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	D5.5 Report with elaborated data overall collected within WP5
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Related Work Package:	WP5
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Author(s)	Dr Valentina Melini
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Summary of Deliverable D5.5 – Integrated Results from WP5	Deliverable D5.5 consolidates the outcomes of three years of food innovation work under WP5, where the project transformed sea fennel into a wide array of fermented and unfermented, shelf-stable prototypes at both laboratory and pilot scale. The report not only documents recipes and processes but also presents detailed analyses of nutritional, functional, microbiological, sensory, and consumer acceptance traits, creating a full picture of sea fennel's potential in the food industry. Among the fermented products, two stood out: a sea fennel-enriched craft beer and a kimchi-like preserve. Both prototypes showed strong functional properties, with significantly higher phenolic content, antioxidant activity, and appealing sensory profiles compared to controls. Beer trials demonstrated that sea fennel could enhance aroma complexity and color, while kimchi formulations achieved safe fermentations and a distinctive herbal character, confirmed by microbial and sensory evaluations. In the unfermented line, partners from Croatia, Türkiye, and Tunisia developed products that combined tradition and modernity. These ranged from dried spice mixes, aromatized oils, patés, and pickled vegetables to extruded snacks, spiced noodles, handmade pasta, harissa, and jams. Shelf-life studies (up to six months) confirmed safety and stability, while nutritional analyses revealed enrichment in vitamin C, carotenoids, tocopherols, and phenolics. Sensory panels and

	consumer tests confirmed good acceptance, especially at moderate inclusion levels that balanced flavor intensity with texture and appearance. Statistical analyses (PCA, PLS, cluster methods) highlighted correlations between bioactive content, sensory appeal, and consumer preference, showing that health-related traits and Mediterranean authenticity strongly drive acceptance.

Versioning and Contribution History

Version	Date	Modified by	Modification reason
v1.0	20/04/2024	Valentina Melini	First version
V2.0	30/03/2025	Valentina Melini	Comments after peer reviewing process

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

Report with elaborated data overall collected within WP5

1 Sea fennel-based fermented shelf stable preserves

1.1 Italian prototypes

1.1.1 Sea fennel-based BEER

Material and Methods

Beer formulation

The brewing test was performed at lab scale and at pilot scale.

Yeast starter formulation

The non-conventional yeast strains, coming from the Department of Life and Environmental Sciences (DiSVA) Collection, and the S. cerevisiae US-05 (Fermentis, Lesaffre, France) starter strain used as control are reported in the following table. The five yeasts strains were selected taking into account the results of a previous work regarding the analytical and sensory profile of beers (Canonico et al., 2023).The yeast strains used in the trials were cultivated and maintained in YPD agar medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 18 g/L agar) at 4 ◦C for short-term storage, while for long-term storage, YPD broth supplemented with 40% (w/v) glycerol at −80 ◦C was used.

Taxonomic classification, strain code and source of isolation of the yeast strains tested and S.cerevisiae commercial strain US-05 (Fermentis, Lesaffre, France).

Species	Strain code	Source of isolation
Lachancea thermotolerans	101	Grapes
Pichia kluyveri	PRMB7	Grapes
Wickerhamomyces anomalus	3003	Backery
Torulaspora delbrueckii	33	Papaya leaves
Saccharomyces cerevisiae	US05	Commercial starter

Brewer's Spent Grain (BSG) and Substrate of Fermentation/Recycled BSG to Produce Worts with Low Sugar Content: BSG +HOP and BSG + SEA FENNEL

The BSG used for the preparation of wort with low sugar content, came from a PILS wort (170 Kg of malt Pils in water at 35 ◦C) added with Cascade hops during the boiling phase. The batch was used to prepare 1000 L of Pils craft beer at the Birra dell' Eremo craft brewery (Assisi, Italy). The wort obtained (1000 L) had the following analytical characteristics: pH 5.5, density 12.3◦P (Plato degree) and 20 IBU (International Bitterness Unit). At the end of the filtration the wort with the characteristics reported above was destined for the craft beer production. At this point, the BSG was further filtered using water at 78 ◦C. Four hundred litres were then collected, and the sugars (glucose, sucrose and maltose) were determined using an enzymatic method (Megazyme, Wicklow, Ireland). Subsequently A total of 15 L of combined wort from exhausted BSG was added with 18 g of Cascade hops and boiled for 1 h. The hopped substrate was divided equally into two batches.

In the first batch, it was kept as is and named recycled BSG+HOP, while, in the second, 5 % sea fennel (BSG+SEA FENNEL). The two worts obtained were used separately as substrates for setting up micro-fermentations.

Micro-fermentations

The micro-fermentations were conducted at a temperature of 20 ◦C ± 2 ◦C using 500 mL flasks equipped with Müller valve to allow the escape of CO2, containing 450 mL of wort from recycled wort. The pre-cultures were prepared using 10% malt extract and incubated for 48 h at 20 ◦C ± 2 ◦C. After incubation, the cells were collected by centrifugation (2000 g for 5 min), resuspended in sterile water and the wort by recycled BSG was inoculated with 1 × 106 cells/mL using the Thoma-Zeiss chamber. The inoculated flasks were placed in thermostat at a temperature of 20 ◦C ± 2 ◦C. A control trial without inoculation was also placed in thermostat. The fermentation evolution was monitored recording the decrease in weight due to the loss of CO2 daily to a constant value. At the end of the resting period at 4 ◦C, the beers underwent refermentation in the bottle by residual and still viable yeasts, adding 1.5 g/L of sucrose during the bottling phase. The sealed bottles were kept at 18–20 ◦C for about 7–10 days, finally stored at 4 ◦C.

Microbiological and chemical analysis

Viable cell counts were carried out at the start of fermentation process.

Ethanol was measured by gas–liquid chromatographic analysis (Canonico, Zannini, Ciani, & Comitini, 2021). Acetaldehyde, ethyl acetate and the higher alcohols were determined by direct injection of FID -GC (GC-2014; Shimadzu, Kyoto, Japan). The samples were injected into a 2 m × 2 mm i.d. glass column, packed with 80/100 Carbopack C/0.2% Carbowax 1500 (Supelco, Sigma Aldrich, Milan, Italy), with an internal standard of the carrier gas. A Shimadzu gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector was used. The oven temperature ranged from 45 to 160 ◦C. The temperature of the injector and the detector was 220 ◦C. The temperature of the injector and the detector was 220 ◦C as reported by Canonico et al. (2015). The solid-phase microextraction (HS-SPME) method was used to determine the concentration of the volatile compounds. Five mL of beer was placed in a vial containing 1 g NaCl closed with a septum-type cap. HS-SPME was carried out with magnetic stirring for 10 min at 25 ◦C. After this period, the internal standard (3-octanol) (Sigma Aldrich, Milan, Italy) at a concentration of 1.6 mg/L was added, and the sample was heated to 40 ◦C. Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/ PDMS) fibre (Sigma-Aldrich, Milan, Italy) was inserted into the vial headspace for 30 min. The compounds were desorbed by inserting the fibre into a Shimadzu gas chromatograph GC injector for 5 min. A glass capillary column was used: 0.25 µm Supelcowax 10 (length, 60 m; internal diameter, 0.32 mm). The fibre was inserted in the split–splitless mode (Canonico, Comitini, & Ciani, 2015). The compounds were identified and quantified by comparisons with external calibration curves for each compound. Specific enzymatic kits (Megazyme, Wicklow, Ireland) were used to determine the concentrations of glucose sucrose, maltose (kit k-masug, Megazyme, Wicklow, Ireland), ethanol (K-ETOH), lact acid (K-LATE) according to the manufacturer instructions.

Physico-chemical and technological parameters evaluated in the prototypes of beer

Total soluble solids, total acidity, pH, alcoholic grade (% v/v), color (EBC), and bitterness (IBUs) were determined as physico-chemical parameters in control and enriched beers. After 6 months of maturation, the samples from three bottles were analysed in duplicate.

The total soluble solids, expressed as °Brix, and pH were measured by using a digital refractometer at 20°C, and a pH meter, respectively.

Total acidity was measured by titration with 0.1 N NaOH up to pH 8.1, using 1 mL of beer in 25 mL of distilled H2O, and results were expressed as % lactic acid (g lactic acid equivalent per 100 mL).

Alcoholic grade was calculated by using the density data of the beers, which were obtained with a hydrometer before and after fermentation.

Colour EBC and Bitterness (IBUs) were determined according with the Official Methods of the Analytical Division of European Brewery Convention, EBC Method 9.6 and 9.8, respectively, using a spectrophotometer (Varian 5000 UV-Vis NIR) (Abellán et al. 2021).

Liters post-boiling, mashing: time and temperature, mashing-out: time and temperature, primary fermentation step: time and temperature, secondary fermentation: time and temperature

Total Phenolic Content (TPC) determination

The total phenolic content (TPC) of the control (HOP) and sea fennel extract enriched beer (SFB) samples was determined according to the Folin–Ciocalteu method. Briefly, 20 µl of the sample was mixed with Folin reagent, incubated, then Na_2CO_3 solution was added and after 30 min in the dark, absorbance was measured at 750 nm in a UV-Vis spectrophotometer (Onda, UV-31 SCAN, Beijing, China). Each sample was analyzed in triplicate and the results were expressed as mg gallic acid equivalents (GAE)·g−1 of the sample, using a calibration curve of gallic acid.

Volatile compounds by means of SPME-GC-MS

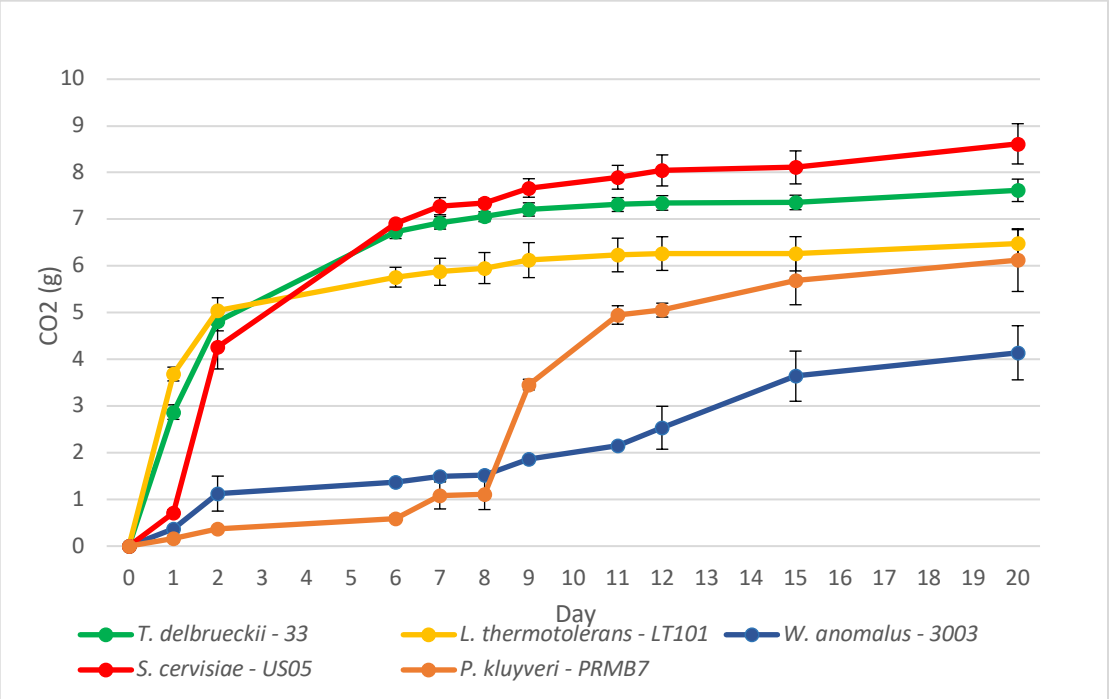
Degassed beer (8 mL) sample and 25 µL internal standard 4-methyl-2-pentanol (534 mg/L) were placed into a 20 mL glass vial for 5 min at 50°C. A HS-SPME fiber (100 µm, DVB/CAR/PDMS, 1 cm, Merck Life Science, Milan, Italy) was exposed for 40 min at 50°C. The fiber was desorbed of volatile compounds in the GC inlet at 250 °C for 5 min. Two columns were used (DBWAX, 60 m × 0.25 mm × 0.25 µm; DB-5MS 30m × 0.25 mm x 0.25 µm) for the untargeted volatile profile of beer for polar and non-polar compounds, respectively. An Agilent 7890 GC equipped with a 5977A MS (New York, USA) was used. The carrier gas helium flowed at the rate of 1 mL/min. For the DBWAX untargeted volatile, the temperature program started at 40 °C, then increased to 120 °C (3 °C/min) and held for 5 min, and finally increased to 230 °C (5 °C/min). For the DB-5MS untargeted volatile analysis, the initial temperature of the oven was 60°C for 1 min, increased to 120°C at 5°C/min rate, held for 3 min and raised to 200°C at a rate of 8°C/min. Finally, the temperature was increased to 250°C at a rate of 10°C/min and held for 1 min. The mass detector was set at 230 °C, and its electron ionization (EI) energy was 70 eV. The mass spectra were obtained in duplicate under full scan acquisition mode with a mass scan range of m/z 33-450 to achieve HS-SPME-GC-MS fingerprints to be used for PCA and PLS-DA elaboration.

Results

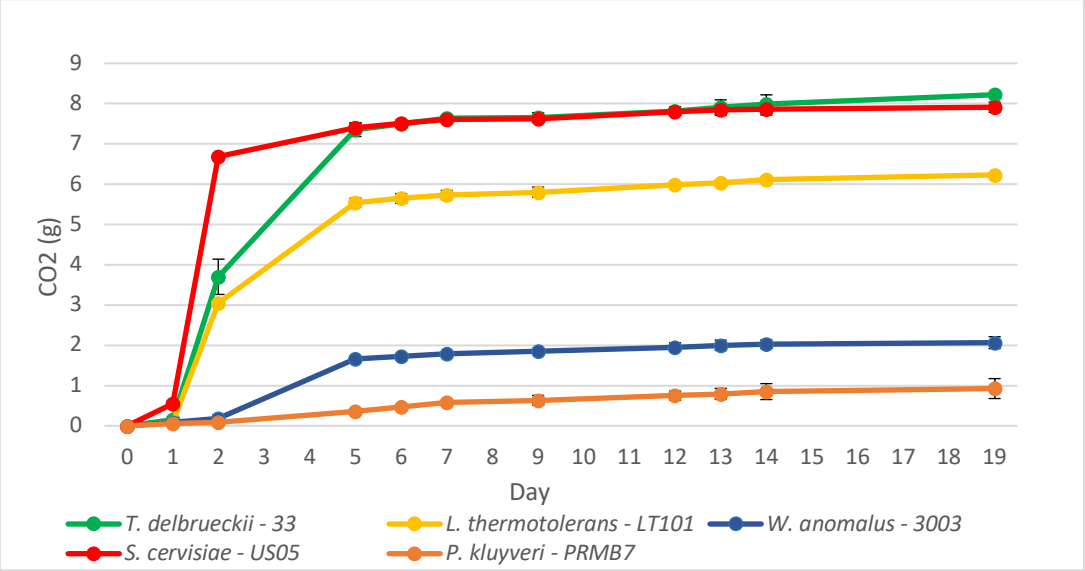
The mashing step started at 62°C (hold 20 min), increased till 68°C in 5 min and hold for 40 min. The mashing out step (78°C) was reached in 10 min and hold for 10 min. Wort was boiled for 60 min with Cascade hop and added of the special ingredient to characterize each different beer sample. After the whirlpool step, wort was filtered and cooled down till 18°C, inoculated with yeast. The fermentation lasted 10 days till the density dropped from around 1.060 till 1.010 g/L. Beer was bottled (750 mL dark glass bottles), added of sugar (2.5 g/L), closed with crown caps, and kept at room temperature for maturation for 6 months.

Fermentation kinetics

The fermentation evolution of the non-conventional yeasts and *S. cerevisiae* commercial strain US-05 on BSG+HOP and BSG+ SEA FENNEL are shown in the following two figures.
The fermentation kinetics of the strain tested on BSG+HOP showed that S. cerevisiae US-05 (starter commerciale) exhibited the highest fermentation evolution (8,61 g di CO_2). A slight reduction in fermentation kinetics was exhibited by *T. delbrueckii 33*, di 7,62 g di CO_2 and *L. thermotolerans LT101* 6.48 g di CO_2.
W. anomalus 3003 and *P. kluyverii PRMB7* exhibited the slower fermentation kinetics. Generally these trends were exhibited by the strain on the BSG+SF (Figure 2)



Fermentation kinetics of the yeasts strain tested on BSG+HOP



Fermentation kinetics of the yeasts strain tested on BSG+ SEA FENNEL

Sugar content analysis

The following table illustrates the results of sugar concentration in the initial substrate.

The results showed improved maltose consumption in the BSG+Sea Fennel substrate compared to BSG+Hop for certain strains, such as W. anomalus 3003 and L. thermotolerans LT101. Regarding residual sucrose, both substrates exhibited generally similar concentrations, except for W. anomalus 3003, which displayed higher residual sucrose levels in BSG+Sea

Fennel. Additionally, glucose utilization was generally complete in both prototypes. Finaly, S. cerevisiae US05 demonstrated consistent patterns in sugar utilization across both conditions.

Sugar content in the initial substrate before fermentation

Sugar (g/L sample)	**BSG+HOP**	**BSG+SF**
Glucose	4,8	5,8
Sucrose	5,3	2,5
Maltose	16,4	17,0

Residual Sugar after fermentation on BSG+HOP

Residual sugar (g/L)	***T. delbrueckii* 33**	***L. thermotolerans* LT101**	***W. anomalus* 3003**	***P. kluyveri* PRMB7**	***S. cerevisiae* US05**
GLUCOSE	0,03 ± 0,00	0,01 ± 0,02	0,20 ±0,23	0,00 ± 0,20	0,02 ± 0,02
SUCROSE	0,05 ± 0,03	0,06 ± 0,07	0,89 ± 0,88	5,13 ± 0,65	0,00 ± 0,14
MALTOSE	1,72 ± 0,12	3,54 ± 2,53	10,11± 0,12	14,13 ± 0,85	0,88 ± 0,02

Residual sugar after fermentation in BSG+ SEA FENNEL

Residual sugar (g/L)	***T. delbrueckii* 33**	***L. thermotolerans* LT101**	***W. anomalus* 3003**	***P. kluyveri* PRMB7**	***S. cerevisiae* US05**
GLUCOSE	0,04 ± 0,02	0,05 ±0,02	0, ±0,92	0,00 ± 0,12	0,00 ± 0,12
SUCROSE	0,00 ± 0,10	0,00± 0,28	4,48 ± 3,03	2,34 ± 3,12	0,03 ± 0,01
MALTOSE	1,36 ±0,39	1,73 ± 0,97	4,32 ± 2,65	13,40 ± 4,70	0,43 ± 0,38

Lactic acid and ethanol content

The addition of sea fennel to the BSG substrate had a minimal impact on both lactic acid and ethanol production. Slight increases in lactic acid and ethanol levels were observed for specific strains such as T. delbrueckii 33 and L. thermotolerans LT101. Overall, the fermentation outcomes were comparable between BSG+HOP and BSG+Sea Fennel, suggesting that sea fennel has a limited effect on the metabolic activity of the tested yeast strains.

Lactic acid and ethanol content in BSG+HOP

Yeast strains	Lactic acid (g/L)	Ethanol (% v/v)
T. delbrueckii 33	0,00 ± 0,00	0,86 ± 0,01
L. thermotolerans LT101	0,06 ± 0,01	0,75 ± 0,00
W. anomalus 3003	0,00 ± 0,00	0,31 ± 0,00
P. kluyveri PRMB7	0,00 ± 0,00	0,3 ± 0,00
S. cerevisiae US05	0,01 ± 0,00	1,45 ± 0,00

Lactic acid and ethanol content in BSG+ SEA FENNEL

Yeast strains	Lactic acid (g/L)	Ethanol (% v/v)
T. delbrueckii 33	0,01 ± 0,00	0,93 ± 0,00
L. thermotolerans LT101	0,08 ± 0,01	0,92 ± 0,00
W. anomalus 3003	0,00 ± 0,00	0,3 ± 0,00
P. kluyveri PRMB7	0,01 ± 0,00	0,3 ± 0,00
S. cerevisiae US05	0,00 ± 0,00	1,37 ± 0,00

Alcohol composition

The analysis revealed differences in volatile alcohol compounds between the BSG+HOP and BSG+Sea Fennel substrates. Acetaldehyde levels were higher in BSG+Sea Fennel for most strains, particularly for T. delbrueckii 33 and S. cerevisiae US05, whereas P. kluyveri PRMB7 exhibited lower levels compared to BSG+HOP. Ethyl acetate production was generally higher in BSG+Sea Fennel, with W. anomalus 3003 and L. thermotolerans LT101 showing the largest increases. n-Propanol levels were consistent across substrates for most strains, though W. anomalus 3003 produced detectable amounts only in BSG+HOP. Isobutanol and amyl alcohol concentrations were elevated in BSG+Sea Fennel for some strains, such as T. delbrueckii 33. However, isoamylic alcohol levels were lower in BSG+Sea Fennel, potentially softening the intensity of fusel alcohol contributions. These variations highlight how the addition of sea fennel may influence the aromatic and flavor profile of the beer.

