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	D5.2 At least 2 pilot scale prototypes of sea fennel-based unfermented foods
Deliverable Lead:	CREA-AN
Related Work Package:	WP5
Related Task:	Task 5.2 (R) Laboratory-scale manufacturing of unfermented shelf-stable preserves
Author(s)	Dr Valentina Melini
Dissemination level	PU
Due Submission Date:	MONTH 24 (30.04.2024)
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Start date of project	30.05.2022
Duration	36 MONTHS
Summary of Deliverable D5.2 – Pilot Scale Prototypes of Sea Fennel-Based Unfermented foods	Across three countries - Croatia, Türkiye, and Tunisia - partners developed and validated a wide portfolio of recipes that combine tradition, innovation, and nutritional value. In Croatia, sea fennel was blended with rosemary, bay laurel, and fennel to produce dried spice mixes rich in antioxidants, while aromatized oils captured its unique herbal notes. A Dalmatian-style paté, combining sea fennel with olives and onions, achieved excellent microbial stability and consumer preference, especially when higher proportions of sea fennel were used. Finally, pickled sea fennel was tested with different vinegars, with alcoholic vinegar formulations proving most successful in preserving texture, green color, and sensory appeal. Türkiye focused on modern snacking and staple foods. Using extrusion technology, researchers created sea fennel-based crunchy snacks with improved antioxidant activity, while controlling crispness and texture through moisture adjustments. Additional prototypes included spiced noodles and handmade pasta enriched with sea fennel powder, bringing functional properties into familiar formats and showing promising sensory acceptability. In Tunisia, sea fennel was introduced into local specialties such as harissa (chili puree) and orange jam, as well as in snack formulations. These prototypes revealed that sea fennel not only enhances nutritional profiles with bioactive compounds but also blends harmoniously with traditional flavors, reinforcing cultural authenticity. Altogether, these prototypes highlight sea fennel's potential as both a traditional and modern food ingredient, capable of enriching condiments, snacks, and main courses alike. They also illustrate pathways for market entry - from artisanal preserves to functional convenience foods - while aligning with consumer demand for healthy, natural, and sustainable Mediterranean products.

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Versioning and Contribution History

Version	Date	Modified by	Modification reason
v1.0	20/04/2024	Valentina Melini	First version
V2.0	30/04/2024	Valentina Melini	Comments after peer reviewing process

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1 Croatian prototypes

Sea fennel edible fresh leaves – sprouts provided by IACKR were exploited by UNIST for development, production, and validation of the following UNFERMENTED FOOD LABORATORY-SCALE PROTOTYPES:

- DRIED SPICES FORMULATED WITH BLENDS OF SEA FENNEL AND OTHER MEDITERRANEAN AROMATIC HERBS
- TYPICAL DALMATIAN PATÈ WITH SEA FENNEL, OLIVES AND ONIONS
- PICKLED SEA FENNEL FORMULATION using different vinegard (apple cider, red wine, alcoholic, etc)

1.1 Dried spices formulated with blends of sea fennel and other Mediterranean aromatic herbs

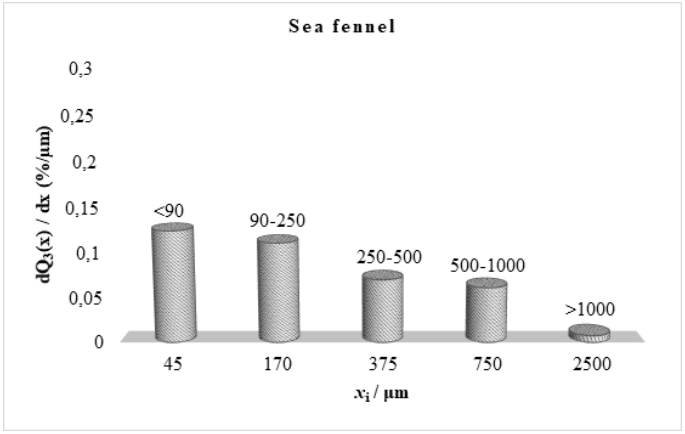
Materials and procedure

With regard to the formulation of dry spices, a selection of the most commonly used aromatic herbs and spices was made based on their most common use for culinary purposes (rosemary, bay laurel, fennel) and with regard to the chemical

composition of the spice blends, their antioxidant and organoleptic properties. The final selection will be made for laboratory-scale prototypes (> 2).

Washed plant materials (sea fennel, bay laurel, fennel and rosemary) were prepared by drying in a convection dryer at 40°C. Dry plant material was homogenized using a stainless-steel mill (A 11 Analytical mill, IKA, Staufen, Germany) and subjected to granulometric analysis.

Dry samples of plant material, a) sea fennel, b) rosemary, fennel and bay laurel



Granulometric analysis

As plant material particle size influence on the efficiency of phenolic extraction three fractions of plant powders were studied (<90 µm, 90-250 µm, 250-500 µm). The prepared sieved samples were extracted using 50% ethanol. The solvent was mixed with the sample in a ratio of 1:10 (1 g of sample in 10 mL of solvent) after which the mixture was placed on a vertical mixer (Bio RS-24 Mini rotator, Biosan, Riga, Latvia) at room temperature for 2 hours. After completion, the samples were centrifuged and filtered and stored at +4°C until analysis.

Samples were subjected to the chemical analysis (spectrophotometric and chromatographic methods). The antioxidant activity of the samples was evaluated by several methods (by FRAP, DPPH), while the application of spices in preventing oxidation spoilage was tested by Rancimat method.

In the FRAP method, the activity of the antioxidants reduced iron(III)-tripyridyltriazine (FeIII-TPTZ) complexes to iron(II) complexes. An aliquot of the samples (10 µL) was added to 300 µL of FRAP reagent. The change in absorbance was measured at 593 nm, and the results of the FRAP assay are expressed in micromoles of Fe2+ equivalents per gram of extract (µM Fe2+/g). The DPPH assay results are expressed as the percentage of DPPH radical inhibition (% inhibition). The free-radical working solution was prepared by dissolving DPPH in ethanol reach an initial absorbance of 1.2 ± 0.02. A 50 µL aliquot of the samples was added to 200 µL of the DPPH solution, the mixture was shaken, and after 60 min the decrease in absorbance was measured.

Spectrophotometric measurements (UV-VIS) were performed using a SPECORD 200 Plus, Edition 2010 (Analytik Jena AG, Jena, Germany). The total phenolic content in extracts was determined by the Folin–Ciocalteu method. Folin–Ciocalteu phenol reagent (125 µL) was added to a cuvette containing a sample (25 µL) and distilled water (1.975 mL), and after 5 min, Na2CO3 solution (10%, w/v) (375 µL) was added. The absorbance of the mixture was measured after 2 h at 765 nm. The results were calculated using the calibration curve for gallic acid and expressed as milligrams of gallic acid equivalents (GAEs) per litre of extract (mg GAE/L).

For the quantification and identification of phenolic compounds, the analytical technique, High-Performance Liquid Chromatography (HPLC), was used, with samples analyzed using a Shimadzu Nexera LC-40 system equipped with a UV/VIS detector (Shimadzu, Kyoto, Japan), and phenolic compounds were separated on a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm, Torrance, California, USA). 0.2% phosphoric acid was used in a 1:1 (v/v) ratio for mobile phase A, and a methanol-acetonitrile mixture (1:1, v/v) was used for mobile phase B. The procedure was performed at a flow rate of 1.0 mL/min and a temperature of 35°C. After the start of elution, the program was set as follows: 0-16 min (linear gradient to 15% B), 16-50 min (linear gradient to 35% B), 50-62 min, (linear gradient to 4% B), 62-65 min (4% B). After establishing the initial conditions, they were maintained for 10 min to equilibrate the column. The obtained peaks were identified by comparing the retention times of the compounds and the absorption spectra at wavelengths of 220 and 320 nm with those measured for phenolic standards tested under the same conditions. Quantification was performed using external standard calibration curves, and the results were expressed as milligrams of compound per liter of extract (mg/L).

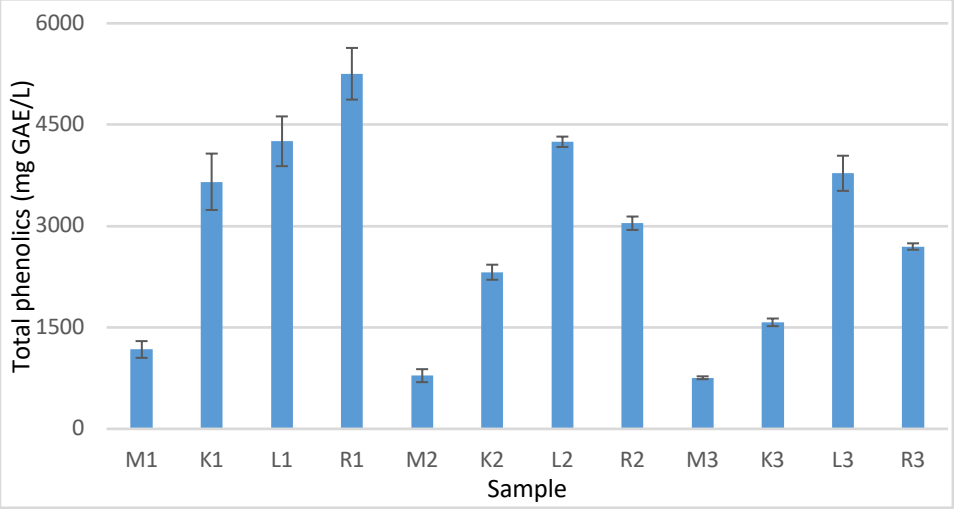
Results

a) Individual plants

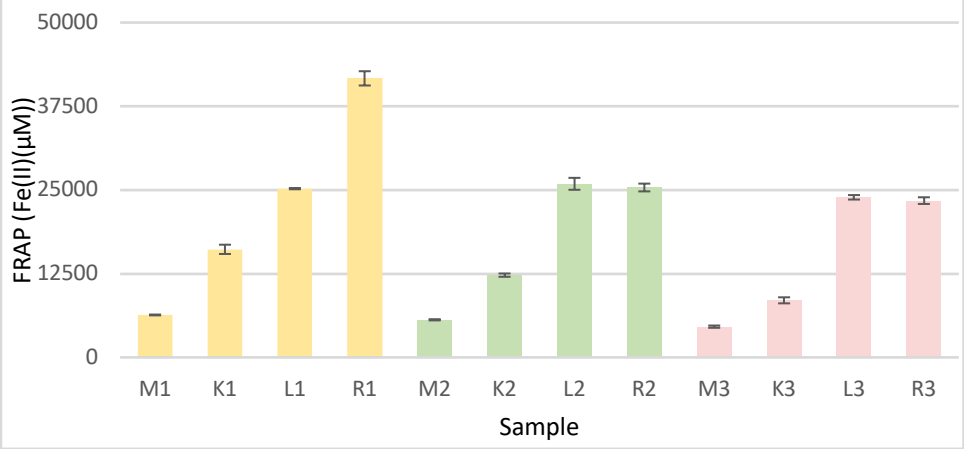
Samples of spices and their marks regarding particle sample size of the powder

Sample mark	Sample	Particle size
M1	Sea fennel	<90 µm
K1	Fennel	
L1	Bay laurel	
R1	Rosemary	
M2	Sea fennel	90-250 µm
K2	Fennel	
L2	Bay laurel	
R2	Rosemary	
M3	Sea fennel	250-500 µm
K3	Fennel	

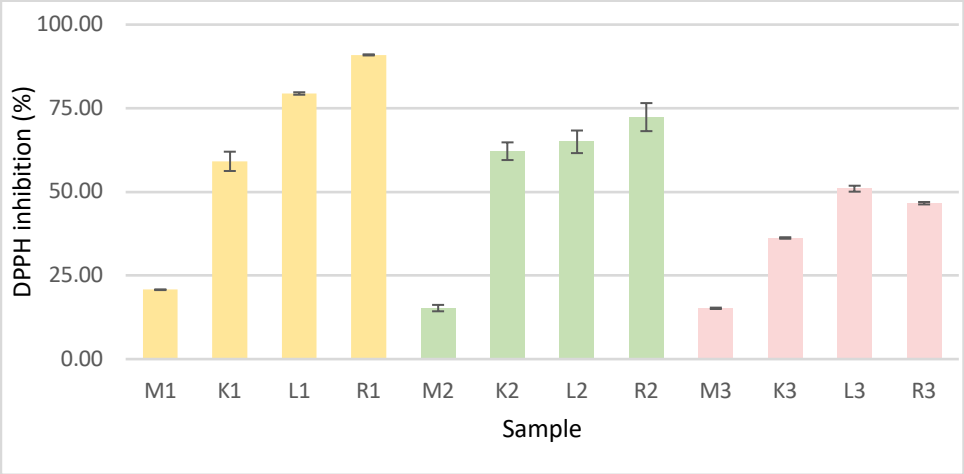
L3	Bay laurel	
R3	Rosemary	



Results for total phenolics in extracts from sea fennel (M), fennel (K), L (Bay laurel), R (Rosemary) from plant material powders with different particle sizes (1= <90 µm, 2= 90-250 µm, 3= 250-500 µm).)



Results for FRAP for extracts from sea fennel (M), fennel (K), L (Bay laurel), R (Rosemary) from plant material powders with different particle sizes (1= <90 µm, 2= 90-250 µm, 3= 250-500 µm).)



Results for FRAP for extracts from sea fennel (M), fennel (K), L (Bay laurel), R (Rosemary) from plant material powders with different particle sizes (1= <90 µm, 2= 90-250 µm, 3= 250-500 µm).)

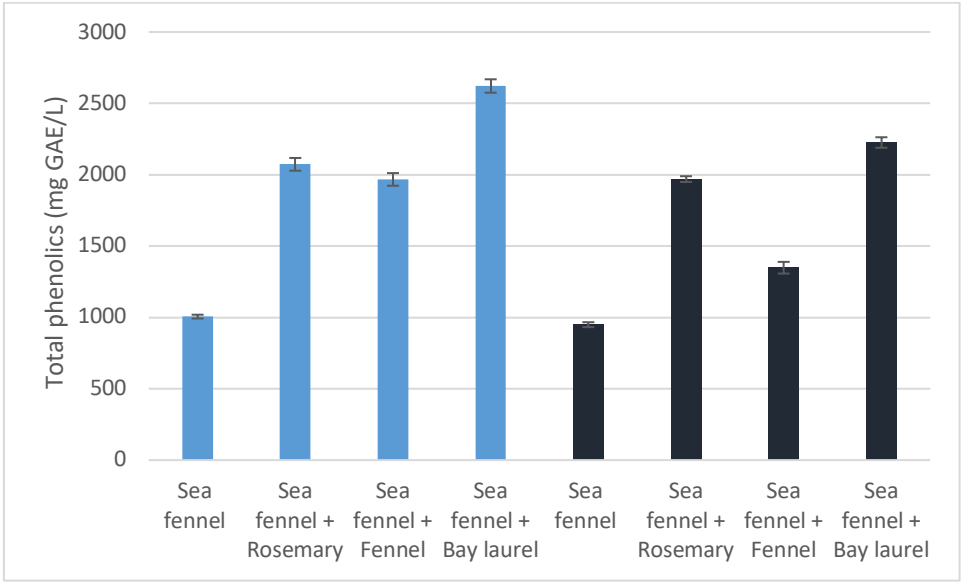
HPLC analysis of spice extracts (µg/mL) neochlorogenic acid (nCGA); Caffeic acid (CA); chlorogenic acid (CGA); cryptochlorogenic acid (cCGA); rosmaric acid (RA); cinnamic acid (CAN); rutin ®; vanilic acid (VA)

	Sea fennel	Fennel	Bay laurel	Rosemary
CA				3.50
CGA	53.70	2.13		
cCGA	5.16	25.50		
cCGA		2.17		
R		14.06	13.07	10.30
CNA			1.64	
RA				45.03
VA				5.54

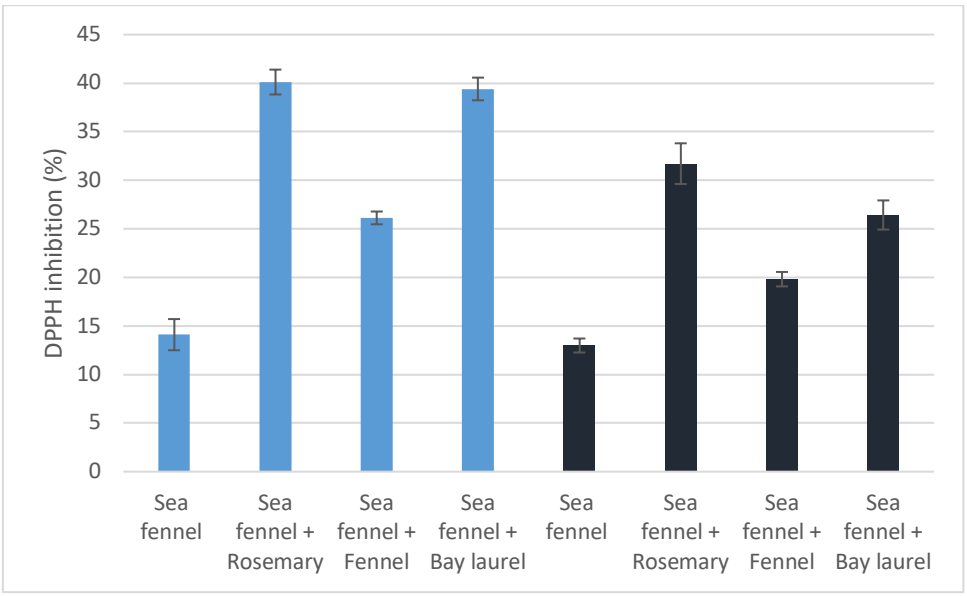
b) Combinations of plants

Samples of spices and their marks regarding particle sample size of the powder

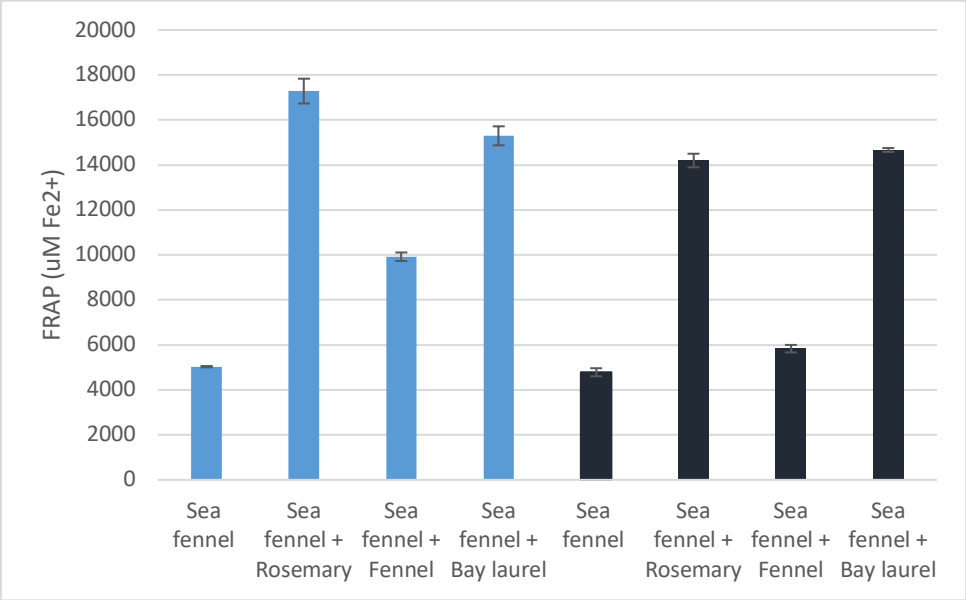
Sample mark	**Sample**	**Particle size**
M	Sea fennel	90-250 µm and 250-500 µm
M+R	Sea fennel + Rosemary	
M+K	Sea fennel + Fennel	
M+L	Sea fennel + Bay laurel	



