and 3D showed the strongest inhibitory effect with MIC and MBC values of 0.62 mg/mL. Samples 3A and 3B were also effective against these pathogenic bacteria, but at twice the concentration (1.25 mg/mL). The strong activity against *E. faecalis* was noticed samples 6C and 3D, while samples 3A and 3B showed no activity. For *B. cereus*, only sample 3A was inactive, while the other samples had MIC values of 1.25 mg/mL.

1.3 INRGREF – Tunisia

1.3.1 Preparation and analysis of extracts from sea fennel crop by-products

Materials and Methods

Woody stems, old leaves, and flowers of sea fennel plants (*Crithmum maritimum L.*) were collected in September 2023. The plant material was dried and ground.

Extracts were prapared by mixing 15 g of the powdered plant material (mixture of leaves, stems and flowers) with 100 mL of solvent. Different solvents were used (ethanol/water (80%), methanol/water 80%, acetone/water 80% and water). After 24 h of maceration, the suspensions were filtered using filter paper, dried and then resuspended in the appropriate solvent and used for further analyses.

Preparation of extracts from sea fennel by-products.

Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu method (1965). 0.5 mL of extract sample was mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10) and 2 mL of 7.5% sodium carbonate. The absorbance was measured at 765 nm after

incubation in the dark for 30 min. Gallic acid was used as standard. The total polyphenol content was expressed as milligrams of gallic acid equivalents (GAE) per gram of the sample.

The total flavonoid content

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method (Quettier Deleu et al. 2000). 1 ml of sample was mixed with 1 ml of 2% aluminum chloride methanolic solution. The mixture was allowed to stand for 15 min, and absorbance was measured at 430 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per G of DM (mg RE/g).

Total condensed tannins

The method described by Broadhurst and Jones (1978) was used to determine the total condensed tannin content in Sea Fennel. 0.5 ml of the extract was mixed with 3 ml of vanillin (4% in methanol) and 1.5 ml of Hydrochloric acid. After incubation for 15 min at 20◦C in the dark, the absorbance was read at 500 nm. The condensed tannin content was calculated from a calibration curve prepared with a solution of catechin (30 ppm). The results were expressed in mg of catechin equivalent per g of DM (mg CE/g).

Antioxidant activity

DPPH Test

The effect of the different extracts on DPPH radical was studied, employing the method described by Brand-Williams et al. (1995). Briefly, 5 ml of DPPH solution (0.004%, in Methanol) was incubated with the extracts. The reaction mixture was shaken well and incubated for 30 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where: Ac—Control reaction absorbance; As—Testing specimen absorbance.

Biological activities

α-amylase inhibitory test

The α-amylase inhibitory potentials were determined using radial diffusion method. A stock solution was prepared with 0.1786 g of α-amylase from Aspergillus oryzae in 1 mL of 20 mM phosphate buffer with 6 mM NaCl, pH 6.9. Twenty-seven plates containing 25 mL of agar-starch were prepared. To each plate, 20 µL of α-amylase solution mixed with 20 µL of extract were inoculated, leading to the formation of clear diffusion zones after 4 hours of incubation at 20 °C. The results were expressed as inhibition (%). The positive control used in this study was α-amylase inhibitor from seeds of wheat (Triticum aestivum).

Antifungal activity

Fungal Strains

The antifungal activity of Acacia oils was tested against three fungal strains: Alternaria alternate, Ulocladium atrum and Aspergillus heteromorphus.

Antifungal test

The culture was made on a PDA medium at the rate of 20 ml per Petri dish. The extract was added in the medium at the rate of 20 µl of extract per Petri dish.

After cooling the medium, a disc of 5 mm in diameter of each fungal strain was placed in the center of the Petri dish while placing the mycelial surface down. The plates were incubated at 22°C for five days. The fungicidal effect was determined by calculating the growth diameter of the strain in question and comparing it to that of a negative control, i.e. an oil-free PDA medium (Cakir et al., 2004).

The results were calculated according to the method of Singh et al. (1993) while calculating the percentage inhibition (I) according to the following formula:

I(%) = [(dC-dE) /dC] x 100
Where:
dC: witness diameter (mm)
dE: diameter in the presence of the tested extract (mm)

Anti-inflammatory activity

To study the potential anti-inflammatory effects of Sea Fennel by product extract, the albumin denaturation assay was utilized as described by Alhakmani et al. (2014), with Aspirin® serving as a control. To perform this assay, a reaction mixture of 500 µl of extract or the standard and 700 µl of phosphate-buffered saline (pH 6.4) was combined with 500 µl of fresh egg albumin. The reaction mixture was then incubated for 15 min at 27 ± 1°C. Denaturation of albumin was induced by heating the mixture in a hot bath at 70°C for 10 min. After cooling, the absorbance at 660 nm was measured using distilled water as a blank. The inhibition percentage of egg Albumin denaturation was calculated using the following formula:
Albumin Inhibition (%) = [(ASample-AControl)/ AControl] x100

Results

Results of yield and total polyphenol, tannins and flavonoids content are summarized the table below.
The aqueous extract showed the highest extract yield while acetone extract showed the lowest value.

Extract	Yield (%)	Total phenols	Condensed Tanins	Total flavonoids
Ethanol	9.68	45,21	141,79	119,97
Methanol	5.03	60,79	233,46	205,26
Acetone	0.29	141,43	980,78	250,03
Water	17.91	40,32	637,51	217,77

Yield and total polyphenol content of sea fennel by-products.

Antioxidant activity

The methanolic extract showed the highest antioxidant activity (80%) while the aqueous extract showed the lowest value (36%).

Biological activities

α-amylase inhibitory test
The ethanolic extract showed the highest inhibition.
No activity was recorded for both acetone and aqueous extracts.

Antifungal activity

All extracts showed an inhibitory effect against all the studied strains.
Alternaria alternate was the most sensitive strain while Ulocladium atrum was the most resistant.

Anti-inflammatory activity

The highest inhibitory effect of albumin was recorded by the aqueous extract (60%).

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1.3.2 Use of sea fennel by-products in animal feed formulas (concentrates or vegetable flours) based on sea fennel or mixed with other products.

Materials and Methods

The experiment consists on assessing the effect of sea fennel by-products on the average daily mass gain of rabbits produced in a semi-automated farm.
Three formulas were considered for the test:

- Control: commercialised concentrate.
- Treatment 1: 5% dried sea fennel was added to commercialised concentrate.
- Treatment 2: 5% dried sea fennel was added to commercialised concentrate.

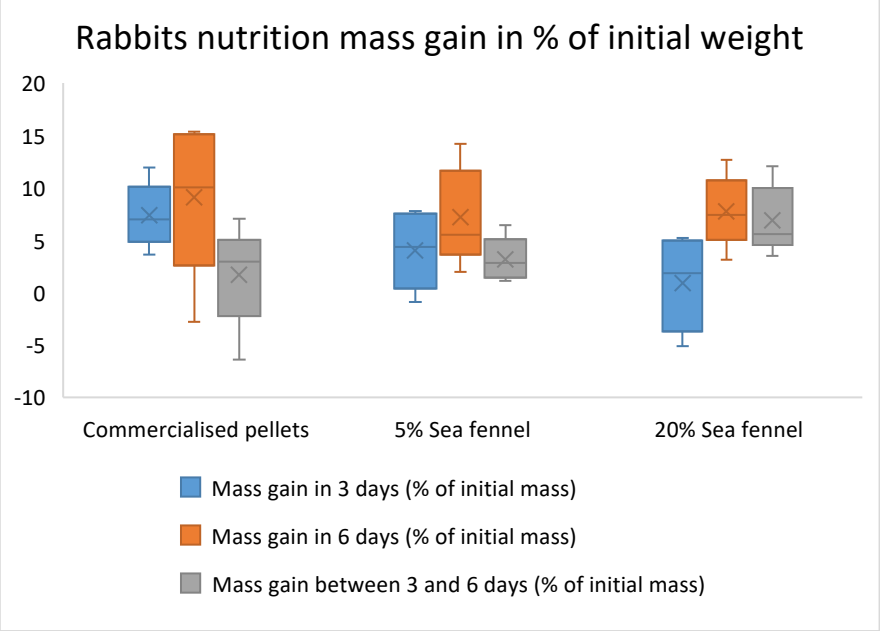
Sea fennel by-products were dried at 40°C, grinded, and mixed with their equivalent mass on pellets.
The mix was then homogenised and reworked. The control was also reworked in order to have the same aspects of the new pellets. Pellets were dried at air temperature for 24 hours.

Production process of pellets

Five rabbits were used for each set, aged between 35 and 40 days. Animals initial weight was considered in order to follow their mass gain.

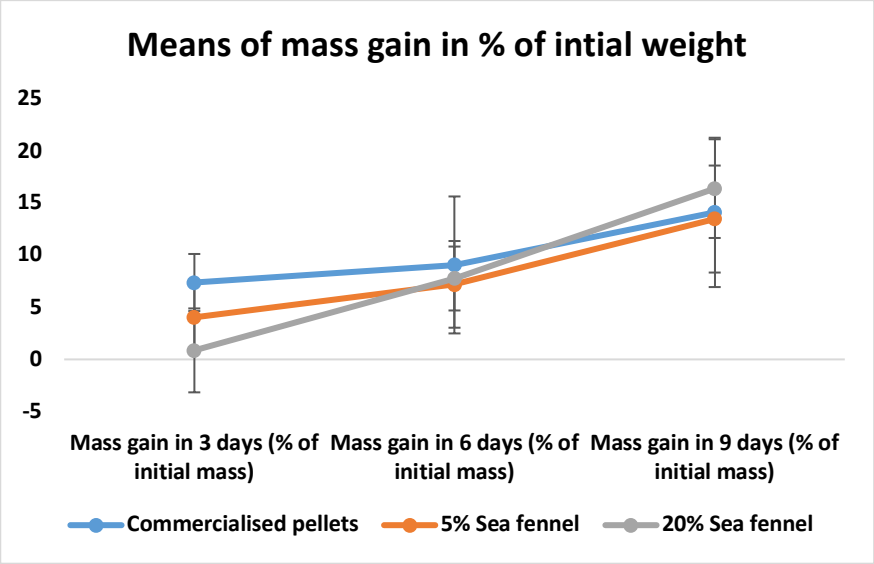
Every rabbit was put in a singular production cage equipped with and automatic water dispenser and a rabbit feeder. Ventilation and luminosity regimes are programmed depending on the age of animals and season.
All rabbits were vaccinated with 1ml of Cubolac-clostridium-vaccine before changing their nutritional regime.
All animals are fed at the same time with an increasing quantity of pellets depending on the age of the animals. A ratio varies between 100g and 150g per day was administrated for each rabbit. The rest of the ratio was quantified and the feeders cleaned every day prior to feeding in order to assess the palatability of the pellets. Animals are weighted every 3 days.

Results
Preliminary observations indicated that the pellets enriched with sea fennel by-products were highly palatable to rabbits, demonstrating good voluntary intake. The preliminary test results of rabbit mass gain could be broken down into three time-based categories: mass gain after 3 days, mass gain after 6 days, and mass gain between days 3 and 6. Rabbits fed with commercialised pellets exhibited the highest average total mass gain after 6 days, with values approaching 15% of their initial weight, although the variation was also quite large. They also showed steady growth from day 3 to day 6. In contrast, rabbits on a diet containing 5% sea fennel experienced moderate weight gain. Their 6-day gain was slightly lower than the commercial diet group, but the gain was more consistent and less variable. Interestingly, the weight gain between day 3 and day 6 was positive but smaller than with the commercial diet, suggesting a more gradual growth. Rabbits fed with 20% sea fennel showed the lowest initial mass gain in the first 3 days, with some animals even losing. However, they had a strong recovery between days 3 and 6. By day 6, their overall weight gain was similar to the 5% sea fennel group, although more variable. In summary, while commercial pellets yielded the highest overall weight gain, 5% sea fennel provided more stable growth, and 20% sea fennel, despite a slow start, led to comparable gains by day 6. This suggests that sea fennel can be a viable dietary supplement, particularly at 5%, but higher concentrations might temporarily slow early growth before compensating later.