Alcohol composition in BSG+HOP

Alcohols (ppm)	***T. delbrueckii 33***	***L. thermotolerans LT101***	***W. anomalus 3003***	***P. kluyveri PRMB7***	***S. cerevisiae US05***
Alcohols					
Acetaldehyde	0,00±0,00	0,00±0,00	5,46±2,04	8,5±0,68	0,00±0,00
Ethyl acetate	121,26±1,36	114,40±0,62	84,70±3,96	5,4±1,38	58,48±2,91
n-propanol	10,73±0,18	0,00±0,00	12,64±0,12	0,00±0,00	26,16±0,35
Isobutanol	5,69±2,42	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00
Amyl alcohol	0,21±1,36	44,81±0,71	1,33±0,04	0,00±0,00	0,00±0,00
Isoamylic alcohol	20,03±0,49	0,00±0,00	15,27±0,03	0,00±0,00	20,40±0,56

Alcohol composition in BSG+SEA FENNEL

Alcohols Mg/l	***T. delbrueckii 33***	***L. thermotolerans LT101***	***W. anomalus 3003***	***P. kluyveri PRMB7***	***S. cerevisiae US05***
Alcohols					
Acetaldehyde	5,93±0,21	0,00±0,00	0,86±0,02	2,06±0,04	3,30±0,14
Ethyl acetate	235,50±5,45	317,87±0,60	539,71±4,10	81±0,26	48,42±24,78
n-propanol	10,41±0,59	10,12±0,30	0,00±0,00	10,19±1,30	19,88±1,10
Isobutanol	30,47±4,65	9,78±0,57	0,00±0,00	0,00±0,00	5,68±0,37
Amyl alcohol	18,53±7,56	0,00±0,00	0,00±0,00	0,00±0,00	16,40±0,65
Isoamylic alcohol	2,04±2,88	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00

Volatile compounds

The inclusion of sea fennel in the BSG substrate led to increases in certain ester compounds, notably isoamyl acetate followed by ethyl hexanoate, which may enhance fruity and floral aroma profiles. However, β-phenyl ethanol production decreased in some strains. Overall, the use of sea fennel as a substrate modifier appears to influence the volatile composition variably across different yeast strains, with notable enhancements in specific aroma-active compounds.

Volatile compounds composition on BSG+HOP

VOLATILE COMPOUNDS (mg/L)	***T. delbrueckii* 33**	***L. thermotolerans* LT101**	***W. anomalus* 3003**	***P. kluyveri* PRMB7**	***S. cerevisiae* US05**
VOLATILES					
Isoamyl acetate	10,92±0,18	7,46±6,98	0,63±0,03	9,67±5,19	0,47±0,00
Ethyl hexanoate	0,03±0,00	0,00±0,00	0,08±0,01	0,04±0,01	0,04±0,01
hexanol	0,01±0,00	0,06±0,00	0,01±0,00	0,01±0,00	0,04±0,00
Ethyl octanoate	0,02±0,01	0,01±0,00	0,00±0,00	0,00±0,00	0,00±0,00
Linalol	0,01±0,00	0,05±0,01	0,00±0,00	0,00±0,00	0,01±0,00
diethylsuccinate	0,01±0,00	0,02±0,00	0,00±0,00	0,03±0,00	0,00±0,00
α - Terpineol	0,02±0,00	0,04±0,01	0,01±0,00	0,04±0,01	0,01±0,00
Citronellol	0,09±0,01	0,03±0,00	0,08±0,00	0,10±0,03	0,04±0,00
phenyl ethyl acetate	0,00±0,00	0,02±0,00	0,03±0,01	0,00±0,00	0,01±0,01
Nerol	0,00±0,00	0,01±0,00	0,00±0,00	0,01±0,00	0,01±0,00
Geraniol	0,00±0,00	0,01±0,00	0,04±0,00	0,00±0,00	0,01±0,00
β-phenyl ethanol	4,14±0,10	7,30±1,00	0,00±0,00	3,30±0,12	13,96±0,90

The main volatile compounds on BSG+SEA FENNEL

VOLATILE COMPOUNDS (mg/L)	*T. delbrueckii* 33	*L. thermotolerans* LT101	*W. anomalus* 3003	*P. kluyveri* PRMB7	*S. cerevisiae* US05
VOLATILES					
Isoamyl acetate	11,35±0,28	19,88±0,90	8,74±0,17	11,76±1,09	1,25±0,07
Ethyl hexanoate	0,08±0,00	0,54±0,01	0,12±0,00	0,04±0,00	0,06±0,00
hexanol	0,00±0,00	0,06±0,00	0,00±0,00	0,01±0,00	0,02±0,00
Ethyl octanoate	0,01±0,00	0,01±0,00	0,01±0,00	0,00±0,00	0,01±0,00
Linalol	0,02±0,00	0,05±0,01	0,01±0,00	0,04±0,01	0,01±0,00
diethylsuccinate	0,01±0,00	0,02±0,00	0,02±0,00	0,02±0,00	0,00±0,00
α - Terpineol	0,05±0,00	0,01±0,00	0,09±0,00	0,03±0,02	0,00±0,00
Citronellol	0,04±0,00	0,01±0,02	0,01±0,00	0,33±0,01	0,10±0,03
phenyl ethyl acetate	0,00±0,00	0.003±0.00	0,03±0,00	0,00±0,00	0,13±0,01
Nerol	0,03±0,00	0,00±0,00	0,00±0,00	0,02±0,00	0,00±0,00
Geraniol	0,00±0,00	0,00±0,00	0,00±0,00	0,01±0,00	0,02±0,01
β-phenyl ethanol	3,45±0,23	0,50±0,00	0,77±0,00	4,45±0,75	8,82±0,07

Color and pH

The Color and pH of control (HOP) and sea fennel extract enriched beer (SFB)

	HOP			SFB		
Before fermentation	pH	Color (EBC)	Bitter (IBUs)	pH	Color (EBC)	Bitter (IBUs)
TREB	5.65±0.04^{c}	15.12±0.12^{c}	11.92±0.62^{a}	5.45±0.01^{c}	38.62±0.12^{c}	20.35±1.1^{d}
After fermentation						
33	4.26±0.03^{a}	12.37±0.12^{b}	20.1±0.3^{c}	4.27±0.04^{a}	45.50±0.75^{c}	18.62±0.47^{cd}
3003	4.28±0.04^{a}	12.50±0.25^{b}	24.22±0.77^{d}	4.69±0.09^{b}	31.87±0.12^{b}	16.92±0.52^{bc}

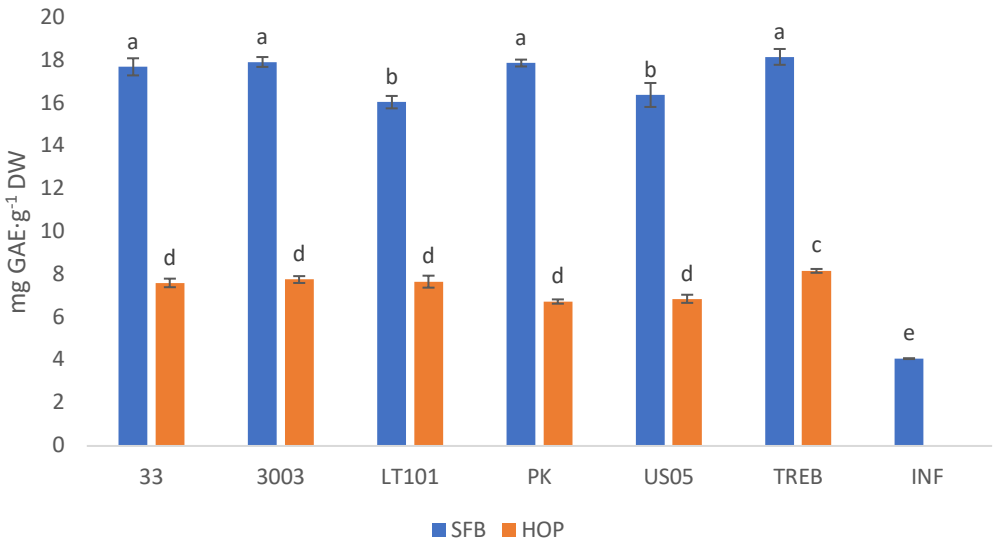
LT101	4.25±0.02^{a}	11.75±1.00^{a}	17.67±0.17^{b}	4.71±0.03^{b}	27.00±0.01^{a}	13.77±0.22^{a}
PK	4.33±0.04^{a}	11.75±0.00^{a}	18.1±0.55^{b}	4.99±0.06^{b}	38.12±0.62^{c}	15.77±0.42^{ab}
US05	4.62±0.05^{b}	19.12±1.87^{d}	24.42±0.02^{d}	4.82±0.00^{b}	31.25±0.75^{b}	16.6±1.25^{bc}

Values are presented as mean of three replicates ± SD. Different letters in each column indicate statistical differences ($p < 0.05$).
EBC, European Brewing Convention
IBUs, International Bitterness Units

The physiochemical results of HOP and SFB beer including pH, color and bitterness across various yeast strains are shown in Table 1. Prior to fermentation, the pH values of the sea fennel extract-enriched beer (SFB) and the control beer (HOP) were higher than those following fermentation. For instance, depending on the yeast strain, SFB's pH varied from 4.27 to 4.99. These values were greater than the 4.25 to 4.62 ranges found in HOP beer. The impact of sea fennel extract may be the cause of this discrepancy.

Higher EBC values were seen in SFB compared to HOP beers, especially where 33 has the highest color intensity (45.50±0.75 EBC), suggesting that the addition of sea fennel extract produced a more intense or dark beer color. All samples showed the same color increase, indicating that the extract had a consistent impact. The bitterness was constantly higher in the control beer condition with maximum value of 24.42±0.02 IBUs, except for TREB, which has higher IBUs under SFB (20.35±1.1 IBUs).

Total phenolic content



TPC (total phenolic content) in control beer (HOP) and sea fennel extract enriched beer (SFB)
Data are expressed as mean ± standard deviation (n= 3) mg gallic acid equivalents/g dw (dry weight).
Different letters indicate statistical differences ($p < 0.05$).

The total phenolic content of the SFB and the HOP across several yeast strains is shown in Figure 1. When compared to HOP beers, SFB consistently exhibit a significantly higher total phenolic content, ranging from 16.06 to 17.94 mg GAE·g−1 DW, with 3003 showing the highest value. In contrast, the range of HOP samples is from 6.74 to 7.77 mg GAE·g^{-1} DW. The TREB sample, which was taken prior to fermentation, had a slightly higher total phenolic content under both HOP and

SFB conditions than the other beer samples. In comparison to the other samples, the sea fennel infusion's (INF) total phenolic content was significantly lower, at just 4.08 mg GAE·g^{-1} DW.

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1.1.2 Kimchi-like preserve

Material and Methods

Microbial starter formulation

The starter culture of lactic acid bacteria used for kimchi product was formulated and selected according to their technological traits. The same strains have been applied in the fermentation of sea fennel sprouts in a brine salt solution (Maoloni et al., 2021). The starter culture was composed of 4 different strains ascribed to: *Lactiplantibacillus plantarum* (strain PB257), *Leuconostoc pseudomesenteroides* (strain PB288), *Pediococcus pentosaceus* (strain FF78), and *Weissella confusa* (strain PB 321). This culture belongs to the Culture Collection of the Department of Agricultural, Food, and Environmental Sciences (D3A, Università Politecnica delle Marche), where they were stored at – 80°C in de Man Rogosa and Sharpe (MRS) broth (VWR, International, Radnor, Pennsylvania, USA) added with glycerol at a 3:2 ratio and sub cultured in MRS broth (VWR) at 30°C for 24 h, prior to their use.

Kimchi production

Started and non-started (control) Kimchi prototypes were prepared. The sea fennel sprouts used for Kimchi prototypes was supplied by the local farm of Rinci company (S.r.l, Castefidardo, Ancona, Italy). All the ingredients including sea fennel sprouts used for Kimchi production are reported in the table below.

Ingredients used for Kimchi production.

	% ingredient	Ingredient (g)
Chinese cabbage	70.53%	3725.34
Onion	4.37%	230.62
Garlic	0.50%	26.61

Ginger	0.50%	26.61
Sea fennel	7.05%	372.53
Red pepper	0.17%	8.87
Paprika	1.85%	97.57
Sugar	0.84%	44.35
Salt	0.76%	39.91
Water	13.43%	709.59

The production was performed according to the steps described as followed: 10 kg of Chinese cabbage (Brassica rapa subsp. pekinensis) were cleansed, cut in 4 parts and then in 4 cm width strips, resulting in a total weight of 9.3 kg.

The vegetable has been washed in water and soaked in a 10.9 % NaCl brine (9.3 kg Chinese cabbage + 40.6 kg water + 5 kg NaCl) for about 14 hours. The cabbage has been washed by immersion in 100 L water, performing two rinses. This process resulted in the obtaining of 7.5 kg cabbage to be used for fermentation. The sauce was prepared using the ingredients reported in Table1.

The Chinese cabbage was divided in two equal portions (3725 g each), and one of them was mixed with a sauce inoculated with the starters mentioned previously, to reach a final concentration of 7 Log CFU/g of microbial load. The other portion was added with non-inoculated sauce and was used as a control. For each Kimchi prototype, started and non-started, three replicates were produced where each one consisted of one glass jar filled with total weight of 1Kg kimchi product and transported to the laboratory where the fermentation was conducted at 5 ± 1°C for 26 days (Figure 2).

Kimchi prototype prepared with sea fennel sprouts.

Physical-chemical analyses

pH values were measured using pH meter equipped with an H2031 solid electrode (Hanna Instruments, Padova Italy) directly immersed in the Kimchi product matrix in aseptic conditions after inoculation (day 0) and during the fermentation

process up until the end of the monitoring period corresponding to 2, 5, 7, 9, 12, 14, 16, 19, 22 and 26 days. For each sample, pH measurements were carried in triplicate for each replicate and values expressed as mean ± standard deviation.

Titratable acidity (TA) was assessed by aliquoting 10 g of each sample and blending with 90mL of distilled water. The resulting mixture was titrated with 0.1 NaOH and the results expressed as % of lactic acid. TA measurements were performed in duplicate for each replicate and the results reported as mean ± standard deviation.

The quantification of organic acids (lactic acetic acid) was performed as previously described by Maoloni et al. (2021). The determination involved processing deproteinized and decolored Kimchi samples. For the deproteinization, a multi-step process was followed: (i) Carrez I solution was prepared by dissolving 3.6g of potassium hexacyanoferrate (II) {K4[Fe(CN)6] × 3H2O} from Sigma Aldrich, Milan, Italy and (iii) sodium hydroxide solution (NaOH, 100 mM), prepared by dissolving 4 g of NaOH in 1 L of distilled water. Then the samples were decolored using 2% (w v^{-1}) polyvinylpolypyrrolidone.

The concentrations of acetic acid and lactic acid in the Kimchi samples were quantified respectively using the Acetic Acid (Acetate Kinase Rapid Manual Format) Assay kit and the D-/L-Lactic Acid (D-/L-Lactate) (Rapid) Assay kit used both sourced from megazyme, Bray, Ireland.

Microbial enumeration

Microbiological analyses were performed by aliquoting 10g of each replicate and adding 90mL sterile 0.1% (w v-1) peptone water. The suspension was then homogenized for 5min at 230rpm in a stomacher machine (400 Circulator, International PBI, Milan, Italy). Enumeration was performed by preparing tenfold serial dilutions for: (i) mesophilic aerobic bacteria on Plate Count Agar (PCA) (VWR International Srl, Milan, Italy), by incubating at 30°C for 48 h; (ii) presumptive mesophilic lactobacilli on De Man, Rogosa, and Sharpe (MRS) agar (VWR) supplemented with cycloheximide (VWR) (100 mg L^{-1}) to inhibit yeasts, by incubating at 37 ◦C for 48–72 h; (iii) presumptive mesophilic lactococci on M17 agar (VWR) supplemented with cycloheximide (VWR) (100 mg L^{-1}), by incubating at 22°C for 72 h; (iv) yeasts on Rose Bengal chloramphenicol Agar (RB) (VWR), by incubating at 25°C for 5 days; (v) Enterobacteriaceae on Violet Red Bile Agar (VRBGA) (VWR), by incubating at 37°C for 24 h, and (vi) Pseudomonadaceae on Pseudomonas Agar Base (PAB) (VWR) supplemented with Cetrimide-Fucidin-Cephalosporin (CFC) selective supplement incubated at 30°C for 24-48h. The results of viable counting were expressed as the mean Log colony forming units (CFU) g^{-1} of three replicates ± standard deviation.

Aliquots of Kimchi were aseptically collected from each replicate of each sample at the end of their fermentation, using sterilized stainless-steel tweezer; the collected samples were subjected to the enumeration of: (i) coagulase-positive staphylococci in accordance with the TEMPO: AFNOR BIO 12/28–04/ 10 standard method and (ii) sulfite-reducing bacteria according to the ISO 15213: 2003 standard method.

Statistical analysis

To assess statistical differences within Kimchi samples, the Tukey-Kramer's Honest Significant Difference (HSD) test (level of significance 0.05) was used by one-way analysis of variance (ANOVA). Tests were performed through JMP v11.0.0 software (SAS Institute Inc., Cary, NC).

Isolation and identification of lactic acid bacteria in control Kimchi

Isolation was performed on non-started Kimchi to identify and characterize the strains that grew during fermentation period in this prototype. Aliquots (10 g) of each replicate were serially diluted in 90 mL of sterile saline peptone water (0.9% NaCl, 0.1% peptone, pH 7.0), and 100 µL of each dilution was streaked on MRS agar under anaerobiosis to count lactobacilli. For each replicate, colonies were selected based on colony morphology. The representative colonies that were selected, corresponding to about 10% of the colonies counted on MRS plates seeded with the highest sample dilution. For each isolate, the cell morphology was examined using a light microscope under oil-immersion (100×). Bacterial isolates were then stored at −80 °C in a mixture of glycerol and MRS (1:1).

DNA extraction was performed to the total isolates of non-started Kimchi that were previously cryopreserved isolates then first cultured on suitable media (MRS) agar. The colonies were suspended in 300 µL of sterile water; the suspension underwent DNA extraction using the method proposed by Hynes et al. (1992) with some modifications suggested and described by Osimani et al. (2015). In fact, after centrifugation of the isolate's suspensions, the cell pellets were resuspended in 1 mL of STE buffer [10 mM Tris–HCl pH 8.0, 100 mM NaCl, 1 mM EDTA pH 8.0,20% sucrose (w/v)] containing 25 mg mL^{-1} of lysozyme. After incubation at 37°C for 3 h, samples were centrifuged at 14,000g for 3 min, and the pellets suspended in 1 mL of lysis buffer consisting of 50 mM KCl, 10 mM Tris–HCl pH 8.0, 0.45% Tween 20 (w/v) 0.45% Triton X (w/v) supplemented with 100 µg mL^{-1} proteinase K. After incubation at 60°C for 3 h, samples were heated at 95°C for 10 min. The DNA quantity and purity were assessed by optical readings at 260, 280 and 234 nm, respectively, using a UV-Vis Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

As previously described by Cardinali et al. (2024) the lactic acid bacteria isolates underwent molecular identification through sequencing the 16S rRNA gene, using universal eubacterial primers P27f (5'-GAG AGT TTG ATC CTG GCT CAG-3') and P1495r (5'-CTA CGG CTA CCT TGT TAC GA-3'). PCR amplification was performed with 100 ng of template DNA in a 50 µL reaction mixture containing 2 U of Taq DNA polymerase (Euroclone, Pero, Italy), 1× reaction buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, and 0.2 µM of each primer. The amplified products were then purified and sequenced by Genewiz (Takaley, UK). The obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) to identify similarities with 16S rRNA sequences of type strains available in the GenBank database (http://www.ncbi.nlm.nih.gov/). Finally, the sequences corresponding to the lactic acid bacteria isolates were deposited in GenBank to receive accession numbers.