Results for total phenolics in extracts prepared from plant material with different particle sizes (blue- 90-250 µm, black- 250-500 µm)



Results for DPPH activity of extracts prepared from plant material with different particle sizes (blue- 90-250 µm, black- 250-500 µm)



Results for FRAP activity of extracts prepared from plant material with different particle sizes (blue- 90-250 µm, black- 250-500 µm)

Oxidative stability (Rancimat) of the oil samples with the addition of extracts

	Fraction 90-250 µm	Fraction 250-500 µm
M	1,13 h	1,14 h
M+R	1,14 h	1,19 h
M+K	1,19 h	1,25 h
M+L	1,21 h	1,18 h

Main conclusions:

- extracts prepared from the smallest particles <90 µm show the highest quantity of phenolic compounds, and thus the best antioxidant capacity
- correlation was observed between the content of isolated phenolic compounds and the results of antioxidant activity.
- highest share of phenolics in sea fennel + bay laurel mixtures (bay laurel > rosemary > fennel)
- different phenolic profiles of the samples – as expected
- domination of chlorogenic and rosmarinic acid
- sea fennel alone did not affect oil oxidative stability, while in combination with herbs did (best results for sea fennel/fennel combination) (Rancimat method)

1.2 Aromatisation of plant-oils by dry sea fennel powder

Material and procedure

Sea fennel (Crithum maritimum L.) were harvested in May of 2023. Woody parts, dried leaves and other debris were removed from fresh plant material, after which the leaves and stems were cleaned with tap water to remove dirt. Clean plant material was frozen and then freeze dried (FreeZone 2.5 L, -50 °C, Labconco, Kanzas City, MO, USA). Part of the dried plant material was grounded using a commercial coffee grinder.
Four types of edible vegetable oils were purchased from Bio&bio store (Split, Croatia): olive oil (EKOZONA, Croatia), linseed oil (EKOZONA, Croatia), sesame oil (EKOPLAZA, Netherlands), and sunflower oil (EKOZONA, Croatia). Olive oil was classified as extra virgin, while the other three were cold pressed oils. The oils were infused with either whole or ground dried plant material; 1 g of dried plant material was used per every 100 mL of oil. The infused oils were kept in dark bottles and shaken at least twice daily for 90 days, and were analysed after 15, 45 and 90 days. Before analyses, plant material was removed from oils by filtration and/or centrifuge.

Phenolic compounds extraction and detection
The phenolic extracts from oils were obtained by a slightly modified procedure described by International Olive Council (COI/T.20/Doc No 29/Rev 2.) [IOC, 2022]. 2 grams of oils were weighed in a screw-cap test tube and then dissolved in 6 mL of 80% methanol. The resulting mixture was vortexed for 2 minutes and then placed in an ultrasonic bath for 15 minutes at room temperature. Finally, the mixture was centrifuged for 25 minutes at 4000 rpm, afterwhich the supernatant phase was separated and analysed.
Total phenolics were determined using the Folin-Ciocalteau method. Total phenolic content (TPC) in the samples was calculated using a chlorogenic acid standard calibration curve and expressed as miligrams of gallic acid equivalents per gram of dry plant material (mg GAE/g DM).

Determination of acidity and free fatty acids in oils
Acidity and free fatty acids in vegetable oils were determined by a modified method desribed by International Olive Council (COI/T.20/Doc No 34/Rev 1.) [IOC, 2017]. To determine the acidity, 5 grams of vegetable oils were weighed and dissolved in 25 mL of ethanol:diethyl ether mixture (1:1) and then titrated with 0,1 M sodium hydroxide solution with 1% phenolphtalein solution as end-point indicator. Free fatty acids were calculated from acidity and expressed as a percentage of oleic acid.

Peroxyde number determination
The vegetable oils' peroxyde numbers were determined by a modified method described by International Olive Council (COI/T.20/Doc. No 35/Rev. 1) [IOC, 2017]. In Erlenmeyer flask, oil samples (3 g) were dissolved in 50 mL of glacial acetic acid and chloroform mixture (3:2). 1 mL of saturated potassium iodide solution was added to the flask and the solution was vigorously shaken. After 1 minute, 100 mL of distilled water was added to the flask and the resulting mixture was titrated by 0,01 M sodium thiosulfite solution with 1% starch solution as an end-point indicator.

Volatiles of the flavored oils
Separation and analysis of the sea fennel essential oil components were conducted using GC-MS. The analysis was performed on a gas chromatograph (model 8890, equipped with an automatic liquid injector model 7693A) coupled with a tandem mass spectrometer (MS) model 7000D GC/TQ (Agilent Inc., Santa Clara, CA, USA). The system was equipped with a non-polar HP-5MS UI column (5% phenylmethylpolysiloxane, 30 m × 0.25 mm, 0.25 µm, Agilent Inc.). Helium was used as the carrier gas at a flow rate of 1 mL/min. The column temperature program was as follows: 3 minutes at 60 °C, followed by a ramp to 246 °C at a rate of 3 °C/min, with an isothermal hold for 25 minutes. The inlet temperature was set to 250 °C, the injection volume was 1 µL, and the split ratio was 1:50. The MS conditions were: ion source temperature of 200 °C, ionization energy of 70 eV, and a full-scan range of 33-350 m/z. Individual peaks were identified by comparing their retention indices with a series of n-hydrocarbons and by matching mass spectra with commercial databases (Wiley 7 MS library, Wiley, NY, USA; NIST02, Gaithersburg, MD, USA). Additional identification was carried out by comparing both mass spectra and retention indices with published literature data (Adams, 2017). All analyses were performed in triplicate, and the percentages of the identified compounds were calculated as the mean ± standard deviation.

Results

Total phenolics in vegetable oil samples and infusions.

	TPC (mg GAE/L)							
	Olive oil		Linseed oil		Sesame oil		Sunflower oil	
	WHOLE SEA FENNEL	GROUND SEA FENNEL	WHOLE SEA FENNEL	GROUND SEA FENNEL	WHOLE SEA FENNEL	GROUND SEA FENNEL	WHOLE SEA FENNEL	GROUND SEA FENNEL
Day 0 (control)	94.55 ± 1.11		16.08 ± 0.73		25.82 ± 0.40		10.85 ± 0.12	
Day 15	91.80 ± 0.09	90.98 ± 0.07	16.12 ± 0.64	16.17 ± 0.14	26.65 ± 1.20	23.53 ± 0.33	11.30 ± 0.24	10.32 ± 0.21
Day 45	82.95 ± 1.16	77.55 ± 1.11	11.88 ± 0.50	13.15 ± 0.02	22.22 ± 0.83	22.45 ± 0.50	7.17 ± 0.05	7.55 ± 0.35
Day 90	71.32 ± 2.05	71.35 ± 1.44	25.33 ± 0.33	23.17 ± 0.05	34.23 ± 1.98	34.37 ± 0.57	21.23 ± 0.05	19.57 ± 0.14

As can be seen in the Table above, the concentration of total phenolics in all four vegetable oils decreased by day 45. For linseed, sesame and sunflower oil samples, total phenolics concentration for day 45 was lower in oils infused with whole sea fennel. However, the opposite was the case with extra virgin olive oil, where oil infused with whole sea fennel had higher phenolics concentration than one infused with ground sea fennel. Interestingly, by day 90, the phenolics concentration in whole and ground sea fennel infused linseed, sesame and sunflower oils increased. Once again, extra virgin olive oil samples were an exception and the concentration of phenolics in both whole and ground sea fennel infused oils decreased by day 90.

Main VOCs of sea fennel flavored plant-based oils (%)

	Flaxseed oil		Olive oil		Sesame oil		Sunflower oil	
Compound	Pure oil	Flavored oil	Pure oil	Flavored oil	Pure oil	Flavored oil	Pure oil	Flavored oil
Hexanal	16.52±2.58	17.77±1.27	8.65±3.15	10.31±0.85	30.00±3.12	30.39±0.13	3.76±0.08	4.30±0.59
Hexan-1-ol	40.02±0.17	26.67±0.94	11.73±0.11	9.50±0.21	26.27±1.46	16.47±0.22	0.83±0.02	0.56±0.05
Limonene	0.29±0.02	7.02±0.04		6.16±0.18	1.91±0.09	14.00±0.10	1.91±0.14	2.42±0.20

Fatty acids profile (%) of plant-based oils (pure oil) and after 90-days infusion with sea fennel powder (flavored oil)

Fatty acid	Flaxseed oil	Olive oil	Sesame oil	Sunflower oil
16:0	5.38±0.33	**11.20±0.47**	**8.10±0.14**	**6.38±0.01**
16:1omega9	0.02±0.02	0.13±0.01	0.03±0.00	0.11±0.00
16:1omega7	0.07±0.01	0.72±0.04	0.11±0.00	
17:0	0.05±0.00	0.06±0.00	0.04±0.00	0.03±0.00
17:1	0.03±0.00	0.08±0.01		0.03±0.00
18:0	3.95±0.06	3.23±0.06	**5.56±0.03**	3.37±0.02

18:1	**19.29±0.06**	**75.47±0.36**	**40.71±0.09**	**31.60±0.07**
18:2	**15.67±0.01**	**7.72±0.01**	**44.33±0.01**	**57.13±0.18**
20:0		0.02±0.01		0.01±0.00
18:3	**55.32±0.20**	1.21±0.05	0.93±0.02	0.46±0.01
20:1	0.03±0.01			
22:0	0.10±0.01	0.12±0.02	0.12±0.00	0.67±0.05
24:0	0.07±0.01	0.04±0.03	0.08±0.01	0.21±0.02

Conclusions:

- GC-MC analysis showed the effect of aromatization
- the addition of sea fennel to vegetable oils leads to changes in their chemical composition

Although the parameters tested varied between the oils used, in most cases the addition of sea fennel had a **negative effect** on oil chemistry and stability during the test period

1.3 Dalmatian paté

Material and procedure

It was aimed to develop two different patés: i) sea fennel and olives (preserved green and black olives, pickled and cooked sea fennel), ii) sea fennel (cooked), olives (pickled green olives) and a domestic/local pickled onion variety (ljutika). The prototypes were subjected to nutritional and sensory analysis.

	1st study					**2nd study**		
Raw material	1	2	3	4	5	1	2	3
Sea fennel cooked	-	-	15%	15%	30%	50%	30%	70%
Sea fennel pickled	20%	20%	-	-	-			
Green olives	70%	-	60%	-	-			
Black olives (brine)	-	70%	-	60%	60%	50%	70%	30%
Onions	-	-	15%	15%	-			

+ olive oil (10%), + salt (1,5%)

Physico-chemical parameters

Salt content

The salt content (g/100 mL) was determined in accordance with the modified method of Mohr by determining the chloride ion concentration by titrating the pickle juice sample (1 mL) diluted with 50 mL of distilled water with silver nitrate solution (0.1 M).

Water activity (a_w)

Water activity (aw) was determined using an AW LabMaster instrument (Novasina AG, Lachen, Switzerland).

Colour (L*, a*, b*, C*, h)

Color analysis was performed with a CIELAB color system (CR-400 Chroma Meter, Konica, Tokyo, Japan) and expressed in terms of parameters lightness L*, a*, b*, C and h.

Microbiology / Shelf-life study

Microbial analyses were conducted at baseline and throughout a six-month storage period. The following microbial groups were evaluated using standard ISO methods: Pseudomonas spp., yeasts, lactic acid bacteria, aerobic mesophilic bacteria, Enterobacteriaceae, Listeria monocytogenes, and coagulase-positive staphylococci.

Oxidative stability - OXITEST

Oxidative stability was measured using the OXITEST method at 90 °C under 6 bar oxygen pressure. A 20 g sample of each paté formulation was analyzed, and the induction period (IP) was recorded in hours and minutes.

Sensory analysis

Sensory evaluation was performed by 12 untrained panelists and samples were scored on a 5-point scale for color, texture, taste, aroma, and overall impression that included visual, textural, taste, and flavor attributes.

Results

Water activity (aw) and moisture content (%)

The water activity values for the sea fennel:black olive patés ranged from 0.974 to 0.975 across formulations. Moisture content increased with higher sea fennel proportion, from 55.6% in the 30:70 blend to 59.9% in the 70:30 blend.

Water activity (aw) and moisture content (%) of sea fennel paté

	water activity (a_w)	moisture content (%)
Sea fennel:black olives paté (30:70)	0.975	55.6±0.0
Sea fennel:black olives paté (50:50)	0.974	59.1±0.3
Sea fennel:black olives paté (70:30)	0.975	59.9±0.2

Colour

The lightness (L*) decreased as sea fennel proportion increased: L* was highest in the 30:70 formulation (30.43) and lowest in the 70:30 formulation (23.36). The chroma (C*) values and hue angles followed similar trends, indicating reduced visual brightness and increased green tone with more sea fennel content.

Colour parameters (L, a*, b*, C*, h)) of sea fennel paté*

	L*	a*	b*	C*	h
Sea fennel:black olives paté (30:70)	30.43	0.308333	12.95333	12.96	88.63667
Sea fennel:black olives paté (50:50)	26.44667	0.698333	9.701667	9.728333	85.86

Sea fennel:black olives paté (70:30)	23.364	1.328	8.82	8.924	81.424

Microbiology / Shelf-life study (6 months)

Throughout the six-month storage period, all samples remained microbiologically stable. No growth of the tested microorganisms was detected, indicating the effectiveness of the preservation method.

Oxidative stability - OXITEST

The oxidative induction period increased with higher sea fennel content. The 70:30 sea fennel:black olive paté showed greater stability (31:18 h) compared to the 30:70 formulation (25:05 h), suggesting a protective antioxidant effect from sea fennel.

Induction periods of the tested pates (Oxitest method)

Sample	Induction period (h)
Sea fennel:black olives pate (30:70)	24:56 / 25:05
Sea fennel:black olives pate (70:30)	30:59 / 31:18

Sensory evaluation

The paté containing 70% sea fennel was the most preferred among panelists, scoring highest in color, texture, taste, aroma, and overall impression. This formulation also received the most favorable scores in the consumer preference test, confirming its superior sensory acceptability.

1.4 Pickled sea fennel

The prototype was formulated by using wild collected samples, since cropped sea fennel is not yet available. In the preliminary study, a prototype of pickled sea fennel was developed using 3 different types of vinegar (apple-cider, red wine, alcoholic vinegar) in different proportions and added with 3% salt and 2% sugar.

Material and methods

Pickling procedure

Fresh, young, and undamaged leaves of wild sea fennel were washed, drained and dried with a paper towel. The fresh plant material (blanching negatively influenced the texture) was placed in clean jars and immersed in brine. According to the traditional recipes and results of preliminary study, different types of brines were prepared using different types of vinegar in different proportions: a) apple cider vinegar, b) wine vinegar, and c) alcoholic vinegar. All brines contain salt (1-3%, w/v) and some of them sucrose (granulated sugar) (1.5-3%, w/v). The filled and sealed jars were subjected to pasteurization treatment (at 95°C, for 10 minutes). After cooling (4 hours), they were checked by lid inspection.

A)

B)

C)

D)

E)

Processing steps of vinegar preserved sea fennel: A) plant material preparation, B), filling jars, C) pasteurization, D) cooling, E) final products

Analysis

Regarding the chemical composition, the pH, titratable acidity, and salinity of the brines were analyzed.

The aromatic or volatile organic compounds (VOCs) of sea fennel pickles were extracted by headspace-solid phase microextraction (HS-SPME) and detected by GC-MS.

Color analysis of the pickled sea fennel leaf was determined with a CIELAB color system (CR-400 Chroma Meter, Konica, Tokyo, Japan) and expressed in terms of parameters lightness L*, a*, b*, C and h.

The texture of sea fennel leaves was analyzed using a texture analyzer (TA Plus; Lloyd Instruments, Fareham, UK) equipped with a 500 N load cell and a Warner-Bratzler blade set with a rectangular slot blade. The parameters measured were hardness (N) and work of cutting (N/mm).

Sensory evaluation was performed by 12 untrained panelists and samples were scored on a 5-point scale for color, texture, taste, aroma, and overall impression that included visual, textural, taste, and flavor attributes. In addition, defects such as off-odor and mechanical damage were evaluated. The same panelists were used for sensory evaluation of the preserved sea fennel samples by 9-point hedonic scale rating.

Results

pH values, salt content and total acidity of the sea fennel samples preserved via the addition of three types of vinegars.