Rabbits nutrition mass gain in % of initial weight

The line chart illustrates the mean mass gain (as a percentage of initial weight) in rabbits over 3, 6, and 9 days, under the same dietary treatments. Initially, rabbits fed with commercialised pellets showed the highest weight gain (~7.5%) at day 3, while those on the 20% sea fennel diet had the lowest (~1%). However, by day 6, all groups reached comparable mass gains (~7–9%), indicating a catch-up effect in sea fennel diets. Notably, by day 9, rabbits on the 20% sea fennel diet surpassed the others with the highest mean weight gain (~17%), followed closely by those on pellets and 5% sea fennel (~13–14%). These trends suggest that while commercial pellets promote faster early growth, higher sea fennel content—despite a slower start—can lead to superior long-term weight gain, reflecting a delayed but potent nutritional effect.



Rabbits nutrition Means mass gain in % of initial weight

1.3.3 Use of sea fennel aqueous extracts for animal nutrition and evaluation of its effect on meat quality.

This part of the study was not conducted due to the limited availability of plant material, which prevented the preparation of sufficient quantities of aqueous extract. As the focus of the work is primarily on the valorization of sea fennel by-products, priority was given to solid residues rather than fresh biomass or large-scale extract production. Future studies may address this aspect once adequate raw material is available.

1.4 UNIEGE - Turkiye

1.4.1 Evaluation of health-beneficial traits of a standardized crude extract

Materials and Methods

Preparation of Standardized Crude Extract

To preserve the integrity of secondary metabolites, the aerial parts of sea fennel were rapidly freeze-dried immediately after harvesting. The freeze-dried samples were subsequently ground into a fine powder to enhance the extraction efficiency of bioactive compounds, as smaller particle size was found to improve extraction outcomes. The plant material was homogenized and sieved through a 1 mm mesh to ensure uniformity. The highest yields of total phenolic and flavonoid contents were obtained using 50% ethanol. Alternatively, a prolonged extraction for 16 hours at low temperature and in darkness was also applied to minimize phenolic degradation. This methodology was adapted from the study by Veršić Bratinčević et al. (2023).

Extraction Yield (EY)

EY was obtained by proportioning the amount required for the extraction process of the sea fennel which was ground into powder, with the amount obtained by lyophilization at the end of the extraction. Expression of the extraction yield as a percentage was calculated by the Eqn. (1) given below.

$$Extraction\ Yield\ (\%) = \frac{weight\ of\ extract}{weight\ of\ sea\ fennel} \times 100 \qquad (1)$$

Total Phenol Content

TPC of the sea fennel (SF) extract was calculated using the Folin-Ciocalteu method. The samples were prepared by diluting with 80% methanol from high to low concentration. Then, 20 µl of each sample was added in triplicate to the wells of a 96-well plate. Folin-Ciocalteu reagent diluted with distilled water at a ratio of 1:10 (Folin:Distilled water) was added to the wells at a volume of 100 µl and incubated in the dark for 5 minutes. After 5 minutes of incubation, 80 µl of 7% sodium carbonate (Na_2CO_3) was added, and the plate was further incubated in the dark at room temperature for 1 hour. Absorbance values were measured at 750 nm wavelength using a microplate reader at the end of the incubation. Miligram gallic acid equivalents (GAE) per gram of dry weight were used to express the TPC. Figure 1 shows the image of plate used during the spectrophotometric assay for the determination of total phenolic content (TPC).

Antioxidant Activity

7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) react at a ratio of 1:1 over the course of 16 hours of dark incubation at room temperature to produce the $ABTS^{\bullet+}$ radical action. Three replicates of 10 µl of the sample were added to each well of 96-well plates. Each well added 200 µl of the ABTS solution, which was then mixed and allowed to incubate at room temperature for 30 minutes in the dark. A microplate reader was used to measure absorbance at 734 nm (Bayraktar et al., 2023). The percentage of scavenging activity of each sample on $ABTS^{\bullet+}$ radical cation was calculated as inhibition (%) using the following Eqn. (2). The IC50 value, at which 50% reduction of the ABTS radical occurred, was determined for each sample. Figure 2 shows the image of plate used during the spectrophotometric assay for the determination of antioxidant activity.

$$Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \qquad (2)$$

where Asample represents the absorbance value of the sample solution at 734 nm and Acontrol represents the absorbance value of the blank (control) at 734 nm.

Alpha Glucosidase Enzyme Inhibition

Alpha-glucosidase enzyme activity of SF extract obtained from the sea fennel were determined. SF extract were dissolved in 1% DMSO at a certain concentrations. Alpha-glucosidase enzyme (1 U/ml) and 5 mM p-nitrophenyl-α-D-glucopyranoside substrate were dissolved in 0.1 M phosphate buffer (Ph 6.9). SF extract were added into 50 µl of 96-well plates. 100 µl of the enzyme was added to each sample and controls and incubated at 37ºC for 10 minutes in the dark. After pre-incubation, 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside substrate in 0.1 M phosphate buffer (pH 6.9) was added to each well. After the substrate was added, it was incubated for 5 minutes at 37ºC in the dark. Absorbance readings were taken on the 405 nm microplate reader device at the 5th minute of incubation (Yao et al., 2010). Instead of the sample, 50 µl of 1% DMSO was added to the well as a control and the samples were compared with the control. Alpha-glucosidase inhibitory activity is expressed as % inhibition and was calculated by the Eqn. (3) given below. Figure 3 shows the image of plate used during the spectrophotometric assay for the alpha-glucosidase enzyme inhibition analysis.

$$\%\ \text{Inhibition} = \left(\left[\frac{A_{405}^{Control} - A_{405}^{Sample}}{A_{405}^{Control}}\right]\right) \times 100 \qquad (3)$$

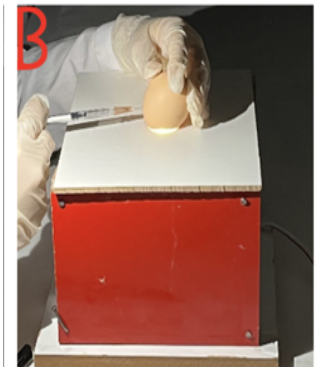
where Asample represents the absorbance value of the sample solution at 405 nm and Acontrol represents the absorbance value of the blank (control) at 405 nm.

Chicken Embryo Model (HET-CAM)

Streptozotocin (STZ) was freshly dissolved in phosphate-buffered saline (PBS, pH 7.4) immediately prior to administration. On the 11th day of incubation, Streptozotocin (STZ) was injected into chicken embryos per group. To confirm the induction of diabetes, blood glucose levels were measured on the 17th day of incubation. Embryos exhibiting blood glucose levels equal to or greater than 200 mg/dL were classified as diabetic and included in the subsequent experimental procedures. Four distinct groups were established to evaluate the antidiabetic activity of the SF extract using the chicken embryo model.

Group 1 (G1): Control, Phosphate Buffer Saline (PBS) injection
Group 2 (G2): Diabetic Control, STZ Injection
Group 3 (G3): Positive Control, Metformin Injection
Group 4 (G4): Treatment with Extract, SF Extract Injection

Eggs were labeled and arranged in quadruple replicates for each experimental group. On the 11th day of incubation, 100 µL (0.1 mL) of the designated solution for each group was injected into four embryos per group (n=4), ensuring four replicates. The injection process was conducted under sterile conditions using a candling device to visualize the interior of the egg and prevent injury to blood vessels, as illustrated in the figure below.



The image of the injection process

Tyrosinase Enzyme Inhibition

Tyrosinase inhibition activity was assessed using a spectrophotometric method with slight modifications based on the procedure described by Yang et al. (2012). In brief, a reaction mixture with a final volume of 140 µL—comprising 20 µL of the test sample at varying concentrations, 20 µL of tyrosinase enzyme solution (250 U/mL), and 100 µL of phosphate buffer (100 mM, pH 6.8)—was added to each well of a 96-well microplate. The plate was preincubated at 25 °C for 10 minutes. Subsequently, 20 µL of substrate solution (3 mM L-tyrosine) was added to each well. After a 30-minute incubation at 25 °C, the absorbance was measured at 492 nm using a microplate reader. Tyrosinase inhibitory activity was expressed as a percentage of inhibition, calculated using Eqn. (4) provided below. Figure 5 shows the image of plate used during the spectrophotometric assay for the tyrosinase enzyme inhibition analysis.

$$\% \text{ Inhibition} = \left(\left[\frac{A_{492}^{Control} - A_{492}^{Sample}}{A_{492}^{Control}}\right]\right) \times 100 \qquad (4)$$

where Asample represents the absorbance value of the sample solution at 492 nm and Acontrol represents the absorbance value of the blank (control) at 492 nm.

Sun Protection Factor (SPF, UV Blocking Activity)

The SPF (Sun Protection Factor) value of the SF extract was determined in vitro using the spectrophotometric method described by Majeed et al. (2020). To calculate the correction factor (CF), commercial sunscreen formulations were purchased and prepared in 96% ethanol. The absorbance of these preparations was measured across wavelengths ranging from 290 to 320 nm at 5 nm intervals. The CF value was then calculated based on Eqn 5.

$$SPF = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times ABS(\lambda) \quad (5)$$

The CF was determined using commercial sunscreens with known SPF values. In this approach, the EEI values were considered constant according to the spectrophotometric method described by Majeed et al. (2020). Standard sunscreens were dissolved in 96% ethanol and their absorbance was recorded in the wavelength range 290-320 nm at 5 nm intervals.

The CF was calculated from the absorbance data obtained and the fixed EEI values and was found to be 15. This CF value was then applied to the absorbance data of the tested extracts and their in vitro SPF values were calculated using the Equation 6. In this way, the sun-protective potential of the SF extract was evaluated.

Results

The bioactive potential of sea fennel (SF) extract was assessed through various analytical methods, including measurements of extraction yield, total phenolic content (TPC), antioxidant activity via the ABTS assay, and inhibitory effects on α-glucosidase and tyrosinase enzymes, as detailed in the table below.

Results of the analysis of the SF extract

	Sea Fennel (SF) Extract
Extraction Yield (%)	17.56
Total Phenol Content (mg GAE g-1 DW)	60.56±0.85
ABTS (IC50, mg ml-1)	72.30±1.79
Alpha Glucosidase Enzyme Inhibition (IC50, mg ml-1)	1.40 ±0.12
Anti-Tyrosinase Enzyme Inhibition (IC50, mg ml -1)	3.44± 0.01

The extraction yield of the SF extract was found to be 17.56%, indicating moderate recovery of phytochemicals from the plant material under the applied conditions. The total phenolic content was determined as 60.56 ± 0.85 mg GAE/g DW, demonstrating a substantial amount of phenolic compounds known for their antioxidant and bioactive properties. The antioxidant capacity measured by the ABTS assay showed an IC_{50} value of 72.30 ± 1.79 µg/mL, which suggests that the extract possesses notable radical scavenging activity. In terms of enzyme inhibition, the SF extract exhibited significant α-glucosidase inhibitory activity (IC_{50} = 1.40 ± 0.12 mg/mL), highlighting its potential role in glycemic control. Furthermore, the anti-tyrosinase activity (IC_{50} = 3.44 ± 0.01 mg/mL) indicates that the extract could be a promising candidate for cosmetic formulations aimed at skin depigmentation or anti-aging applications. These results collectively support the multifunctional bioactivity of sea fennel extract and its suitability for nutraceutical or cosmeceutical development.

Chicken Embryo Model (HET-CAM)

The blood glucose levels of chicken embryos in each experimental group were measured on the 17th day of incubation to evaluate the antidiabetic effect of the sea fennel (SF) extract. The results are presented in the table.

Blood glucose levels of chicken embryos in different treatment groups

	Blood Glucose Level (mg/dL)
G1 (Control)	160 ± 6.1
G2 (Diabetic Control, STZ Injection)	197 ± 6.4
G3 (Positive Control, Metformin Injection)	156± 11.7
G4 (Treatment with Extract, SF Extract Injection)	141 ± 1.3

To investigate the antidiabetic potential of sea fennel (SF) extract, a chicken embryo model (HET-CAM) was employed. Diabetes was experimentally induced on the 11th day of incubation via injection of streptozotocin (STZ), and blood glucose levels were assessed on the 17th day. Embryos exhibiting blood glucose levels ≥200 mg/dL were classified as diabetic and included in the analysis. As shown in the table above, the diabetic control group (G2), which received only STZ, exhibited a significantly elevated blood glucose level of 197 ± 6.4 mg/dL, confirming successful diabetes induction. In contrast, both the metformin-treated group (G3, 156 ± 11.7 mg/dL) and the group treated with SF extract (G4, 141 ± 1.3 mg/dL) showed notably lower glucose levels, suggesting hypoglycemic effects. Interestingly, the blood glucose level in the

SF-treated group was even lower than in the metformin group and the non-diabetic control group (G1, 160 ± 6.1 mg/dL), implying that the SF extract may possess strong antidiabetic activity. This effect may be attributed to the phenolic and flavonoid compounds present in the extract, which are known to contribute to glucose regulation and α-glucosidase inhibition. These findings support the potential application of sea fennel extract as a natural antidiabetic agent.