RNA extraction, cDNA synthesis, and metataxonomic analysis

Aliquots of 1.5 mL of each kimchi homogenates (dilution 10^{-1}) were centrifuged at 14,000 rpm for 10 min (Centrifuge 5420, Eppendorf, Hamburg, Germany). The supernatant was carefully removed to obtain cell pellets. For each sample, the pellet was preserved in RNA later® Stabilization Solution (Ambion, Foster City, CA, USA) and stored at −80 °C for subsequent RNA extraction. Total microbial RNA was extracted using the Quick-RNA™ Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. The extracted RNAs were checked for the quantity, purity and the absence of DNA contamination. Then, SensiFAST cDNA Synthesis Kit (Meridian Bioscience Inc., Cincinnati, Ohio, USA) was used for the synthesis of the cDNA from 10 µL of RNA per sample, following the manufacturer's instructions.
The bacterial community was analyzed by amplifying the V3–V4 hypervariable region of the 16S rRNA gene using primers and procedure described by Klindworth et al. (2013). The fungal population was studied by the amplification of the D1 domain of the 26S rRNA gene according to Mota-Gutierrez et al. (2018). PCR amplicons were purified following the Illumina metagenomic pipeline (Illumina Inc., San Diego, CA, United States). Pair-end sequencing (2X250bp) was performed with a MiSeq platform (Illumina Inc., San Diego, CA, United States) using V2 chemistry according to the manufacturer's instructions.
Raw reads were analyzed by using QIIME2 software; in detail, primers and adapters were first trimmed by using Cutadapter, and then quality filtered using the DADA2 algorithm. Amplicon sequence variants (ASVs) generated through DADA2 were used for taxonomic assignment against the Greengenes database for bacteria, and a manually built database for fungi. When the taxonomy assignment was not able to reach species level, the family or genus name was displayed.
The sequence data were processed and analyzed using Microbiome Analyst (Chong et al., 2020) to assess microbial diversity indices, specifically the Shannon index for alpha diversity and the Bray-Curtis dissimilarity index for beta diversity, following rarefaction to the minimum read depth across all samples. Alpha diversity differences among samples were evaluated using the Kruskal-Wallis test applied to the Shannon index. Beta diversity was visualized through Principal Coordinate Analysis (PCoA) based on the Bray-Curtis dissimilarity index, with significant differences between groups determined using Permutational Multivariate Analysis of Variance (PERMANOVA) ($p < 0.05$).
ASV tables were further analyzed to identify taxa with statistically significant differences among samples. This was achieved using one-way ANOVA and Kruskal-Wallis test ($p < 0.05$). The results were visualized using box plots, specifically

highlighting the taxa with statistically significant differences. All statistical analyses and visualizations were performed within the R environment (version 4.4.3).

Determination of volatile compounds via Headspace/Solid Phase MicroExtraction–Gas Chromatography/Mass Spectrometry (HS/SPME-GC/MS)

Ten grams of each homogenized sample were treated with a solution of calcium chloride dihydrate (87 g in 100 mL deionized water) and homogenized for 1 minute using an Ultra Turrax. The resulting mixture was then centrifuged at 2,000 rpm for 15 min at 5°C, and the supernatant was filtered through a Whatman No. 41 filter. The isolation of leaf volatiles was performed using the Headspace Solid Phase Microextraction (HS-SPME) technique on the obtained filtrate. Five mL of the filtrate were placed in a 15 mL vial with a magnetic stir bar for SPME, which was then sealed with a PTFE/silicone septum. The vial was immersed in a water bath maintained at 40 °C. The HS-SPME extraction was conducted by exposing a 2-cm 50/30 µm DVB/CAR/PDMS fiber to the headspace of the filtrate for 60 min, at 40 °C under stirring (400 rpm). After the extraction, the fiber was immediately inserted into the GC split-splitless injection port for desorption, and the GC analysis was initiated. The same fiber was used for all analyses.

GC/MS analyses were performed using an Agilent 6890 GC 5973N MS system equipped with a quadrupole mass filter for mass spectrometric detection (Agilent Technologies, Palo Alto, CA). Desorption of the extracted volatiles from the fiber was carried out for 3 minutes in the GC injector, operating at 240 °C in splitless mode. Following desorption, the fiber was kept in the injector for an additional 5 minutes under purge mode (75 mL min^{-1} purge flow) to remove any residual substances adsorbed on the fiber and minimize carry-over effects. A 0.75 mm liner suitable for SPME analyses was installed in the injector. GC separation was achieved using a DB-Wax column (0.25 mm i.d. × 60 m, 0.5 µm film thickness) from Agilent Technologies. The GC operating conditions were as follows: inlet temperature set to 240 °C; oven temperature programmed from 40 °C (held for 10 min) to 235 °C (held for 7 min) at a rate of 4 °C min^{-1}, with a total run time of 65.7 min. The carrier gas flow rate was set at 2.0 mL min^{-1}, corresponding to a linear velocity of 36.3 cm/s. The transfer line temperature was 240 °C. The MS detector operated in electronic ionization mode at 70 eV, with source and quadrupole temperatures set at 230 and 150 °C, respectively. Detection was performed in full scan mode, covering the mass range 33-300 amu. Compound identification was based on the comparison of mass spectra and linear retention indices (LRI) from chromatograms of kimchi samples with those of authentic standards when available. In the absence of authentic standards, tentative identification was achieved by comparison with data from the NIST/EPA/NIH Mass Spectra Library or relevant literature. LRI were determined for each column using a series of linear alkanes (C7-C30) injected under the same chromatographic conditions. For semi-quantitative determination of volatiles, duplicate extractions of the same filtrate were performed, and GC separation was repeated. The levels of VOCs were estimated based on the area of the chromatographic peaks (Fu et al., 2013).

Sensory analysis

The sensory analysis was performed using the method proposed by Maoloni et al., 2022 with some modifications.

This was carried out at the end of fermentation by a panel consisting of 8 non-smokers, made up of 5 females and 3 males aged between 25 and 45. Panelists were preliminarily trained to describe the attributes of Kimchi and sea fennel. A discussion was planned by the panel to find out the most appropriate sensory attributes of Kimchi together with sea fennel. Regarding the evaluation of the prototypes started and non-started Kimchi consisting of two samples, these were presented to the panelists at room temperature and coded with random numbers. The sensory test was divided into sessions, in which each panelist evaluated 2 samples one at each time, working in individual booths and equipped with still bottled water and crackers biscuits to cleanse the olfactory palate between and after the evaluations.

The two-prototype started, and non-started Kimchi were evaluated by the trained panelists for (i) six olfactory descriptors being fermented, garlic, pungent, chilly, vegetable and sea fennel, (ii) six aromas' descriptors being fermented, garlic, spicy, chilly, vegetable and sea fennel, (iii) four flavor descriptors being acidity, bitterness, salty, and sweet, (iv) three textural descriptors being hardness, fibrousness, and crunchiness, and (v) global acceptance. Aliquots (10g) of each

sample per panelist were placed in white plastic cups, blindly labelled with numbers. For each descriptor, the panelists were asked to assign a score ranging from 1 to 9 where 1 expresses the lowest and 9 the highest intensity. They were also invited to express their degree of liking with a 9-point hedonic scale, where 1 presents the lowest and 9 the highest degree of liking (Peryam and Pilgrim, 1957).

Results

Physical-chemical analysis

The results of pH and total titratable acidity of started and non-started Kimchi are shown in the figures below. The two analyzed Kimchi had similar trends, although both samples had a faster decrease in pH and oppositely an increase of TTA. In fact, at day 0, the pH values were 4.87± 0.08 and 4.92 ± 0.08 for non-started and started Kimchi, respectively. A drop in pH is observed at day 12 for started kimchi whereas a slight increase is reported for non-started Kimchi with values of 5.16 ± 0.17 and 4.4 ± 0.13, respectively. The drop of pH during the fermentation period is mostly reported between day 12 and day 19 when started Kimchi had a fast decrease whereas non-started Kimchi had a progressive decrease. Instead on day 26 the results are similar, the values reported are 3.97 ± 0.01 and 3.86 ± 0.04. The decrease of pH is accompanied by an increase of TTA similarly for started and non-started samples from day 0 to day 26. At the end of fermentation on day 26, TTA reached 0.30 ± 0.03 for non-started Kimchi while for started Kimchi the value reported is 0.50 ± 0.04.

Results of pH measurements of started and naturally fermented (control) kimchi during fermentation.

Sampling time (t_{day})	**Prototypes**	
	started kimchi	control kimchi
t_0	$4.90 \pm 0.08^{b,A}$	$4.87 \pm 0.08^{c,A}$
t_2	$5.20 \pm 0.07^{a,A}$	$5.30 \pm 0.17^{ab,A}$
t_5	$5.30 \pm 0.07^{a,B}$	$5.51 \pm 0.13^{a,A}$
t_7	$4.90 \pm 0.10^{b,B}$	$5.46 \pm 0.13^{ab,A}$
t_9	$4.70 \pm 0.15^{bc,B}$	$5.49 \pm 0.07^{ab,A}$
t_{12}	$4.40 \pm 0.13^{cd,B}$	$5.16 \pm 0.17^{bc,A}$
t_{14}	$4.20 \pm 0.03^{d,B}$	$4.97 \pm 0.08^{c,A}$
t_{16}	$4.10 \pm 0.19^{def,B}$	$4.57 \pm 0.08^{d,A}$
t_{19}	$4.00 \pm 0.05^{ef,B}$	$4.19 \pm 0.08^{e,A}$
t_{22}	$3.90 \pm 0.02^{f,B}$	$3.99 \pm 0.01^{e,A}$
t_{26}	$3.90 \pm 0.02^{f,B}$	$3.97 \pm 0.01^{e,A}$

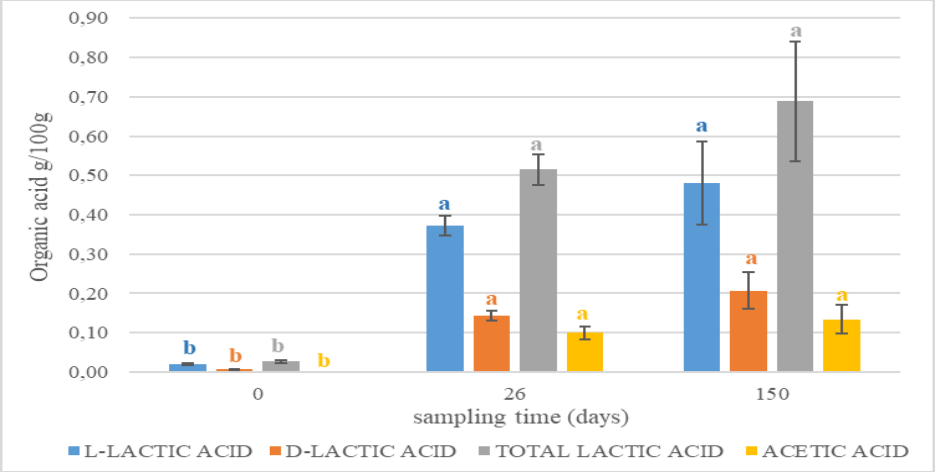
Values are expressed as means ± standard deviations. For each sample, overall means with different superscript letters within the same column are significantly different ($p < 0.05$). For each sampling time, overall means with different small letters within the same row are significantly different ($p < 0.05$).

Results of titratable acidity (TA) measurements of started and naturally fermented (control) kimchi during fermentation.

Sampling time (t_{day})	**Prototypes**	
	started kimchi	control kimchi

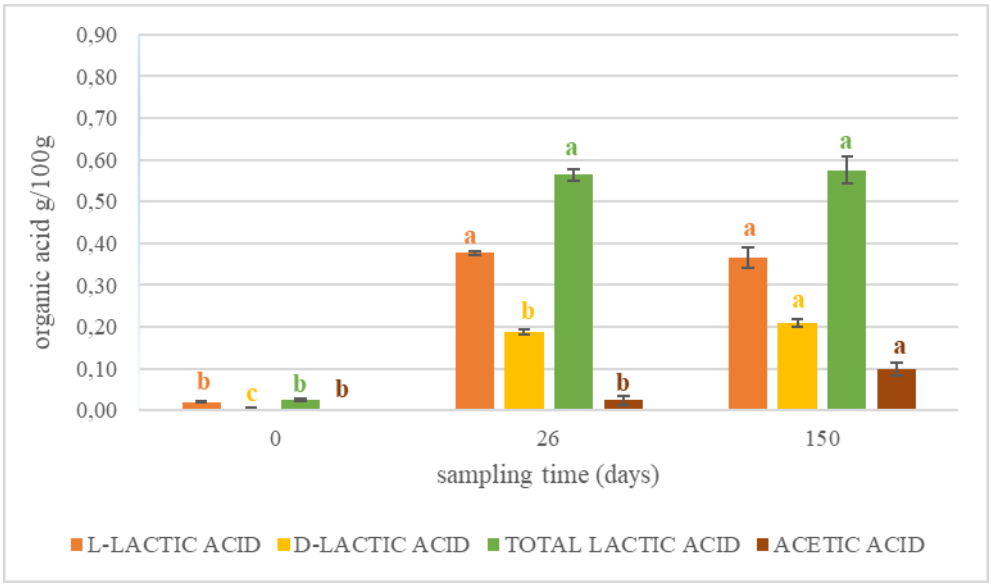
t_0	$0.09 \pm 0.01^{b,A}$	$0.09 \pm 0.01^{b,A}$
t_{26}	$0.53 \pm 0.05^{a,A}$	$0.34 \pm 0.03^{a,B}$

Results are expressed as % of lactic acid equivalent. For each sample, overall means with different superscript letters within the same column are significantly different ($p < 0.05$). For each sampling time, overall means with different small letters within the same row are significantly different ($p < 0.05$).



Results of the organic acids quantification of the laboratory scale prototype of started Kimchi made with sea fennel at the beginning, the end of fermentation and after 105 days from the start of fermentation (FK).

The results are expressed as mean of three biological replicates ± standard deviation.



Results of the organic acids quantification of the laboratory scale prototype of non-started Kimchi made with sea fennel at the beginning, the end of fermentation and after 105 days from the start of fermentation (CK).

The results are expressed as mean of three biological replicates ± standard deviation.

The results of the organic acids quantification are presented in figures 5 and 6. In details, the total lactic acid content in both started (FK) and non-started (CK) Kimchi increased during fermentation process. For CK samples, values ranged from 0.02 ± 0.00 to 0.56 ± 0.01 g/100g, while for FK, they ranged from 0.03 ± 0.00 to 0.52 ± 0.04 g/100 g. However, after 150 days, the total lactic acid concentration only slightly increased in started Kimchi, reaching 0.69 ± 0.15 g/100g. In almost all samples, the isomer L-lactic acid was more abundant than D-lactic acid.

In contrast, regarding acetic acid, by the end of fermentation, the concentration in started Kimchi was higher than in non-started Kimchi, with values of 0.10 ± 0.02 and 0.02 ± 0.01 g/100g, respectively. However, after 150 days, the acetic acid concentration in non-started Kimchi significantly increased reaching 0.10 ± 0.02 g/100g, whereas it only slightly increased in started Kimchi to 0.13 ± 0.04 g/100g.

In conclusion, both prototypes exhibited a higher yield of lactic acid compared to acetic acid, both at the end of fermentation, and 150 days after the start of fermentation.

Microbiological analyses

The results of viable counts performed on the two prototypes of Kimchi made with sea fennel are presented in the table below.

Microbial group	Sampling time (days)	Started kimchi	Control kimchi
Mesophilic lactobacilli	t_0	$7.3 \pm 0.1^{b,A}$	$1.9 \pm 0.1^{d,B}$
	t_2	$7.5 \pm 0.1^{b,A}$	$3.5 \pm 0.3^{c,B}$
	t_5	$7.6 \pm 0.2^{b,A}$	$5.1 \pm 0.5^{b,B}$
	t_{12}	$8.3 \pm 0.0^{a,A}$	$8.2 \pm 0.1^{a,A}$
	t_{19}	$8.4 \pm 0.1^{a,A}$	$8.5 \pm 0.1^{a,A}$
	t_{26}	$8.4 \pm 0.1^{a,A}$	$8.5 \pm 0.0^{a,A}$
Mesophilic lactococci	t_0	$7.3 \pm 0.1^{b,A}$	$4.9 \pm 0.1^{b,B}$
	t_2	$7.4 \pm 0.0^{b,A}$	$5.9 \pm 0.3^{a,B}$
	t_5	$7.6 \pm 0.2^{b,A}$	$5.8 \pm 0.3^{a,B}$
	t_{12}	$8.3 \pm 0.0^{a,A}$	$6.6 \pm 0.3^{a,B}$
	t_{19}	$8.4 \pm 0.1^{a,A}$	$6.0 \pm 0.5^{a,B}$
	t_{26}	$8.3 \pm 0.1^{a,A}$	$6.1 \pm 0.4^{a,B}$
Yeasts	t_0	$< 1.0^{a,A}$	$< 1.0^{a,A}$
	t_2	$< 1.0^{a,A}$	$< 1.0^{a,A}$

	t_5	< 1.0^{a,A}	< 1.0^{a,A}
	t_{12}	< 1.0^{a,A}	< 1.0^{a,A}
	t_{19}	< 1.0^{a,A}	< 1.0^{a,A}
	t_{26}	< 1.0^{a,A}	< 1.0^{a,A}
Enterobacteriaceae	t_0	4.4 ± 0.5^{a,A}	4.3 ± 0.4^{a,A}
	t_2	5.6 ± 0.2^{a,A}	5.7 ± 1.0^{a,A}
	t_5	5.5 ± 0.8^{a,A}	5.1 ± 0.2^{a,A}
	t_{12}	4.4 ± 0.8^{a,B}	5.7 ± 0.2^{a,A}
	t_{19}	1.9 ± 0.7^{b,A}	3.1 ± 0.7^{b,A}
	t_{26}	< 1.0^{c,A}	< 1.0^{c,A}
Mesophilic aerobic bacteria	t_0	7.2 ± 0.0^{c,A}	5.4 ± 0.1^{b,B}
	t_2	7.5 ± 0.1^{bc,A}	6.2 ± 0.7^{b,B}
	t_5	7.6 ± 0.3^{b,A}	5.8 ± 0.3^{b,B}
	t_{12}	8.3 ± 0.0^{a,A}	8.3 ± 0.1^{a,A}
	t_{19}	8.4 ± 0.1^{a,A}	8.6 ± 0.1^{a,A}
	t_{26}	8.4 ± 0.0^{a,A}	8.4 ± 0.2^{a,A}
Pseudomonadaceae	t_0	5.2 ± 0.3^{a,A}	5.2 ± 0.1^{a,A}
	t_2	5.0 ± 0.3^{a,A}	5.7 ± 0.6^{a,A}
	t_5	4.9 ± 0.3^{a,A}	5.1 ± 0.6^{a,A}
	t_{12}	3.4 ± 0.3^{b,B}	5.2 ± 0.2^{a,A}
	t_{19}	2.5 ± 0.6^{bc,A}	2.8 ± 0.3^{b,A}
	t_{26}	2.1 ± 0.4^{c,A}	2.7 ± 0.6^{b,A}

Values are expressed as Log CFU g^{-1} ± standard deviation of three biological replicates. Within each row, for each microbial group at the same sampling time, overall means with different capital superscript letters are significantly different ($p < 0.05$). For each microbial group and sample, overall means with different small superscript letters in the same column are significantly different ($p < 0.05$).