	Apple Cider Vinegar (1:5, *v/v*)	Wine Vinegar (1:4, *v/v*)	Alcoholic Vinegar (1:5, *v/v*)	*p*-Value
pH value	3.55 ± 0.05	3.64 ± 0.09	3.49 ± 0.06	0.09
Titratable acidity (g/100 mL)	1.62 ^{a} ± 0.01	1.65 ^{a} ± 0.01	2.48 ^{b} ± 0.01	<0.01
Salt (g/100 mL)	1.16 ^{a} ± 0.02	1.16 ^{a} ± 0.02	1.25 ^{b} ± 0.01	<0.01

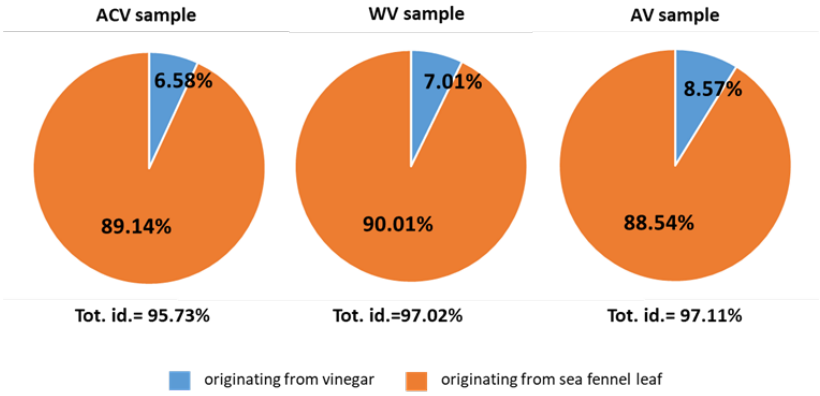
Mean values within a row with different letters in superscript differ significantly ($p < 0.05$).

The volatile organic compounds (VOCs, %) of preserved sea fennel leaf in apple cider, wine and alcoholic vinegar.

No.	Compound	Apple Cider Vinegar (1:5, *v/v*)	Wine Vinegar (1:4, *v/v*)	Alcoholic Vinegar (1:5, *v/v*)
1.	Acetic acid *	6.10 ^{a} ± 0.30	6.41 ^{a} ± 0.08	8.40 ^{b} ± 0.54
2.	α-Pinene	4.69 ± 0.26	5.17 ± 0.34	5.04 ± 0.35
3.	Camphene	0.06 ± 0.03	0.07 ± 0.03	0.10 ± 0.05
4.	(*E*)-Hept-2-enal	0.07 ± 0.02	0.04 ± 0.01	-
5.	Benzaldehyde *	-	-	0.02 ± 0.00
6.	Sabinene	0.20 ^{a} ± 0.00	0.14 ^{b} ± 0.01	0.10 ^{c} ± 0.01
7.	6-Methylhept-5-en-2-one	0.01 ± 0.00	-	-
8.	β-Myrcene	1.16 ^{a} ± 0.09	1.69 ^{b} ± 0.10	2.06 ^{c} ± 0.13
9.	(*E,Z*)-Hepta-2,4-dienal *	0.01 ± 0.00	-	-
10.	Octanal	0.09 ± 0.02	0.05 ± 0.02	0.04 ± 0.02
11.	α-Phellanderene	0.40 ^{a} ± 0.05	0.38 ^{a} ± 0.01	0.48 ^{b} ± 0.02
12.	α-Terpinene	0.17 ^{a} ± 0.00	0.43 ^{b} ± 0.03	0.90 ^{c} ± 0.06
13.	Limonene	52.64 ^{a} ± 2.21	43.75 ^{b} ± 2.22	43.31 ^{b} ± 2.58
14.	(*E*)-β-Ocimene	5.99 ^{a} ± 0.04	8.30 ^{b} ± 0.41	8.50 ^{b} ± 0.42
15.	(*Z*)-β-Ocimene	0.59 ^{a} ± 0.04	0.94 ^{b} ± 0.01	1.23 ^{c} ± 0.01
16.	γ-Terpinene	6.26 ^{a} ± 0.08	8.04 ^{b} ± 0.953	7.81 ^{b} ± 0.52
17.	α-Terpinolene	0.28 ^{a} ± 0.00	0.44 ^{b} ± 0.02	0.86 ^{c} ± 0.05
18.	Linalool	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
19.	(3*E*)-6-Methylhepta-3,5-dien-2-one *	0.08 ^{a} ± 0.00	0.06 ^{b} ± 0.00	0.07 ^{b} ± 0.00
20.	*p*-Mentha-1,3,8-triene	-	-	0.04 ± 0.01
21.	2-Phenylethanol *	0.05 ^{a} ± 0.03	0.32 ^{b} ± 0.01	0.07 ^{a} ± 0.02
22.	(3*E*,5*E*)-2,6-dimethylocta-1,3,5,7-tetraene	0.29 ^{a} ± 0.04	0.21 ^{b} ± 0.00	0.17 ^{b} ± 0.00
23.	(*Z*)-Alloocimene	1.22 ^{a} ± 0.08	2.04 ^{b} ± 0.14	2.23 ^{b} ± 0.15
24.	β-Terpineol	0.09 ^{a} ± 0.00	0.12 ^{b} ± 0.01	0.11 ^{c} ± 0.00
25.	4-prop-1-en-2-ylcyclohexene *	0.33 ± 0.01	0.16 ± 0.00	-
26.	(*Z*)-Non-2-enal	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
27.	*p*-Mentha-1,5-dien-8-ol	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
28.	Terpinen-4-ol	10.03 ^{a} ± 0.32	12.85 ^{b} ± 0.95	9.40 ^{a} ± 0.64
29.	α-Terpineol	1.18 ^{a} ± 0.02	1.68 ^{b} ± 0.07	1.37 ^{c} ± 0.06

30.	(*E*)-Carveol	0.36 ^{a} ± 0.03	0.21 ^{b} ± 0.01	0.16 ^{b} ± 0.00
31.	(*Z*)-Carveol	0.09 ^{a} ± 0.02	0.06 ^{ab} ± 0.01	0.03 ^{b} ± 0.01
32.	Thymol methyl ether	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
33.	Carvone	1.16 ^{a} ± 0.12	0.58 ^{b} ± 0.04	0.60 ^{b} ± 0.04
34.	α-Ionene	0.02 ^{a} ± 0.00	0.05 ^{b} ± 0.00	0.02 ^{a} ± 0.00
35.	2-Phenylethyl acetate *	0.02 ^{a} ± 0.00	0.06 ^{b} ± 0.01	0.02 ^{a} ± 0.00
36.	(*E*)-Dec-2-enal	0.07 ^{a} ± 0.01	0.04 ^{b} ± 0.00	0.03 ^{c} ± 0.00
37.	o-Thymol	0.06 ^{a} ± 0.00	0.05 ^{a} ± 0.00	0.03 ^{b} ± 0.00
38.	β-Caryophyllene	0.13 ^{a} ± 0.00	0.35 ^{b} ± 0.02	0.35 ^{b} ± 0.02
39.	γ-Elemene	0.10 ^{a} ± 0.04	0.15 ^{a} ± 0.05	0.44 ^{b} ± 0.05
40.	α-Bergamotene	0.14 ± 0.03	0.17 ± 0.03	0.20 ± 0.04
41.	Aromadendrene	0.04 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
42.	α-Curcumene	0.18 ± 0.02	0.19 ± 0.00	0.18 ± 0.00
43.	(*E*)-β-Ionone	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
44.	α-Zingiberene	0.17 ^{a} ± 0.00	0.38 ^{b} ± 0.01	0.79 ^{c} ± 0.03
45.	β-Bisabolene	0.26 ^{a} ± 0.02	0.32 ^{b} ± 0.00	0.44 ^{c} ± 0.01
46.	β-Sesquiphellanderene	0.41 ^{a} ± 0.03	0.49 ^{a} ± 0.03	0.70 ^{b} ± 0.04
47.	Selina-3,7(11)-dien	0.02 ^{a} ± 0.00	0.03 ^{b} ± 0.00	0.09 ^{c} ± 0.00
48.	Germacrene B	0.04 ^{a} ± 0.00	0.07 ^{b} ± 0.00	0.22 ^{c} ± 0.02
49.	Caryophyllene oxide	0.15 ^{a} ± 0.01	0.15 ^{a} ± 0.01	0.11 ^{b} ± 0.00
50.	α-Guaiol	0.21 ± 0.02	0.23 ± 0.00	0.20 ± 0.01

** originated from vinegar; RI—retention index. Results are expressed as mean ± standard deviation (SD); the average of two results was used as the third repetition in the ANOVA. Mean values within a row with different letters in superscript differ significantly ($p < 0.05$).*



Distribution of origin of compounds in preserved sea fennel samples. ACV—apple cider vinegar; WV—wine vinegar; AV—alcoholic vinegar; tot. id.—total identified.

Color and texture parameters of the sea fennel persevered via the addition of apple cider, wine and alcoholic vinegar.

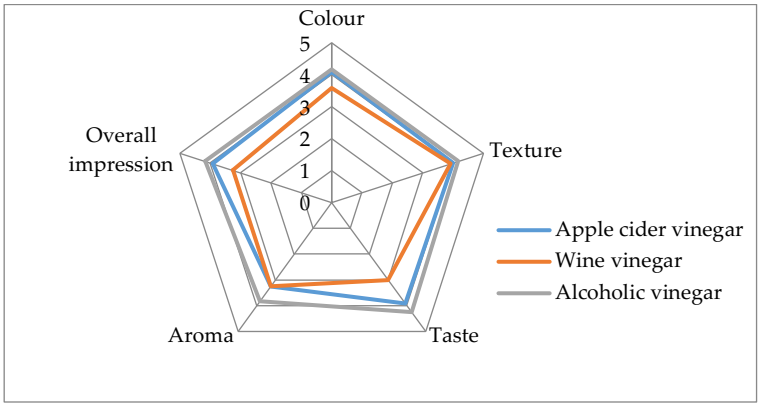
	Apple Cider Vinegar (1:5, *v*/*v*)	Wine Vinegar (1:4, *v*/*v*)	Alcoholic Vinegar (1:5, *v*/*v*)
Color parameters			

L*	38.48 ^{a} ± 0.33	35.30 ^{b} ± 0.32	36.21 ^{b} ± 0.33
a*	−0.27 ^{a}± 0.01	−0.84 ^{ab} ± 0.01	−1.45 ^{b} ± 0.02
b*	20.62 ^{a} ± 0.60	23.03 ^{b} ± 0.59	15.28 ^{c} ± 0.61
C	20.64 ^{a} ± 0.58	23.05 ^{b} ± 0.57	15.36 ^{c} ± 0.56
h	90.88 ^{a} ± 0.43	91.22 ^{a} ± 0.43	95.03 ^{b} ± 0.42
Texture parameters			
Load at Maximum Load (N)	8.22 ± 1.79	10.47 ± 0.45	8.56 ± 1.58
Work of shear (N/mm)	14.19 ^{a} ± 3.52	19.62 ^{b} ± 1.72	13.91 ^{a} ± 1.05

Results are expressed as mean ± standard deviation; mean values within a row with different letters in superscript differ significantly ($p < 0.05$).

(**a**) (**b**) (**c**)

*Samples of pickled sea fennel leaves in (**a**) apple cider vinegar, (**b**) wine vinegar and (**c**) alcoholic vinegar.*



Sensory profiles of sea fennel preserved in pickle juices prepared using different vinegars (5: excellent, 4: good, 3: moderate, 2: poor, and 1: extremely poor).

Sensory parameters of the sea fennel preserved via the addition of apple cider, wine and alcoholic vinegar.

	Apple Cider Vinegar (1:5, *v/v*)	Wine Vinegar (1:4, *v/v*)	Alcoholic Vinegar (1:5, *v/v*)	*p*-Value
Color	4.08 ± 0.38	3.58 ± 0.29	4.17 ± 0.52	0.25
Texture	4.00 ± 0.66	3.92 ± 0.29	4.17 ± 0.28	0.81
Taste	3.92 ^{a} ± 0.80	3.00 ^{b} ± 0.50	4.25 ^{a} ± 0.25	<0.05
Aroma	3.25 ± 0.87	3.25 ± 0.25	3.83 ± 0.52	0.44
Overall impression	3.92 ± 0.63	3.25 ± 0.50	4.17 ± 0.38	0.16

Results are expressed as mean ± standard deviation; mean values within a row with different letters in superscript differ significantly ($p < 0.05$

Conclusion:

- pH- from 3.49 (alcoholic vinegar) to 3.64 (wine vinegar)
- titratable acidity and salinity were higher in the alcoholic vinegar
- reddish color of the wine vinegar negatively affected the sea fennel color and was also negatively evaluated by the panelists,
- alcoholic vinegar maximally preserved the green tones of the leaf (a*).
- all sensory parameters of alcoholic vinegar sample (namely color, texture, taste, aroma and overall impression) were given the highest scores
- wine vinegar sample received the lowest scores
- intense aroma of the wine vinegar was a negative characteristic (off-flavor)

The results showed that alcoholic vinegar received the highest score in the sensory evaluation, while the sample preserved with wine vinegar, which is mainly used in the traditional preservation of sea fennel in the Croatian coastal region, received the lowest scores and the negative perception of consumers, mainly due to the strong aromatic vinegar notes and negative attributes for color and firmness.

2 Turkish prototypes

Sea fennel edible fresh leaves – sprouts provided by WP4 were exploited by UNIEGE for development, production, and validation of the following UNFERMENTED FOOD LABORATORY-SCALE PROTOTYPES.

2.1 Snacks obtained by extrusion starting from (a) doughs made with sea fennel and other ingredients and (b) stuffed with sea fennel cream

Snacks, often manufactured through methods like deep-fat frying, extrusion, baking, or toasting, have gained significance in modern diets due to changes in consumer behavior and the constraints of time for meal preparation. However, despite their widespread consumption, it is commonly recognized that these snacks tend to lack proper nutritional balance, mainly due to their high fat content and insufficient levels of protein and dietary fiber (Cuj-laines et al., 2018). Hence, to enhance the nutritional quality of snacks, various components such as legumes, vegetables, fruits, and by-products of food processing can be integrated into their recipes (Gomes et al., 2023). Extrusion, a hydrothermal process characterized by high temperatures and short durations, induces physical and chemical alterations in materials by causing gelatinization and starch breakdown, dissolution of dietary fiber, and aggregation of proteins due to thermal and mechanical stresses generated by hot barrels and rotating screws (Yagci et al., 2022; Costantini et al., 2021; Patil et al., 2016).

During the reporting period, under Task 5.2 of the project, the exploitation of sea fennel edible parts for manufacturing of innovative sea fennel-based foods (Laboratory-scale manufacturing of unfermented shelf-stables preserves), the following study has been carried out by Ege University team.

Material and Methods

Materials

The raw material utilized was corn grits obtained from Semolina Azteca Milling Turkey in Samsun, Turkey (Batch number S21-034), with a moisture content of 11.66%, protein content of 5.40%, ash content of 0.49%, oil content of 1.42%, and particle distribution indicating that for every 100 grams of grit, 50-65 grams were larger than 500 µm and 35-50 grams were larger than 300 µm. The sea fennel was dried to a moisture content of 9.27% using hot air at 40°C. Following drying, the sea fennel was then ground and sieved to a particle size smaller than 5 mm. Total phenolic content and total antioxidant activity of the sea fennel were 8.793 mg Gallic acid equivalent/g sample, and 14.003 mg Trolox equivalent antioxidant capacity/g sample, respectively.

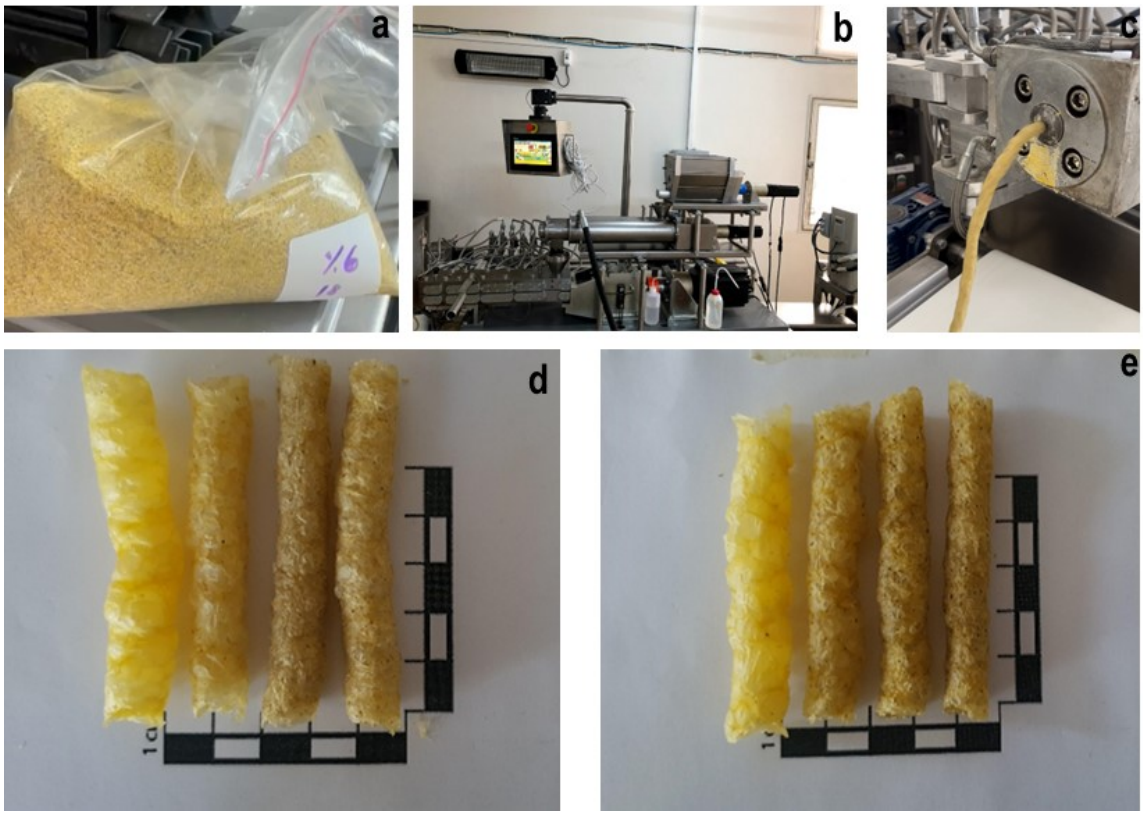
Methods

Composite flour preparation

To prepare the samples, flour blends were prepared by blending different amounts of sea fennel (SF) with corn grits (CG) in ratios of 100:0, 98:2, 96:4, and 94:6 (w/w). Distilled water was added to the blend and thoroughly mixed to adjust the moisture content to either 16% or 18%. The moistened flour blends were sieved to ensure a particle size smaller than 5 mm and then placed in polyethylene bags overnight at 25°C to reach moisture equilibrium. Prior to the extrusion moisture content of the samples was assessed again.