Sun Protection Factor (SPF, UV Blocking Activity)

The absorbance values and the calculated Sun Protection Factor (SPF) of the SF extract, as determined by the UV spectrophotometric method, are presented in the table.

SPF determination of sea fennel extract

λ (nm)	Absorbance (λ)	EE (λ) x I (λ)
290	1.2917 ± 0.0361	0.0150
295	1.3178 ± 0.0432	0.0817
300	1.3203 ± 0.0456	0.2874
305	1.3152 ± 0.0457	0.3278
310	1.3300 ± 0.0464	0.1864
315	1.3814 ± 0.0492	0.0839
320	1.4558 ± 0.0534	0.0180
ΣABS (λ) x EE(λ) x I (λ)	1.33 ± 0.05	
SPF=CF x ΣABS (λ) x EE (λ) x I (λ)	19.91 ± 0.69	

EE (l)= erythemal effect spectrum; I (l)= solar intensity spectrum; ABS (l)= absorbance of sea fennel extract; CF=correction factor (-15).

As shown in the table above, the SPF value of the sea fennel extract was calculated to be 19.91 ± 0.69, based on absorbance values measured at wavelengths ranging from 290 to 320 nm, and weighted using the erythemal effect and solar intensity spectra. This value places the extract in the category of moderate to high protection, according to SPF classification standards. Ultraviolet (UV) radiation, particularly in the UVB range (290–320 nm), is known to cause erythema (sunburn), premature skin aging, and increases the risk of skin cancer. SPF is a critical indicator of a substance's ability to protect against UV-induced damage. The significant absorbance observed especially at 315 and 320 nm highlights the extract's effectiveness in the upper UVB region. With an SPF close to 20, the sea fennel extract demonstrates strong potential as a natural, plant-derived sun-protective agent. This aligns with the increasing demand in the cosmetic industry for eco-friendly and bioactive ingredients derived from sustainable sources. The presence of phenolic compounds in the extract, known for their UV-absorbing and antioxidant properties, likely contributes to the observed SPF. These results suggest that sea fennel extract could be effectively utilized in sunscreen formulations, moisturizers, or other dermatocosmetic products designed to protect the skin from harmful solar radiation. Its natural origin also addresses the needs of environmentally conscious consumers seeking safer alternatives to synthetic UV filters.

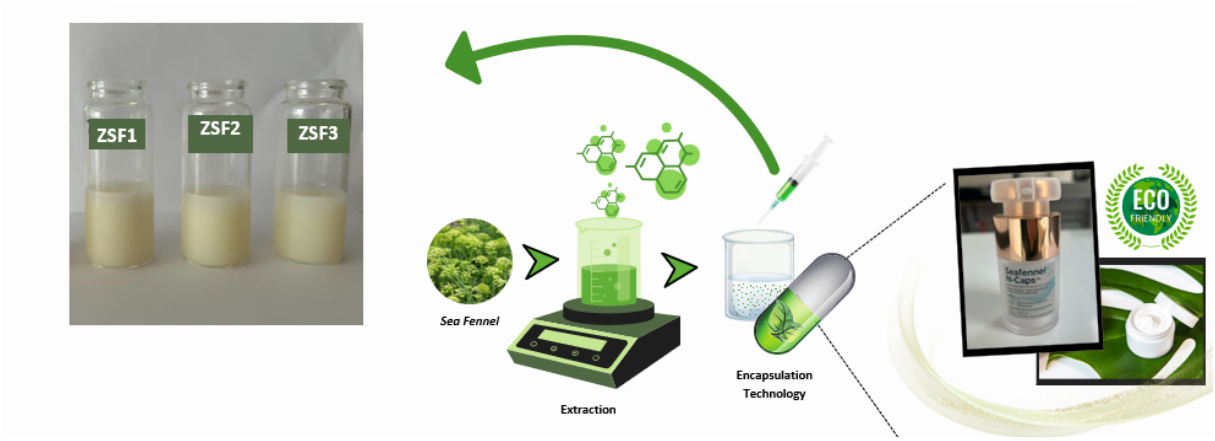
1.4.2 Encapsulation of Sea Fennel (SF) Extract

Materials and Methods

Preparation of SF Extract loaded Zein nanoparticles

SF extract loaded zein nanoparticles were prepared by a antisolvent precipitation method. Zein (1 g) was dissolved in 100 mL 80% (v/v) aqueous ethanol solution with magnetic stirring at 600 rpm for 2 h. SF extract was added to the prepared zein solution at different Zein/SF mass ratios (5:1, 10:1, and 20:1), and the resulting solutions were stirred using a magnetic

stirrer for 2 hours. The dispersions were adjusted to pH 4.0 using 0.1 mol/L hydrochloric acid (HCl) or sodium hydroxide solution (NaOH). Approximately 3 mL zein- aqueous ethanol solution was added dropwise slowly into 30 ml of deionized water using an ultrasonic bath to form dispersions. The dispersion was then evaporated at 45 °C to remove the existing ethanol using a rotary evaporator. To compensate for the removed ethanol, an equal volume of deionized water was added to the dispersion. Particles were recovered by centrifugation at 6000 rpm for 30 min at 20 °C. The nanoparticle suspensions were frozen and lyophilized for 48 h under a freeze-drier system to obtain the final nanoparticles. The effect of Zein/SF mass ratio, on particle size, polydispersity index, and stability was examined.Cosmetic formulation prototypes were prepared using the selected extract and nanoparticle. Figure 6 illustrates the preparation process and potential application of Sea Fennel (SF) extract-loaded zein nanoparticles, which were developed using the antisolvent precipitation method for use in eco-friendly cosmetic formulations.



Preparation and application of Sea Fennel (SF) extract-loaded zein nanoparticles via the antisolvent precipitation method.

Results

The particle size and polydispersity index (PDI) values of sea fennel (SF) extract-loaded zein nanoparticles prepared at different Zein/SF mass ratios are presented in the table below.

Particle size and PDI velues of the Sea Fennel (SF)-loaded nanoparticles with different Zein/Sea Fennel mass ratios

Zein/Sea Fennel Mass Ratios	Particle Size (nm)	PDI
ZSF1 (5:1)	159±2.59	0.27±0.039
ZSF2 (10:1)	114±2.82	0.54±0.012
ZSF3 (20:1)	146±1.45	0.45±0.017

In this study, sea fennel (SF) extract-loaded zein nanoparticles were successfully prepared using the antisolvent precipitation method at three different Zein/SF mass ratios (5:1, 10:1, and 20:1). Zein is widely used in pharmaceutical, food, and cosmetic applications due to its biodegradable and biocompatible nature. According to the results, although the smallest particle size was obtained with the ZSF2 (10:1) formulation, the lowest polydispersity index (PDI = 0.27 ± 0.039) was observed in the ZSF1 (5:1) formulation. This indicates that ZSF1 exhibits a more homogeneous and stable structure. While all three formulations had similar particle sizes, due to the higher PDI values of ZSF2 and ZSF3, ZSF1 nanoparticles were considered the most suitable candidate for use in product formulations. Consequently, ZSF1 nanoparticles were identified as the most suitable candidate for product development, and cosmetic formulation prototypes were prepared using the ZSF1 formulation.

Image of cosmetic formulation prototypes

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1.5 UNIBRE - France

1.5.1 NMR analysis of aqueous extracts from sea fennel leaves

Materials and methods

Sampling of wild *C. maritimum* populations

Leaves (L; n= 3) and flowers (F; n= 1) were collected at the plant flowering period in August 2023 from 3 wild sea fennel populations growing spontaneously along Brittany shoreline: Landunvez, Plouha, and Primelin. Samples were kept at -20°C for several days, then freeze-dried for long-term storage until further analysis. Finally, dry samples were groung to a fine powder and stored in hermetically-closed flasks at 20°C in the dark.

Preparation of hydro-alcoholic extracts from leaf and flower samples

An aliquot (0.5 g) of dried powder from wild sea fennel (leaves or flowers) was extracted with 10 mL of 50% ethanol in water, following the ratio 1:20 w/v, as reported by Méot-Duros et al. (2009), at room temperature in dark conditions, under agitation for 20 min. The mixture was centrifuged (3500 rpm, 5 min), then pellet was resuspended in 50% ethanol. A three-time sequence of extractions was carried out, and the three relevant supernatants were gathered and concentrated under vacuum at 35°C. Extracts were freeze-dried, weighed and stored at -20 °C before further analysis.

Total phenol contents in sea fennel extracts

Secondary metabolites were analysed through the Total Phenol Content (TPC). TPC was evaluated with Folin-Ciocalteu reagent as previously described by Gao et al. (2019), and the tissue concentrations were expressed as mg gallic acid equivalents (GAE)/g dry weight (DW).

Radical scavenging activities

The radical scavenging activity was tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and (ABTS) radicals as previously reported by Marwah et al. (2007) and Re el al. (2008). Results were expressed as IC50 (the concentration of plant extract that quenches 50% of the initial radical amount in the assay) and Trolox equivalents, respectively.

Ferric Reducing Ability Power (FRAP)

The assay used was based on the reaction of Fe^{2+} with 2,4,6-tri(pyridyl)-s-triazine (TPTZ) to form a violet-blue colour with maximal absorbance at 593 nm, according to Bolanos de la Torre et al. (2015). Results were expressed as EC50, the concentration of plant extract that results in a 50% efficacy.

Identification of major soluble compounds in plant extracts

Fractionation of sea fennel raw extract was performed by solid-liquid partition chromatography on C18-bound silica gel (GRACE Davisil RP18). The elution of polar compounds was made with increasing methanol concentrations (successively 0, 20, 40, 60, 80, 100%) and finally ethyl acetate. The fractions were then concentrated by rotary evaporation at 40°C and resuspended in the corresponding solvent.

When necessary, solute purification from one fraction was performed by HPLC using Shimadzu UFLC XR device equipped with PDA detector (SPD-M20A, Shimadzu). A Spherisorb ODS2 column (5 µm, 250 x 4,6 mm, Waters) was used for solute separation, and mobile phase consisted of a mixture of acetonitrile 100% (A) and ultrapure water (B). The following linear gradient was applied: t=0 min 100% B; t=10 min 100% A; t=12 min 100%. Compounds were detected at 254 nm, and collected for acid hydrolysis treatment (1 N HCl, 110°C for 1 h) before structural elucidation.

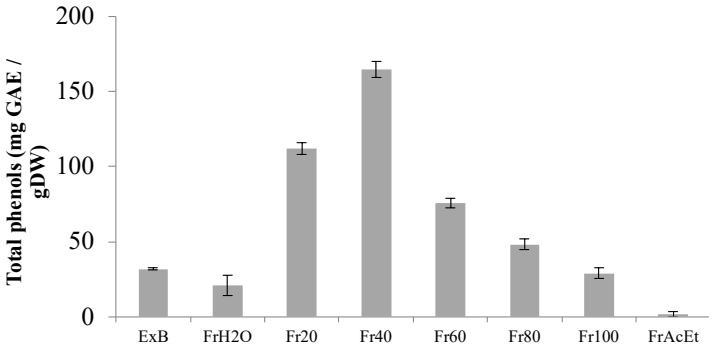
For bioactive compound characterization, an aliquot of crude extract and each fraction was concentrated by rotary evaporation at 35°C, and the dry residue was solubilized in 700 µL of 99.5% deuterated- water (D_2O) or methanol (MeOD) for NMR analyses. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance DRX-400 spectrometer (400 MHz), equipped with a 5 mm dual $^{1}H/^{13}C$ probe head, using standard pulse sequences available in the Bruker Software (Brüuker, Wissembourg, France). A typical 1D ^{1}H NMR spectrum consisted of 32 scans, and 2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propanoic acid sodium salt was used as an internal standard. The determination of major solutes present in sea fennel extract or fractions was made on NMR spectra in comparison with external standards. For ^{13}C (J-mod) and 2D Homo- and heteronuclear NMR analyses (COSY, HMBC, HMQC, TOCSY), experiments were performed at 298°K on a Brüker Avance III HD500 spectrometer equipped with an inverse 5 mm TCI cryoprobe (^{1}H, ^{13}C, ^{15}N) with z gradient. Data were processed using TopSpin® software, version 4.0 (Bruker).