The counts of mesophilic aerobic bacteria showed a slight difference during the first week 5.4 ± 0.1 and 7.2 ± 0.0 Log CFU g^{-1} for non-started and started Kimchi respectively, and the counting was stable until the end of the fermentation

showing a same trend in both prototypes, with no significant differences at the end of fermentation where the microbial load reported was 8.4 Log CFU g^{-1} in both of them. .
Regarding mesophilic lactococci, a progressive increase in the load was reported during fermentation, where the highest counts were observed between day 5 and day 26 for both prototypes. Moreover, mesophilic lactococci counting was higher in started Kimchi than the non-started prototype at the end of fermentation with 8.3 ± 0.1 and 6.1 ± 0.4 Log CFU g^{-1}.
Regarding yeasts, counts reported were < 1.0 Log CFU g^{-1} in both prototypes.
Enterobacteriaceae counts decreased during fermentation period for both prototypes to reach <1.0 Log CFU g^{-1} at the end of fermentation.
As for Mesophilic aerobic bacteria, a similar trend is observed for both Kimchi prototypes described by an increase from day 5 to end of fermentation while the results showed that at the beginning of fermentation the counts in started Kimchi were higher than the non-started Kimchi.
Regarding Pseudomonadaceae, there were no significant differences between the two prototypes during the fermentation and the counts progressively decreased to reach 2.7 ± 0.6 and 2.1 ± 0.4 at the end of fermentation for started and non-started Kimchi respectively.
Finally, Coagulase-positive staphylococci and sulfite-reducing bacteria were under the detection limit (<1 Log CFU g^{-1}) in both prototypes.
coagulase-positive staphylococci and sulfite-reducing bacteria were not detected in both prototypes at the end of fermentation.

Identification of lactic acid bacteria

68 isolates were identified during the different sampling time. Among them, 54 were identified as lactic acid bacteria. The closest relatives, percentage identities, and accession numbers of the sequences obtained from the 54 lactic acid bacteria isolated from control Kimchi are reported in the table below.
In detail, the results showed that *Weissella koreensis* was the most abundantly detected species (22), followed by *Leuconostoc mesenteroides (17), Latilactobacillus graminis, (5), Leuconostoc citreum (4), Enterococcus hirae (3), Pediococcus pentosaceus (1),* and *Leuconostoc holzapfelii (1).*
The isolates were subjected to various characterization tests to identify the most effective strains for potential application in vegetable-based fermentations.

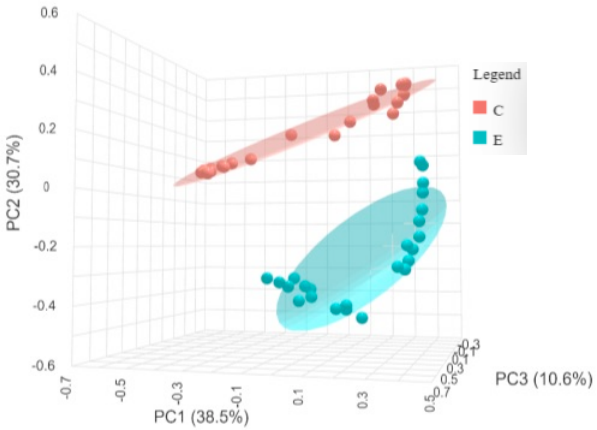
Identification of non-starter lactic acid bacteria (NSLAB) isolated from non-started Kimchi

Isolation source	Isolate code	Closest relative	% identity*	Accession number**
Kimchi	CK13	*Enterococcus hirae*	99.42%	NR_114783.2
Kimchi	CK14	*Enterococcus hirae*	99.89%	NR_114743.1
Kimchi	CK15	*Enterococcus hirae*	98.05%	NR_114783.2
Kimchi	CK18	*Pediococcus pentosaceus*	98.91%	NR_042058.1
Kimchi	CK28	*Weissella koreensis*	98.90%	NR_029041.1
Kimchi	CK29	*Weissella koreensis*	97.98%	NR_029041.1
Kimchi	CK30	*Weissella koreensis*	99.34%	NR_029041.1
Kimchi	CK32	*Weissella koreensis*	99,05%	NR_029041.1
Kimchi	CK31	*Weissella koreensis*	99.65%	NR_029041.1
Kimchi	CK33	*Weissella koreensis*	99.33%	NR_029041.1
Kimchi	CK35	*Weissella koreensis*	98.10%	NR_029041.1
Kimchi	CK36	*Weissella koreensis*	99.47%	NR_029041.1
Kimchi	CK46	*Leuconostoc mesenteroides*	98,57%	NR_074957
Kimchi	CK47	*Leuconostoc citreum*	98.28%	NR_041727.1
Kimchi	CK48	*Leuconostoc mesenteroides*	99.6%	NR_074957
Kimchi	CK49	*Weissella koreensis*	99.28%	NR_029041.1
Kimchi	CK51	*Weissella koreensis*	97.89%	NR_029041.1
Kimchi	CK52	*Leuconostoc citreum*	98.88%	NR_041727.1
Kimchi	CK53	Leuconostoc holzapfelii	98.63%	NR_042620

Kimchi	CK54	*Weissella koreensis*	99.61%	NR_029041.1
Kimchi	CK64	*Leuconostoc mesenteroides*	99.91%	NR_074957
Kimchi	CK65	*Leuconostoc mesenteroides*	99.42%	NR_074957
Kimchi	CK66	*Leuconostoc mesenteroides*	99.91%	NR_074957
Kimchi	CK67	*Leuconostoc mesenteroides*	97.45%	NR_074957
Kimchi	CK69	*Leuconostoc mesenteroides*	99.92%	NR_074957
Kimchi	CK70	*Leuconostoc mesenteroides*	97.36%	NR_074957
Kimchi	CK71	*Leuconostoc mesenteroides*	99,43%	NR_074957
Kimchi	CK72	*Leuconostoc mesenteroides*	98.74%	NR_157602
Kimchi	CK82	*Weissella koreensis*	99.63%	NR_029041.1
Kimchi	CK84	*Leuconostoc mesenteroides*	99.83%	NR_074957
Kimchi	CK85	*Weissella koreensis*	99,65%	NR_029041.1
Kimchi	CK86	*Leuconostoc mesenteroides*	97,76%	NR_113912.1
Kimchi	CK88	*Weissella koreensis*	99.34%	NR_029041.1
Kimchi	CK89	*Weissella koreensis*	99.08%	NR_029041.1
Kimchi	CK90	*Leuconostoc mesenteroides*	98.28%	NR_074957
Kimchi	CK93	*Leuconostoc citreum*	99,74%	NR_041727.1
Kimchi	CK94	*Leuconostoc mesenteroides*	97.65%	NR_074957
Kimchi	CK96	*Leuconostoc mesenteroides*	100.00%	NR_118557
Kimchi	CK100	*Leuconostoc mesenteroides*	99.82%	NR_074957
Kimchi	CK102	*Leuconostoc mesenteroides*	99.32%	NR_074957
Kimchi	CK103	*Leuconostoc mesenteroides*	99,91%	NR_113912.1
Kimchi	CK109	*Weissella koreensis*	97.66%	NR_029041.1
Kimchi	CK110	*Weissella koreensis*	98.68%	NR_029041.1
Kimchi	CK113	*Weissella koreensis*	99.55%	NR_029041.1
Kimchi	CK114	*Leuconostoc citreum*	99.66%	NR_041727.1
Kimchi	CK200	*Latilactobacillus graminis*	99.50%	NR_042438.1
Kimchi	CK201	*Latilactobacillus graminis*	99.92%	NR_042438.1
Kimchi	CK202	*Weissella koreensis*	99.65%	NR_029041.1
Kimchi	CK203	*Weissella koreensis*	98.47%	NR_029041.1
Kimchi	CK205	*Latilactobacillus graminis*	100.00%	NR_042438.1
Kimchi	CK204	*Latilactobacillus graminis*	98.12%	NR_042438.1
Kimchi	CK206	*Weissella koreensis*	99.73%	NR_029041.1
Kimchi	CK207	*Latilactobacillus graminis*	99.7%	NR_042438.1
Kimchi	CK208	*Weissella koreensis*	99.05%	NR_029041.1

Microbiota composition

A total of 136,483 bacterial reads were analyzed, with an average of approximately 2,904 reads per sample. For the fungal biota, a total of 2,332,367 reads were analyzed, with an average of 72,886 reads per sample. No statistically significant differences were observed in the diversity indices ($p > 0.05$), except for the Bray-Curtis dissimilarity index of bacterial ASVs, which differed significantly between the control (C) and experimental (E) kimchi groups ($p < 0.05$) as sh.

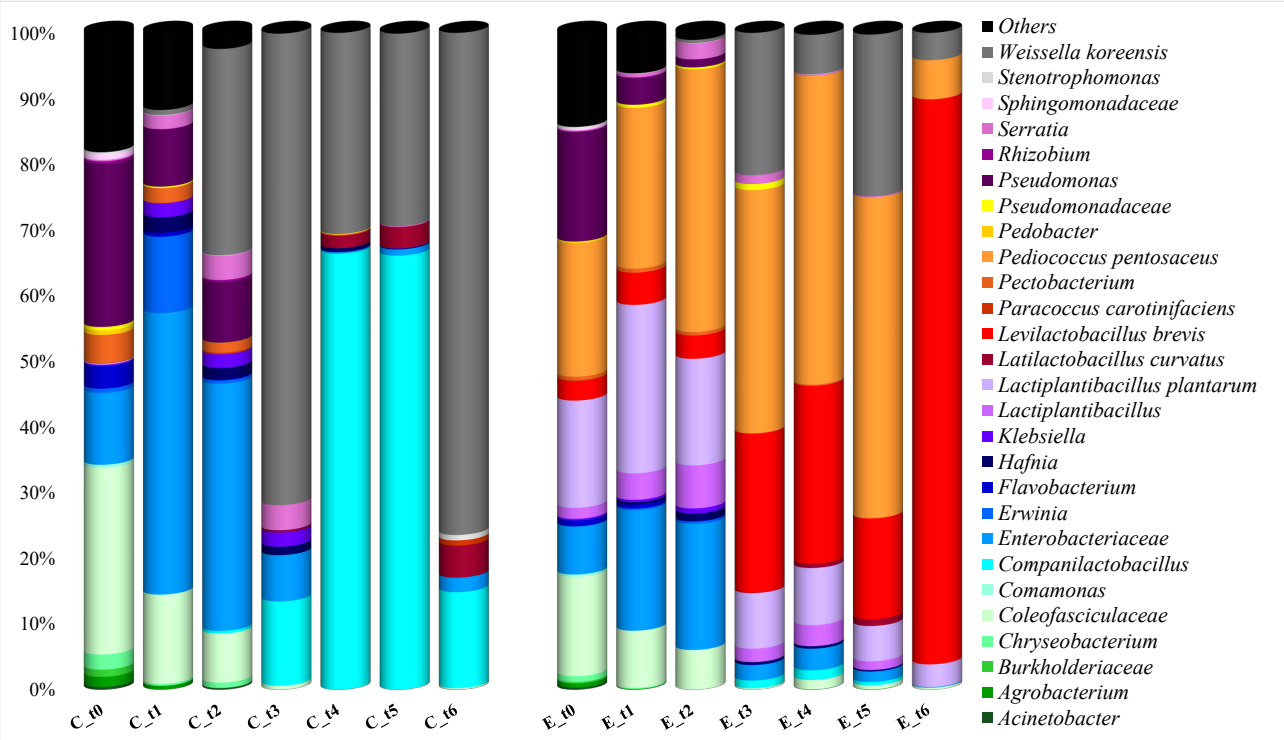


PCoA based on Bray-Curtis index of bacterial ASVs.

The bacterial biota composition of control and experimental kimchi is shown in Figure 2. Figure 3 illustrates box plots of taxa exhibiting statistically significant differences among samples ($p < 0.05$).

During the early fermentation stages (t0–t2), both control (C) and experimental (E) kimchi samples showed the occurrence of Enterobacteriaceae, *Pseudomonas* spp., Coleofasciculaceae, *Erwinia* spp., *Serratia* spp., and *Klebsiella* spp.. However, these taxa declined sharply after t2 and were consistently more abundant in the control group (overall mean of 30.37% *vs* 14.89% for *Enterobacteriaceae*; 14.07% *vs* 7.25% for *Pseudomonas* spp.; 16.25% *vs* 9.91% for Coleofasciculaceae; 4.24% *vs* 0.23% for Erwinia spp.; 1.99% *vs* 1.06% for *Serratia* spp.; 1.47% vs 0.45% for *Klebsiella* spp. in C and E, respectively). Several minor taxa, including *Acinetobacter* spp., *Agrobacterium* spp., *Chryseobacterium* spp., *Comamonas* spp., *Flavobacterium* spp., *Pectobacterium* spp., *Pedobacter* spp., and Sphingomonadaceae, were also more abundant in the control group during early fermentation, while being significantly lower or nearly absent in the experimental group.

As fermentation progressed, lactic acid bacteria (LAB) became dominant in both control and experimental kimchi. However, their succession and composition differed markedly. The experimental kimchi (E) exhibited an earlier and more stable LAB colonization. In detail, *Pediococcus pentosaceus*, *Lactiplantibacillus plantarum*, and *Levilactobacillus brevis* were predominant, with mean relative abundances of 31.90%, 11.99%, and 23.44%, respectively, from t_0 through t_6. Notably, *L. brevis* became the dominant species in the final stage of fermentation, reaching 86.03% of the relative abundance.

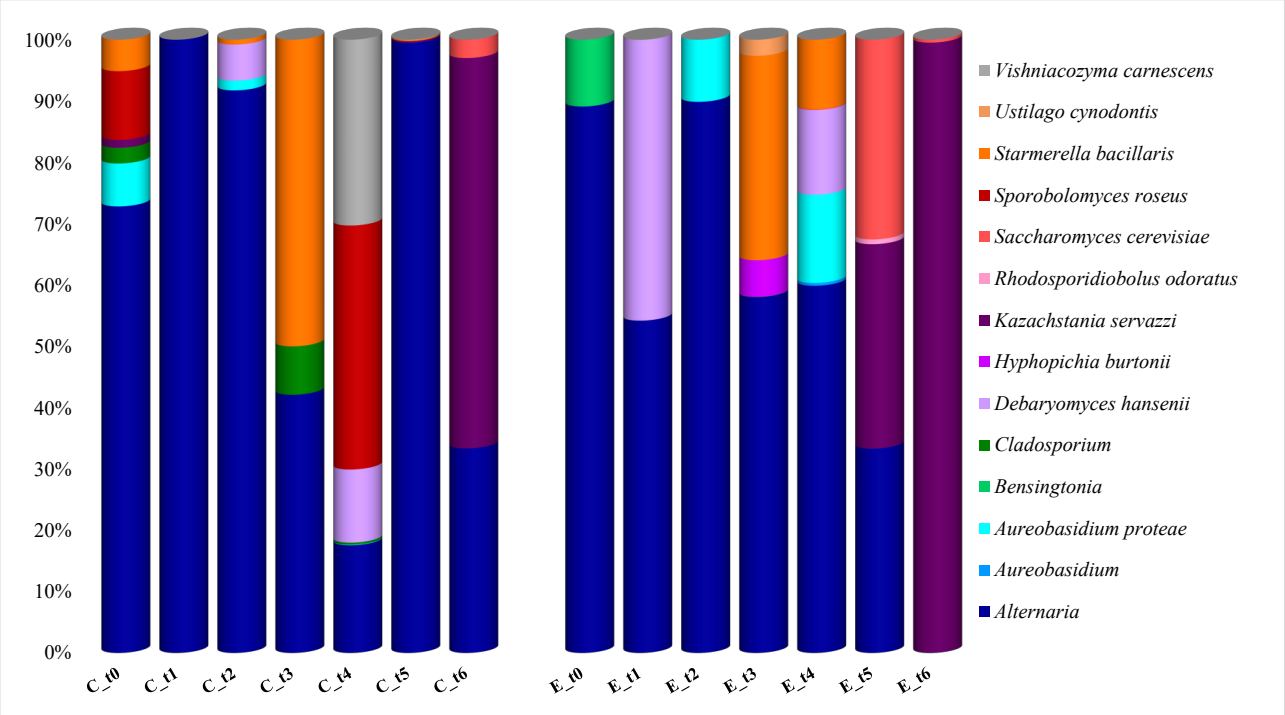


Bar plots illustrating the relative abundance of bacterial taxa at the finest taxonomic resolution across kimchi samples. Samples labeled "C" represent control kimchi, while "E" denotes experimental kimchi. Sampling times are as follows: t_0 – immediately after preparation; t_1 – 2 days; t_2 – 5 days; t_3 – 12 days; t_4 – 19 days; t_5 – 26 days; t_6 – 150 days

In contrast, the control group showed a delayed LAB succession. LAB dominance emerged at t_2, with *Weissella koreensis* reaching 31.43% of the relative abundance. This species persisted in both C and E groups through t_6 but remained significantly more abundant in the control samples (47.90% in C *vs* 11.39% in E). *Companilactobacillus* spp. emerged at t3 in the control group (12.83%), peaked at t4–t5 (66%), and settled at 14.60% by t_6. *Latilactobacillus curvatus* also

increased slightly from t_3 to t_6, reaching 4.84% by the end of fermentation. These taxa appeared only sporadically in the experimental samples during t_3–t_5, each accounting for less than 2% of the relative abundance.

Alternaria spp. was the most prevalent taxon across all samples, with overall mean relative abundances of 65.30% and 54.91% in control and experimental kimchi, respectively.
While the overall fungal profiles exhibited considerable variability depending on both fermentation type (C *vs* E) and sampling time, statistical analysis revealed significant differences ($p < 0.05$) for only two taxa. In detail, *Bensingtonia* spp. was detected primarily during the early stages of fermentation, with the highest relative abundance observed in experimental samples at t_0 (E_t_0). In contrast, *Kazachstania servazzii* became prominent in the later stages, particularly in the inoculated (E) kimchi. Several taxa, including *Aureobasidium proteae*, *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, and *Starmerella bacillaris*, appeared sporadically in both control and experimental samples without a consistent pattern. The control group also exhibited variable occurrences of *Cladosporium* spp., *Sporobolomyces roseus*, and *Vishniacozyma carnescens*, whereas the experimental group showed only rare detection of *Aureobasidium* spp., *Hyphopichia burtonii*, *Rhodosporidiobolus odoratus*, and *Ustilago cynodontis*.



Bar plots illustrating the relative abundance of fungal taxa at the finest taxonomic resolution across kimchi samples. Samples labeled "C" represent control kimchi, while "E" denotes experimental kimchi. Sampling times are as follows: t_0 – immediately after preparation; t_1 – 2 days; t_2 – 5 days; t_3 – 12 days; t_4 – 19 days; t_5 – 26 days; t_6 – 150 days.

Volatile components analysis

Results of semi-quantitative analysis of Volatile Organic compounds (VOCs) detected in the static headspace of the two prototypes of Kimchi made with sea fennel are reported in Table 6. The analysis allowed the identification of 38 compounds including (4) Aldehydes: Acetaldehyde, Pentanal, Geranial, Neral; (3) Esters: Ethyl Acetate, 1-Butanol-3-methyl acetate, 1-Methoxy-2-propyl acetate; (6) Alcohols: Ethanol, 1-Penten-3-ol, 3-Methyl-1-butanol, (Z)-3-Hexen-1-ol, 1-Pentanol, Phenylethyl alcohol; (3) Carboxylic Acids: Acetic acid, Hexanoic acid, Octanoic acid; (15) Terpenes: Camphene, α-Pinene,

β-Myrcene, Sabinene, γ-Terpinene, D-Limonene, β-Phellandrene, p-Cymene, α-Terpineol, Terpinene-4-ol, Borneol, 1,8-Cineol, Camphor, Thymol methyl ether, Dill apiole; (2) Sulfur compounds: Dimethyl trisulfide, Dimethyl disulfide; (2) Nitriles: 5-Cyano-1-pentene, Benzene propanenitrile; (1) Phenols: Carvacrol; (1) Heterocyclic compounds: unknown Thiazole; (1) Isothocyonates: Phenethyl Isothiocyanate.

Results showed a generally similar trend between control and started kimchi made with sea fennel, with only minor variation in specific compounds. Acetaldehyde, detected at the beginning of fermentation (t_0), was absent at the end of fermentation and after 150 days. Similarly, sabinene showed high concentration at the early stage of fermentation but decreased significantly, reaching negligible level in both prototypes.