Extrusion process

A 25 mm barrel diameter co-rotating twin-screw extruder (Feza Machine Co. Ltd., Istanbul, Türkiye) was used for the extrusion process. It was equipped with a single, 3 mm circular die and had a screw length-to-diameter ratio (L/D) of 25: The first three regions of the barrel from the feeder toward the die were 60°C, 100°C, and 130°C respectively. In addition, the temperatures of the fourth zone and die were set at 150°C. Additionally, the screw configuration, screw speed (200 rpm), and feed rate (20 kg/s) were kept constant during extrusion. Following extrusion, the extrudates were cooled to room temperature (25 °C), sealed, and stored in plastic polyethylene terephthalate (PET) jars. The picture of the extrusion process and extrudates is given in the figure below.



Processing steps of sea fennel added extruded snacks: a) composite flour , b), extruder, c) exit of the die during extrusion process, d) the picture of extruded product with 16% moisture content with substitution of 0, 2, 4, and 6% sea fennel powder, respectively, e) the picture of extruded product with 18% moisture content with substitution of 0, 2, 4, and 6% sea fennel powder, respectively.

Physio-chemical properties of extruded snacks

Moisture and Water Activity

The AACC (2000) approach was followed in determining the moisture (Method: 44–15A). The extrudates had a moisture content between 9.25% and 10.49%. Furthermore, a water activity measurement apparatus (Testo AG 400, Lenzkirch, Germany) was used to measure the water activity of the extrudes.

Functional properties

In order to calculate the samples' water absorption index (WAI) (Equation 3) and water solubility index (WSI) (Equation 4), 2.5 g of the ground sample were suspended in 30 mL of distilled water at 30°C and agitated for 30 minutes. After that, it was centrifuged for 20 minutes at 2500 g using a Hettich Universal 320 R. Until the weight stabilized to a consistent value, the supernatant was kept at 105°C (Anderson et al., 1969). The following formulae were used to calculate the WSI and WAI.

$$WAI\ (g/g) = (m_{sediment})/ (m_{dry\ solid}) \quad (3)$$

$$WSI\ (\%) = (m_{dissolved\ solid\ in\ supernatant})/ (m_{dry\ solid}) \times 100 \quad (4)$$

where $m_{dry\ solid}$ indicates the sample weight at beginning weight, $m_{dissolved\ solid\ in\ supernatant}$ is the weight of the solids that have dissolved in the supernatant, and $m_{sediment}$ is the weight of the gel that is left over after centrifugation.

Physical properties

Expansion ratio and Apparent density

By using a digital vernier caliper, the diameters of the samples were measured by taking an average of at six ten samples. In addition, the expansion ratio (ER) (Equation 1) of the samples was calculated as the ratio of the extrudate to the die diameter (Thymi et al., 2005).

$$ER = d_{extrudate}/d_{die} \quad (1)$$

where the diameter of the die is denoted by d_{die} and the diameter of the extrudate by $d_{extrudate}$.

The snacks' dimensions were measured to calculate the apparent density (AD) (Equation 2). To calculate the AD, measurements were made of the samples' diameter and length per unit weight (g) (Lazou & Krokida, 2010).

$$\rho_{app} = \frac{4.m}{\pi.d_{extrudate}^2.L} \quad (2)$$

where ρ_{app}, $m_{extrudate}$,and L stand for AD, extrudate mass (g), extrudate diameter (cm), and extrudate length (cm), respectively.

Textural properties

The hardness (H) and crispiness (CR) of the samples were determined using the TA-XT2i Texture Analyzer (StableMicrosystems, Surrey, UK) equipped with a five-blade Kramer cutting cell. The analysis was conducted in the "compression force measurement" mode, employing a 50-kg load cell and a single layer of the product. Parameters for the analysis included a 48-mm probe distance and pretest, test, and post-test speeds of 1, 2, and 10 mm/s, respectively. The initial compression peak force (N) required to cut the specimen was recorded as the H value, while the total number of peaks was recorded as the CR value (Oliveria et al., 2017).

Color

After grinding, the color values of the extrudates were determined using HunterLab Colorflex (HunterLab, CFLX 45-2 Colorimeter, Reston). For each sample, CIELAB space parameters L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness) color values were measured.

Total phenolic content and antioxidant activity

The extract of the sample for the total phenolic content (TPC) and antioxidant analysis were performed according to Shevkani et al. (2019) Samples prepared at room temperature (25 °C) by combining dried sea fennel, corn grits, and extrudates (1.0 g) with 80% methanol (10 ml) for 2 hours using a magnetic stirrer (200 rpm), followed by centrifugation at 7000 g for 10 minutes. The resulting supernatants were collected, while the sediments were resuspended in 80% methanol, stirred, and centrifuged again. The supernatants from both rounds of extraction were combined and filtered to remove any insoluble particles. All extracts were prepared in triplicate and immediately analyzed for total phenolic content and antioxidant activity.

Total phenolic content was determined using a modified version of the method developed by Singleton et al. (1999). Specifically, 100 µl of the extract was diluted with distilled water to a final volume of 4.8 ml. Then, 300 µl of Folin–Ciocalteau reagent was added, and the mixture was incubated for 8 minutes. Following this, 900 µl of 20% sodium carbonate solution was added, and the solutions were allowed to stand for 1 hour at room temperature before measuring absorbance at 765 nm using a UV/Vis spectrophotometer. Gallic acid was used as a standard, and the results were expressed as mg GAE (gallic acid equivalents) per gram of dry matter.

Total antioxidant activity was assessed using the ABTS free radical scavenging test. ABTS free radicals were generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate in the dark for 12 hours. The resulting working solution was prepared by diluting the ABTS stock solution with 80% methanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. A mixture of 3 ml ABTS working solution and 100 µl of extract was prepared, and absorbance was measured at 734 nm after 6 minutes. The calibration curve was generated by plotting percentage inhibition against the concentration of Trolox in both assays and antioxidant activity was expressed as mg Trolox equivalents per gram of dry matter.

Statistics

The SPSS Version 22.0 program was used to perform statistical analyses on the experimental data (SPSS Inc., Chicago, IL). The experiment's results are presented as the mean ± standard deviation. The analysis of variance (ANOVA) was used to examine the impact of each individual component on the sample attributes. At a significance level of $p \leq 0.05$, variance analysis and Duncan's multiple range tests were utilized to identify variations between the extrudates' characteristics.

Results and Discussion

Moisture content and water activity of the extrudates

The moisture content and water activity results of the extruded snacks are given in the table below. The results show that both the moisture content of the flour and the addition of the sea fennel affect the final product properties. The increase in the feed moisture led to an increase in the moisture content of the extrudates. Furthermore, the addition of sea fennel into the formulation caused an increase in the final moisture content of the extruded snacks. The water activity of the samples increased as the feed moisture levels increased.

Effect of extrusion parameters on the functional properties

WSI is frequently regarded as a measure of molecular component breakdown (Medina-Rendon et al., 2021), reflecting the amount of soluble constituents released during processing (Pardhi et al., 2019). The WSI of the extrudates exhibited a significant correlation ($p < 0.05$) with each parameter, as illustrated in Table 3.1.1. WSI increased as the sea fennel content and feed moisture increased. This can be attributed to the dextrinization and depolymerization of starch and the degradation of starch into smaller chain components resulting in higher solubility (Beigh et al., 2020). Furthermore, the augmentation of the soluble dietary fiber content in sea fennel through the process of extrusion may also contribute to this phenomenon.

WAI serves as an indicator of the water absorbed by the extrudate, providing insight into the volume occupied by starch following water absorption, with retention indicating the starch gelatinization index (Lucas et al., 2018). As depicted in Table 1, WAI values were notably influenced ($p < 0.05$) by both the sea fennel content and feed moisture. Increasing feed moisture resulted in an elevation of WAI. This augmentation is attributed to water acting as a plasticizer in the feed, thereby mitigating the degradation of starch granules and consequently yielding higher WAI values (Pardhi et al., 2019).

Consequently, samples with elevated feed moisture content exhibited higher WAI values. Conversely, the addition of sea fennel led to a reduction in WAI due to the decreased starch content.

Effect of extrusion parameters on the physical properties

Expansion ratio and apparent density

The ER of the snacks significantly decreases ($p < 0.05$) with increasing sea fennel content. According to Yağcı and Göğüş (2008), the insoluble fiber in sea fennel decreases the dough's elasticity and plasticity, while the increased fiber content breaks down the cell wall to prevent the development of gas bubbles during extrusion (Pérez-Navarrete et al., 2006; Wójtowicz et al., 2018). Besides, the steam at high pressure during the extrusion could cause the cellular matrix to collapse, which lowers the ER with the addition of the sea fennel (Bisharat et al., 2013; Yu et al., 2013). The decreased starch content could be another factor contributing to the expansion's reduction. On the other hand, the initial moisture content of the flour mixture also affected ER ($p < 0.05$) because water has a plasticizing effect during the extrusion process which allows the starch to gain glassy characteristics. Thus, it encourages dough formation and decreases expansion, which results in reducing the ER of the product.

The AD is a significant physical feature that impacts the quality of the extrudates (Banki et al., 2021). According to the results, the initial moisture content and sea fennel content in the formulation have an impact on the AD. While comparing the product without sea fennel and the product with sea fennel, it can be seen that sea fennel addition increased the AD properties of extrudates. The reason for this could be decreasing in the gelatinization due to an increment in the dietary fiber content of the flour mixture (Pérez-Navarrete et al., 2006).

Physico-chemical and functional properties of the extrudates

Sample	Moisture Content (%)	Water Activity (a_w)	WAI (g/g)	WSI (%)
S0M16T140	10.177 ± 0.074^{j}	0.5880 ± 0.0006^{k}	5.0597 ± 0.2904^{def}	33.6768 ± 2.9018^{bc}
S2M16T140	9.391 ± 0.178^{fg}	0.5243 ± 0.0047^{h}	5.2063 ± 0.3277^{def}	35.4464 ± 2.6626^{bcd}
S4M16T140	9.765 ± 0.052^{hi}	0.5277 ± 0.0058^{h}	4.7236 ± 0.1652^{de}	39.7045 ± 1.5350^{cd}
S6M16T140	9.865 ± 0.071^{ij}	0.5577 ± 0.0033^{j}	5.3505 ± 0.2311^{def}	34.4893 ± 0.8516^{bc}
S8M16T140	9.359 ± 0.327^{efg}	0.5377 ± 0.0032^{i}	4.9193 ± 0.1239^{def}	35.8585 ± 0.3764^{bcd}
S0M18T140	9.109 ± 0.117^{bcdef}	0.5007 ± 0.0023^{ef}	5.6809 ± 0.2202^{fg}	24.1590 ± 0.8983^{a}
S2M18T140	8.791 ± 0.056^{abd}	0.4893 ± 0.0007^{bc}	5.5214 ± 0.1096^{efg}	30.9481 ± 3.7954^{ab}
S4M18T140	8.737 ± 0.086^{a}	0.4860 ± 0.0006^{abc}	5.5953 ± 0.0074^{efg}	30.6458 ± 0.6146^{ab}
S6M18T140	8.999 ± 0.114^{abcd}	0.5050 ± 0.0010^{f}	4.9407 ± 0.7404^{def}	35.1772 ± 4.3553^{bcd}
S8M18T140	8.919 ± 0.038^{abcd}	0.4977 ± 0.0019^{ef}	5.1752 ± 0.1864^{def}	34.8627 ± 0.6721^{bc}
S0M16T155	9.972 ± 0.033^{ij}	0.5250 ± 0.0006^{h}	4.4872 ± 0.8118^{cd}	41.1334 ± 9.0335^{cd}
S2M16T155	9.758 ± 0.009^{hi}	0.5197 ± 0.0026^{gh}	4.9653 ± 0.0275^{def}	40.0610 ± 0.6534^{cd}
S4M16T155	9.536 ± 0.048^{gh}	0.5210 ± 0.0021^{gh}	4.5227 ± 0.2669^{cd}	42.9393 ± 2.5366^{de}
S6M16T155	9.109 ± 0.051^{bcdef}	0.4853 ± 0.0022^{abc}	2.2672 ± 0.0246^{a}	62.4325 ± 0.1413^{g}
S8M16T155	9.016 ± 0.081^{abcde}	0.4793 ± 0.0018^{a}	3.7429 ± 0.4446^{bc}	49.2430 ± 3.4865^{ef}

S0M18T155	9.116±0.055^{bcdef}	0.5260±0.0067^{h}	6.2749±0.2672^{g}	26.0128±0.5967^{a}
S2M18T155	9.228±0.042^{cdefg}	0.5150±0.0047^{g}	5.4676±0.2182^{efg}	35.9916±2.2206^{bcd}
S4M18T155	9.327±0.049^{defgd}	0.4937±0.0019^{ce}	3.6411±0.1537^{b}	50.0923±1.8458^{ef}
S6M18T155	8.942±0.081^{abc}	0.4880±0.0008^{abc}	3.5774±0.1245^{b}	50.8034±0.9609^{f}
S8M18T155	9.088±0.038^{bcdef}	0.4837±0.0003^{ab}	3.4101±0.2500^{b}	51.6994±1.1803^{f}

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences (p < 0.05) according to Duncan's multiple comparison test.*

Textural properties

Crispiness and hardness were utilized as parameters to assess the textural characteristics of the extrudates. Crispiness values ranged from 59.85 to 207.39, with the sample lacking sea fennel and containing 16% feed moisture exhibiting the highest crispiness value. Both sea fennel content and feed moisture significantly influenced ($p < 0.05$) the crispiness (CR) values. This phenomenon can be ascribed to the formation of a microstructure characterized by fewer cells and thicker cell walls, resulting in extrudates that are less expanded and harder overall as fiber content increases.

Both feed moisture and sea fennel content presented a significant impact ($p < 0.05$) on hardness. An increase in sea fennel content led to an increase in sample hardness. This increase in hardness can be attributed to the presence of dietary fiber in sea fennel, which disrupts the viscoelastic structure during extrusion, thereby diminishing product expansion and consequently elevating hardness. Moreover, an augmentation in feed moisture resulted in increased hardness due to the plasticization and softening of the protein-starch matrix. The compression of air bubbles formed within the product reduces expansion, leading to the formation of extrudates with higher density and a firmer structure (Sahu & Patel, 2020; Altaf et al., 2020).

Physical properties of the extrudates

Sample	ER	AD	Hardness (kg)	Crispiness (kg.s)	Color		
					L*	a*	b*
S0M16T140	4.5462^{n}	0.0917^{cd}	159.0410±11.6897^{ab}	31.8750±4.6117^{g}	73.67^{n}	6.50^{gh}	38.71^{k}
S2M16T140	4.2862±0.0289^{m}	0.0874±0.0019^{b}	165.3233±9.2996^{b}	28.3750±4.4381^{fg}	66.37±0.11^{k}	4.7±0.06^{a}	32.92±0.28^{def}
S4M16T140	3.8953±0.0169^{i}	0.0931±0.0035^{cd}	230.2149±13.5556^{d}	26.3571±3.0027^{fg}	63.73±0.10^{h}	4.41±0.06^{a}	31.83±0.06^{bc}
S6M16T140	3.5867±0.0229^{gh}	0.1016±0.0030^{g}	230.8451±10.9419^{d}	23.5000±3.2071^{ef}	55.90±0.1^{a}	5.83±0.14^{f}	32.20±0.16^{cd}
S8M16T140	3.5824±0.0192^{g}	0.0989±0.0029^{fg}	271.5087±13.5514^{f}	13.8235±1.9117^{abc}	59.39±0.08^{d}	5.55±0.01^{de}	32.99±0.04^{efg}
S0M18T140	4.1956±0.0613^{l}	0.0954±0.0053^{def}	317.7058±35.6095^{h}	68.3333±5.9554^{h}	74.01±0.08^{n}	8.35±0.05^{j}	44.37±0.08^{m}
S2M18T140	3.9971±0.0761^{j}	0.0980±0.0037^{efg}	256.1096±10.6866^{e}	23.6000±4.3932^{ef}	70.37±0.04^{l}	4.77±0.01^{b}	35.25±0.03^{h}
S4M18T140	3.5033±0.0216^{f}	0.1190±0.0043^{i}	328.5780±11.3402^{h}	11.4000±1.1402^{ab}	64.63±0.09^{i}	4.57±0.03^{ab}	33.34±0.02^{fg}
S6M18T140	3.0938±0.278^{c}	0.1374±0.0029^{j}	420.5983±20.8250^{i}	12.6667±2.3381^{ab}	60.78±0.02^{f}	4.52±0.01^{a}	32.33±0.01^{cde}
S8M18T140	3.0281±0.0677^{b}	0.1389±0.0065^{j}	511.8113±21.8315^{j}	10.0000±1.4142^{a}	58.18±0.06^{c}	5.42±0.02^{cd}	33.63±0.02^{g}
S0M16T155	4.0514±0.0357^{k}	0.0791±0.0025^{a}	146.7559±5.2434^{a}	86.0000±10.1136^{i}	73.19±0.02^{m}	9.08±0.09^{k}	39.92±0.31^{l}
S2M16T155	3.9871±0.0208^{j}	0.0795±0.0034^{a}	145.9818±10.4369^{a}	31.8889±3.8224^{g}	70.02±0.6^{l}	6.75±0.05^{i}	38.88±0.41^{k}

S4M16T155	3.8952±0.0318^{i}	0.0782±0.0025^{a}	181.7138±7.0476^{c}	29.3333±4.2269^{g}	65.43±0.10^{j}	6.38±0.13^{g}	35.97±0.37^{i}
S6M16T155	3.6257±0.0297^{h}	0.0812±0.0046^{a}	191.0192±7.9588^{c}	28.8571±2.7946^{fg}	62.68±0.06^{g}	5.74±0.05^{ef}	33.09±0.17^{fg}
S8M16T155	3.2529±0.0250^{e}	0.0891±0.0036^{bc}	250.1093±7.4686^{e}	19.7273±2.7236^{de}	58.11±0.20^{c}	5.76±0.11^{ef}	31.21±0.16^{b}
S0M18T155	3.2529±0.0550^{e}	0.0860±0.0037^{b}	248.7240±19.4615^{e}	104.6667±12.6689^{j}	73.02±0.16^{m}	6.33±0.02^{g}	39.97±0.81^{l}
S2M18T155	3.6219±0.0286^{gh}	0.0939±0.0036^{de}	230.9712±14.0617^{d}	18.8333±2.1370^{cde}	66.36±0.11^{k}	6.66±0.15^{hi}	37.56±0.88^{j}
S4M18T155	3.2014±0.0293^{d}	0.1013±0.0037^{g}	288.1484±25.9254^{g}	16.6000±2.5100^{bcd}	62.89±0,06^{g}	6.39±0.04^{g}	35.34±0.04^{h}
S6M18T155	3.0014±0.0261^{b}	0.1065±0.0037^{h}	293.9180±12.5033^{g}	15.8750±1.4577^{bcd}	57.69±0.07^{b}	6.25±0.67^{g}	32.29±0.42^{cde}
S8M18T155	2.8400±0.0205^{a}	0.1099±0.0028^{h}	295.1385±20.9764^{g}	13.4286±1.9024^{abc}	60.01±0.26^{e}	5.28±0.15^{c}	30.46±0.74^{a}

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$) according to Duncan's multiple comparison test.*

Color

A snack food product's color is a major determinant of its quality and the customers choose snack food items depending on how they seem, judging them primarily on color brightness (Al-Subbi, 2020). The Hunter color values of the extruded products are displayed in Table 2.2.. The added concentration of sea fennel powder resulted in a decrease in both lightness (L* value), redness (a*), andyellowness (b* value) as shown in Table 2.2. It was evident from the results that the addition of sea fennel powder concentration reduced the amount of lightness (L* value), redness (a), and yellowness (b value). The results show that the L* value decreased with the addition of sea fennel and moisture content The results show that the green color of the chlorophylls in the sea fennel increased the greenness of the extruded products, whereas decreasing the lightness and the yellowness of the final product. Besides, the moisture content of the flour mixture was an important parameter for color and the L*, a*, and b* values were reduced with the moisture content.