Results

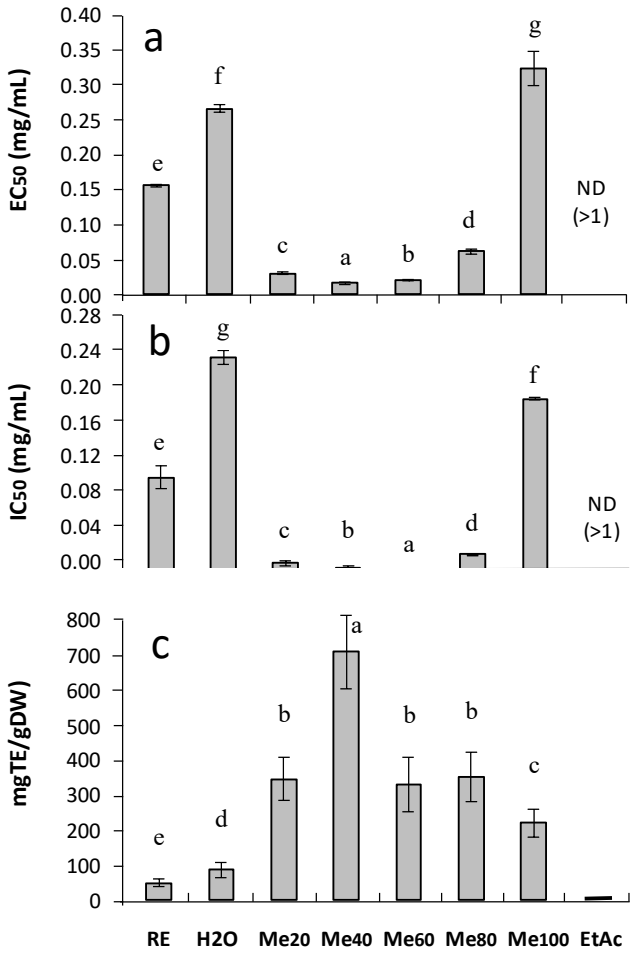
The leaf and flower extract yields were about 48% and 41%, respectively.

Total phenolic content

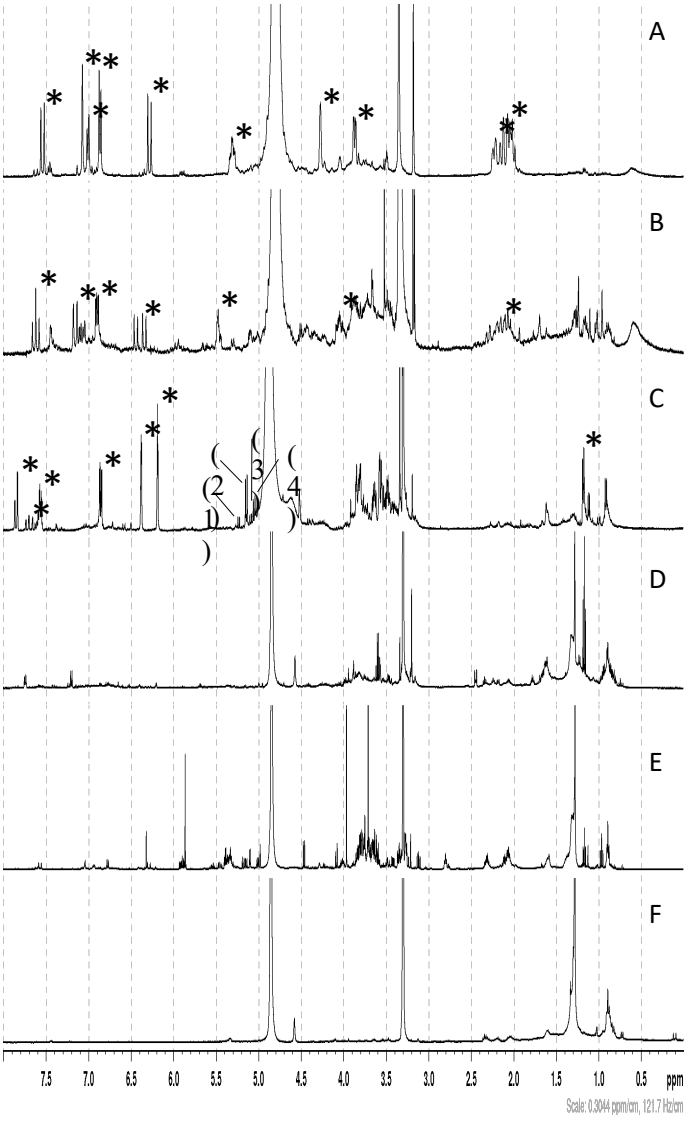
The crude extract of C. maritimum exhibited a high phenolic amount, with 33.3 mg GAE.g^{-1}DW. After fractionation of sea fennel leaf extract, phenolics were detected in the three fractions eluted with 20, 40, and 60% MeOH which exhibited 3.4, 5, and 2.3 times more phenolic compounds than crude extract, respectively.



Antioxidant activities



The crude extract (RE) of sea fennel exhibited high antioxidant activities with EC_{50} value of 0.152 mg.mL^{-1} for FRAP bioassay (Fig. a above). After fractionation of this extract, $MeOH_{20}$, $MeOH_{40}$, $MeOH_{60}$, and $MeOH_{80}$ fractions exhibited a strong reducing capacity, with FRAP EC_{50} values 5.1, 7.2, 6.3 and 2.7 times lower than that of the crude extract, respectively. The same trend was obtained with the DPPH assay, where $MeOH_{20}$, $MeOH_{40}$, $MeOH_{60}$, and $MeOH_{80}$ fractions showed IC_{50} values 3.6, 4.4, 6.7, and 3 times lower than that of the crude extract (0.136 mg.mL^{-1}), respectively (Fig. b above). For these 2 bioassays, medium polar fractions (namely $MeOH_{40}$ and $MeOH_{60}$ fractions) were the most active ones. ABTS radical scavenging capacity was distributed in almost all the fractions since every fraction eluted with methanol solution exhibited a strong activity (higher than 200 mg TE.g^{-1}DW) (Fig. c above). Thus, $MeOH_{20}$, $MeOH_{40}$, $MeOH_{60}$, $MeOH_{80}$, and $MeOH_{100}$ fractions showed 7, 10.8, 6.8, 7.1 and 4.6 times higher activities than the crude extract (51.7 mg GAE.g^{-1}DW). Conversely, the first and last fractions (namely those eluted with H_2O and ethyl acetate) showed very low or hardly detectable activities using every antioxidant bioassay.



^{1}H-NMR spectrum

Following purification of the extract, the ^{1}H-NMR spectrum of the $MeOH_{20}$ fraction showed mainly the presence of chlorogenic acid signals (Fig. A above). Characteristic signals assigned to dicaffeoylquinic acids were found in the MeOH40 fraction, including 3,5-di-O-caffeoylquinic acid (Fig. B). The ^{1}H-NMR spectrum of the $MeOH_{60}$ fraction exhibited several signals which could mark the presence of glycosylated quercetins (Fig. C). Further purification by HPLC, followed with acid hydrolysis and ^{13}C- and 2D NMR experiments allowed us to identify unequivocally 4 glycosylated quercetins, namely quercetin 3-O-rutinoside (= rutin), quercetin 3-O-galactoside, quercetin 3-O-glucoside, and quercetin 3-O-robinobioside. The $MeOH_{80}$ fraction showed small signals in the 6-8 ppm region and more pronounced ones between 1-1,5 ppm on its ^{1}H-NMR spectrum, corresponding to aromatic and aliphatic protons, respectively (Fig. D). The last two fractions, eluted with $MeOH_{100}$ and ethyl acetate, exhibited apolar compounds with aliphatic protons, as shown by the intense signals in the 1-2 ppm region (Figs. E and F, respectively).

1.5.1.1 Health-beneficial activity

Materials and methods

Anti-aging activities

Antityrosinase activity was evaluated using L-tyrosine as substrate and mushroom tyrosinase (SIGMA) according to the method described by Masuda et al. [33] slightly modified. The samples were dissolved in DMSO (50%, v/v). Then, 40 µL of each sample was mixed with 80 µL phosphate buffer (0.1 M, pH 6.8), 40 µL tyrosinase (30 units/mL in phosphate buffer, pH 6.5) and 40 µL 2.5 mM L-tyrosine in a 96-well microplate. The absorbance of the sample was monitored kinetically at 475 nm for 15 minutes, taking readings every 30 seconds, and compared to a blank containing all ingredients except extracts. Inhibition (%) of catalysis of L-tyrosine to L-dopa and then to dopaquinone was calculated using the following formula:

where At and A0 are the absorbances of the sample at t and t=0, respectively, and Ct and C0 are the absorbances of the control at t and t=0, respectively. The anti-tyrosinase activity was expressed as kojic acid equivalents (mg KAE/g DW).

Antielastase was assessed using elastase from porcine pancreas Type IV E0258 (Sigma) and N-Succinyl-Ala-Ala-Ala-p-nitroanilide (SANA) as substrate according to the method of Kalyana Sundaram et al. [34]. Twenty-five µL of extracts at different concentrations in DMSO (5%, v/v) (3 replicates) were mixed with 175 µL of Tris-HCl buffer (0.2 M, pH 8) and 25 µL of enzyme (10 µg/mL) in each well of a microplate. After incubation (30 min at 37°C), the reaction was initiated by adding 25 µL of SANA (1 mg/mL). Catalysis of SANA in p-nitroaniline was monitored for 15 min at 410 nm with a reading every 30 s. The negative control consisted of DMSO (5%, v/v) instead of the extracts, and the percentage of inhibition of elastase Inhibition (%) was calculated according the following formula:

where Ss and Sc are the slopes in the sample and control assays, respectively. Finally, the IC_{50} was determined using the regression curve of the inhibition percentage.

The anticollagenase activity of the extracts was investigated using the Enzo Life Sciences MMP-1 kit (Colorimetric Drug Discovery Kit BML-4K404). Each microplate well contained 50 µL of buffer (50 mM HEPES, 10 mM $CaCl_2$, 0.05% Brij-35, 1 mM DTNB, pH 7.5), 20 µL of samples diluted in the buffer at various concentrations (3 replicates) and 20 µL of enzyme at 637.5 mU/µL. The reaction was initiated by adding the chromogenic substrate Thiopeptide (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC_2H_5) (10 µL at a concentration of 1 mM). The catalysis of thiopeptide to 2-nitro-5-thiobenzoic acid was monitored kinetically at 412 nm for 15 minutes, with a measurement every 30 seconds. The reaction rate was determined using the linear part of the kinetics (from 0 to 10 minutes).

Anti-diabetic, anti-inflammatory and neuroprotective activities

Sea fennel extract and fractions, at concentrations ranging from 1 to 5 mg/mL, were evaluated for their capacity to inhibit a-amylase and a-glucosidase according to Zengin [32]. Moreover, extract and fractions were tested against porcine lipase according to McDougall et al. [53]. Acarbose was used as a positive control for a-amylase and a-glucosidase, and orlistat was used as a positive control for lipase inhibition. Results were expressed as IC_{50} relative to a control containing DMSO.

Nitric oxide (NO) production by LPS-stimulated RAW 264.7 macrophages was assessed as described by Rodrigues et al. [4]. RAW 264.7 cells were cultured in RPMI 1640 culture medium, enriched with 10% heat-inactivated FBS, 1% L-glutamine (2 mM), and 1% penicillin (50 U/mL) / streptomycin (50 µg/mL), and kept at 37ºC in 5% CO_2 humidified atmosphere. Murine cells were seeded in a 96-well plate at 2.5 x 10^5 cells/well and allowed to adhere overnight. Then, they were co-treated with 100 ng/mL of LPS and sea fennel raw extract (at concentrations that allowed cellular viability higher than 80%) for 24 h. NO production was assessed using the Griess assay. Results were expressed as a percentage of inhibition of NO production, relative to a control containing DMSO (0.5%, v/v), and compared to the positive control L-NAME.