During fermentation, the level of dimethyl disulfide, acetic acid, dill apiole, α-terpineol, borneol, benzene propanenitrile, p-cymene, 4-ethyl-5-methylthiazole, terpinene-4-ol and phenethyl isothiocyanate increased significantly from t_0 to t_{26}. Among these, p-cymene and terpinene-4-ol were the major compounds. Terpinene-4-ol reached the highest chromatographic peak area of 239.30 ± 41.02 and 249.58 ± 28.29 ×10^5 at t_{26}, for control and started Kimchi, respectively. However, dillapiole was more abundant in control Kimchi, whereas acetic acid was higher in the started Kimchi.

Additionally, compounds such as ethanol, 1-pentanol, carboxylic acids (hexanoic acid, octanoic acid), 3-methyl-1-butanol, ethyl acetate and terpinene-4-ol increased significantly after 150 days since the start of fermentation. Ethanol, 3-methyl-1-butanol, and terpinene-4-ol concentrations reached chromatographic peak areas of 175.14, 275.84, 451.32 ×10^5, respectively, for control Kimchi, and 124.80, 200.60, 333.30 ×10^5 for started kimchi, respectively.

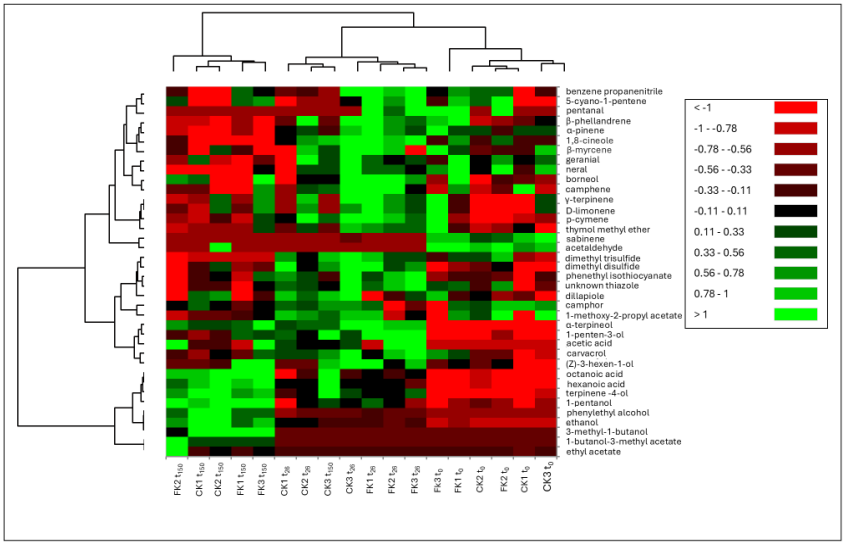
Terpenes such as γ-terpinene and β-myrcene, camphor, thymol methyl ether and D-limonene, along with other compounds, like 1-methoxy-2-propyl acetate, remained stable throughout fermentation process. Phenolic compounds like Carvacrol showed minimal variation over monitoring time in both treatments.

The heatmap illustrates the concentration of VOCs across replicates and fermentation time in both prototypes. Very low concentrations correspond to red, while very high concentrations correspond to light green colour. The analysis revealed a separation of samples based on sampling time. In fact, samples from t_0 were clustered together, as did those from t_{26} and t_{150} except CK5 (t_{150}). At the beginning of fermentation (t_0), sabinene and acetaldehyde, were more abundant regardless of the use of starter. At t_{26}, Kimchi prototypes produced significantly higher concentration of terpenes such as β-phellandrene, α-pinene, D-Limonene and p-Cymene along with acetic acid in most of the replicates, indicating its accumulation over time. At t_{150}, the VOC profile in started and control Kimchi was enriched with terpinene-4-ol and alcohols such as (Z)-3-hexen-1-ol, 1-pentanol, 3-methyl-1-butanol and ethanol, as well as carboxylic acids like octanoic and hexanoic acid in samples FK1, FK2, FK3, CK1 and CK2 t_{150}. Other noteworthy compounds were also observed at this stage of the monitoring period, such as 3-methyl-1-butanol and ethyl acetate.

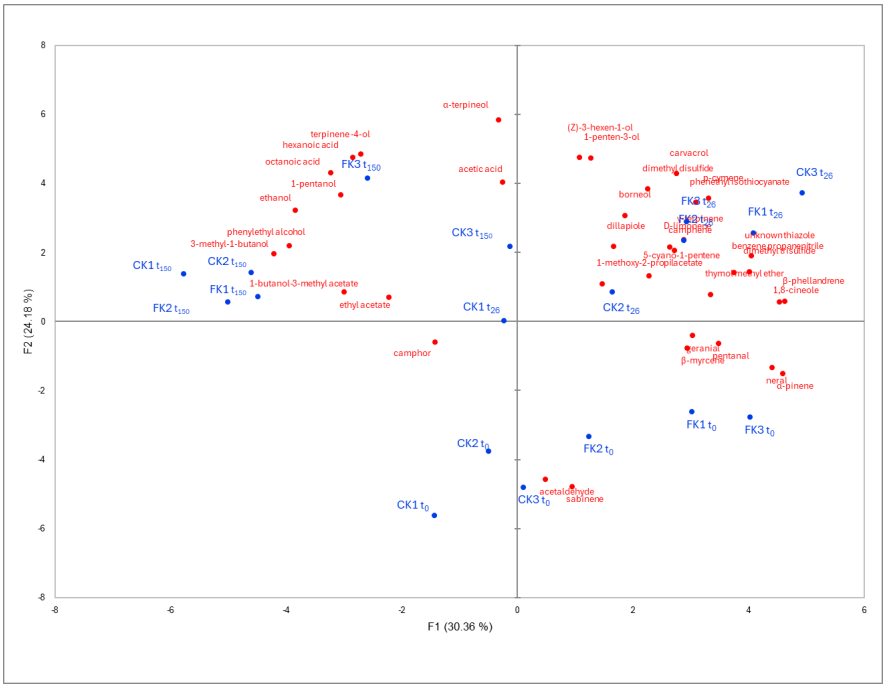
To confirm the correlation between the volatile compounds, fermentation time and treatment, a PCA analysis was conducted as shown in the figure below. The figure displays the biplot of the score plot and the loading plot from the PCA performed on the quantitative data of VOCs. PC1 and PC2 explained 30 and 24% of the total variance accounting for a cumulative 54.54% of the variability in the dataset. PC1 neatly separated samples analyzed at t_{150}, CK1(t_{26}), CK2 and CK1 (t_0), all characterized by negative scores, from all the other samples collected at t_{26} and t_0, characterized by positive scores. PC2, distinguished samples from early fermentation (t_0) exhibiting negative scores from those sampled at t_{26} and t_{150}. Samples clustered according to their fermentation stage, rather than the application of starter culture, forming distinct clusters in different quadrants and indicating a significant difference between them. Regarding metabolite distribution, Acetaldehyde and monoterpenes were prominent at t_0, while compounds such as phenols, alcohols, esters and carboxylic acids were strongly associated with samples from later stages of fermentation (t_{26} and t_{150}).

In more detail, at the beginning of fermentation (t_0), the samples CK1, CK2, CK3, FK1, FK2, and FK3 were associated with aldehydes and monoterpenes. By the end of fermentation, the replicates CK2, CK3, FK1, FK2 and FK3 t_{26} were linked to terpenes, alcohol, sulfur-containing compounds and phenols. Conversely, the replicate CK2t_{26} showed a distinct VOC

profile, compared to other control Kimchi samples at t_{26}. However, the VOC profile shifted at t_{150}, characterized by abundance of carboxylic acids and alcohols including acetic acid, ethanol, octanoic and hexanoic acid.



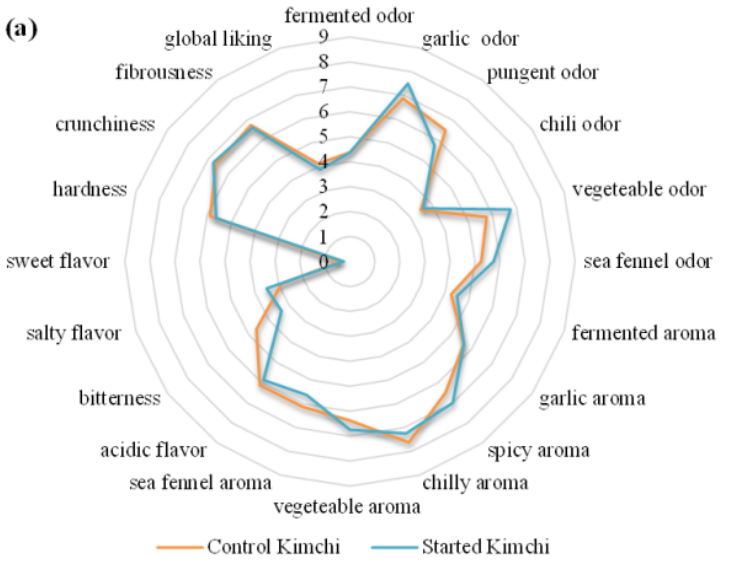
Plot of Heat Map analysis performed on control and started Kimchi made with sea fennel (38 VOCs in 18 Kimchi samples).
Note. Labels of Kimchi samples represent replicates of started Kimchi (FK) and control Kimchi (CK), followed by replicate numbers (1; 2; 3) and sampling time (t_0; t_{26}; t_{150}).

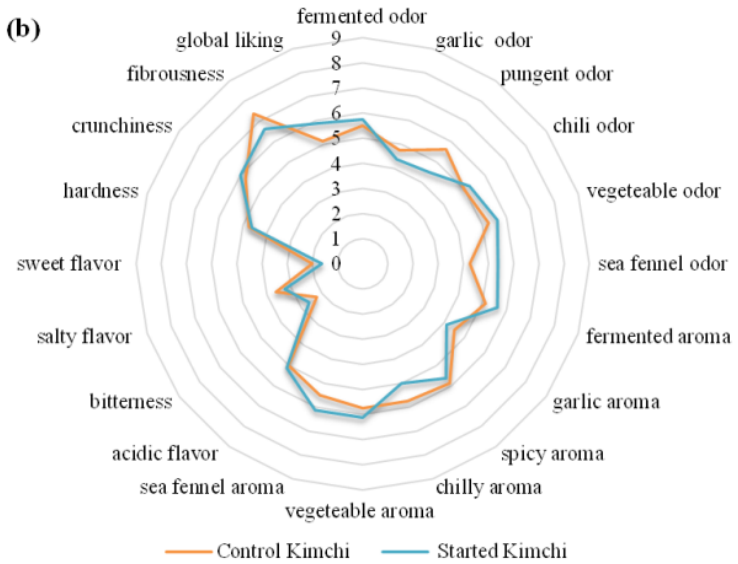


Principal Component Analysis bi-plot of the first two PCs obtained on VOCs whole dataset.
Note. Observations, in blue labels, refer to Kimchi samples: labels indicate the type of Kimchi (FK for started Kimchi, CK for control Kimchi), followed by replicate numbers (1; 2; 3) and sampling time (t0; t26; t150). Variables, in red labels, refer to individual VOCs.

Sensory analyses

Overall, most sensory attributes were perceived similarly between the two prototypes, regardless of the use of a starter culture. Spiciness, chili aroma, and garlic odor were more strongly perceived in all samples at day 26 (t26) (Figure 3, panel a) compared to day 150 (t150) (Figure 3, panel b). No significant differences in crunchiness were observed between the two samples at either point, although fibrousness slightly increased in both after 150 days of storage.The sea fennel flavor was perceived as slightly more intense in the starter-inoculated kimchi than in the control at t150. Both samples received relatively low scores for sweetness and saltiness at both time points. Regarding overall acceptability, the starter-inoculated kimchi was rated more favorably than the naturally fermented counterpart at t150, with the highest score being 5.88 ± 0.64.





Results of sensory analysis performed on started and control kimchi. (a) Sensory analyses performed after 26 days of fermentation; (b) sensory analyses performed after 150 days since the start of fermentation. Each sample was evaluated by a trained panel consisting of 8 non-smoker tasters aged between 25 and 48 for the presence and intensity of (i) six olfactory descriptors, being fermented, garlic, pungent, chilly, vegetable and sea fennel; (ii) six aroma descriptors, being fermented, garlic, spicy, chilly, vegetable and sea fennel; (iii) four flavor descriptors, being acidity, bitterness, salty, and sweet; (iv) three textural descriptors, being hardness, fibrousness, and crunchiness; (v) global acceptance. Each descriptor was evaluated by attributing a score comprised between 1 and 9, with 1 expressing the lowest and 9 the highest intensity. Results are reported as mean values ± standard deviation.

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1.1.3 Fermented sea fennel sprouts in vinegar

Material and Methods

Starter culture

The same starter strains used for the fermentation of kimchi have been used for the manufacture of laboratory-scale prototypes of fermented pickles.

Preparation of fermented sea fennel sprouts in vinegar

Laboratory scale prototypes of fermented sea fennel were produced by collecting a 5 kg sea fennel sprouts from the local farm (Rinci S.r.l, Castefidardo, Ancona, Italy) which cultivates sea fennel crops for food use industry. They were washed and rinsed with tap water, blanched at 95°C for 30s, and drained for 10 min by air-drying. Then 3 replicates were prepared, and each one consists of one plastic container containing a mix of 1795 g of blanched sea fennel with 5385 mL brine composed of autoclaved 7 % NaCl solution and 1 % fructose sterilized by filtration. All the replicates were inoculated by the 4 strains previously mentioned to reach 7 Log CFU mL^{-1} in brine. Two batches have been performed in the same way separately during November 2022 and July 2023.

Laboratory scale prototype of fermented sea fennel preserves produced by mixing blanched sea fennel sprouts and sterile brine.

Physical-chemical analyses (pH and TTA)

Aliquots (1 mL) of brine of each replicate were aseptically collected immediately after inoculation and during the fermentation until the end of the monitoring period corresponding to day 0, 1, 3, 6, 8, 10, 13, 15, 17 and 20. The pH measurement was accomplished with a pH meter model 300 (Hanna Instruments, Padova, Italy). The results were expressed as the mean of the replicates ± standard deviation.

Concerning TTA, the analyses were performed by aliquoting and blending 10 g of sea fennel sprouts for each replicate with 90mL of distilled water. The final suspension was titrated with 0.1 NaOH and the results expressed as % of lactic acid. TTA analyses were reported as mean of the 3 replicates ± standard deviation.

Microbial counting

Microbiological analyses were performed in brine samples. In more detail, counting of: (i) mesophilic aerobic bacteria, (ii) presumptive mesophilic lactobacilli, (iii) yeasts, and (iv) Enterobacteriaceae were performed as described previously for Kimchi in 1.2.1.4.

Tenfold serial dilutions were prepared from the brine of each inoculated sea fennel preserves replicate. The results of viable counting were expressed as the mean Log colony forming units (CFU) mL^{-1} of sea fennel preserves of three replicates ± standard deviation.

Statistical analysis

To assess statistical differences within Kimchi samples, the Tukey-Kramer's Honest Significant Difference (HSD) test (level of significance 0.05) was used by one-way analysis of variance (ANOVA). Tests were performed through JMP v11.0.0 software (SAS Institute Inc., Cary, NC).

Results

Physical-chemical characterization

The results of physical-chemical characterization of the analyzed prototype of fermented sea fennel preserve in batch 1 and 2 are reported in tables below.

Results of the pH determination of prototypes of fermented sea fennel.

Sampling time (t, days)	Prototypes	
	Batch 1	Batch 2
	Fermented Sea Fennel in brine	Fermented sea fennel in brine
t_0	6.05 ± 0.02^{a}	6.28 ± 0.05^{a}
t1	5.86 ± 0.06^{a}	5.32 ± 0.07^{b}
t_3	5.43 ± 0.10^{b}	5.08 ± 0.03^{c}
t_6	4.78 ± 0.19^{c}	4.91 ± 0.05^{c}
t_8	4.25 ± 0.20^{d}	4.86 ± 0.04^{cd}
t_{10}	3.83 ± 0.16^{e}	4.66 ± 0.09^{d}
t_{13}	3.80 ± 0.05^{e}	4.25 ± 0.08^{e}
t_{15}	3.62 ± 0.08^{e}	4.17 ± 0.09e
t_{17}	3.58 ± 0.12^{e}	4.07 ± 0.09^{e}
t_{20}	3.59 ± 0.06^{e}	4.04 ± 0.14^{e}

The results were expressed as the means of two independent measurements for each of the three replicates ± standard deviation. Within each row, for each batch overall means with different lowercase superscript letters are significantly different ($p < 0.05$).

Results of the titratable acidity determination of prototypes of fermented sea fennel.

Sampling time (t, days)	Prototypes	
	Batch 1	Batch 2
	Fermented sea fennel	Fermented sea fennel
t_0	0.12 ± 0.04^{a}	0.03 ± 0.00^{b}

t_{26}	0.18 ± 0.02[a]	0.32 ± 0.03[a]

The results are expressed as means % lactic acid of three replicates ± standard deviations. Within each row, for each batch overall means with different lowercase superscript letters are significantly different ($p < 0.05$).

The pH value decreased during the monitoring period in batch 1 and 2 where it was significantly lower at the end of fermentation which passed from 6.05 ± 0.02 to 3.59 ± 0.06 and from 6.28±0.05 to 4.04±0.14 respectively in **batch** 1 and 2, contrarily to TTA that increased from 0.12 ± 0.04 to 0.18 ± 0.02 and from 0.03±0.00 to 0.32±0.03 % lactic acid equivalent.

Microbial Viable Counts

Microbiological analyses were performed in brine samples of fermented sea fennel in batches 1 and 2 and theresults are reported below.

Microbial counting of fermented sea fennel preserves brine during fermentation process for Batch 1 and 2.

Microbial group	Sampling time (t, days)	Prototypes	
		Batch 1	Batch 2
Mesophilic lactobacilli (Log CFU mL^{-1})		Fermented sea fennel preserves	
	t_0	7.3 ± 0.0[a]	7.2 ± 0.1[a]
	t_1	6.4 ± 0.1[b]	6.8 ± 0.0[ab]
	t_3	6.4 ± 0.1[b]	6.3 ± 0.0[c]
	t_6	6.2 ± 0.2[b]	6.5 ± 0.4[bc]
	t_{13}	7.3 ± 0.1[a]	6.7 ± 0.2[abc]
	t_{20}	6.4 ± 0.2[a]	6.9 ± 0.2[ab]
Yeasts (Log CFU mL^{-1})			
	t_0	< 1.0[c]	0.0 ± 0.2[b]
	t_1	< 1.0[c]	2.7± 0.1[b]
	t_3	1.7 ± 0.4[c]	4.6 ± 0.4[a]
	t_6	4.1 ± 0.7[b]	5.6 ± 0.1[a]
	t_{13}	6.7 ± 0.2[ab]	5.2 ± 1.2[a]
	t_{20}	6.4 ± 0.1[a]	5.3 ± 0.6[a]
Enterobacteriaceae (Log CFU mL^{-1})			
	t_0	< 1.0b	0.4 ± 0.8[c]
	t_1	< 1.0[b]	0.3 ± 0.6[c]
	t_3	4.5 ± 1.5[a]	6.7 ± 0.5[a]
	t_6	5.4 ± 1.2[a]	6.5 ± 0.4[a]
	t_{13}	< 1.0[b]	3.6 ± 0.7[b]
	t_{20}	< 1.0[b]	2.4 ± 0.5[b]
Mesophilic aerobic bacteria (Log CFU mL^{-1})			
	t_0	7.3 ± 0.0[a]	7.1 ± 0.0[a]
	t_1	6.3 ± 0.1[bc]	6.7 ± 0.0[ab]
	t_3	5.8 ± 0.3[c]	6.9 ± 0.2[ab]
	t_6	6.5 ± 0.4[b]	7.1 ± 0.3[a]
	t_{13}	6.3 ± 0.2 [bc]	6.9 ± 0.2[ab]
	t_{20}	5.9 ± 0.3[bc]	6.6 ± 0.1[b]

The results are expressed as means of three replicates ± standard deviation. Within each row, for each microbial group in each batch, overall means with different lowercase superscript letters are significantly different ($p < 0.05$).

During the monitoring period of fermentation of fermented sea fennel preserves, the mesophilic lactobacilli slightly decreased at the end of fermentation for Batch 1 and 2 which passed from 7.3 ± 0.0 to 5.9 ± 0.3 Log CFU mL^{-1} and from 7.2 ± 0.1 to 6.9 ± 0.2 Log CFU mL^{-1} similarly to Mesophilic aerobic bacteria which had similar trend between the beginning and day the end of fermentation time.