Total phenolic content and antioxidant activity

Total phenolic content and total antioxidant activity were determined for the extruded snacks. Both sea fennel content and feed moisture significantly influenced ($p < 0.05$) the total phenolic content and total antioxidant activity of the samples. An increase in sea fennel content led to an increase in the sample's total phenolic content and antioxidant activity. This can be attributed to the high total phenolic content and total antioxidant activity of the sea fennel.

Total phenolic content and antioxidant activity of the extrudates

Sample	Total phenolic content (mg GAE/g sample)	Total antioxidant activity (mg TEAC/g sample)
S0M16T140	0.7644±0.0038^{b}	0.2434±0.0046^{b}
S2M16T140	0.8044±0.0079^{b}	0.6709±0.0092^{f}
S4M16T140	0.9271±0.0203^{b}	0.7946±0.0034^{g}
S6M16T140	11.0697±0.0229^{g}	0.8702±0.0148^{hi}
S8M16T140	12.4008±0.0598^{h}	0.9222±0.0603^{i}
S0M18T140	0.1108±0.0277^{a}	0.1646±0.0030^{a}
S2M18T140	1.9503±0.0052^{cd}	0.2391±0.0423^{b}

S4M18T140	4.9553±0.0177^{f}	0.2236±0.0101^{ab}
S6M18T140	13.9488±0.2105^{i}	0.6754±0.0072^{f}
S8M18T140	16.3124±0.1073^{j}	1.0654±0.0140^{j}
S0M16T155	1.6198±0.0173^{cd}	0.2130±0.0229^{ab}
S2M16T155	2.0172±0.0546^{d}	0.3096±0.0081^{c}
S4M16T155	3.7348±0.0198^{e}	0.5473±0.0045^{e}
S6M16T155	14.2631±0.0665^{i}	0.9116±0.0147^{i}
S8M16T155	33.1789±1.6409^{k}	1.4645±0.0083^{k}
S0M18T155	0.7182±0.0050^{ab}	0.3614±0.0045^{cd}
S2M18T155	0.8115±0.0043^{b}	0.4154±0.0027^{d}
S4M18T155	1.3238±0.0606^{bc}	0.6045±0.0036^{e}
S6M18T155	15.9597±0.1294^{j}	0.6920±0.1075^{f}
S8M18T155	54.1683±0.0618^{l}	0.8306±0.0810^{gh}

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$) according to Duncan's multiple comparison test.*

Moreover, an increase in feed moisture resulted in elevated total phenolic content and antioxidant activity within the samples. This increase in both total phenolic content and antioxidant activity could arise from the damage to cell structures during thermal processing, facilitating the easier extraction or release of soluble phenolic compounds from the samples (Tepsongkroh et al.,2019).

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2.2 Spiced noodles with sea fennel

Material and Methods

Materials

Wheat flour was purchased from Soke Un (Aydin, Turkey) and potato starch (12% moisture content) was purchased from Değirmen Inc. (Izmir, Türkiye).

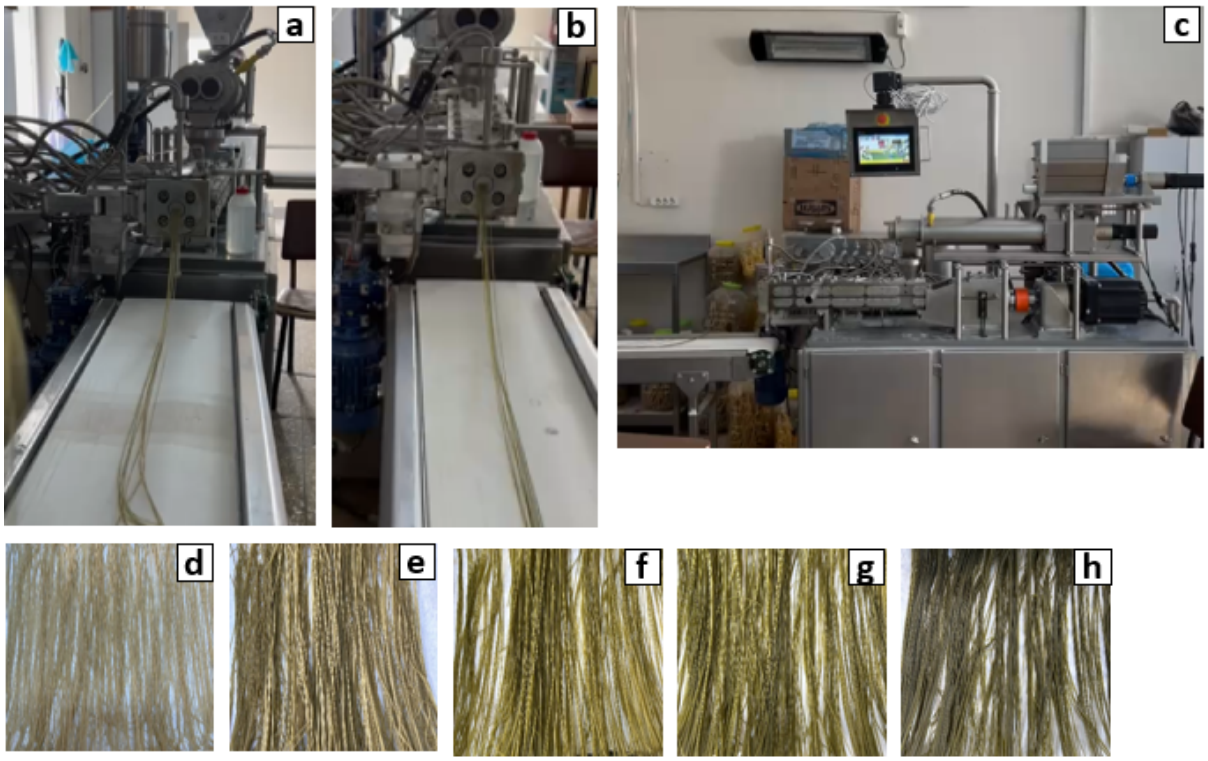
Methods

Preparation of samples

Powder blends (PBs) were prepared by mixing wheat flour, potato starch, salt, and sea fennel powder. Sea fennel powder was added 0% (control), 2%, 4%, 6% and 8% w/w of wheat flour and potato starch weight. All raw materials were blended at a speed of 250 rpm for 5 min using a KitchenAid mixer (KitchenAid, St. Joseph, MI, USA).

Extrusion process

Extrusion was carried out using a lab-scale, corotating twin-screw extruder (Feza Machine Co. Ltd., Istanbul, Turkey) equipped with a cylindrical preconditioner. The screw was 25mm in diameter, with a length-to-diameter ratio of 25:1.The extruder barrel was divided into four zones, each with their own electrical heating and cooling system. The prepared dry mix was fed into the preconditioner via a twin-screw volumetric feeder connected to the extruder. Samples were fed into the extruder at a rate of 55 ± 1 g/min. A liquid pump was used to feed water into the extruder at a rate of 16-28 mL/min, resulting in a moisture content of 30%-40% in the finished product. The screw speed stayed constant at 100 rpm. The barrel temperatures were adjusted to 40 °C, 70 °C, 85 °C, and 80 °C in the first, second, third, and fourth zones (die temperature was 80 °C). Noodles were extruded through a circular die with 7 1mm diameter openings. Following preparation, the noodles were dried in a tray dryer at 95°C for 1 hour.



Spiced Noodle with Sea Fennel: a), b) exit of the die during extrusion process, extruder, c), extruder, d), e), f), g), and h) picture of noodle substitution of 0, 2, 4, 6 and 8% sea fennel powder, respectively.

Moisture and Water Activity

The AACC (2000) approach was followed in determining the moisture (Method: 44–15A). A water activity measurement apparatus (Testo AG 400, Lenzkirch, Germany) was used to measure the water activity of the noodles.

The Expansion Ratio

The expansion ratio is calculated by dividing the cross-sectional area of the noodles by the diameter of the die. The equation is as follows:

$$ER = D/d$$

where D is the noodle diameter (mm) and d is the die diameter. The noodles' diameter was measured using a digital caliper.

Textural properties

The textural properties namely hardness, springiness and chewiness of the instant noodles were measured using a TA.XT Express Texture Analyzer (Stable Microsystems, Godalming, Surrey, United Kingdom). The noodle strands were first cooked to the optimal cooking time, then five cooked strands were arranged parallel on a flat metal plate. The compression mode settings (pre-test, test, and post-test) were as follows: speed of 2.0 mm/s, strain of 75%, trigger type of auto-10 g, and a 35-mm cylinder probe (Stable Micro Systems).

Color

The color values of the extrudates were determined using HunterLab Colorflex (HunterLab, CFLX 45-2 Colorimeter, Reston). For each sample, CIELAB space parameters L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness) color values were measured.

Water Absorption Index and Water Solubility Index

The water absorption index (WAI) and water solubility index (WSI) were measured following the procedure established by Anderson et al. (1969). Ground samples weighing 2.5 grams were mixed with 30 mL of distilled water in centrifuge tubes at a temperature of 30 °C for 30 minutes and then centrifuged at 2500 g for 20 minutes. Subsequently, the supernatant was poured into an evaporating dish that had a known weight. WAI is defined as the weight of the gel obtained after the supernatant is removed, relative to the original weight of the dry solids. WSI is defined as the weight of dry solids found in the supernatant, expressed as a percentage of the original sample weight. All measurements were carried out in triplicate.

Total phenolic content and antioxidant activity

The extract of the sample for the total phenolic content (TPC) and antioxidant analysis were performed according to Shevkani et al. (2019). Samples prepared at room temperature (25 °C) by combining dried sea fennel, corn grits, and extrudates (1.0 g) with 80% methanol (10 ml) for 2 hours using a magnetic stirrer (200 rpm), followed by centrifugation at 7000 g for 10 minutes. The resulting supernatants were collected, while the sediments were resuspended in 80% methanol, stirred, and centrifuged again. The supernatants from both rounds of extraction were combined and filtered to remove any insoluble particles. All extracts were prepared in triplicate and immediately analyzed for total phenolic content and antioxidant activity.

Total phenolic content was determined using a modified version of the method developed by Singleton et al. (1999). Specifically, 100 µl of the extract was diluted with distilled water to a final volume of 4.8 ml. Then, 300 µl of Folin–Ciocalteau reagent was added, and the mixture was incubated for 8 minutes. Following this, 900 µl of 20% sodium carbonate solution was added, and the solutions were allowed to stand for 1 hour at room temperature before measuring absorbance at 765 nm using a UV/Vis spectrophotometer. Gallic acid was used as a standard, and the results were expressed as mg GAE (gallic acid equivalents) per gram of dry matter.

Total antioxidant activity was assessed using the ABTS free radical scavenging test. ABTS free radicals were generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate in the dark for 12 hours. The resulting working solution was prepared by diluting the ABTS stock solution with 80% methanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. A mixture of 3 ml ABTS working solution and 100 µl of extract was prepared, and absorbance was measured at 734 nm after 6 minutes. The calibration curve was generated by plotting percentage inhibition against the concentration of Trolox in both assays and antioxidant activity was expressed as mg Trolox equivalents per gram of dry matter.

Statistics

The SPSS Version 22.0 program was used to perform statistical analyses on the experimental data (SPSS Inc., Chicago, IL). The experiment's results are presented as the mean ± standard deviation. The analysis of variance (ANOVA) was used to examine the impact of each individual component on the sample attributes. At a significance level of $p \leq 0.05$, variance analysis and Duncan's multiple range tests were utilized to identify variations between the samples' characteristics.

Results

Moisture content and water activity of the extrudates

The moisture content and water activity results of the extruded snacks are given in the table. The results show that both the feed moisture content and the addition of sea fennel significantly influenced the final properties of the extrudates. As the sea fennel ratio increased (from S0 to S8), the moisture content of the extruded snacks also increased, with the highest value observed in sample S2 (8.54%) and the lowest in the control sample S0 (5.24%). Similarly, water activity increased with both higher feed moisture and sea fennel content, ranging from 0.3247 in S0 to 0.5463 in S2. These results indicate that sea fennel addition contributes to a moister and more active water environment in the final product, potentially affecting shelf life and texture.

Effect of extrusion parameters on the functional properties

The WAI values of the extruded snacks are presented in the table below. WAI indicates the amount of water retained by the product after hydration and reflects the degree of starch gelatinization during extrusion. The results showed that WAI increased with both the feed moisture and the addition of sea fennel. The lowest value was observed in the control sample S0 (3.68 g/g), while the highest was in S8 (4.51 g/g). The increase in WAI with feed moisture can be attributed to water acting as a plasticizer, enhancing starch swelling and gelatinization (Alam et al., 2016). However, a slight reduction in WAI in sample S6 (3.83 g/g) suggests that excessive fiber content from sea fennel may reduce the starch ratio in the formulation, thereby limiting water absorption capacity.

The WSI of the samples, which indicates the level of molecular degradation and the amount of soluble components released during extrusion, increased significantly with both sea fennel content and feed moisture. The WSI ranged from 4.89% (S0) to 7.09% (S8). This trend can be attributed to the dextrinization and breakdown of starch into smaller soluble fragments under high temperature and shear forces during extrusion (Ding et al., 2006). Additionally, the presence of soluble dietary fibers in sea fennel, which may become more extractable during processing, likely contributed to the increase in WSI.

Physico-chemical and functional properties of the extrudates spiced noodle

Sample	Moisture Content (%)	Water activity, a_w	WAI (g/g)	WSI (%)
S0	5.2387±0.2471a	0.3247±0.0037a	3.6804±0.0277a	4.8988±0.5703a
S2	8.5427±0.1056d	0.5463±0.0034d	4.1017±0.0073ab	5.1560±1.1632a
S4	6.6940±0.1284b	0.4167±0.0007b	4.1509±0.1161ab	5.2066±1.9106a
S6	7.3553±0.0596c	0.4633±0.0148c	3.8384±0.2161ab	6.1946±1.2158a
S8	6.8547±0.1248b	0.4547±0.0022c	4.5069±0.5651b	7.0943±1.1811a

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$) according to Duncan's multiple comparison test.*

Effect of extrusion parameters on the physical properties

Expansion ratio

The Expansion Ratio (ER) of the extruded snacks significantly varied with the addition of sea fennel and changes in feed moisture levels. The ER ranged from 1.30 (S8) to 1.54 (S4). The highest expansion was observed in S4, while the lowest was recorded in S8, which contained the highest amount of sea fennel. This decreasing trend in ER with increased sea fennel content can be attributed to the high fiber content of sea fennel, which disrupts the formation of a cohesive starch matrix and interferes with gas bubble formation during extrusion. Moreover, excessive fiber can absorb water and reduce the available moisture for starch gelatinization, further limiting expansion. Additionally, increased feed moisture initially promotes ER due to enhanced plasticization of starch, but excessive moisture can reduce melt viscosity and expansion (Ding et al., 2006).

Textural properties

As shown in the table below, the textural attributes of the extrudates, including hardness, crispiness, and chewiness, were significantly influenced by the addition of sea fennel and feed moisture levels. Hardness refers to the force required to compress the product and is a key indicator of structural integrity. Among the samples, the highest hardness value was observed in S2 (430.49 kg), whereas the lowest value was recorded in S8 (298.22 kg). This trend suggests that increased

sea fennel content led to a softer structure, likely due to fiber interfering with the continuous starch matrix, which weakens the overall mechanical strength (Altan et al., 2008). Crispiness, reflects the product's brittleness and resistance to fracture. All samples showed relatively similar crispiness values, ranging from 0.978 to 1.011 kg·s, with a slight increase in S8, indicating that higher sea fennel content maintained or slightly enhanced crisp texture. According to Ding et al. (2005), feed moisture and temperature significantly influenced the crispness and hardness of extrudates (Ding et al., 2006). While increased moisture reduced crispness, higher temperature slightly enhanced it. Chewiness measures the energy required to chew a sample before swallowing and is influenced by both hardness and cohesiveness. The chewiness values decreased significantly with sea fennel addition, dropping from 1839.35 (S0) to 821.95 (S2) and remained relatively low in other enriched samples.