The neuroprotective property of sea fennel extract was evaluated through the in vitro inhibition of acetylcholinesterase (AChE) according to Custódio et al. [31]. Samples (20 µL at concentrations of 1, 5, and 10 mg/mL) were mixed with 140 µL of sodium phosphate buffer (0.1 mM, pH 8.0) and 20 µL of AChE solution (0.28 U/mL) in a 96-well microplate. The mixture was incubated for 15 min at room temperature and the reaction was initiated by the addition of 10 µL of 4 mg/mL ATChI and 20 µL of 1.2 mg/mL DTNB. The absorbance was read at 405 nm and results were expressed as IC_{50} relative to a control containing water instead of extract. Galantamine was used as a positive control.

Results

Anti-aging activities

Sea fennel crude extract exhibited an appreciable activity against tyrosinase, with a value of 235 mg KAE.g^{-1} DW. Of the 7 fractions eluted from this extract, the last four exhibited a significantly higher activity than that of the crude extract. Among them, the $MeOH_{100}$ and ethyl acetate fractions appeared the most active with 600 mg KAE.g^{-1} DW. Moreover, neither raw extract nor fractions of sea fennel aerial parts showed antielastase, anticollagenase or anti-melanogenic properties on B16 4A5 melanoma cells.

Anti-diabetic, anti-inflammatory and neuroprotective activities

Pancreatic lipase is the enzyme responsible for digestion and absorption of triglycerides, and its inhibition is one of the widest studied methods to determine the potential activity of natural products to prevent and treat obesity. Here, neither extracts nor fractions showed inhibitory activity on rat lipase enzyme in vitro. Then, their capacity to inhibit a-amylase and a-glucosidase was assessed. Though sea fennel raw extract was inactive on these enzymes, two fractions strongly inhibited a-glucosidase. Thus, $MeOH_{20}$ and $MeOH_{80}$ fractions exhibited a hundred-fold higher inhibition than the standard glucosidase inhibitor acarbose. However, no amylase inhibition could be detected in these fractions.

Although sea fennel raw extract did not show any capacity to inhibit NO production by RAW 264.7 macrophages, two of its fractions were able to reduce this indicator of inflammation (Table 1). Noteworthy, the fraction eluted with 60% MeOH exhibited the strongest NO inhibitory power, with IC_{50} value (6.41 ± 0.37 µg/mL) four times lower than that of the positive control L-NAME (27.81 ± 1.93 µg/mL).

Sea fennel extract and fractions were evaluated for their capacity to inhibit AChE. However, neither raw extract nor its fractions exhibited any inhibitory effect on AchE.

Anti-tyrosinase (diphenolase inhibition), anti-inflammatory (inhibition of NO production in RAW 264.7 macrophages), and anti-diabetic (alpha-glucosidase inhibition) activities of sea fennel polar extract and its fractions. Means ± SD of three replicates are presented, and different letters indicate significantly different means (P<0.05). ND, not determined

	Anti-tyrosinase (mgKAE/gDW)	NO inhibition (IC_{50}, µg/mL)	Anti-α glucosidase (IC_{50}, mg/mL)
Raw extract	234.29 ± 48.56 c	ND	ND
$MeOH_{20}$	151.12 ± 74.89 c	ND	0.02 ± 0.01 b
$MeOH_{40}$	226.37 ± 91.23 c	89.54 ± 2.16 a	ND
$MeOH_{60}$	531.46 ± 68.42 b	6.41 ± 0.37 c	ND
$MeOH_{80}$	563.14 ± 26.94 b	ND	0.04 ± 0.00 b
$MeOH_{100}$	637.02 ± 29.23 a	ND	ND
EtAc	626.19 ± 41.13 a	ND	ND
L-NAME		27.81 ± 1.93 b	
Acarbose			3.14 ± 0.09 a

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2 PREPARATION AND ANALYSIS OF ESSENTIAL OILS FROM SEA FENNEL CROP BY-PRODUCTS

2.1 UNIST - Croatia

2.1.1 Preparation and analysis of essential oils from sea fennel crop by-products

a) Different isolation methods - EOs

Materials and methods

Essential oils from sea fennel were extracted from 100 g of plant material using two methods: conventional hydro-distillation (HD) and microwave-assisted hydro-distillation (MHD). In both cases, a solvent trap (pentane/diethyl ether, 2:1 v/v) was used to retain lipophilic compounds and prevent their migration into the water phase. After distillation, the oils were dried over anhydrous sodium sulfate and stored at −20 °C until analysis. The aim was to evaluate the impact of the two isolation methods on the chemical composition of sea fennel essential oils. The chemical profiles of EOs and hydrolates were analysed using gas chromatography coupled with mass spectrometry (GC-MS), while headspace-solid phase microextraction (HS-SPME) was employed for hydrolate extraction. High-performance liquid chromatography (HPLC) was used to determine the phenolic composition of residual wastewater.

Parameters used in EOs isolation methods

	Hydro-Distillation	Microwave-Assisted Hydro-Distillation
Equipment	Clevenger-type apparatus	MAE Ethos X, (Milestone Srl, Italy)
Heating time *	15 min	3 min
Temperature	100 °C	98 °C
Duration	3 h	30 min
Soaking	Yes	Yes
Other		Microwave power 500 Hz

* time needed to obtain the distillation of the essential oil first droplet.

Chemical composition of sea fennel essential oils isolated by hydro-distillation (HD) and microwave-assisted hydro-distillation (MHD)

No.	Component	Rt	LRI	Literature LRI	HD (%)	MHD (%)	Mode of Identification
1.	α-thujene	5.989	926	924	0.69 ± 0.13	0.50 ± 0.09	GC, MS
2.	α-pinene	6.195	932	932	4.26 ± 0.84	2.33 ± 0.44	GC, MS
3.	camphene	6.608	946	946	tr	nd	GC, MS
4.	sabinene	7.410	972	969	25.24 ± 2.64	27.81 ± 4.30	GC, MS
5.	ß-pinene	7.986	991	974	1.77 ± 0.43	1.14 ± 0.26	GC, MS
6.	α-terpinene	8.897	1016	1014	1.12 ± 0.05	0.72 ± 0.09	GC, MS
7.	*p*-cymene	9.175	1023	1020	1.84 ± 0.65	2.53 ± 0.09	GC, MS
8.	limonene	9.337	1027	1024	51.38 ± 4.42	53.05 ± 5.20	GC, MS
9.	(*E*)-ß-ocimene	9.698	1037	1044	4.04 ± 0.59	2.96 ± 0.38	GC, MS
10.	γ-terpinene	10.506	1057	1054	7.12 ± 0.73	6.54 ± 0.43	GC, MS
11.	(*Z*)-sabinene hydrate	10.820	1065	1065	0.14 ± 0.13	0.41 ± 0.06	GC, MS
12.	terpinolene	11.689	1088	1086	0.38 ± 0.08	0.15 ± 0.03	GC, MS
13.	linalool	12.073	1097	1095	nd	0.38 ± 0.06	GC, MS
14.	(*E*)-*p*-mentha-2,8-dien-1-ol	13.024	1120	-	tr	nd	GC, MS
15.	terpinen-4-ol	15.438	1177	1174	1.76 ± 0.04	1.30 ± 0.31	GC, MS
Total identified (%)					*99.82*	*99.74*	
Total non-oxygenated compounds					*97.92*	*97.73*	
Total oxygenated compounds					*1.90*	*2.09*	

Results

The MHD method resulted in a higher yield of sabinene and limonene in the essential oil, and also led to a higher phenolic content in the wastewater, particularly an increased concentration of chlorogenic acid. Significant differences were observed between the EO and hydrolate compositions. The MHD hydrolate contained a higher amount of terpinen-4-ol, whereas certain other compounds were found in greater amounts in the HD hydrolate. Overall, the isolation method significantly influenced not only the EO composition but also the chemical profiles of the hydrolates and wastewater. These by-products, rich in bioactive compounds, show promising potential for further valorization and application in food, cosmetic, or pharmaceutical industries in accordance with sustainable and green extraction practices.

b) Different plant parts (flowers, stems, leaves) – EOs + residual water (wastewater)

Materials and methods

The aerial parts of wild-grown sea fennel (*Crithmum maritimum* L.) were collected in Central Dalmatia (43°39'44"N, 15°56'40"E, Croatia) during the full-flowering stage. Approximately 1 kg of plant material was collected from more than 10 increments within the same site (covering an area of around 10 m²). Prior to essential oil isolation, the plant material was air-dried for 15 days at room temperature and subsequently separated into plant parts: flowers, leaves, and stems. Essential oils (EOs) from the dried plant parts (flowers, leaves, and stems) were isolated by hydrodistillation using a Clevenger-type apparatus. The isolated oils were dried over anhydrous sodium sulfate, and the yields were calculated based on the dry weight of the plant material. The hydrodistillation wastewater was collected, cooled, filtered, and freeze-dried. The resulting crude extracts were dissolved in water at a concentration of 10 mg/mL and used for further analysis. The chemical composition of essential oils (EOs) obtained from different parts of sea fennel (flowers, leaves, and stems) was analysed using gas chromatography coupled with mass spectrometry (GC-MS).

Chemical composition of sea fennel essential oils from different plant part material

No.	Component	RI	Flower	Leaf	Stem	Mode of Identification
	Monoterpene Hydrocarbons					
1.	α-Pinene	938	1.76	0.08	0.35	RI, MS
2.	Sabinene	977	44.94	51.47	42.55	RI, MS
3.	β-Pinene	993	0.07	0.89	0.80	RI, MS
4.	α-Terpinene	1019	0.86	0.98	1.47	RI, MS
5.	*p*-Cymene	1028	n.d.	0.09	0.27	RI, MS
6.	Limonene	1032	43.58	36.28	36.45	RI, MS
7.	(*E*)-β-Ocimene	1042	0.77	0.07	1.15	RI, MS
8.	γ-Terpinene	1062	2.79	3.49	5.28	RI, MS
10.	Terpinolene	1090	n.d.	0.37	0.47	RI, MS
	Oxygenated Monoterpenes					
9.	*cis*-Sabinene hydrate	1070	0.11	0.10	0.38	RI, MS
11.	*trans*-Sabinene hydrate	1098	0.08	n.d.	0.15	RI, MS
12.	*trans*-*p*-Menth-2-en-1-ol	1122	n.d.	0.10	0.13	RI, MS
13.	Terpinen-4-ol	1179	3.53	5.35	10.35	RI, MS
14.	α-Terpineol	1190	n.d.	n.d.	0.07	RI, MS
	Total identified (%)		98.49	99.27	99.87	
	Oil yield (%, *w/w*)		1.35	0.63	0.62	

n.d.—not detected; RI = retention indices on HP-5MS UI column; MS = mass spectra.

Results

A total of 14 different compounds were identified, with variations in both composition and abundance among the samples.

The highest EO yield was obtained from the flowers (1.35%), while significantly lower yields were recorded from the stems and leaves, with more than a two-fold difference. All EO samples were predominantly composed of non-oxygenated monoterpenes, ranging from 88.79% in the stem oil to 94.77% in the flower oil. The main compounds within this group

included sabinene (42.55–51.47%) and limonene (36.28–43.58%), followed by γ-terpinene (2.79–5.28%). Among oxygenated monoterpenes, terpinen-4-ol was found in all samples, with the highest level in the stem oil (10.35%).

Table 2. GC-MS chemical composition of French (FRA) and Croatian (CRO) sea fennel hydrolates.