Regarding Enterobacteriaceae, they disappeared at the end of fermentation in Batch 1 although they increased between day 3 and day 6 then decreased from day 6 to day 20 in batch 2 with reported values of 6.5 ± 0.4 to 2.4 and 5.3 ± 0.5 Log CFU mL^{-1}. The enumeration of the yeasts showed an increase during the monitoring period that reached 6.4 ± 0.1 and 5.3 ± 0.6 Log CFU mL^{-1} in batch 1 and 2 respectively.

1.1.3.1 PICKLES

The fermented sea fennel in brine has been processed into 24 pickles samples composed each of 300g vinegar and mineral water and 200g of fermented sea fennel leaves and sprouts with different acidity percentages. Two types of vinegar are used: wine and apple vinegar with 12 samples for each type including three replicates for each acidity level applied. The different acidity percentages are as follows: 0.05%, 0.2%, 0.5% and 0.7%.

The 24 samples have been subjected to the pasteurization mild thermal treatment equivalent to 74°C for 3min to kill and eliminate vegetative pathogenic organisms and to extend the shelf life of food product for a limited period.

The pickles are analyzed for a period of 6 months starting from May until November to assess their shelf-life and determine the optimal acidity level for preservation and organoleptic quality They are sampled during the monitoring period for pH, TTA, microbial enumeration, color and volatile compounds. The two batches were produced with two types of vinegar.

Pickles sample containing fermented sea fennel, water and vinegar.

Material and Methods

Physical-chemical analyses (pH and TTA)

Aliquots (1 mL) of brine of each replicate from each sample were aseptically collected immediately after pasteurization and during the monitoring period at the following months: 0, 1, 2, 3, 4, 5, and 6. The pH measurement was accomplished with a pH meter model 300 (Hanna Instruments, Padova, Italy). The results were expressed as mean of the three biological replicates for each technical ± standard deviation.

Concerning TTA, the analyses were performed by aliquoting 10 ml of pickle brine for each replicate with 90mL of distilled water. The final product was titrated 0.1 NaOH and the results expressed as % of lactic acid equivalent. TTA analyses were reported as mean of the 3 replicates ± standard deviation.

Microbial Viable Counts

Microbiological analyses were performed in brine pickle samples. In more details, counting of: (i) mesophilic aerobic bacteria, (ii) presumptive mesophilic lactobacilli, (iii) yeasts, and (iv) Enterobacteriaceae were performed as described previously for Kimchi.

Tenfold serial dilutions were prepared from the brine of each pickle replicate. The results of viable counting were expressed as the mean Log colony forming units (CFU) mL^{-1} of three replicates ± standard deviation.

Color assessment

The colorimetric test of pickles was performed on sea fennel leaves in each replicate, bounding together at least three leaves with the same size to create a homogenous sample. Color parameters in the CIELab color space, lightness (L), redeness-greeness (a*: + red; − green), and yellowness-blueness (b*: + yellow; − blue) were measured using a Chroma Meter CR-200 (Minolta Japan). In addition, the hue angle h* (h°) was calculated using the formula h° = 180 + arctg (b*/a*) (Mclellan et al., 1995), and the chroma (C) was calculated using the formula $C = [(a^{*2} + b^{*2})]^{1/2}$. The results were expressed as the mean of three replicates per sample ± standard deviation.

Salt determination

Sea fennel leaves (5 g) were homogenized in 20 g of distilled water using an Ultra-Turrax machine at an estimated speed of 10,000 RPM for 2 minutes, ensuring thorough mixing for subsequent analysis. Then, Salt measurements were conducted at two time points: the initial month (Month 0) and six months later (Month 6). However, samples prepared with 0.05% acidity were excluded from the comparison, as they were contaminated and could not be reliably evaluated against the initial month.

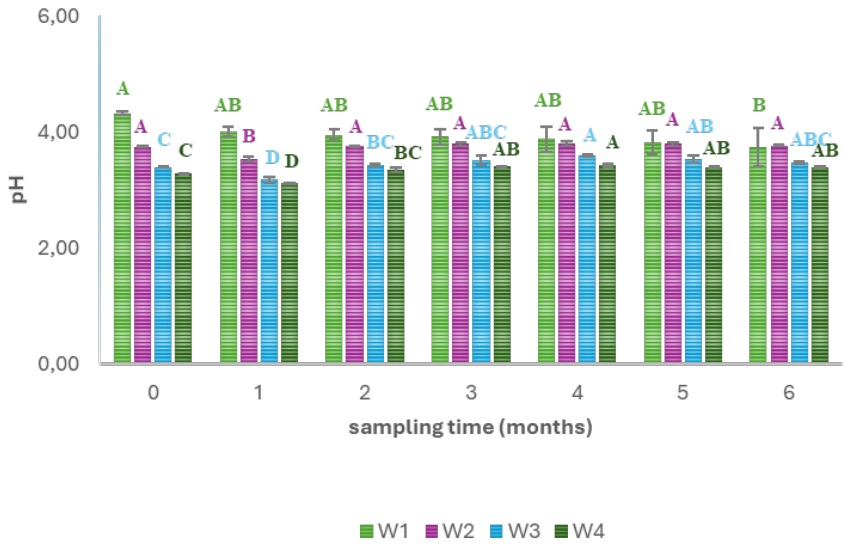
Sensory analysis

Sensory analysis was conducted to evaluate the organoleptic properties of the sea fennel pickles samples over time. attributes such as herbal, woody, acidic, and sea fennel odors; herbal, woody, acidic, and sea fennel aromas; acidic flavor, bitterness, salty flavor, and sweet flavor; as well as textural attributes like hardness, fibrosity, and crunchiness, were analyzed alongside an overall assessment of global liking. Samples with 0.05% acidity were excluded from the analysis due to contamination, which prevented reliable comparisons.

Results

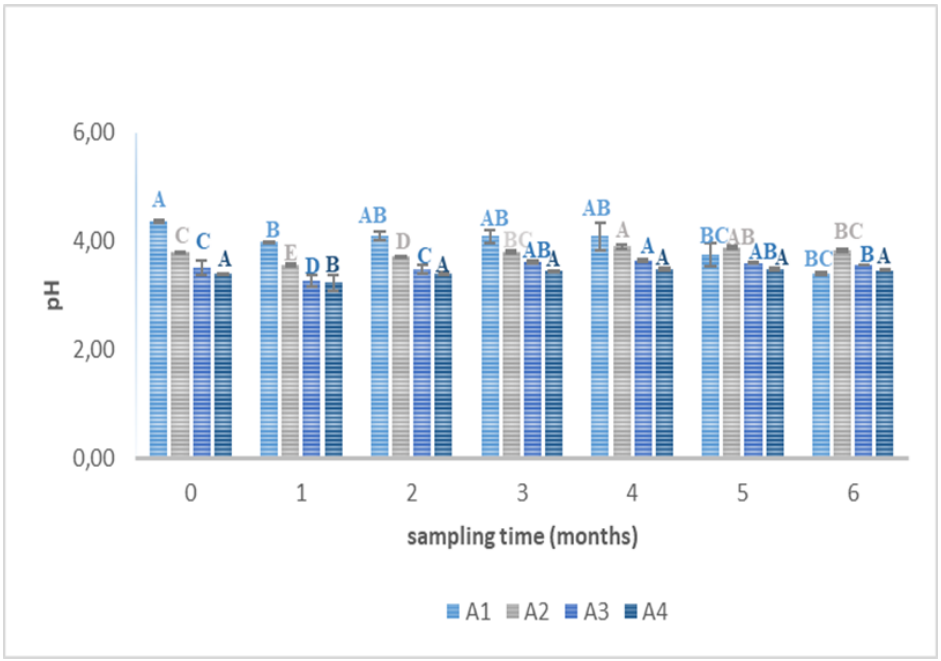
Physical-chemical characterization

The results of pH determination of the analyzed prototype of pickles made of wine and apple vinegar in batch 1 are demonstrated in the figures below.



Results of the pH determination of pickles prototypes made of sea fennel and wine.

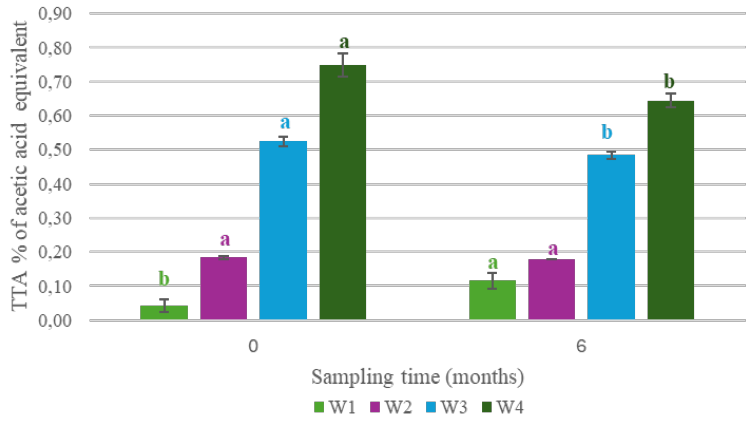
W1: samples with 0.05% acidity (total acetic acid in the final product)
W2: samples with 0.2% acidity
W3: samples with 0.5% acidity
W4: samples with 0.7% acidity
Results are expressed as mean of three biological replicates for each sample ± standard deviation.



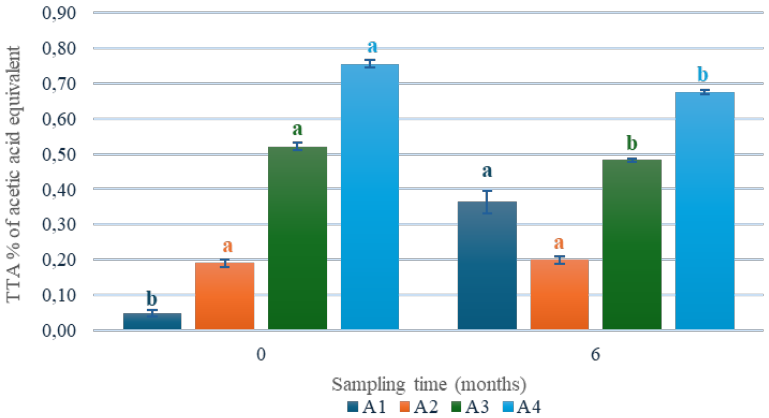
Results of the pH determination of pickles prototypes made of sea fennel and apple vinegar.

A1: samples with 0.05% acidity (total acetic acid in the final product)
A2: samples with 0.2% acidity
A3: samples with 0.5% acidity
A4: samples with 0.7% acidity
Results are expressed as mean of three biological replicates for each sample ± standard deviation.

The pH values remained relatively stable for both prototypes, whether using wine or apple vinegar during the monitoring period except the samples with the lowest acidity (0.05%) where the pH values decreased from t0 to t6, from 4.32 ± 0.02 to 3.73 ± 0.34 and from 4.36 ± 0.02 to 3.41 ± 0.02 for W1 and A1, respectively. As for the remaining samples made with the wine vinegar, the pH at the end of monitoring period (t6) ranged from 3.39 ± 0.02 to 3.77 ± 0.01, varying according to the acidity level applied. However, in the samples made with apple vinegar, pH ranged from 3.46 ± 0.02 to 3.83 ± 0.02.



Results of TTA in sea fennel pickles made with wine vinegar.



Results of TTA measurements in sea fennel pickles made with apple vinegar.
The results were expressed as the means of three biological replicates ± standard deviation.

Based on the reported results in table 2, the total titratable acidity (TTA) increased with higher acidity levels of the vinegars used. Moreover, minimal variation was observed among the different prototypes except for W1 and A1 where TTA values progressively increased. At t0, TTA ranged from 0.06 ± 0.02 to 1.12 ± 0.03 % of acetic acid equivalent in wine vinegar

pickles and from 0.07 ± 0.01 to 1.13 ± 0.01 % of acetic acid equivalent in apple vinegar pickles, correlating with the acidity levels. However, at t6, TTA values ranged from 0.17 ± 0.02 to 0.97 ± 0.02 for pickles made with wine vinegar and from 0.54 ± 0.03 to 1.01 ± 0.01 % of acetic acid equivalent, for apple vinegar pickles.

In conclusion, except for the samples with 0.05% acidity, the pH of the samples made with either apple wine vinegar exhibited an opposite trend to the acidity level applied. Additionally, the Total Titratable Acidity (TTA) values increased proportionally with the acidity of the vinegar used.

Microbial enumeration

Overall, mesophilic lactobacilli were generally not detected in batch 1 and 2 in most of the samples during the six months monitoring period. Mesophilic aerobic bacteria showed limited growth in the samples made with acidity higher than 0,05%, while the growth reached 4.61 to 4.69 Log CFU/mL in both batches in W1 and A1. Similarly, Yeasts showed a gradual growth from month 3 in samples A1 and W1 regardless of number of batches. However, Enterobacteriaceae were absent throughout the shelf-life study.

In summary, both batches demonstrated microbial stability, with specific aerobic and yeast growth linked to acidity levels, and no presence of harmful bacteria was observed.

Coagulase-positive staphylococci (*Staphylococcus aureus* and other species), reducing anaerobic bacteria, Spores of sulfite-reducing anaerobic bacteria, and *Listeria monocytogenes* were not detected in both batches at time 0 and 6.

Microbial counting of pickled sea fennel in vinegar Batch 1.

Microbiological group	Sampling time (months)	Prototypes							
Mesophilic lactobacilli (Log CFU mL^{-1})		W1	W2	W3	W4	A1	A2	A3	A4
	t0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t2	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t3	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t4	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t5	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t6	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Mesophilic aerobic bacteria (Log CFU mL^{-1})		W1	W2	W3	W4	A1	A2	A3	A4
	t0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t2	2.48 ± 25	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t3	2.46 ± 23	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t4	2.89 ± 2.54	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t5	4.04 ± 06	< 1.0	< 1.0	< 1.0	2.67 ± 0.06	< 1.0	< 1.0	< 1.0
	t6	4.69 ± 03	< 1.0	< 1.0	< 1.0	4.61 ± 0.05	< 1.0	< 1.0	< 1.0
Yeasts (Log CFU mL^{-1})		W1	W2	W3	W4	A1	A2	A3	A4
	t0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t2	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

	t3	3.48 ± 02	< 1.0	< 1.0	< 1.0	18 ± 2.50	< 1.0	< 1.0	< 1.0
	t4	3.59 ± 00	< 1.0	< 1.0	< 1.0	1.72 ± 2.44	< 1.0	< 1.0	< 1.0
	t5	3.47 ± 0.07	< 1.0	< 1.0	< 1.0	3.52 ± 0.04	< 1.0	< 1.0	< 1.0
	t6	3.56 ± 0	< 1.0	< 1.0	< 1.0	3.61 ±0.05	< 1.0	< 1.0	< 1.0
Enterobacteriaceae (Log CFU mL^{-1})		W1	W2	W3	W4	A1	A2	A3	A4
	t0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t2	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t3	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t4	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t5	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t6	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

Microbial counting of pickled sea fennel in vinegar Batch 2.

Microbiological group	Sampling (t,months)	Prototypes							
Mesophilic lactobacilli (Log CFU mL^{-1})		W1	W2	W3	W4	A1	A2	A3	A4
	t0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	2.13 ± 3.01	< 1.0	< 1.0
	t2	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	1.56 ± 2.21	< 1.0	< 1.0
	t3	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	1.54 ± 2.17	< 1.0	< 1.0
	t4	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	1.54 ± 2.17	< 1.0	< 1.0
	t5	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	0.85 ± 1.20	< 1.0	< 1.0
	t6	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Mesophilic aerobic bacteria (Log CFU mL^{-1})		W1	W2	W3	W4	A1	A2	A3	A4
	t0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	2,14 ± 3,03	< 1.0	< 1.0
	t2	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	1,52 ± 2,15	< 1.0	< 1.0
	t3	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	1,54 ± 2,18	< 1.0	< 1.0
	t4	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	1,55 ± 2.19	< 1.0	< 1.0
	t5	1.29 ± 1.82	< 1.0	< 1.0	< 1.0	2.67 ± 0.06	1,25 ± 1,76	< 1.0	< 1.0
	t6	< 1.0	< 1.0	< 1.0	< 1.0	4.61 ± 0.05	< 1.0	< 1.0	< 1.0
Yeasts (Log CFU mL^{-1})		W1	W2	W3	W4	A1	A2	A3	A4
	t0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
	t1	1,36 ± 1,93	< 1.0	< 1.0	< 1.0	< 1.0	1,87 ± 2,64	< 1.0	< 1.0
	t2	1,87 ± 2,64	< 1.0	< 1.0	< 1.0	< 1.0	1,18 ± 1,67	< 1.0	< 1.0
	t3	1,25 ± 1,76	< 1.0	< 1.0	< 1.0	1.18 ± 2.50	1,00 ± 1,41	< 1.0	< 1.0
	t4	1,24 ± 1,75	< 1.0	< 1.0	< 1.0	1.72 ± 2.44	2,42 ± 3,42	< 1.0	< 1.0
	t5	1,21± 1,72	1,42 ± 2,01	< 1.0	< 1.0	3.52 ± 0.04	1,52 ±2,16	< 1.0	< 1.0
	t6	0,98 ± 1,38	< 1.0	< 1.0	< 1.0	3.61 ± 0.05	0,76 ±1,07	< 1.0	< 1.0
Enterobacteriaceae (Log CFU mL^{-1})		W1	W2	W3	W4	A1	A2	A3	A4

t0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
t1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
t2	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
t3	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
t4	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
t5	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
t6	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

Color measurement

The color parameters of sea fennel pickles from batches 1 and 2 were measured at three sampling times: immediately after preparation (t_0), after 3 months (t_3), and after 6 months of storage (t_6). The measured parameters included L* (lightness), a* (red-green coordinate), b* (yellow-blue coordinate), hue angle (h°), and chroma (C), which collectively describe the color characteristics of the samples.

For Batch 1, lightness (L*) generally showed a slight increase over time in most prototypes, indicating that the pickles became somewhat lighter during storage. In more detail, the sample W2 showed an increase of L* from 32.25 at t0 to 37.24 at t6. The a* values remained consistently negative across all prototypes and times, indicating a stable greenish hue that did not significantly change with storage. The b* values, representing yellowness, tended to decrease over time in most samples, suggesting a reduction in yellow coloration as storage progressed. Hue angle (h°) remained relatively stable across time points, indicating that the overall color tone did not significantly change. Chroma (C), which reflects color saturation, generally decreased over storage, suggesting a loss of color intensity or dulling of the samples.

In Batch 2, a similar pattern was observed, with some differences in the magnitude of changes. Lightness (L*) generally increased from t_0 to t_3 and then slightly decreased or stabilized by t_6. For instance, W2 increased from 35.08 at t_0 to 42.12 at t_3, then decreased to 38.17 at t6. The a* values remained negative, showing a persistent greenish color, although some fluctuations were noted. The b* values varied but tended to decrease over time in some prototypes, indicating a slight reduction in yellowness. Hue angles were relatively stable, and chroma showed a moderate decrease in some samples, reflecting a slight loss in color vitality during storage.

Overall, the results suggest that the pickles undergo gradual lightning and slight loss of color saturation over six months, while the green hue remains stable. Differences between batches and prototypes were observed but were generally minor. The color changes might be influenced by factors such as acidity level, vinegar type, and storage conditions. These findings are important for understanding the visual quality and consumer appeal of sea fennel pickles during shelf life.

Color analyses of pickled sea fennel in vinegar Batch 1 and 2.