Physical properties of the extrudates spiced noodle

Sample	ER	Hardness (kg)	Crispiness (kg.s)	Chewiness	Color		
					L*	a*	b*
S0	1.4011±0.0721a	357.7244±38.9510b	0.98167±0.055200a	1839.3468±164.4456c	77.77±0.06e	0.9533±0. 0217d	12.96±0.16a
S2	1.4334±0.0395b	430.4912±40.2865c	0.99714±0.038282a	821.9538±653.4806a	67.87±0.08d	-0.1733±0.*123c	16.64±0.04b
S4	1.5375±0.0693a	327.0220±30.4574ab	0.97833±0.000577a	1722.4312±226.9045c	64.44±0.33c	-0.9467±0.0145b	18.71±0.16d
S6	1.3867±0.0490a	316.6152±28.0331a	0.99100±0.018547a	1583.8314±262.5910bc	61.63±0.69b	-1.0400±0.0158a	20.33±0.19e
S8	1.3009±0.0378a	298.2244±37.4395a	1.01171±0.083590a	1106.4680±697.7347ab	54.27±2.45a	-0.9850±0.0223b	18.06±0.12c

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences (p < 0.05) according to Duncan's multiple comparison test.*

Color

The color parameters of the extruded snacks were significantly influenced by the addition of sea fennel. As shown in Table 2.5. The L* values (lightness) decreased from 77.77 (S0) to 54.27 (S8), indicating that the snacks became darker with increasing sea fennel content. This can be explained by chlorophyll degradation and thermal browning reactions during extrusion, which are also observed in other drying and thermal processes applied to sea fennel (Renna et al., 2017). The a* values (red-green axis) shifted slightly, with the highest a* observed in S6 (1.04) and the lowest in S2 (0.17). This modest increase in redness may relate to the formation of Maillard pigments or interactions of phenolic compounds with proteins during high-temperature extrusion (Renna & Gonnella, 2012). The b* values (yellow-blue axis) increased from 12.96 (S0) to 20.33 (S6), indicating enhanced yellowness. This increase may be attributed to the presence of carotenoids and phenolic compounds naturally abundant in sea fennel (Renna et al., 2017), which are known to influence yellow hue, especially after drying and thermal processing.

Total phenolic content and antioxidant activity

The effect of sea fennel addition on the total phenolic content (TPC) and total antioxidant activity (TAA) are shown in Table 2.6. The results clearly demonstrated that increasing the sea fennel ratio enhanced both parameters statistically significantly ($p<0.005$). In control sample, which contained no sea fennel, the TPC and TAA were 0.5484 mg GAE/g and 0.00942 mg TEAC/g, respectively, which were lower than others. With the addition of sea fennel, both values increased markedly. With the addition of sea fennel, especially, the S6 and S8 samples, there was a significant rise in TPC and TAA values and S6 and S8 exhibit the highest TPC values 20.5116 and 18.9936 mg GAE/g, respectively. While S6 exhibited the highest TPC, S8 showed a higher TAA despite a slightly lower TPC. This discrepancy suggests that the antioxidant capacity is not solely dependent on the total quantity of phenolics but also influenced by the specific composition and reactivity of individual phenolic compounds present in sea fennel. This observation is consistent with prior findings indicating that different phenolic structures exhibit varying antioxidant potentials depending on factors such as hydroxylation pattern, glycosylation, and polymerization degree (Dai & Mumper, 2010; Ignat et al., 2011). Being a rich source of phenolic compounds, sea fennel enhances the functional properties of noodle through its antioxidant potential. Like TPC, TAA showed similar trend increasing with sea fennel and TAA values were between 0.00942 and 0.12965 TEAC/g. The similar results were obtained previous studies highlighting sea fennel as potential antioxidant source owing to its rich content of chlorogenic acid, caffeic acid derivatives, and flavonoids (Correia et al., 2024). The plant's potential as a natural component to improve the health-promoting qualities of food products is supported by the biological activity found in this study. Furthermore, the antioxidant activity plateau between S6 and S8 indicates that, after a certain point, additional sea fennel content increases might not correspondingly boost bioactivity; this is probably because of matrix limitations, compound interactions, or saturation effects (Souid et al., 2021).

Total phenolic content and antioxidant activity of the extrudates spiced noodle

Sample	Total phenolic content (mg GAE/g sample)	Total antioxidant activity (mg TEAC/g sample)
S0	0.5484±0.0417a	0.00942±0.00076a
S2	4.1363±0.3949b	0.02510±0.00441b
S4	3.9507±0.3278b	0.02488±0.00497b
S6	20.5116±0.9695d	0.12836±0.00173c
S8	18.9936±0.4705c	0.12965±0.00313c

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$) according to Duncan's multiple comparison test.*

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2.3 Handmade pasta incorporated with sea fennel powder

Due to its attractive sensory qualities and abundance of essential oils, sea fennel (Crithmum maritimum L.) is a classic fresh food in cuisine in many countries, especially in Mediterranean cuisine (Özcan et al., 2001; Renna et al., 2017). However, the consumption of sea fennel is not sufficient for commercial cultivation (Renna et al., 2017). Therefore, the sea fennel is included in unfermented food product formulations so that the usage and consumption of the sea fennel will increase.

During the reporting period, under Task 5.2 of the project, the exploitation of sea fennel edible aerial parts for manufacturing of innovative sea fennel-based foods (Laboratory-scale manufacturing of unfermented shelf-stables preserves), the following study has been carried out by Ege University team.

Material and Methods

Materials

Durum wheat flour (Tellioglu Gida, Balikesir) and fresh sea fennel were used to produce hand-made pasta. Sea fennel has been dried so that it can be part of the food product ingredient. The drying process was performed based on the study

of Renna et al. (2017). The fresh sea fennel was supplied by the local producer (NEBBA Tarim Ürünleri Dağitim PAZ.TİC.LTD.ŞTİ., Antalya) and the samples were kept at -80°C before drying. The drying process was carried out using conventional air drying using an oven (Memmert Oven, Schwabach, Germany) at 45°C for 48 hours. The reason for selecting this temperature is that it was found that the sensory evaluation and physical analysis results were close to the fresh sea fennel among the conventional drying method at different temperatures. After the drying process, it was aimed that the water activity of the sample decreased to 0.6 to prevent microbial growth (Syamaladevi et al., 2016). Renna et al.'s (2017) concluded that the water activity of sea fennel dried at 45°C for 72 hours with conventional air drying was around 0.3. On the other hand, in this study, the water activity of sea fennel dried at 45°C for 48 hours with 50% flap was measured as 0.19. The reason for this difference could be the flap ratio of the conventional oven. In this way, the humidified air could be easily removed from the inside of the oven (Alfred Watzl & Martin Rückert, 1998). After drying, the samples were ground with waring grinder machine (Waring Blender, Stamford, CT, USA), and the sieving was performed to obtain a powder with a diameter≤1 mm.

Methods

The edible parts of the sea fennel (fresh leaves and sprouts) were dried to be used to produce unfermented food products, and the dried sea fennels were grounded. After the grinding process, the dried samples were used in the hand-made pasta formulation. The sea fennel powders were substituted at the level of 5 and 10% (w/w) based on durum flour. The quality analyses, namely color parameters, cooking properties (optimum cooking time, water absorption, cooking loss, swelling index), and texture parameters were determined for each sample.

Preparation of pasta

Doughs were prepared with 0, 5, or 10 g of sea fennel and mixed with durum semolina to obtain a 100 g mixture. A Kitchen Aid mixer (KitchenAid Commercial, Benton Harbor, MI, USA) was used to stir the ingredients at 135 rpm for 480 s. The mixed crumbly dough was kneaded by hand for 2 min to receive slick stretchy dough. The dough samples were wrapped with plastic wrap to rest for 20 min at room temperature and then pressed by a laboratory pasta machine 14 times until the thickness was 1.00 ± 0.05 mm. The prepared sheets were then cut into strips (width 1.00 mm, thickness 1.00± 0.05 mm). The pasta strands were dried in an oven (Memmert Oven, Schwabach, Germany) at 60 °C for 2 h, then the pasta strands were packed in plastic bags till further use for analysis.

Color parameters

Color parameters of the pastas were analyzed using a CIE lab color analyzer (Konica Minolta, Japan) for the determination of L*, a*, and b* parameters. The five pasta strands were randomly analyzed for color parameters.

Cooking properties

To evaluate the cooking quality of the pastas, various parameters such as cooking time (when the white core of the pasta center just disappears), cooking loss, swelling index, and water absorption were assessed using the AACC-approved method 66-50 (2000). The cooking loss, which indicates the loss of solid substances during cooking, was determined by cooking 10 g of the sample in 300 mL of boiling distilled water at the optimal cooking time. The cooking water was collected in a beaker and subsequently evaporated in an oven (Memmert Oven, Schwabach, Germany) at 105°C until a constant weight was reached. The residue was weighed and reported as a percentage of the starting material. The swelling index was determined by cooking 10 g of the sample at the optimal cooking time and then drying it at 105 °C until a constant weight was reached (Cleary and Brennan, 2006). The water absorption (WA%) of the drained cooked pastas was calculated as [(weight of cooked samples) – (weight of raw sample)]/(weight of raw sample). All measurements were performed in at least triplicate.

Texture analysis

The methodology for analyzing texture properties was reported by Ma et al. (2019) using a texture analyzer (TA-XTExpress, Stable Micro Systems, London, England). The samples were cooked in boiling water for their respective

optimal cooking times: 570 s for the control and 5% sea fennel-enriched pastas, and 540 s for the 10% sea fennel-enriched pastas. Texture profile analysis (TPA) was performed immediately after cooking, using a cylindrical probe with a 25 mm diameter, and compression was selected as the test mode. The pre-test, test, and post-test speeds were set to 3.00 mm/s, 1.00 mm/s, and 1.00 mm/s, respectively. A compression strain of 75% and a triggering force of 5.0 g were utilized.

Sensory Analysis

New sustainable food products (noodle) containing sea fennel was produced within the scope of the research at Ege University Food Engineering Department the day before the panel. The sensory evaluation was held in the "Sensory Test" room of the same department. Sensory evaluation was carried out according to the study of Biadge (2021) and Koh et al. (2022). The samples are coded with random numbers and given to the panelist. Panelists were asked to evaluate the products in terms of color, flavor, appearance, taste, texture (chewiness and stickiness), and overall acceptability using a 9-point hedonic scale, from 9=extremely like to 1=extremely dislike. The panelists were instructed to use water for palate cleansing after each sample was evaluated.

Results

Color parameters

The color parameters of the samples were shown in Table 3.3.1. The lightness values of the pastas were decreased as the sea fennel substitution ratio was increased. In addition, a shift of the color toward green was determined. This decrease in lightness and increase in greenness may arise because of the own color of the sea fennel.

Color parameters of the pasta

Sample	L*	a*	b*
Control	83.38±0.83^{a}	1.05±0.072^{a}	19.14±0.26^{b}
5%	70.46±0.32^{b}	-0.35±0.13^{b}	18.56±0.33^{b}
10%	68.42±0.51^{b}	-0.34±0.08^{b}	20.56±0.39^{a}

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$) according to Duncan's multiple comparison test.*

Cooking properties

The cooking properties such as water absorption, cooking loss, and swelling index were determined (Table 3.3.2). Water absorption and swelling index values decreased while increasing seafennel content. On the contrary, cooking loss values were increased with increasing sea fennel substitution ratio. This decrease in water absorption values could be attributed to the amount of insoluble material content and water absorption capacity of the gluten. With the increasing sea fennel substitution ratio, the gluten content of the formulations was decreased which led to lower water absorption values.

As the substitution ratio increased, the cooking loss increased which may arise because of the dilution of gluten content. The reduction of gluten content in the formulation may have led to the formation of a weaker starch-gluten network resulting in the decrease of the structural integrity of the samples. Therefore, a higher level of solids leaches into the water during cooking.

Cooking properties of the pasta

Sample	Water Absorption	Cooking loss	Swelling Index
	%	%	g water/g dry pasta

Control	141.76±8.37[a]	6.02±0.68[b]	0.483±0.037[a]
5%	134.49±6.31[a]	7.31±0.16[ab]	0.477±0.030[ab]
10%	116.36±1.11[a]	8.04±0.08[a]	0.446±0.004[b]

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences (p < 0.05) according to Duncan's multiple comparison test.*

Texture analysis

Hardness and adhesiveness values of the sea fennel-added samples (Table 3.3.3) were determined higher than control samples and it is positively correlated with the sea fennel substitution ratio. The reduction in the insoluble material and increasing soluble material with the increasing sea fennel substitution might lead to decreasing water absorption which causes a harder structure. Another reason for increasing hardness values could be decreasing gluten content in the formulation which led to lower water absorption values.

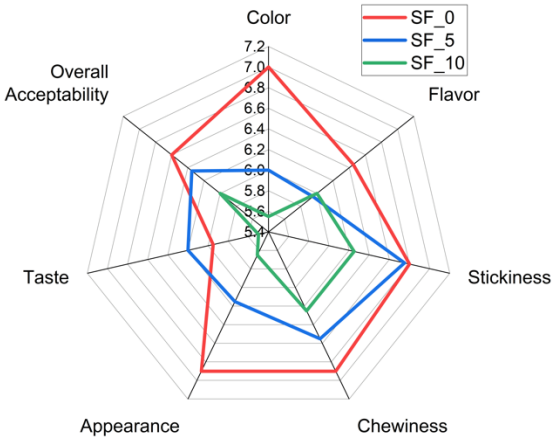
Texture properties of the noodles

Sample	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
	N	N.sec					
Control	77.85±4.51[a]	-4.69±0.70[a]	0.8806±0.0452[a]	0.5618±0.0385[a]	4567.39±721.76[a]	4002.83±515.51[a]	0.3628±0.0462[b]
5%	85.28±6.65[ab]	-5.05±0.55[a]	0.8976±0.0519[a]	0.5324±0.0351[b]	4647.10±664.06[a]	4148.05±395.76[a]	0.3324±0.0348[ab]
10%	88.03±3.98[b]	-5.06±0.87[a]	0.8725±0.0439[a]	0.4735±0.0304[b]	4236.42±757.55[a]	3674.64±527.67[a]	0.3008±0.0320[a]

**Data have been expressed as mean values of replicates ± standard deviation. Different letters within the same column indicate significant differences (p < 0.05) according to Duncan's multiple comparison test.*

Sensory Evaluation

The sensory evaluation result of three noodle samples is shown in figure below in terms of a spider plot. The quality of the noodle, such as color, flavor, appearance, texture (stickiness and chewiness), taste, and overall, acceptability were assessed. The highest score was observed in the noodles sample SF_0 which was the substitution ratio of the sea fennel is zero (control); on the other hand, the one with the lowest score was SF_10, which has a substitution ratio of 10. It can be understood that the color changes in the noodles were affected by formulation. However, the noodle which has 5 % substitution ratio had a higher score in terms of taste. Although only the taste of the noodle that use sea fennel was liked more than control, the result will guide on how to proceed in next step. In other words, the formulation of extruded product is gone consider these sensory results.



Sensory evaluation (scale 1-9) for noodles using sea fennel powder different substitution ratios based on semolina (0, 5 and 10 % (w/w))

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3 Tunisian prototypes

Sea fennel edible fresh leaves – sprouts, collected from spontaneous populations, were dried and exploited by INRGREF for development, production and validation of the following UNFERMENTED FOOD LABORATORY-SCALE PROTOTYPES:

- Chili Puree (HARISSA)
- Orange jam
- Snack

3.1 Chili puree (Harissa)

Material and methods

Chili puree formulation

The formulation of chili puree was made based on a popular traditional recipe. The main ingredients used were dried pepper (50%), garlic (35%), preparation (salt, coriander, caraway) (15%).

Different doses of sea fennel powder were added to harissa in order to determine the best concentration to be used. Treatments were considered as follow:

- A negative control with no salt (T-)
- A positive control with 2% salt (T+)
- Treatment 1 with 2% sea fennel powder (2%CM)
- Treatment 2 with 3% sea fennel powder (3%CM)

pH

The measurement of pH is essential in the food industry, as a significant variation can signal a change in the food product, indicating potential spoilage. For measure pH, a calibrated pH meter electrode was inserted into the sample. Once a constant value is obtained, the pH can be read directly on the pH meter scale with an accuracy of at least 0.05 pH units.

Acidity

The aim of titratable acidity determination is to measure all available H+ ions in the medium, whether dissociated or not, by potentiometric titration with a sodium hydroxide solution NaOH sodium hydroxide solution. The procedure begins by weighing 25 g of the sample into a 250 ml beaker, then adding 50 ml boiled and cooled distilled water and mixing until a homogeneous liquid is obtained. The contents of the beaker are then transferred to a 250 ml volumetric flask, adding boiled and cooled distilled water up to the mark, then filtered through filter paper. Approx. 50 ml of the filtrate is pipetted into a beaker fitted with a stirrer. The pH is controlled using a NaOH burette until a final pH of approximately 8.1. The titratable acidity, expressed in milliequivalents per 100 g of product, is then calculated according to formula:

Acidity = (250/m)*(V1/10)*(100/V0)

with

- m: is the mass, in grams of product weighed (25g in this case)
- V0: is the volume, in ml, of the test sample (50ml)
- V1: is the volume, in ml, of the NaOH solution (0.1 N) used.

The titratable acidity of harissa is often expressed in grams of citric acid citric acid per 100 grams of product by multiplying the value obtained by 0.07. According to Tunisian standard NT 52.07 (2005), which defines the requirements for harissa, the acidity level must not exceed 3%.

Dry matter

A method commonly used for this purpose is oven drying. In this approach, a 30g quantity of harissa was placed in a container and weighed. The container was then placed in a ventilated oven set at a temperature of 103°C ±2, until its mass remained constant. During this process, the water in the sample evaporates. After drying, the container was removed from the oven, cooled in a desiccator to prevent any absorption of moisture and then reweighed. The difference in weight before and after drying process represents the weight loss due to water evaporation. Using this weight difference and the initial mass of the sample, the dry matter (DM) content of Berber harissa was calculated.

Determination of "Brix" soluble dry residue (NT ISO 2173, 2003)

Determination of the soluble dry residue involves measuring the sucrose concentration of an aqueous solution with the same refractive index as the sample being analysed, under identical preparation and temperature conditions. At a temperature of 20°C, the refractive index of the various harissa samples was measured and converted into dry residue soluble residue. To determine the Brix values of traditional harissa, 40 g of the sample was ground and 100-150 ml of water was added. The mixture was brought to the boil and simmered gently for 2 minutes. After cooling (approx. 20 minutes), the filtrate was collected for determination of the Brix value. Brix value, expressed as a percentage, is calculated using formula:

$$\boldsymbol{Brix}(\%) = \boldsymbol{P}^{*}(\boldsymbol{m1}/\boldsymbol{m0})$$

With :

- P: the mass fraction of soluble solids in the diluted solution in %
- m0 : the mass in grams of the sample before dilution
- m1: mass in grams of sample after dilution.

Colorimetry

Color is determined using a CR-300 chroma meter color analyzer colorimeter, which must be calibrated with a PCR reference plate. The colorimeter converts all colors in space into a code (L*a*b*) with :

- Luminance (L) or brightness: expressed as a percentage (0: black; 100: white)

- a* and b*: are two chrominance parameters ranging from green (a: -60) to red (a: +60) and from blue (b: -120) to yellow (b: +120).