RI	Compounds	FRA (%)	CRO (%)
921	α-thujene	0.40 ± 0.02	nd
934	α-pinene	1.36 ± 0.02	nd
970	sabinene	5.64 ± 0.07	nd
986	2,3-dehydro-1,8-cineole	tr	nd
989	β-myrcene	0.61 ± 0.07	nd
998	octanal	tr	nd
1001	α-phellandrene	tr	nd
1012	α-terpinene	0.62 ± 0.04	nd
1020	*p*-cymene	6.40 ± 0.07	nd
1024	β-phellandrene	0.19 ± 0.01	nd
1039	(*Z*)-β-ocimene	2.10 ±0.01	nd
1041	benzeneacetaldehyde	0.77 ± 0.15	5.06 ± 0.03
1056	γ-terpinene	9.34 ± 0.24	nd
1065	*cis*-sabinene hydrate	tr	nd
1086	terpinolene	0.35 ± 0.02	nd
1095	*trans*-sabinene hydrate	tr	4.73 ± 0.04
1097	linalool	nd	tr
1118	*cis*-*p*-menth-2-en-1-ol	0.25 ± 0.04	4.26 ± 0.02
1138	*trans*-*p*-menth-2-en-1-ol	tr	3.37 ± 0.05
1176	terpinen-4-ol	2.12 ± 0.22	41.93 ± 2.99
1183	*p*-cymen-8-ol	tr	nd
1188	α-terpineol	0.21 ± 0.01	5.71 ± 0.51
1205	*trans*-pipertiol	nd	2.46 ± 0.01
1218	*trans*-carveol	0.20 ± 0.01	3.36 ± 0.03
1222	*cis*-carveol	nd	3.75 ± 0.02
1232	thymyl methyl ether	26.30 ± 1.85	nd
1242	carvacryl methyl ether	0.28 ± 0.02	nd
1293	thymol	0.32 ± 0.03	tr
1301	carvacrol	tr	1.07 ± 0.02
1312	*p*-vinylguaiacol	0.76 ± 0.04	nd
1327	myrtenyl acetate	nd	0.99 ± 0.01
1384	(*E*)-β-damascenone	nd	1.03 ± 0.09
1390	10-(acetylmethyl)-3-carene	nd	13.80 ± 2.01
1422	dihydrodehydro-β-ionone	nd	5.88 ± 0.09
1498	bicyclogermacrene	0.23 ± 0.01	nd
1521	myristicin	0.48 ± 0.00	nd
1557	elemicin	tr	nd
1563	germacrene B	0.39 ± 0.02	nd
1620	dillapiole	36.66 ± 5.66	1.52 ± 0.01
	TOTAL	95.98	98.92

RI = retention indices on HP-5MS UI column, tr—traces (<0.1%), nd—not detected.

In addition to EO composition, the hydrodistillation by-products (residual water) were also analyzed. Twelve phenolic compounds were detected in the residual water, with chlorogenic acid and its isomers (cryptochlorogenic and neochlorogenic acids) being the most abundant. The total phenolic, flavonoid, and tannin contents followed the order: flowers > leaves > stems. Despite compositional differences between plant parts, all samples demonstrated high levels of bioactive compounds. Particularly, the residual water showed relatively high concentrations of key phenolics with recognized biological activity, indicating a strong potential for valorization and re-use in line with principles of green processing and circular economy.

Phenolic profile (mg/g of dry extract) of residual wastewater extracts after essential oil extraction by hydro-distillation (HD) and microwave-assisted hydro-distillation (MHD)

No.	Component	HD	MHD
1.	gallic acid	0.03 ± 0.01	0.02 ± 0.00
2.	protocatechuic acid	0.05 ± 0.02	0.04 ± 0.00
3	neochlorogenic acid	7.92 ± 0.04	4.48 ± 0.11
4.	*p*-hydroxybenzoic acid	0.78 ± 0.06	0.53 ± 0.02
5.	chlorogenic acid	13.67 ± 0.03	22.18 ± 0.03
6.	cryptochlorogenic acid	7.75 ± 0.05	5.17 ± 0.02
7.	caffeic acid	0.04 ± 0.01	0.03 ± 0.02
8.	ferulic acid	0.11 ± 0.00	0.22 ± 0.00
9.	sinapic acid	0.16 ± 0.01	0.11 ± 0.00
10.	rutin	0.35 ± 0.00	0.34 ± 0.01

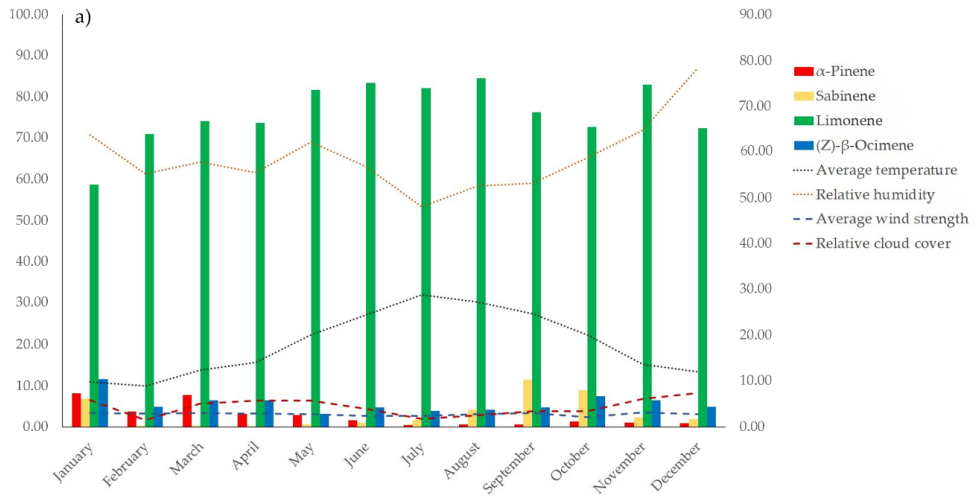
c) Annual changes of phytochemicals in EOs

Materials and methods

Fresh leaves material was collected, chopped, and accurately weighed (100 g per sample). The samples were then subjected to hydrodistillation using a Clevenger-type apparatus for 3 hours. The isolated essential oils were collected, their mass or volume was measured, and the samples were stored in dark vials at +4 °C until analysis by gas chromatography–mass spectrometry (GC-MS).

Results

The main results for essential oils showed that the yield from sea fennel flowers ranged from 0.19% to 1.87% (w/w), depending on the sampling location. Gas chromatography–mass spectrometry (GC-MS) analysis revealed that the essential oils were dominated by limonene and sabinene, with limonene comprising up to 62.2% and sabinene up to 43.3% of the total oil, depending on the sample origin. Other notable compounds included γ-terpinene, p-cymene, and α-pinene, but their proportions varied depending on the sampling time. Overall, the essential oil composition was predominantly characterized by monoterpene hydrocarbons, with significant variability observed throughout the year among samples collected from the same location.



d) Croatian and French EOs

Table 1. GC-MS chemical composition of French (FRA) and Croatian (CRO) sea fennel essential oils.

RI	Compounds	FRA (%)	CRO (%)
921	α-thujene	0.15 ± 0.02	nd
934	α-pinene	0.84 ± 0.13	tr
970	Sabinene	4.24 ± 0.10	51.47 ± 3.22
992	β-pinene	0.17 ± 0.02	0.89 ± 0.02
1012	α-terpinene	0.20 ± 0.02	0.98 ± 0.03
1020	*p*-cymene	2.97 ± 0.43	tr
1039	(*Z*)-β-ocimene	0.80 ± 0.17	nd
1032	Limonene	nd	36.28 ± 2.99
1042	(*E*)-β-ocimene	nd	tr
1056	γ-terpinene	9.88 ± 1.10	3.49 ± 0.07
1065	*cis*-sabinene hydrate	nd	0.10 ± 0.01
1086	Terpinolene	0.12 ± 0.01	0.37 ± 0.05
1118	*cis*-*p*-menth-2-en-1-ol	nd	0.10 ± 0.01
1176	terpinen-4-ol	0.25 ± 0.01	5.35 ± 0.04
1232	thymyl methyl ether	0.21 ± 0.01	nd
1242	carvacryl methyl ether	18.00 ± 2.40	nd
1620	Dillapiole	62.10 ± 1.83	nd
	TOTAL	99.93	99.03

RI = retention indices on HP-5MS UI column, tr—traces (<0.1%), nd—not detected.

EOs

- 13 compounds in the French, 12 compounds in the Croatian sample
- French sea fennel EO: dillapiole (62.10 ± 1.83%), carvacryl methyl ether (18.00 ± 2.40%) and γ-terpinene (9.88 ± 1.10%)
- Croatian sea fennel EO: sabinene (51.47 ± 3.22%) and limonene (36.28 ± 2.99%)

Hydrolates

- 32 components in the French sample, 17 compounds in the Croatian sample
- French sea fennel hydrolate: dillapiole (36.66 ± 5.66%), thymyl methyl ether (26.30 ± 1.85%) and γ-terpinene (9.34 ± 0.24%)
- Croatian sea fennel hydrolate: terpinen-4-ol (41.93 ± 2.99%) and 10-(acetylmethyl)-3-carene (13.80 ± 2.01%)

Table 3. Antioxidant activity of the French (FRA) and Croatian (CRO) sea fennel essential oils (EOs) and hydrolates.

Antioxidant Assay	FRA EO	CRO EO	FRA Hydrolate	CRO Hydrolate	Gallic Acid (Standard)
FRAP (μM Fe^{2+}/L)	1.34 ± 0.17 c	1.11 ± 0.08 b	0.77 ± 0.19 a	1.16 ± 0.15 b	2485.09 ± 3.73 *
DPPH (% inhibition)	1.99 ± 0.16 a	2.00 ± 0.17 a	2.39 ± 0.25 b	2.17 ± 0.13 ab	95.35 ± 0.33 **
NO (% inhibition)	10.11 ± 0.14 a	11.30 ± 0.24 b	38.94 ± 0.13 d	35.20 ± 0.19 c	11.56 ± 0.90 **
ORAC (μM Trolox equivalents/L)	115.77 ± 0.35 bc	95.48 ± 3.82 b	36.22 ± 2.86 a	138.05 ± 0.41 c	-

* at concentration 0.1 mg/mL; ** at concentration 1 mg/mL. In each line, values followed by different letter are statistically significant ($p < 0.05$, by Tukey's test).

Table 4. Antimicrobial activity of the French (FRA) and Croatian (CRO) sea fennel essential oils (EOs) and hydrolates, expressed as inhibition diameter (mm).

Bacterial Strains	FRA EO	CRO EO	FRA Hydrolate	CRO Hydrolate	Streptomycin
Escherichia coli	Ø	18.0 ± 1.3^{b}	Ø	Ø	28.5 ± 2.1^{a}
Staphylococcus aureus	8.0 ± 0.5^{b}	24.8 ± 3.3^{a}	Ø	Ø	22.3 ± 1.0^{a}
Pseudomonas aeruginosa	Ø	8.3 ± 0.6^{b}	Ø	Ø	26.6 ± 1.5^{a}

Data are means of inhibition diameter (mm) ± S.D. of three replicates. In each line, values followed by different letter are statistically significant ($p < 0.05$, by Tukey's test); Ø no inhibition activity.

Table 5. Antiageing activities of the French (FRA) and Croatian (CRO) sea fennel essential oils (EOs) and hydrolates, as well as of positive controls. Antityrosinase, anticollagenase, and antielastase are expressed as IC_{50} in µg/mL.

Enzymes	FRA EO	CRO EO	Arbutin	EGCG	Ursolic Acid
Tyrosinase	Ø	649 ± 54	137 ± 6	/	/
Collagenase	Ø	2571 ± 334	/	51 ± 7	/
Elastase	Ø	Ø	/	/	238 ± 13

EGCG–epigallocatechin gallate. Data are means of IC50 ± S.D. of three replicates. Ø, no inhibition activity.

Conclusion: different chemotypes, different bioactivity (low antioxidant activity of both samples, high antimicrobial activity and high tyrosinase and collagenase activity of Croatian EO, French samples are low active or inactive)

2.1.2 Addition of EOs to different vegetable oils to prevent their degradation (oxidation) and as flavor components.

Materials and Methods

Rancimat method:

The oxidative stability of the oils after addition of EO was evaluated using Rancimat 743 (Metrohm, Herisau, Switzerland) instrument to monitor the progress of accelerated oxidation at high temperatures. The oil samples (3 g) were tested at a temperature of 120 °C (ΔT = 1.4 °C) with the constant air flow of 20 L/h. The conductivity was measured as a function of time and the results are expressed as induction time (in hours). All determinations were performed in triplicate, and the results are presented as mean value ± standard deviation.

Results

Induction periods (h) for oil/fat with and without addition of sea fennel EO

	Induction period (h)
Pure oil	2.37 ± 0.03
Oil with + 0,1 mL EO	2.32 ± 0.04
Pure lard	0.80 ± 0.01
Lard with + 0,1 mL EO	0.84 ± 0.06
Lard with + 0,5 mL EO	0.73 ± 0.01

EO addition was tested on oil and lard samples. In case of oil, addition of 0.1 mL of the sample caused accelerated oxidation of the oil, while in case of lard, the effect was opposite. The lard was used in second experiment where higher amount of oil was added (0.5 mL) and that procedure also caused decreased oxidative stability of lard, compared to the control sample.
Conclusion: the EO addition to the lipid medium had negative effect on its oxidative stability.