Batch	Prototype	Sampling time (t, months)	Color Parameter				
			L	a*	b*	h°	C
B1	W2						
		t0	32.25 ± 2.20^{a}	-2.49 ± 0.04^{a}	14.89 ± 1.10^{a}	103.26 ± 0.72^{a}	17.70 ± 1.08^{a}
		t3	34.79 ± 1.47^{ab}	-3.03 ± 1.07^{a}	11.92 ± 3.21^{a}	104.08 ± 1.95^{a}	12.30 ± 3.36^{a}
		t6	37.24 ± 1.66^{ab}	-2.19 ± 0.43^{a}	10.17 ± 1.17^{b}	102.18 ± 2.36^{a}	10.41 ± 1.18^{b}
	W3						
		t0	36.34 ± 3.03^{a}	-3.27 ± 1.46^{a}	17.79 ± 3.36^{a}	100.08 ± 2.64^{a}	18.10 ± 3.57^{a}
		t3	34.35 ± 0.81^{ab}	-2.04 ± 0.25^{a}	8.23 ± 0.46^{a}	103.99 ± 2.38^{a}	8.49 ± 0.39^{a}
		t6	37.02 ± 3.06^{ab}	-2.23 ± 0.50^{a}	9.10 ± 1.06^{b}	103.67 ± 1.61^{a}	9.37 ± 1.15^{b}
	W4						
		t0	35.43 ± 1.90^{a}	-3.14 ± 0.96^{a}	15.58 ± 3.59^{a}	101.27 ± 1.61^{a}	15.90 ± 3.69^{a}
		t3	36.20 ± 1.21^{a}	-2.37 ± 0.89^{a}	9.59 ± 2.50^{a}	103.58 ± 2.34^{a}	9.88 ± 2.63^{a}
		t6	33.86 ± 1.50^{b}	-2.75 ± 0.84^{a}	8.91 ± 2.04^{b}	106.92 ± 2.06^{a}	9.33 ± 2.19^{b}
	A2						
		t0	33.51 ± 1.31^{a}	-2.77 ± 0.13^{a}	13.31 ± 1.88^{a}	101.95 ±2.30^{a}	13.60 ± 1.81^{a}
		t3	34.27 ± 2.12^{ab}	-2.67 ± 0.90^{a}	12.30 ± 3.50^{a}	102.15 ± 0.74^{a}	12.59 ± 3.61^{a}

		t6	40.76 ± 1.96^{a}	-2.89 ± 1.59^{a}	14.34 ± 1.42^{a}	101.12 ± 5.02^{a}	14.65 ± 1.70^{a}
	A3						
		t0	33.29 ± 1.92^{a}	-2.36 ± 1.45^{a}	13.66 ± 2.32^{a}	99.27 ± 4.77^{a}	13.89 ± 2.51^{a}
		t3	33.31 ± 0.38^{ab}	-1.82 ± 0.60^{a}	7.85 ± 1.27^{a}	102.77 ± 2.45^{a}	8.06 ± 1.36^{a}
		t6	35.16 ± 2.06^{ab}	-2.65 ± 0.17^{a}	9.26 ± 0.85^{b}	105.99 ± 0.55^{a}	9.63 ± 0.86^{b}
	A4						
		t0	33.72 ± 1.48^{a}	-3.52 ± 0.88^{a}	14.95 ± 2.67^{a}	103.16 ± 0.99^{a}	15.36 ± 2.79^{a}
		t3	32.33 ± 0.88^{b}	-1.83 ± 0.49^{a}	8.89 ± 2.00^{a}	101.78 ± 2.75^{a}	9.08 ± 2.00^{a}
		t6	33.33 ± 0.79^{b}	-1.60 ± 0.09^{a}	7.32 ± 0.38^{b}	102.32 ± 0.12^{a}	7.49 ± 0.39^{b}
B2	W2						
		t0	35.08 ± 0.36^{b}	-5.48 ± 1.58^{a}	19.29 ± 2.21^{a}	105.70 ± 2.64^{a}	20.06 ± 2.56^{a}
		t3	42.12 ± 1.70^{a}	-3.40 ± 1.07^{a}	17.71 ± 1.22^{a}	100.85 ± 0.83^{a}	18.03 ± 1.29^{a}
		t6	38.17 ± 0.55^{b}	-4.56 ± 0.02^{a}	15.79 ± 0.04^{a}	106.10 ± 0.11^{a}	16.43 ± 0.03^{b}
	W3						
		t0	35.80 ± 0.96^{b}	-5.55 ± 0.05^{a}	17.20 ± 0.59^{a}	107.88 ± 0.73^{a}	18.07 ± 0.55^{a}
		t3	38.91 ± 0.07^{a}	-4.28 ± 0.01^{a}	17.56 ± 3.13^{a}	103.90 ± 2.43^{a}	18.08 ± 3.04^{a}
		t6	36.03 ± 1.45^{ab}	-2.95 ± 1.10^{a}	14.06 ± 1.94^{a}	101.63 ± 2.72^{a}	14.37 ± 2.13^{b}
	W4						
		t0	37.37 ± 1.88^{a}	-3.40 ± 0.13^{a}	14.34± 0.04^{a}	103.34 ± 0.04^{a}	13.34 ± 0.47^{a}
		t3	36.20 ± 1.21^{a}	-2.37 ± 0.89^{b}	9.59 ± 2.50^{b}	103.58 ± 2.34^{a}	9.88 ± 2.63^{b}
		t6	35.12 ± 2.91^{a}	-3.20 ± 0.59^{b}	13.46 ± 1.12^{b}	103.50 ± 3.44^{a}	13.84 ± 0.95^{b}
	A2						
		t0	38.26 ± 1.50^{a}	-5.71 ± 0.14^{a}	20.44± 0.24^{a}	105.62 ±0.55^{a}	21.22 ± 0.19^{a}
		t3	40.07 ± 2.56^{a}	-3.30 ± 0.50^{b}	12.98± 1.09^{b}	104.35 ± 3.23^{a}	13.40 ± 0.93^{b}
		t6	32.75 ± 0.94^{a}	-3.51 ± 0.66^{b}	14.90 ± 2.82^{ab}	103.25 ± 0.01^{a}	15.30 ± 2.90^{ab}
	A3						
		t0	32.24 ± 0.63^{a}	-3.65 ± 0.29^{a}	14.70 ± 0.44^{a}	103.95 ± 1.47^{a}	15.15 ± 0.36^{a}
		t3	37.66 ± 1.06^{a}	-3.41 ± 0.94^{a}	14.13 ± 1.33^{a}	103.45 ± 2.40^{a}	14.54 ± 1.51^{a}
		t6	37.04 ± 2.61^{a}	-4.10 ± 0.12^{a}	15.35 ± 1.18^{a}	105.00 ± 1.54^{a}	15.89 ± 1.11^{a}
	A4						
		t0	35.82 ± 2.29^{a}	-4.43 ± 1.38^{a}	16.67 ± 3.28^{a}	104.70 ± 1.68^{a}	17.25 ± 2.79^{a}
		t3	37.12 ± 0.07^{a}	-3.44 ± 0.30^{a}	14.37 ± 0.27^{a}	103.46 ± 1.38^{a}	14.78 ± 0.19^{a}
		t6	36.27 ± 2.13^{a}	-3.22 ± 0.98^{a}	13.42 ± 2.10^{a}	103.33 ± 1.96^{a}	13.81 ± 2.27^{a}

The results are expressed as the means of three replicates (± standard deviation). Within each row, for the same batch and each color parameter, overall means marked with different lowercase superscript letters are significantly different ($p < 0.05$).

Salt measurement

A general decrease in salt content was observed across the monitoring period from month 0 to month 6 in both batches. Initially, the salt content was close to the residual salt in the fermented sea fennel after washing and before pasteurization (3%), with minor differences likely attributed to the application of vinegar and varying acidity levels. Regarding the type of vinegar used, no significant differences were noted between wine and apple vinegar. No differences were observed between the different acidity levels applied as salt concentration decreased in all samples over time.

Salt Content in Pickles with Sea Fennel in Batches 1 and 2 at Months 0 and 6.

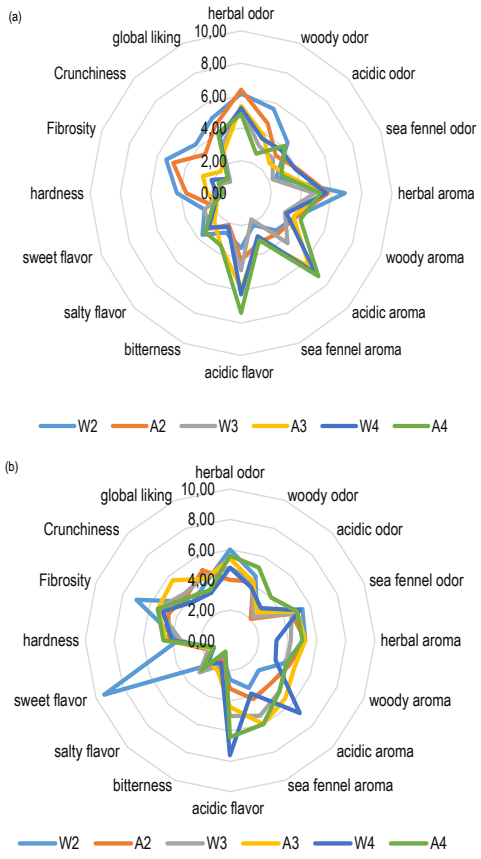
Sampling time (t, months)	Sample	Batch1	Batch2
	W2	2,62 ± 0,12^{a}	2,45 ± 0,57^{a}
T_0	W3	2,25 ± 0,28^{a}	2,15 ± 0,21^{a}
	W4	2,43 ± 0,28^{a}	2,23 ± 0,28^{a}
	A2	2,05 ± 0,26^{a}	2,10 ± 0,00^{a}
	A3	2,68 ± 0,06^{a}	2,33 ± 0,28°
	A4	2,61 ± 0,32^{a}	2,33 ± 0,40^{a}
T_6			
	W2	1,05 ± 0,00^{b}	2,00 ± 0,00^{a}

W3	1,65 ± 0,13^{b}	1,20 ± 0,07^{b}
W4	1,48 ± 0,08^{b}	1,45 ± 0,07^{b}
A2	1,32 ± 0,06^{b}	1,20 ± 0,00^{b}
A3	1,70 ± 0,28^{b}	1,28 ± 0,11^{b}
A4	1,70 ± 0,21^{b}	1,28 ±0,18^{b}

The results are expressed as the means of three replicates (± standard deviation). Within each row, for the same, overall means marked with different lowercase superscript letters are significantly different ($p < 0.05$).

Sensory analyses

The sensory analysis of pickles made with sea fennel showed that Batch 2 maintained better textural stability, with higher scores for hardness, fibrosity, and crunchiness, compared to Batch 1. This difference in stability may be attributed to environmental factors, as Batch 1 was produced in summer 2023 and stored at room temperature, while Batch 2 was made during the cooler winter months of 2024. In more detail, an increase in acidity led to a decline in herbal and woody odors and aromas, suggesting that higher acidity levels may suppress these characteristics. Notably, sea fennel odor and aroma were most highly detected in samples with 0.5% acidity, regardless of the type of vinegar used, indicating that moderate acidity enhances these sensory attributes. On the other hand, acidic aroma and flavor intensified with higher acidity levels, negatively affecting overall acceptability due to the perception of an overly acidic flavor and textural degradation. Preference trends also differed by vinegar type, with wine vinegar at 0.5% acidity being favored in Batch 1 and apple vinegar in Batch 2. However, type of vinegar did not highly affect the individual sensory attributes These findings suggest that moderate acidity (0.5%) provides a balance that preserves sensory quality and highlights the characteristic profile of sea fennel.



Results of sensory analysis performed on pickles made with sea fennel. (a) Sensory analyses performed after 6 months: Batch 1; (b) sensory analyses performed after 6 months: Batch2.

2 Sea fennel-based unfermented shelf stable preserves

2.1 Croatian prototypes

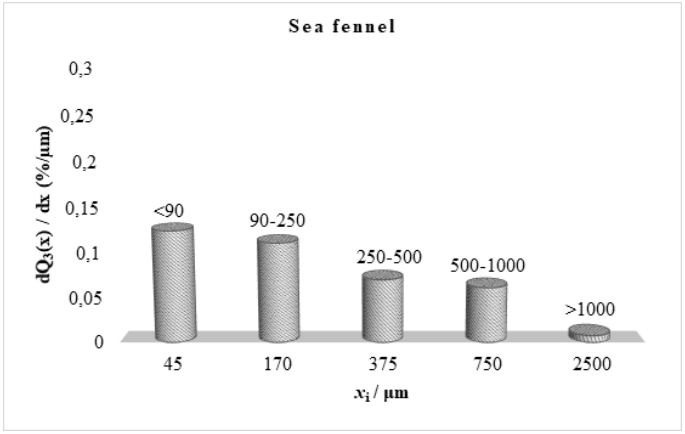
2.1.1 Dried spices formulated with blends of sea fennel and other Mediterranean aromatic herbs

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).

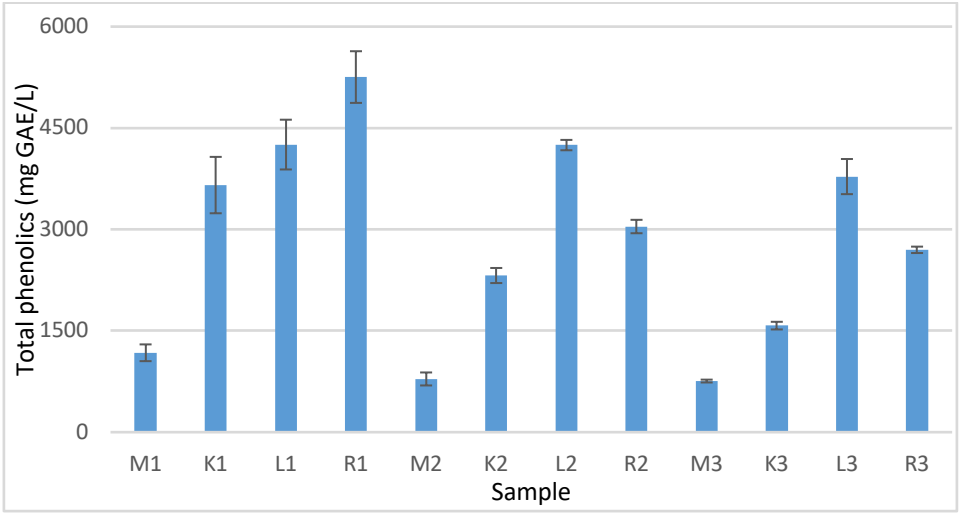
For the quantification and identification of phenolic compounds, the analytical technique, High-Performance Liquid Chromatography (HPLC), was used, with samples analyzed using a Shimadzu Nexera LC-40 system equipped with a UV/VIS detector (Shimadzu, Kyoto, Japan), and phenolic compounds were separated 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

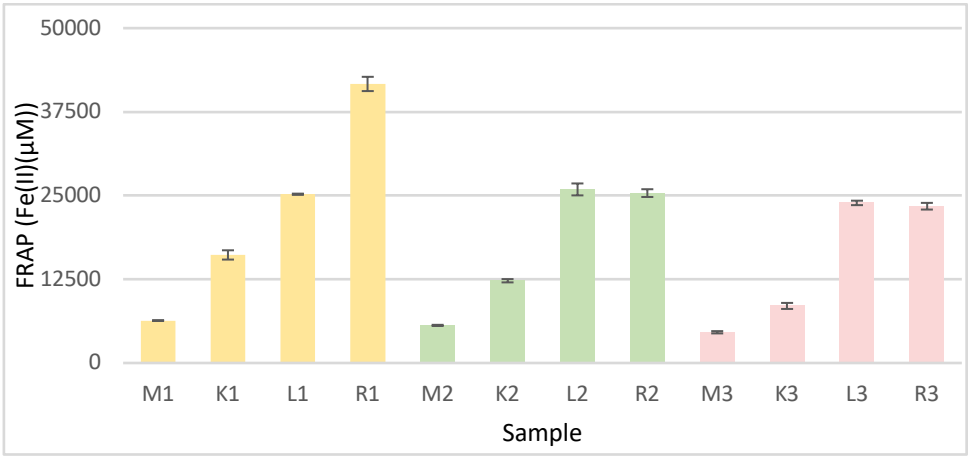
a) Individual plants

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

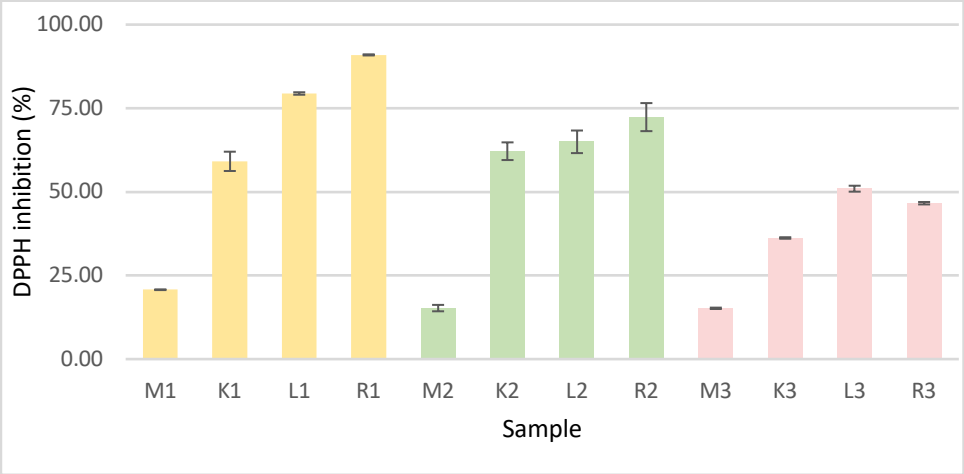
Sample mark	Sample	Particle size
M1	Sea fennel	<90 µm
K1	Fennel	
L1	Bay laurel	
R1	Rosemary	
M2	Sea fennel	90-250 µm
K2	Fennel	
L2	Bay laurel	
R2	Rosemary	
M3	Sea fennel	250-500 µm
K3	Fennel	
L3	Bay laurel	
R3	Rosemary	



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).)