Energetic value

Determining the energy value of harissa involves calculating its calorie content based on its composition of the main macronutrients: carbohydrates, lipids and proteins (FAO, 2003). Since harissa is mainly composed of of chillies, garlic and spices, its energy value will depend mainly on its carbohydrate content. The carbohydrates may come in part from the chillies, while lipids and proteins may be present in varying quantities depending on ingredients and manufacturing methods. The calorie content of harissa was calculated by multiplying the amount of carbohydrates, lipids and proteins by their specific conversion factor (4 kcal/g for carbohydrates and proteins, and 9 kcal/g for lipids). However, it should be noted that harissa may also contain other components, such as dietary fiber, which may have an impact on its total energy value.

Preparation of stock solutions (NT.53.09, 1999)

Using a sterile spatula, 10 g of shredded material was suspended in 90 ml of sterile physiological water. A 1/10 diluted suspension was obtained.

Enumeration of sulfite-reducing anaerobes (NF V 08-061, 1996)

After preparation of the stock solution, 1ml was transferred to each sterile Petri dish. Next, 15ml of Tryptone-Sulfite-Neomycin (TSN) agar were aseptically poured into each Petri dish, carefully mixing the inoculum. with the molten medium to obtain a homogeneous, bubble-free distribution. After solidified, a new layer of TSN was added to cover the previous layer. Finally, the Petri dishes were turned upside down and incubated at 37°C for 24 hours to 48h. After 48 h of incubation, the plates were examined for the presence of colonies characteristic of sulfite-reducing anaerobes. Black or grey colonies indicating sulfite reduction by bacteria were counted. Results are expressed as the number of colony-forming units per gram of product (CFU/g), taking dilutions into account.

Yeast and mold enumeration (NT.16. 16, 1983)

The principle of the yeast and mold enumeration method is based on surface seeding of 0.1 ml of the mother suspension on Sabouraud culture medium, cooled to 47°C. After incubation at 25°C for 5 days, the number of colonies was counted and the number of microorganisms per gram of sample was calculated. The plates selected should contain no more than 150 colonies. Yeasts appear as whitish, hairless dots medium-sized. Molds appear as large, hairy colonies of various colors (white, green or black).

Sensory analysis

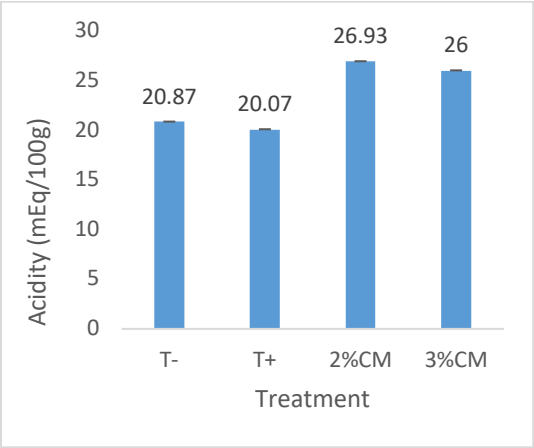
Harissa is a complex, spicy food product whose sensory analysis proved difficult. Indeed, during the tasting sessions, participants were confronted with four recipes with different formulations. We presented the taster with a small piece of bread and olive oil, to reduce the taste of spiciness. We set up an initial tasting group made up of staff from INRGREF and students in order to reach different age groups. A second tasting group was organized during a seminar, including participants of various nationalities and African nationalities. Finally, a third hedonic tasting group was formed by a hotel kitchen service team. Thanks to these three groups, we were able to collect the opinions of different types of consumers.

Hedonic panel: Hedonic methods aim to explore consumer preferences by comparing the overall hedonic appreciation of different products, focusing on individual feelings of pleasure or displeasure aroused by the food. Unlike descriptive sensory analysis, these methods call on uninformed subjects with no prior experience of experience of sensory analysis In addition, the recruitment of subjects is generally targeted at a specific group of consumers from the universe of the products in the samples tested. According to AFNOR standards (NF V09-500 December 2012), the recommended number of subjects for this type of test is 60 consumers.

Harissa preparation

Results

The analysis of acidity (expressed in mEq/100g) shows a clear increase following the incorporation of sea fennel (Crithmum maritimum). Both the negative (T−) and positive (T+) controls exhibited similar acidity values, 20.87 and 20.07 mEq/100g respectively, indicating no significant effect in the absence of the plant extract. In contrast, the addition of sea fennel at 2% led to a marked increase in acidity, reaching 26.93 mEq/100g, the highest value among all treatments. At 3%, acidity remained elevated (26.00 mEq/100g), though slightly lower than at 2%. These results suggest that the sea fennel extract significantly influences the acidity of the product, with the most pronounced effect observed at the 2% concentration.



Acidity levels of different treatments

The pH values of the different formulations ranged between 4.60 and 4.92, indicating a mildly acidic profile across all treatments. The negative control (T−) had the highest pH at 4.92 ± 0.06, while the positive control (T+) showed the lowest pH (4.60 ± 0.09), suggesting a more pronounced acidifying effect in this formulation. The addition of Crithmum maritimum at 2% and 3% resulted in pH values of 4.83 ± 0.07 and 4.84 ± 0.03, respectively, both slightly lower than the T− control but higher than T+. These findings suggest that sea fennel extract slightly reduces the pH compared to the negative control but does not acidify the medium as strongly as the positive control. Overall, the incorporation of sea fennel maintains the pH within a stable, acceptable acidic range for cosmetic or food applications.

Recette	pH
T-	4,92±0,06
T+	4,60±0,09
2% CM	4,83±0,07
3% CM	4,84±0,03

pH levels of different treatments

The incorporation of Crithmum maritimum extract significantly darkened the product (lower L*), enhanced the red tones (higher a*), and reduced the yellow component (lower b*), especially at 2% and 3% concentrations. These changes are likely due to the natural pigmentation and bioactive compounds present in sea fennel.

Treatment	L* (63,14)		a* (3,73)		b* (25,35)	
	$M \pm SD$	T (p)	$M \pm SD$	T (p)	$M \pm SD$	T (p)
T-	73,4±2,47	7,18 (0,019)	3,81±0,45	0,32 (0,776)	27,6 ± 0,90	4,25 (0,051)
T+	70,5±2,79	4,55 (0,045)	4,04±0,39	1,40 (0,296)	27,3 ± 0,60	5,52 (0,031)
2% CM	53,2 ± 0,21	-80,83 (0,000)	7,24 ± 0,12	51,0 (0,000)	21,8 ± 0,12	-52,17 (0,000)
3% CM	51,0 ± 1,08	-19,55 (0,003)	7,27 ± 0,10	58,7 (0,000)	21,2 ± 0,30	-23,98 (0,002)

M: Mean, *SD:* Standard deviation, *T:* Student's t, *p:* Probability

Colorimetric criteria of different treatments

The highest anthocyanins content was recorded by 3% CM sample with about 18.18 mg/100g while the lowest value was reached by the T+ sample. The increase of the proportion of sea fennel in the chili puree was accompanied with the increase of anthocyanins content.

Recipe	Anthocyanins content (mg/100g)
T-	11,34±1,87
T+	8,52±0,38
2% CM	15,51±0,53
3% CM	18,18±0,01

Anthocyanins content of different treatments

The energetic value (in Kcal per 100g) of the different recipes shows slight variations. The control sample without any additive (T–) recorded an energy value of 172.41 ± 3.77 Kcal/100g, while the positive control (T+) showed a slightly higher value of 173.54 ± 17.80 Kcal/100g. When 2% of sea fennel was incorporated, the energy value increased to 177.08 ± 2.89 Kcal/100g. Similarly, the recipe containing 3% CM presented a comparable energetic value of 176.78 ± 0.47 Kcal/100g. These results suggest that the inclusion of sea fennel slightly increases the caloric content of the recipes compared to the controls.

Recipe	Energetic value (Kcal/100g)
T-	172.41±3.77
T+	173.54±17.80
2%CM	177.08±2.89
3%CM	176.78±0.47

Energetic value of different treatments

Incorporating sea fennel leaves in the Harissa recipes have a positive impact on the stability of the mix and its resistance to mold and yeast. The sulfite-reducing bacteria decreased from 1.33 UFC/g for the negative control to 0.33 UFC/g for the sample containing 2% of sea fennel.

Recipe	**sulfite-reducing bacteria (UFC/g)**
T-	1,33±1,52
T+	0±0,00
2% CM	0,33±0,57
3% CM	0,67±1,54

Sulfite-reducing bacteria of different treatments

The analysis of yeast and mold counts (expressed in CFU/g) shows a significant decrease with the addition of sea fennel. The control sample without treatment (T−) exhibited the highest microbial load (6 ± 2.46 CFU/g), while the positive control (T+) showed a reduced count (2.66 ± 3.7 CFU/g). The incorporation of 2% of sea fennel led to a further reduction (0.33 ± 1.15 CFU/g), and no yeast or mold growth was detected in the sample containing 3% of sea fennel (0.00 ± 0.00 CFU/g). These results suggest that C. maritimum has a strong antifungal effect, especially at higher concentrations.

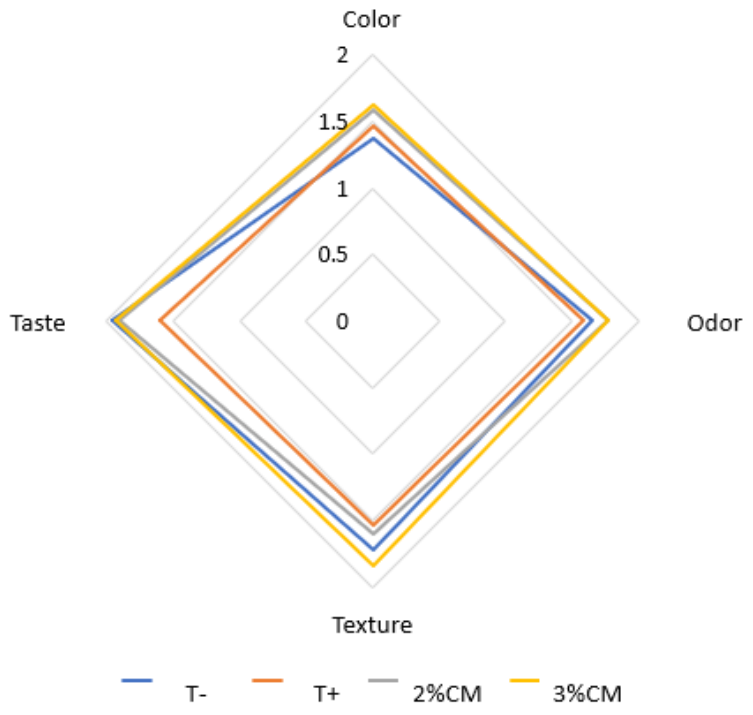
Recipe	Yeast and mold (UFC/g)
T-	6±2.46
T+	2.66±3.7
2%CM	0.33±1.15
3%CM	0,00±0

Yeast and mold of different treatments

High levels of plant used in manufacturing the Harissa were the most appreciated by consumers especially for salinity, colour, texture, and taste.

Recipe	Sensory criteria								
	Color		Taste		Odor		Texture		Ranking
	M	(c)	M	(c)	M	(c)	M	(c)	(c)
T-	1,37	(4)	1,95	(1)	1,65	(3)	1,72	(2)	3
T+	1,46	(3)	1,60	(4)	1,58	(4)	1,53	(4)	4
2% CM	1,58	(2)	1,90	(3)	1,76	(1)	1,60	(3)	2
3% CM	1,62	(1)	1,93	(2)	1,76	(1)	1,83	(1)	1

Sensory analysis of different treatments



3.2 Orange jam

Material and methods

A prototype of an orange jam added with sea fennel was developed. Sea fennel was incorporated as a powder at different doses (0.5%, 1%, 1.5% and 2%) with the aim of assessing the effect of sea fennel on the stability and the taste of the orange jam.

Jam production stages

Raw materials: To make the jam, we chose oranges of the Maltaise variety from Tunisia. This variety is known for its sweetness, tender, juicy flesh and pleasant flavor (GIF, 2020). The oranges were supplied by a plot commissioned by INRGREF. With regard to sea fennel, the plants were collected from Cap Negro. Plants were dried in dehydrators for 48 hours and then ground.

Sorting and washing: The oranges were sorted to eliminate any non-conformities (deformed oranges, contaminated oranges, etc.), then washed with water and a few drops of bleach to get rid of dirt and impurities (dust, stains, etc.).

Peeling and cutting: At this stage, the oranges are peeled so as to remove only the outer part of the peel (flavedo) responsible for the bitter taste. The albedo part must be left intact, since it is this part that is rich in pectin and which will contribute to the jam's gelling process. After peeling, the oranges are cut into small pieces and the seeds removed.

Cooking: The oranges are mixed with sugar. We used 500 g of sugar for 1 kg of oranges, in order to produce a low-sugar jam. The mixture is then cooked over a low heat in a granite pot for a maximum of 40 min. During cooking, the mixture should be simmered occasionally, and any seeds removed. Once cooking is complete, use an immersion blender to obtain a smooth jam.

Filling: The jars are hot-filled by hand, using a piping bag. During this operation, it is important to avoid the formation of air bubbles, which are responsible for the growth of yeast and mold. Before filling, the jars are sterilized in an autoclave at 121.1°C for 20 minutes.

Incorporating sea fennel: Sea fennel is incorporated into the jam in powder form at the following levels (0.5%, 1%, 1.5% and 2%). During this stage, the powder is homogenized with the jam by hand, using a spoon and an immersion blender. After this stage, the jars are hermetically sealed by hand.

Appertizing: The jam jars are sterilized in a water bath at 65°C for 30 minutes. Once sterilization is complete, the samples are left to cool to room temperature.

Physico-chemical analysis

Measurement of titratable acidity (NF V 05-101)

The method consists in weighing 25 g of the sample to the nearest 0.01 g, then adding 50 ml of boiled and cooled distilled water and mixing until a homogeneous liquid is obtained. The resulting mixture is transferred to a 250 ml volumetric flask and made up to the mark with boiled and cooled distilled water. The mixture is then filtered with filter paper. Once filtration is complete, pipette 25 ml of the filtrate obtained, add a few drops of phenolphthalein and titrate the solution with sodium hydroxide NaOH (0.1 N). Titration is completed when a persistent pink coloration is obtained for 30 s. For reliable results, take three readings on the same sample.

The titratable acidity, expressed in milliequivalents, for 100 g of product = (250/m)*(V_1/10)*(100/V_0)

With:

- m is the mass in g of the product weighed
- V0 is the volume in ml of the test sample
- V1 is the volume in ml of the NaOH 0.1 N solution

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 soluble dry residue °Brix

The Brix scale is used to determine the percentage of soluble dry matter, i.e. the amount of sugar in a product. The sweeter the product, the higher the Brix level. The Brix degree is measured by an Anton Paar electronic refractometer used in accordance with the NF standard (NF V05-109, 1970; Nielsen S.S, 2017).

Color measurement

Color is determined using a KONICA MINOLITA colorimeter, which must be calibrated with a PCR reference plate. The colorimeter converts all colors in space into a code (L*a*b*) with :

- Luminance (L) or brightness: expressed as a percentage (0: black; 100: white)

- a* and b*: are two chrominance parameters ranging from green (a: -60) to red (a: +60) and from blue (b: -120) to yellow (b: +120).

Measuring water activity (WA)

WA is measured using a ROTRONIC hygrometer fitted with a specific WA cell. 1 g of the sample (jam) is placed in a saucer at room temperature (25°C), which is then covered by the probe. Measurement is completed in around ten minutes.

Microbiological analysis

Yeast and mold enumeration

Yeasts and molds belong to the fungal kingdom. They generally thrive in acidic environments and tolerate low temperatures. Several culture media can be used to count yeasts and molds: Sabouraud medium and PDA (Potato Dextrose Agar). Enumeration is performed as follows:

- Inoculate 1ml of the suspension on the surface of Sabouraud medium using a flamed spreader.
- Incubation of petri dishes at 25-28°C for 3 to 5 days
- Count the number of colonies formed and calculate the number of microorganisms per gram or ml of product using the following formula:
- $N=\sum C/[(n1+(0.1*n2))*d]$
    - With:
        - C: sum of colonies on all plates retained at the level of the two dilutions
        - n1: number of plates retained for the first dilution
        - n2: number of plates retained for the second dilution
        - d: dilution at which the first counts are obtained

Counting the total aerobic mesophilic flora (FMAT)

This involves counting microorganisms with an optimum growth temperature of 30°C, growing on ordinary media (nutrient agar, PCA (Plate Count Agar)). The FMAT count gives a general idea of the microbial contamination of the product. This involves inoculating petri dishes with 1ml of the stock solution and dilutions in PCA medium. The plates are then incubated at 30°C for 72 hours. Colonies are counted using the same method as for yeasts and molds, and the result is expressed in CFU/gram.

Sensory analysis

Two tests were carried out with 2 panels of tasters (a trained panel and a naïve panel) to determine the most appreciated incorporation rate of sea fennel.

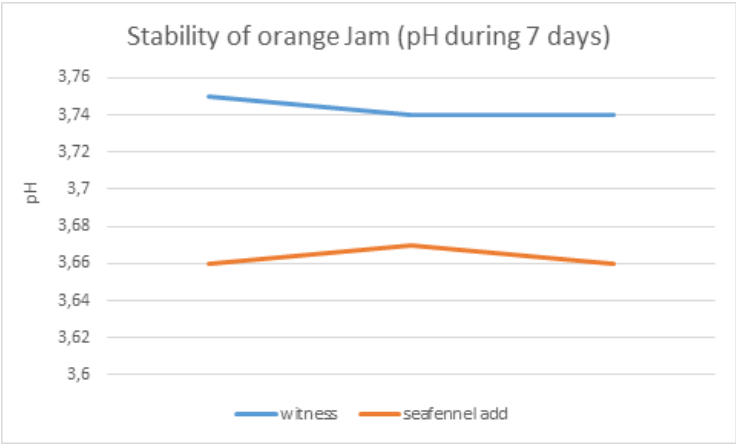
The first was a rating scale test to determine the intensity of the desired criteria (Color, Odor, Bitterness, Taste (bitter/acidic), Flavor and Texture). This test was carried out by rating on a scale with ten trained tasters. Following this test, two recipes were selected for hedonic evaluation.