2.2 INGREF - Tunisia

2.2.1 Use of sea fennel by-products' essential oil in manufacturing jelly candy.

Materials and Methods

Essential oil extraction from sea fennel

Essential oils were extracted from dried sea fennel plants collected from five Tunisian populations. Identification of the essential oils was performed using a Hewlett Packard HP5890 series II GC-MS equipped with an HP5MS column (30 m ×0.25 mm). Helium was used as carrier gas at 1.2mL.min−1. The sample (1µL) was injected in the split mode (1:20), the program used was isothermal at 70°C, followed by 50 to 240°C at a rate of 5°C.min−1, then held at 240°C for 10 min. The mass spectrometer was an HP 5972. The total electronic impact mode at 70 eV was used. The components were identified by comparing their relative retention times and mass spectra with the data from the library of essential oils constituents, Wiley, Mass Finder, and Adams GC-MS libraries and by comparing their RI calculated with C8 to C40 Alkanes Calibration Standard (40147-U, Supelco, Germany).

Preparation of jelly candies

Jelly candy was prepared as described by Ali et al. (2021) using strawberry juice as basic flavouring and colouring agent. First of all, seeds were removed from the juice and the gelatine was soaked in a sufficient
amount of water for 5 min then dissolved at 70 °C for 5 min in a water bath. Then, sugar solution and gelatine were mixed and manually stirred for 20 min at 110°C. Just prior to completing the preparation, essential oil was added to the mix. A control and 3 treatments were prepared considering different doses (Trt1 - 0,02%; Trt 2 - 0,2%; Trt3 - 0,5%) of sea fennel EO. The endpoint of cooking was identified using a hand refractometer for measuring the concentration of total soluble solids (TSS) which ranged from 61 to 66%. The final product was moulded into a silicone mould and kept in a refrigerator at 4 °C for 6 h. The jelly candy was then un-moulded and was packed in glass jars and stored at 4 °C until further analysis.

Jelly candies with sea fennel

Dry matter, ash, and organic matter

Dry matter was determined by oven drying 5g samples of different treatments at 103°C until constant weight. The same samples were then calcinated in an oven at 550°C for 7 hours to determine organic matter and ash levels of the sample.

pH and TSS (Total soluble solids)

To measure pH and TSS levels, a solution was prepared, containing 5 grams of Jelly candy suspended and homogenized in 50ml of distilled water.

pH measurement: prior to any measurement, the METLER TOLEDO pH meter is calibrated in standard solutions (pH=2, pH=7 and pH=9). Then dip the probe into the product and read the pH value on the display. The probe must be cleaned with distilled water each time it is changed from one sample to another. In general, three readings are taken for each sample.

Measurement of total soluble solids: the Brix scale is used to determine the percentage of soluble dry matter. The Brix degree was measured by an Anton Paar electronic refractometer used in accordance with the NF de standard (NF V05-109, 1970; Nielsen S.S, 2017).

Total Phenolic Content

To measure phenols, flavonoids, and antioxidant activity, a solution was prepared, mixing 2 grams of jelly candy was suspended in 20ml ethanol:water (80:20) solution. The mix was stirred for 1h and filtered with a Whatman N°1 filter paper.

For phenols, a mix of 0.4 mL of extract is introduced into a test tube and 2 mL of Folin-Ciocalteu reagent (1N) is added. 4 minutes later, 1.6 mL of Na_2CO_3 solution (7%) is added. The resulting mixture is incubated at room temperature for 2 hours. The absorbance is then measured spectrophotometer at 765 nm against a blank. The results obtained are expressed in milligram equivalents of gallic acid per gram of dry matter (mg EAG/ g DM).

Total Flavonoid Content

Total flavonoids were quantified by the aluminum trichloride ($AlCl_3$) method. And the absorption was measured at 510 nm. To do so, 0.75 ml of ethanol was added to 0.25 ml of extract and a 0.05 ml of $AlCl_3$ solution. Afterwords, 1.4 ml of distilled water was added and the mix was vortexed and left to incubate for 30min. Results are expressed in

milligrams equivalent of quercetin per gram of dry matter (mg EQ/g DM). A calibration curve is constructed using standard prepared at different concentrations.

Antioxidant activity

The method used to assess the antioxidant activity was based on the inhibition of DPPH free radical. 3.9 ml of DPPH solution (2.4mg/100 ml ethanol) were mixed and read at 517nm. The mix was incubated for 1 hour. A control was prepared using ethanol instead of extract. Percentage of Inhibition (of free radical scavenging DPPH) is calculated by the formula:

% of DPPH inhibition = ((Absorbance of the blank - Absorbance of extracts)/ Absorbance of the blank) * 100

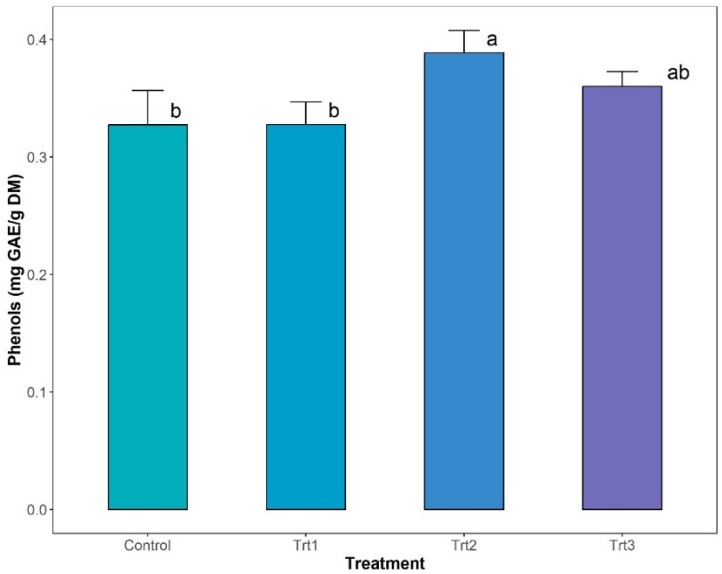
Sensory analysis

Sensory analysis was carried out on a testing panel composed of 40 people aged between 20 and 65 years old. Three parts were considered in this test: visual (color, aspect, form and odor), textural (appreciation of the quality of candy, gumminess), and taste (appreciation of sweetness, Bitterness, acidity, Flavor). Tasters rate their appreciation of the product on a scale from 1 (highly appreciate) to 4 (not appreciate)

Results

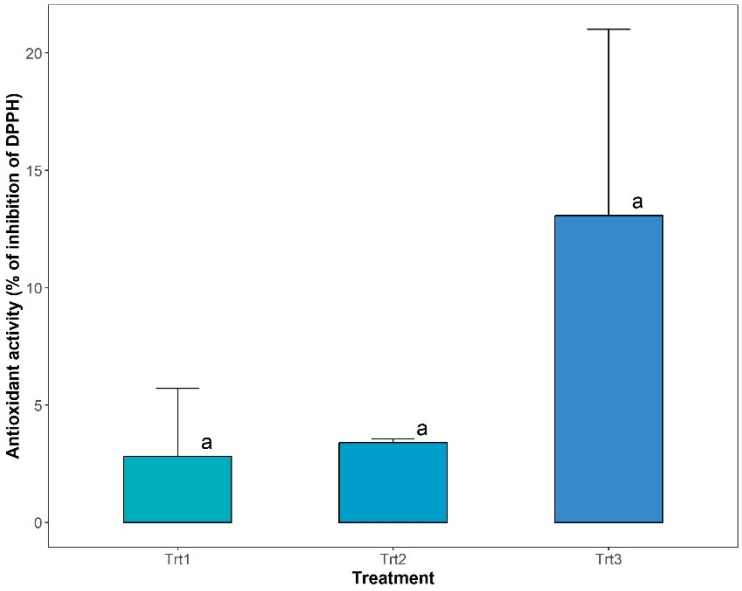
Phenols

Phenolic content was lowest in the Control group, ranging from 0.297 to 0.356. Treatment 1 (Trt1) slightly increased phenols (~0.306–0.343), while Treatment 2 (Trt2) had the highest phenol levels across the board (up to 0.410), suggesting improved antioxidant potential. Treatment 3 (Trt3) also had elevated phenols (~0.346–0.369), but not as high as Trt2. Overall, Trt2 appears most effective at enhancing phenolic content.



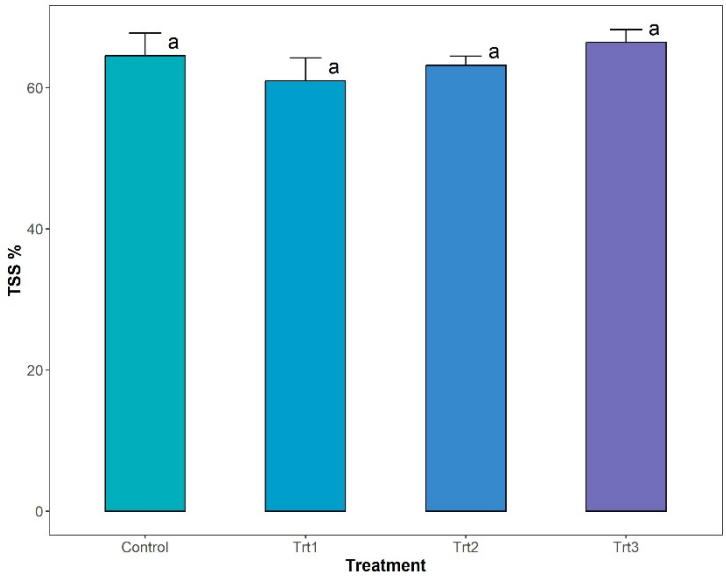
Antioxidant Activity (AA)

In Trt1, values ranged widely from 0.94 to 6.15, indicating moderate but inconsistent response. Trt2 showed moderate and consistent AA values (~3.2–3.5), while Trt3 displayed a dramatic increase ranging from 4.9 to 20.75. This suggests Trt3 had the strongest effect on inhibiting DPPH action indicating a high antioxidant response.



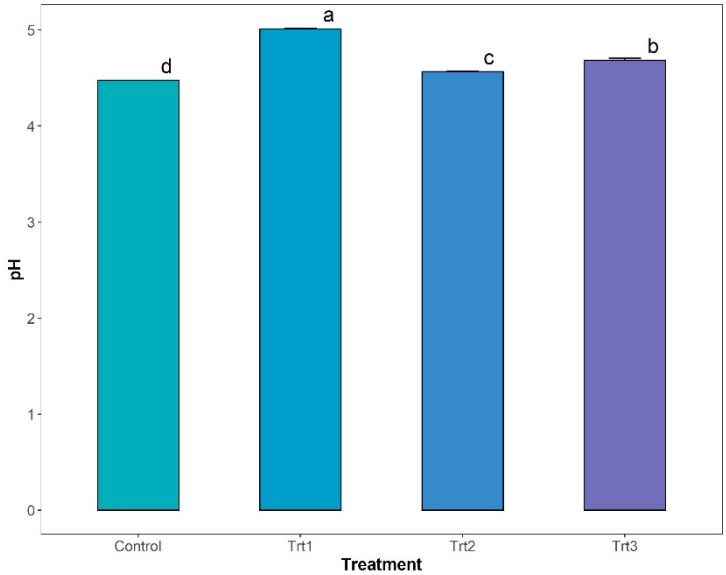
TSS (Total Soluble Solids)

TSS was lowest in Trt1 (57.3–63.6), indicating lower sugar or soluble nutrient content. Control and Trt2 had comparable mid-range values (~61–65), but Trt3 showed the highest TSS values (up to 68.5), suggesting better quality of the candy. Trt3 likely enhanced nutrient accumulation or sugar content most effectively.



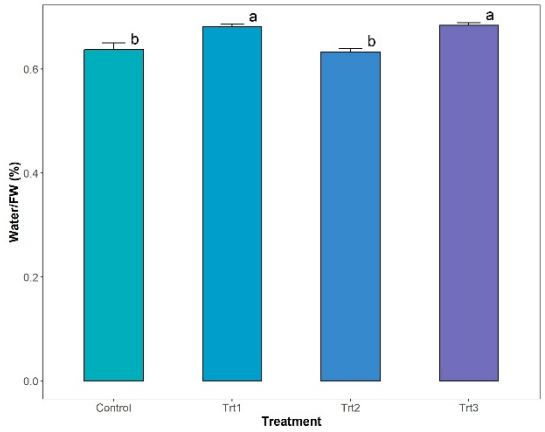
pH

Control samples maintained a stable, slightly acidic pH around 4.47, while Trt1 increased the pH to around 5.01, making it more alkaline. Trt2 and Trt3 remained in a moderate range (~4.56–4.70), but Trt1 clearly had the strongest alkalizing effect.