The second is a hedonic test. Tasters rate their appreciation of the product on a scale from 1 (I certainly hate it) to 5 (I love it). The criteria assessed by the test are color, texture, taste, odor and flavor. The test was carried out with 50 naive tasters.

Preparation of the orange jam prototype at INRGREF laboratory

Results

As shown in the graphic below, the incorporation of sea fennel in orange jam influences its stability. The pH of the jam added with sea fennel was lower than the control. The shelf life of the product has also been increased.

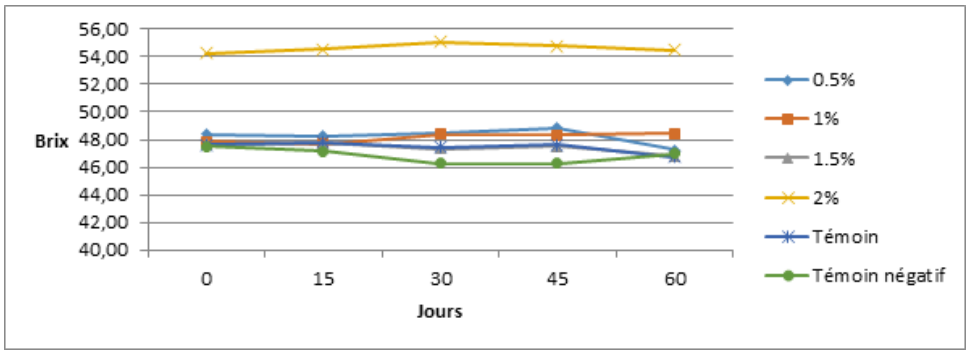


Physical characteristics of orange jam prototype

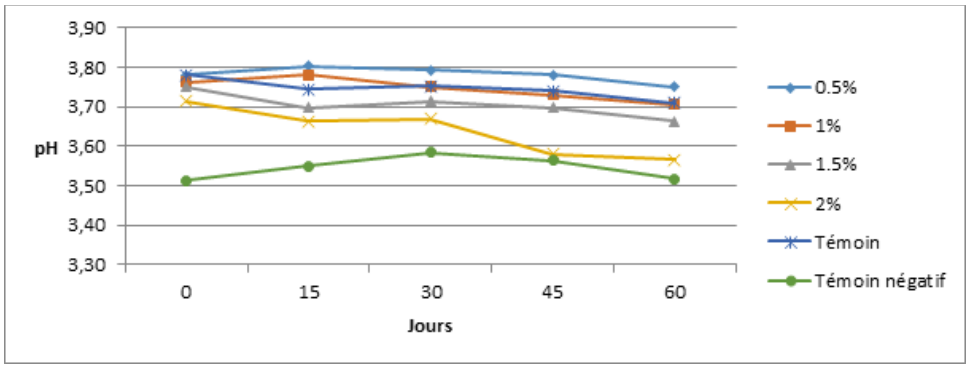
To assess the effect of sea fennel on product stability, physico-chemical parameters (Brix, pH, titratable acidity, etc.) were monitored for 60 days at two storage temperatures (20°C and 37°C). The results showed a drop in pH from 3.78 to 3.71 and in water activity from 0.89 to 0.77 for the different incorporations compared with the standard. These results were confirmed by the microbiological analyses, since all the samples were compliant.

Recipies	0.5%	1%	1.5%	2%	Control	Negatif control
Brix	48,40±0,27b	47,87±0,12a	47,67±0,35c	54,27±0,15c	47,61±0,08c	47,52±0,2c
pH	3,78±0,00a	3,76±0.006b	3,75±0,00c	3,71±0,006d	3,78±0,00a	3,51±0,006e
Titratable acidity (méq/100g)	6,00±0,4d	6,07±0,3d	6,73±0,12c	7,60±0,2b	7,67±0,3b	9,87±0,6a
Water activity (aw)	0,83±0,00d	0,84±0,00c	0,77±0,00f	0,78±0,00e	0,89±0,00a	0,85±0,00b

Effect of incorporating sea fennel on the color of orange jam



Brix evolution over 60 days at 20 and 37°C

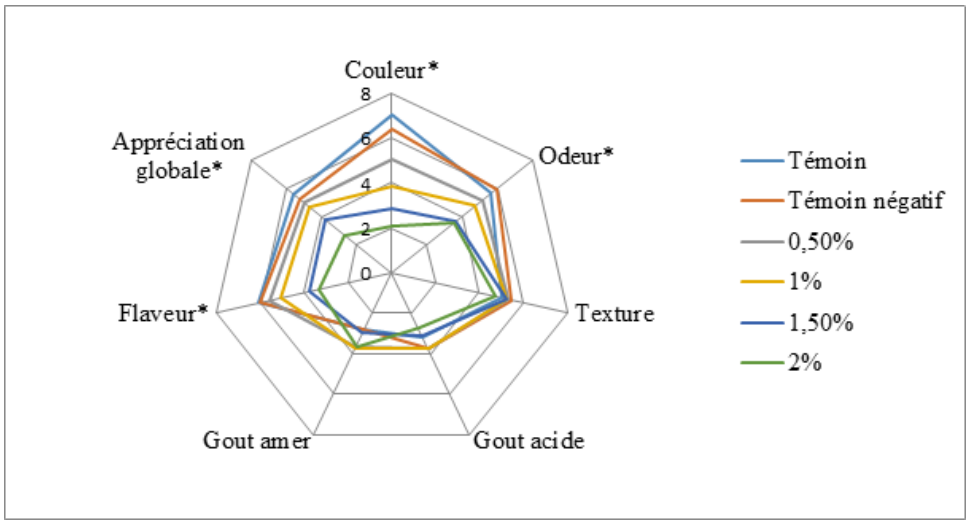


pH trends for various recipes.

The microbial analysis revealed that yeast and mold counts remained below detectable levels (N<10 CFU/g) for all samples, except for the 2% formulation, which showed a slight increase (2 × 10² CFU/g). Both the control and the negative control samples exhibited no detectable yeasts and molds (N<10 and 0 CFU/g, respectively). Similarly, the total mesophilic flora was undetectable (N<10 CFU/g) in most samples, with the exception of the 1% concentration, which recorded a value of 2 × 10² CFU/g. These findings indicate overall microbiological stability at lower concentrations, with a slight microbial development observed at 1% and 2% levels.

Evaluation of microbiological quality after 60 days storage at 20°C

	Control	Negatif control	0,50%	1%	1,50%	2%
Yeasts and molds (UFC/g)	N<10	0	N<10	N<10	N<10	2. 10²
Total mesophilic flora (UFC/g)	N<10	0	N<10	2 . 10²	N<10	N<10



*Analytical sensory characterization of jam recipes (*p<0.05)*

Results show that the incorporation of sea fennel in jam in lower doses is appreciated by consumers. Higher values of plant incorporated while preparing the jam increase acidity and bitterness in samples making the preparation less appreciated.

Sensory evaluation results indicated a slight improvement in color perception with the 0.5% formulation (2.50 ± 0.91), compared to the control (2.07 ± 0.71), with a statistically significant difference (*p < 0.05). Smell, texture, and taste scores showed minimal variation between the two samples. The 0.5% sample recorded values of 2.31 ± 0.96 for smell, 2.29 ± 1.02 for texture, and 2.25 ± 1.02 for taste, compared to the control values of 2.16 ± 0.81, 2.13 ± 0.75, and 2.22 ± 0.97, respectively. These results suggest that the addition of 0.5% of the tested ingredient slightly enhances color without adversely affecting the other sensory attributes.

*Hedonic appreciation of the control recipe and the recipe with 0.5% sea fennel (n=50; *p<0.05)*

	Color	Smell	Texture	Taste
Control	2,07±0,71*	2,16±0,81	2,13±0,75	2,22±0,97
0 ,5%	2,50±0,91*	2,31±0,96	2,29±1,02	2,25±1,02

3.3 Snack

Material and Methods

The preparation of the breadsticks

The preparation of the breadstick's dough was carried out in two main steps. First, a part of dry and wet ingredients was mixed thoroughly until a homogeneous dough was obtained. This initial mixing phase ensured proper hydration of the flour and even distribution of the added components. After the dough was formed, it was left to rest for 15 minutes at room temperature. This resting period allowed the gluten network to relax, making the dough easier to shape and improving its final texture.

Following the resting phase, the rest of ingredients was added, mixed, and the dough was shaped into uniform breadsticks using a manual rolling technique to ensure consistency in size and thickness. The shaped dough pieces were then transferred onto a baking tray lined with parchment paper.

The breadsticks were baked in a preheated oven at 120°C for 60 minutes. This relatively low-temperature, long-duration baking process was used to achieve a crispy texture without overbrowning. After baking, the breadsticks were allowed to cool completely at room temperature before further analysis or packaging.

Three kinds of snack were tested:

- Control: with salt and without sea fennel
- Trt1: without salt and containing 6% of dried sea fennel leaves
- Trt2: With salt and containing 6% of dried sea fennel leaves

	Control	Trt1	Trt2
First step			
Flour	100	100	100
Water	150	150	150
Sugar	4.5	4.5	4.5
Yeast	1.5	1.5	1.5
Second step			
Flour	150	150	150
Oil	10.5	10.5	10.5
Salt	3.5	0	3.5
Dried leaves	0	15	15

Preparation of sea fennel breadsticks

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 breadsticks crashed and homogenized in 50ml of distilled water, and the mix was then filtered.

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 phenols

To measure phenols, flavonoids, and antioxidant activity, a solution was prepared, mixing 0.5 grams of breadsticks crashed and homogenized in 20ml ethanol:water (60:40) solution. The mix was centrifuged at 2000rpm for 10min and the supernatant was recuperated.

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 Na2CO3 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 flavonoids

Total flavonoids were quantified by the aluminum trichloride (AlCl3) 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 AlCl3 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

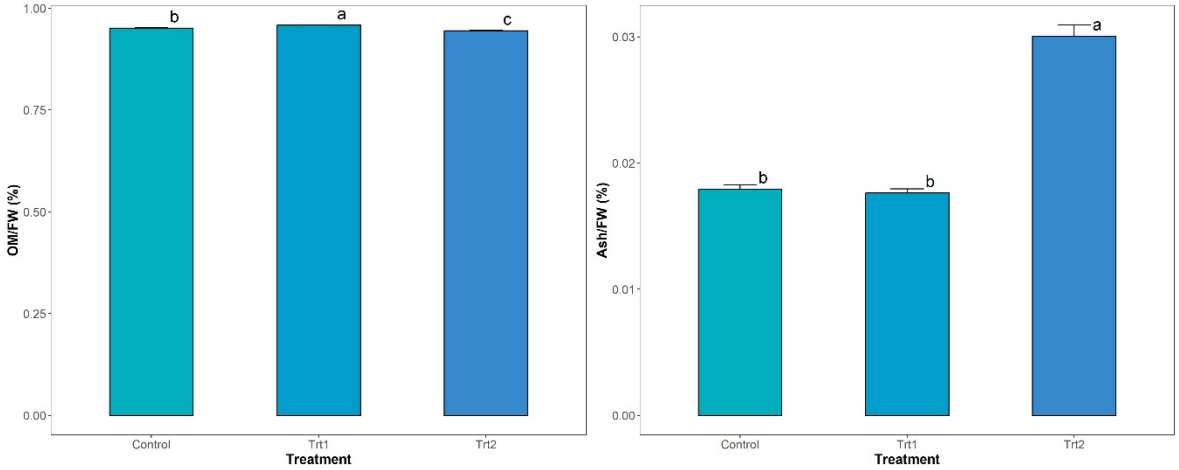
Sensory analysis

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 breadsticks), and taste (appreciation of Bitterness, acidity, Flavor). Tasters rate their appreciation of the product on a scale from 1 (I certainly hate it) to 4 (I love it)

Results

Ash and Organic Matter

Significant differences were recorded between the three snacks. The highest value of organic matter was observed in Trt1 (about 90%).

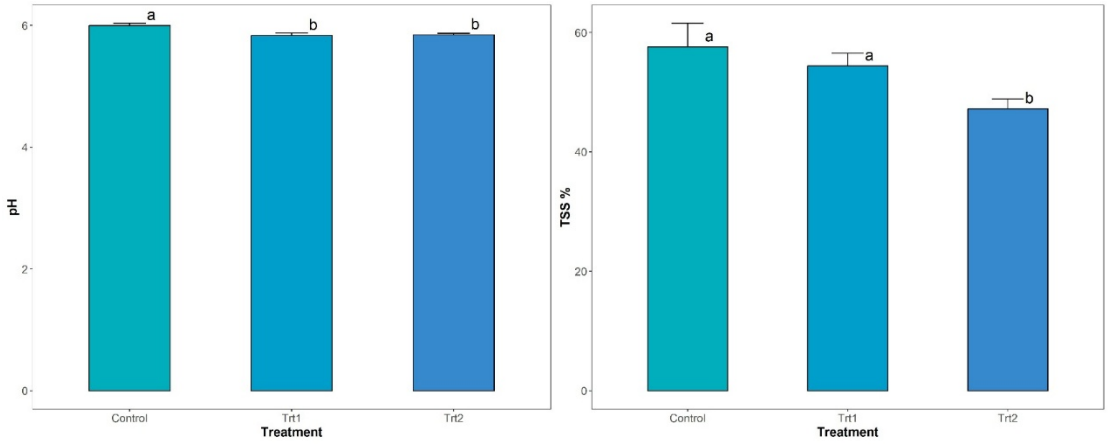


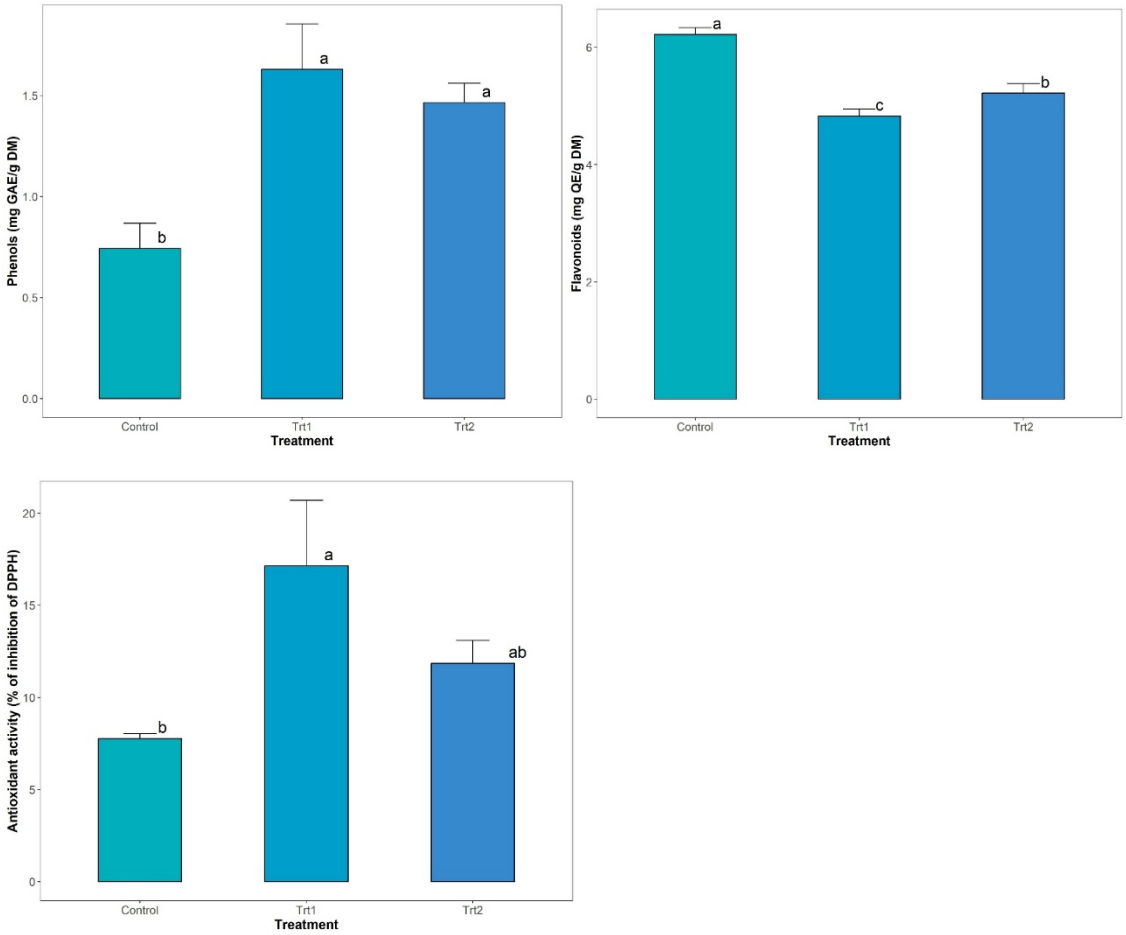
Biochemical analysis

The control samples, which did not contain sea fennel, exhibited higher values for pH, total soluble solids (TSS), and flavonoid content. These elevated levels suggest a stable and relatively less reactive matrix in the absence of added bioactive plant material.

In contrast, the incorporation of dried *Crithmum maritimum* (sea fennel) leaves into the dough formulation led to notable biochemical changes. Specifically, snacks enriched with sea fennel showed a marked increase in total phenolic content, indicating that the herb contributed additional polyphenolic compounds to the matrix. As a result of this enrichment, antioxidant activities were significantly enhanced compared to the control.

These findings suggest that sea fennel acts as a natural functional ingredient, capable of improving the nutritional and health-promoting properties of baked snacks by increasing their antioxidant potential. The observed changes also support the potential of using sea fennel as a source of bioactive compounds in functional food development.





Sensory performances

The sensory evaluation conducted on the different snack formulations revealed no statistically significant differences in overall acceptability between the control and the sea fennel-enriched treatments. This suggests that the incorporation of dried *Crithmum maritimum* leaves into the dough did not negatively impact consumer perception in terms of key sensory attributes such as taste, texture, odor, and color.

Furthermore, demographic variables such as age and gender had only a limited influence on the panelists' evaluations, indicating a generally uniform perception of the products across different consumer profiles.

Interestingly, the perceived saltiness was rated higher in the two sea fennel containing treatments compared to the control sample. This suggests that sea fennel, known for its naturally salty and aromatic character, may act as a flavor enhancer. Its inclusion could potentially reduce the need for added salt while maintaining consumer satisfaction with the taste. These findings highlight the potential of sea fennel as a natural salt substitute in the development of healthier, reduced-sodium snack products.