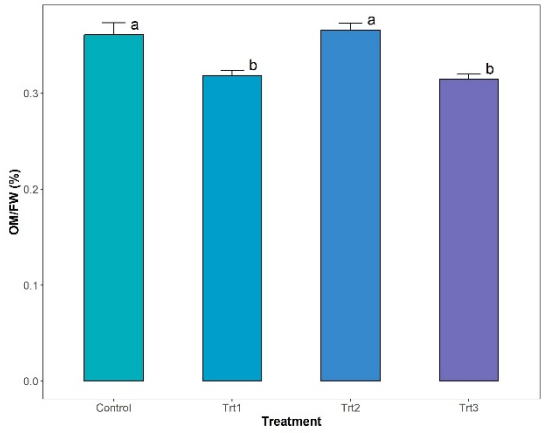
Water Content

Control samples had water content between 62–64%. Trt1 and Trt3 had the highest water content (up to ~68.8%), while Trt2 had the lowest (~62.7–64%). High water content in Trt1 and Trt3 may affect the conservation process of the jelly candy in future.



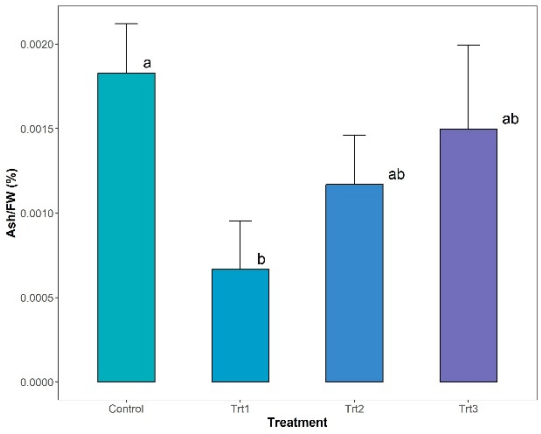
Organic Matter

Trends were very similar to dry matter. Trt2 and Control showed higher values (35–37%), whereas Trt1 and Trt3 had lower organic matter. Again, Trt2 stands out for maintaining high solid content while not being overly dry.



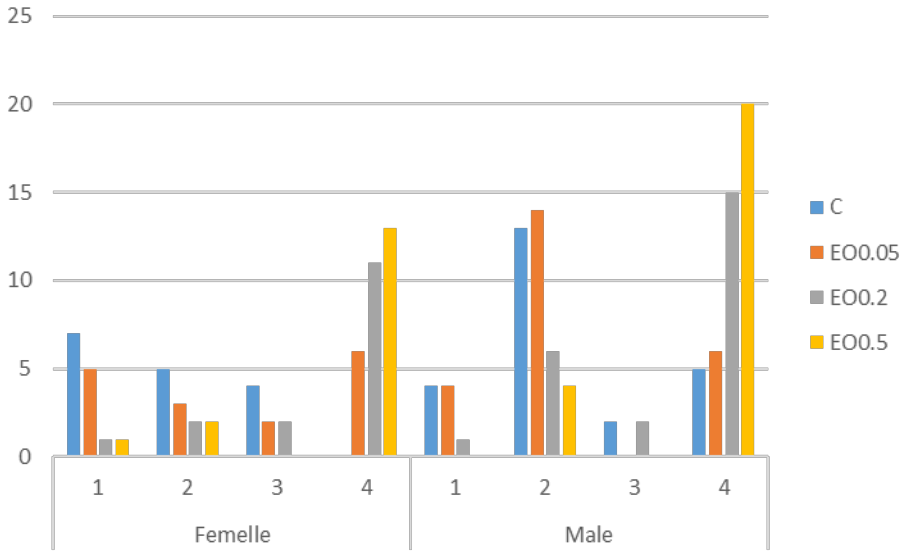
Ash Content

Ash content, representing inorganic mineral residues, was relatively low across all samples. Control had the highest ash content (~0.002), while Trt1 had the lowest (~0.0005). Trt2 and Trt3 were intermediate, suggesting minor variations in mineral content.



Sensory analysis

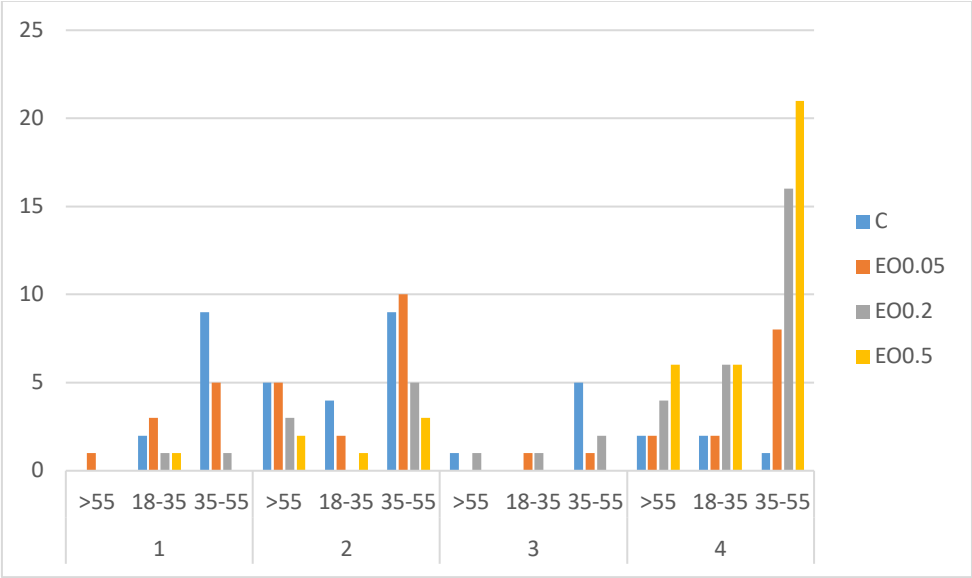
Considering appreciation levels between 1 and 4 (1 highly like and 4 highly dislike), 31% and 19% of females highly appreciate and appreciate the use of a dose of 0.05% EO in jelly candy, respectively. 17% and 58% of males highly appreciate and appreciate the use of a dose of 0.05% EO in jelly candy, respectively. 60% of the panelists have an age between 35 and 55 years, 20% have an age between 18 and 35 years, and 20 % have an age higher than 55 years.



For control, appreciation levels are relatively low for 18–35 and 35–55 groups, indicating good appreciation of jelly candy. The >55 group consistently shows higher values, meaning lower appreciation. This suggests that under standard conditions, jelly candy is best appreciated by younger and middle-aged adults.

The first treatment (EO 0.05) has an improved appreciation compared to the control, especially for the 35–55 group. The 18–35 group also shows strong appreciation under this treatment. The >55 group's values remain high, indicating

minimal improvement in appreciation. Overall, EO 0.05 appears to enhance appreciation, especially for adults under 55.
The second treatment (EO 0.2) results in moderate values across most age groups. It doesn't significantly enhance appreciation compared to EO 0.05 or Control. In general, results imply reduced appreciation especially for age groups 35–55 and >55. Thus, EO 0.2 may have a neutral or slightly negative effect on candy appreciation.
For the third treatment (EO 0.5), it yields the highest values, especially for the 35–55 group, indicating strong aversion or very low appreciation. All age groups show a significant increase in values under this treatment, suggesting that EO 0.5 negatively impacts appreciation across all ages, most notably in older adults.
Overall, The EO 0.05 treatment appears to enhance appreciation for jelly candy the most, particularly in younger and middle-aged adults. In contrast, EO 0.5 drastically reduces appreciation, making it the least favorable treatment. The control condition (C) and EO 0.2 fall in between, with Control being more favorable. This analysis suggests that the addition of low doses of essential oil (EO 0.05) may enhance consumer satisfaction, while stronger doses may be off-putting.



Appreciation of jelly candy across different age groups

3 EXPLOITATION OF SEA FENNEL RESIDUES AS SOIL AMENDMENTS

3.1 IACKR - Croatia

3.1.1 Soil amendment produced by composting of sea fennel crop residual biomass

Materials and Methods
Composting was done at the Institute for Adriatic Crops at the end of August.
Three types of material were used:

- sea fennel plants after harvest at the end of August
- whole processing tomato plants after harvest (green/N material)

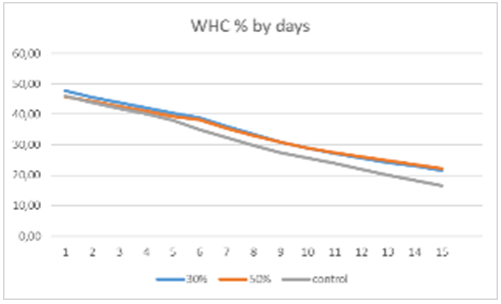
material from pruning cherry, olives… (brown/C material).

Composting was done in compost bins with two treatments mixing 3 types of materials volume:volume:
Treatment 1: 50% seafennel + 15% tomato + 35% pruned material
Treatment 2: 30% seafennel + 20% tomato + 50% pruned material

Temperature was measured in compost piles with sensors connected with data logger WatchDog series 1000. Temperature reached maximum level of 60°C in both treatments and piles were warmer than outside temperature for more than 100 days.
During composting process samples collected from the piles were taken for pH, EC and other analyses.
Compost pH had increasing trend through the time, while dry matter percentage was decreasing.

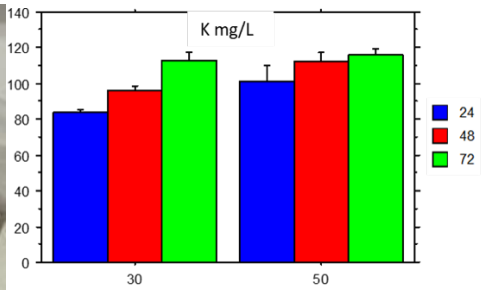
Results

- Composts were taken from bins after 6 months, sieved and prepared for further analyses
- Water holding capacity was similar to commercial peat substrate
- Compost with 30% seafennel had average pH 9,14 and EC 1350 mS/cm, while 50% compost had pH 9,4 and EC 1550 mS/cm (prepared in 1:5 - compost:water solution)

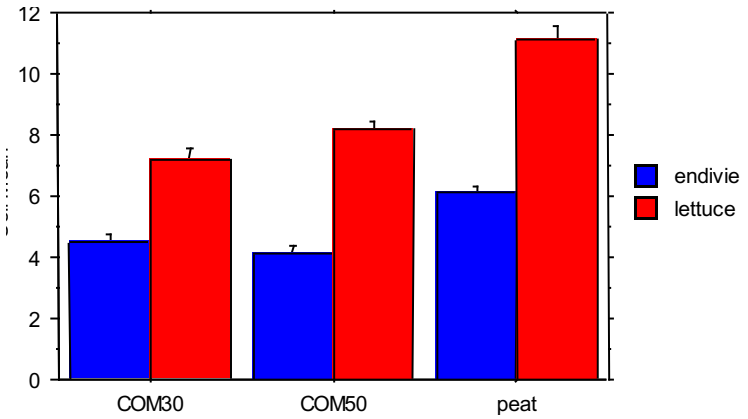


- Compost tea was prepared in 10:1 aerated water solution for 24, 48 or 72 hours

- Tea solution analyses by ionic cromatography showed that seafennel ratio and time had significant influence on K and Mg concentrations, while for other nutrients no differences were found. It can be noted that in all solutions nitrate or ammonium ions were not detected.



- Seafennel composts and commercial substrate were used for vegetable seedlings production in ebb and flow system.
- Lettuce, endivie, tomato, pepper and cucumber seeds were sown in commercial substrate and 2 ours prepared composts with 30 and 50 % initial seafennel plant parts.
- All species had better germination and growth in commercial substrate.
- For possible future use of compost derived from seafennel plants it can be recommended to make mix with peat or peat based substrates to lower pH. Additional nitrogen input should be done.



Fresh weight of lettuce and endivie seedlings grown in seafennel compost (COM 30 and COM 50) and peat commercial substrate