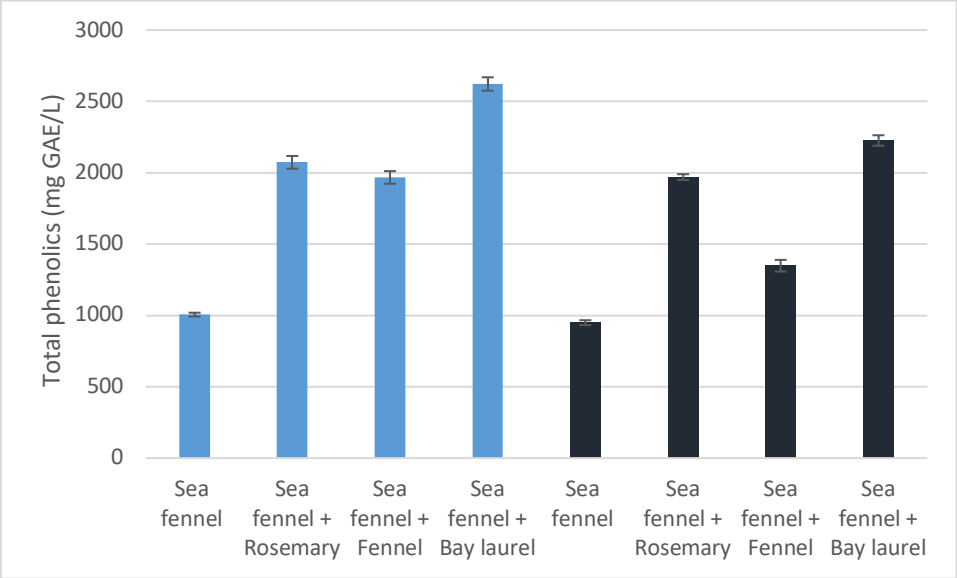
HPLC analysis of spice extracts (µg/mL) neochlorogenic acid (nCGA); Caffeic acid (CA); chlorogenic acid (CGA); cryptochlorogenic acid (cCGA); rosmaric acid (RA); cinnamic acid (CAN); rutin ®; vanilic acid (VA)

	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

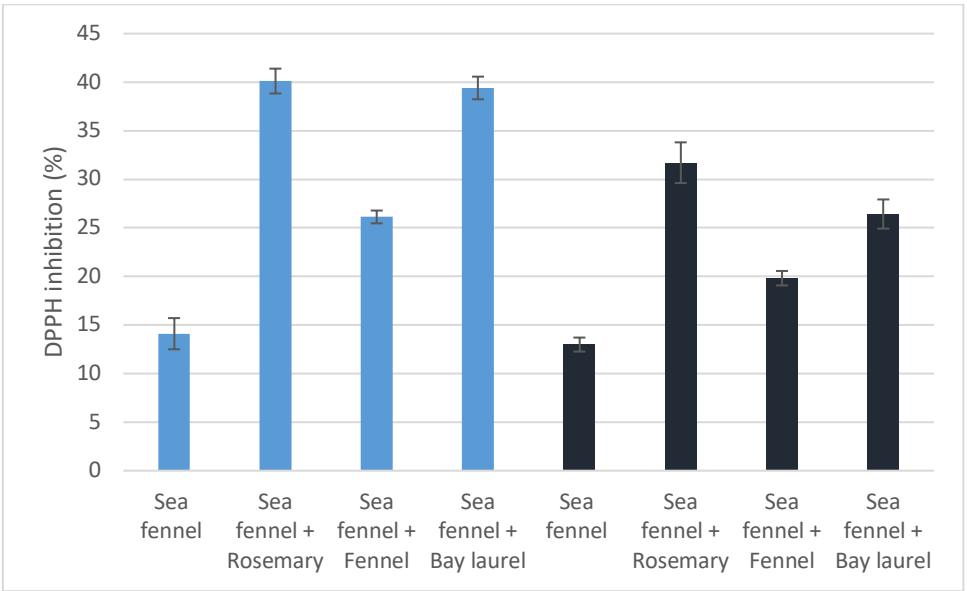
b) Combinations of plants

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

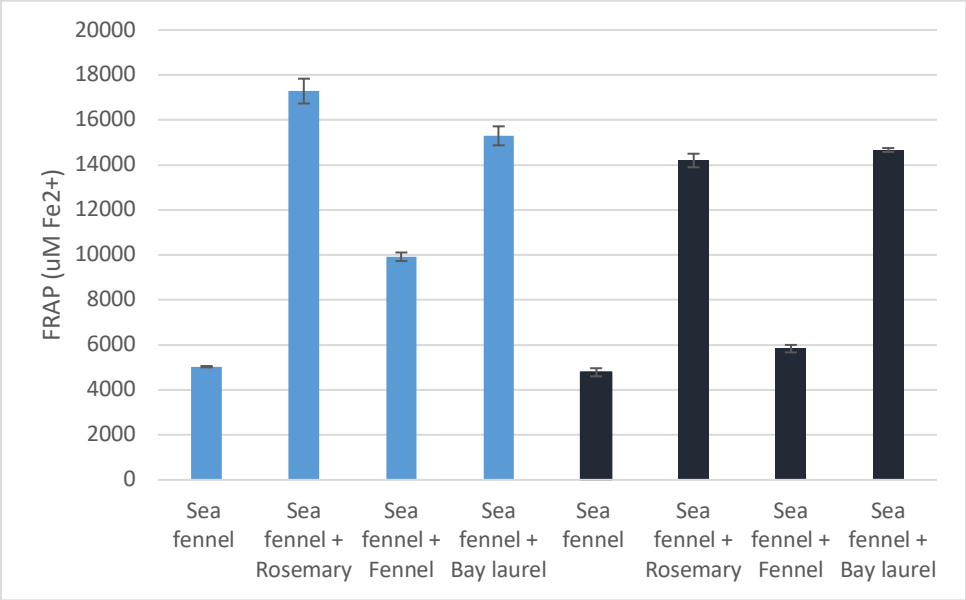
Sample mark	**Sample**	**Particle size**
M	Sea fennel	90-250 µm and 250-500 µm
M+R	Sea fennel + Rosemary	
M+K	Sea fennel + Fennel	
M+L	Sea fennel + Bay laurel	



c) *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)

2.1.2 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

2.1.3 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.

2.1.4 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

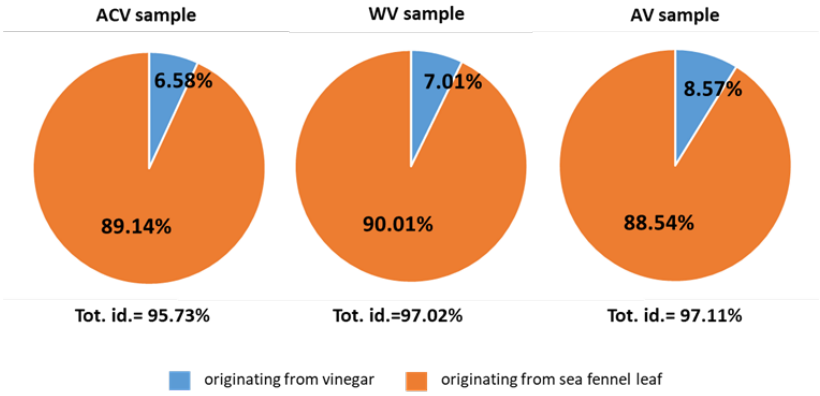
Mean values within a row with different letters in superscript differ significantly ($p < 0.05$).

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.

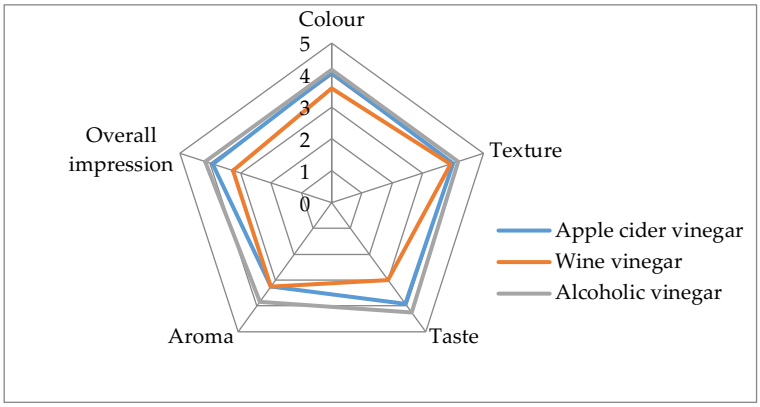
	Apple Cider Vinegar (1:5, *v*/*v*)	Wine Vinegar (1:4, *v*/*v*)	Alcoholic Vinegar (1:5, *v*/*v*)

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.

2.2 Turkish prototypes

2.2.1 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.

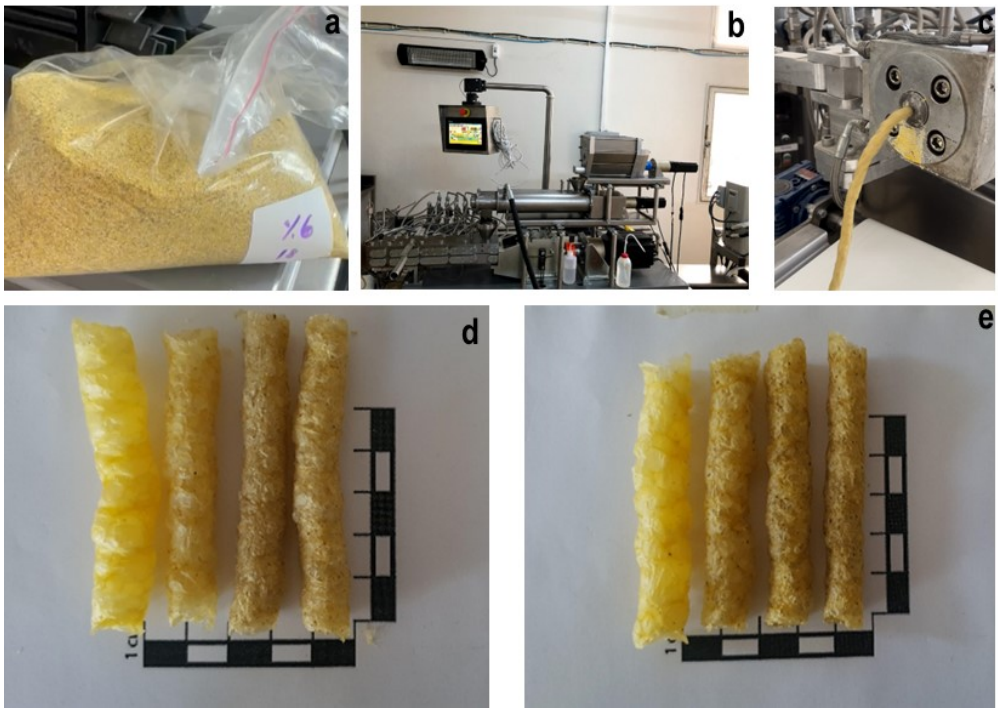
Methods

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^{j}
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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2.2.2 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).

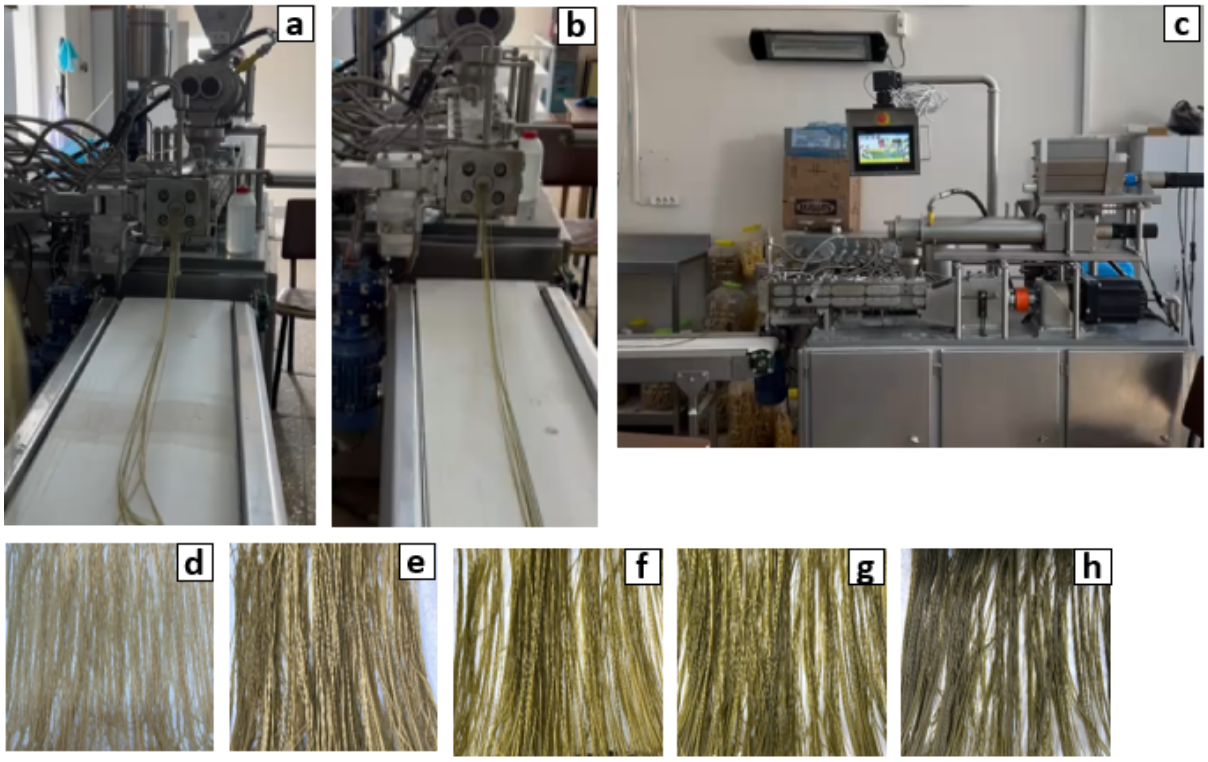
Methods

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*	Color a*	Color 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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2.2.3 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.

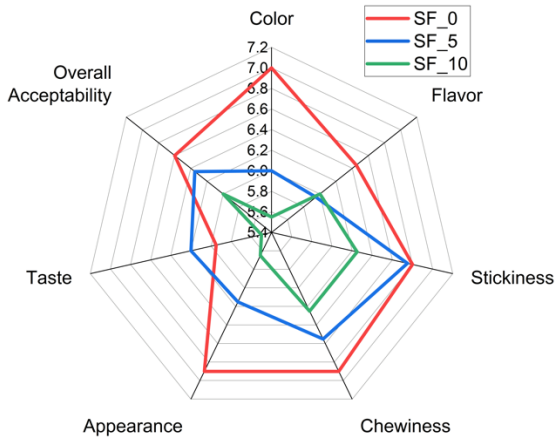
Texture properties of the noodles

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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2.3 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

2.3.1 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

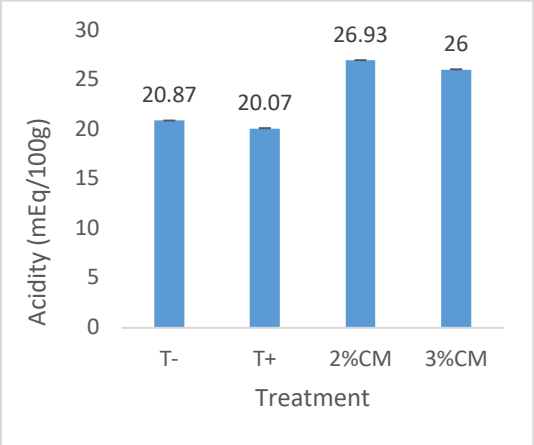
participants of various nationalities and African nationalities. Finally, a third hedonic tasting group was formed by a hotel kitchen service team. Thanks to these three groups, we were able to collect the opinions of different types of consumers.

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.

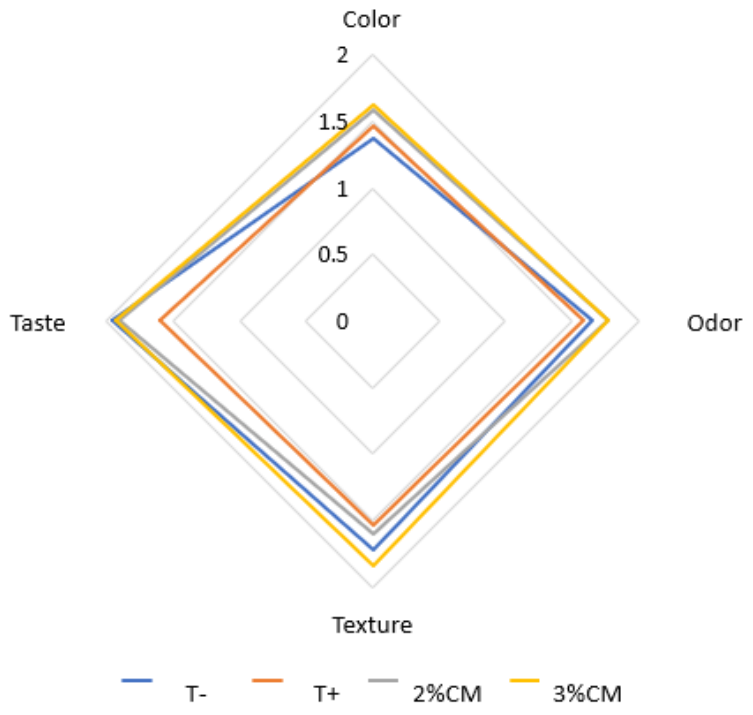
Recipe	Yeast and mold (UFC/g)
T-	6±2.46
T+	2.66±3.7
2%CM	0.33±1.15
3%CM	0,00±0

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



2.3.2 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.

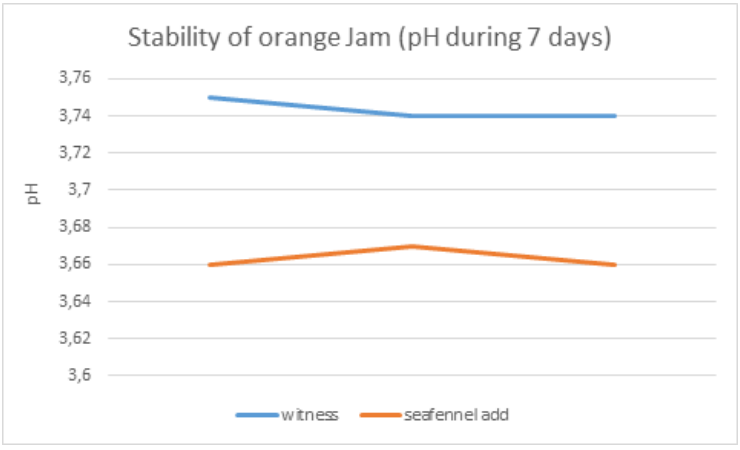
The first was a rating scale test to determine the intensity of the desired criteria (Color, Odor, Bitterness, Taste (bitter/acidic), Flavor and Texture). This test was carried out by rating on a scale with ten trained tasters. Following this test, two recipes were selected for hedonic evaluation.

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.



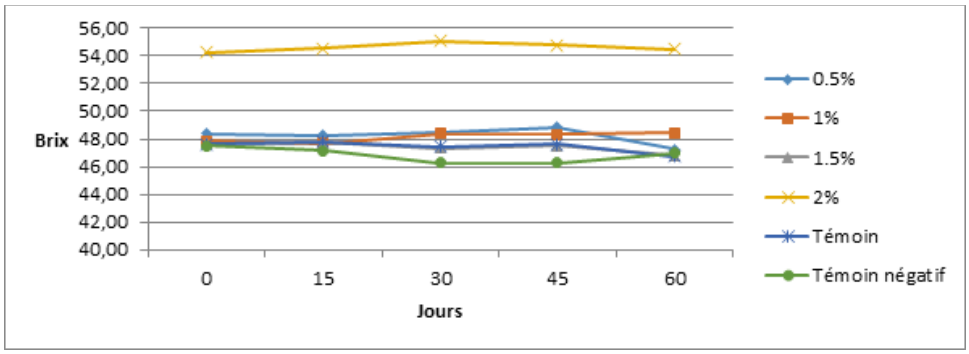
Physical characteristics of orange jam prototype

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

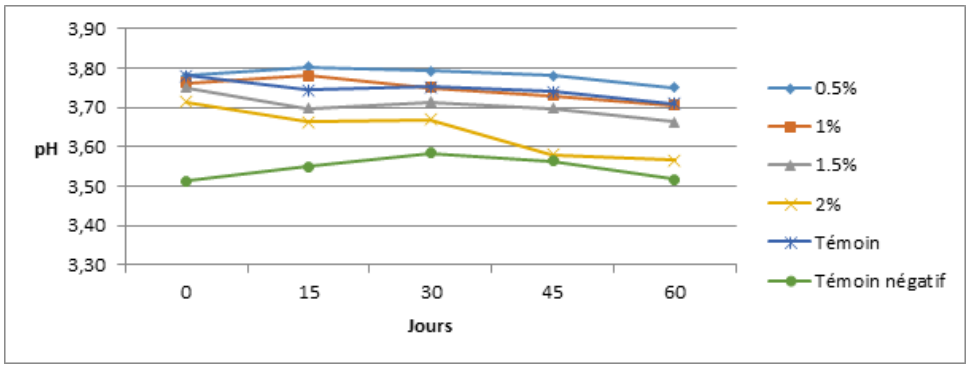
and in water activity from 0.89 to 0.77 for the different incorporations compared with the standard. These results were confirmed by the microbiological analyses, since all the samples were compliant.

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

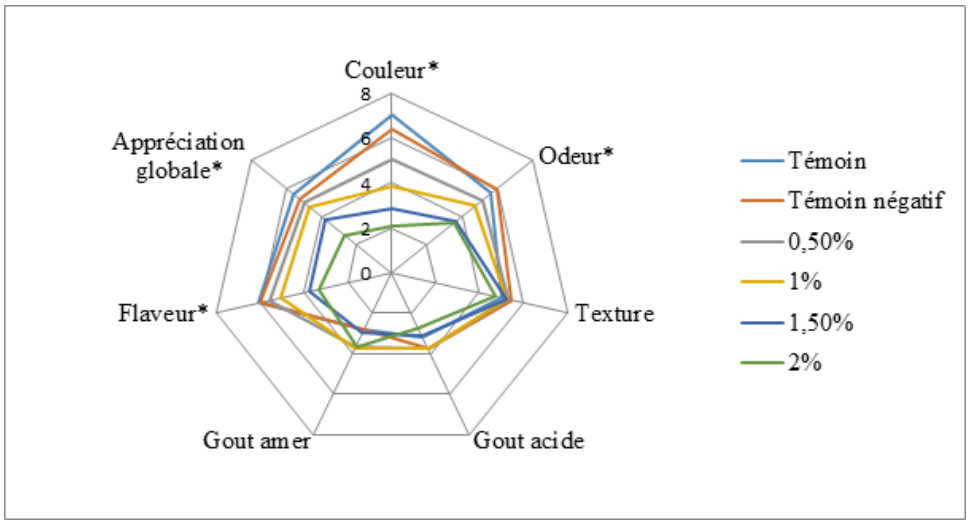


pH trends for various recipes.

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

2.3.3 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

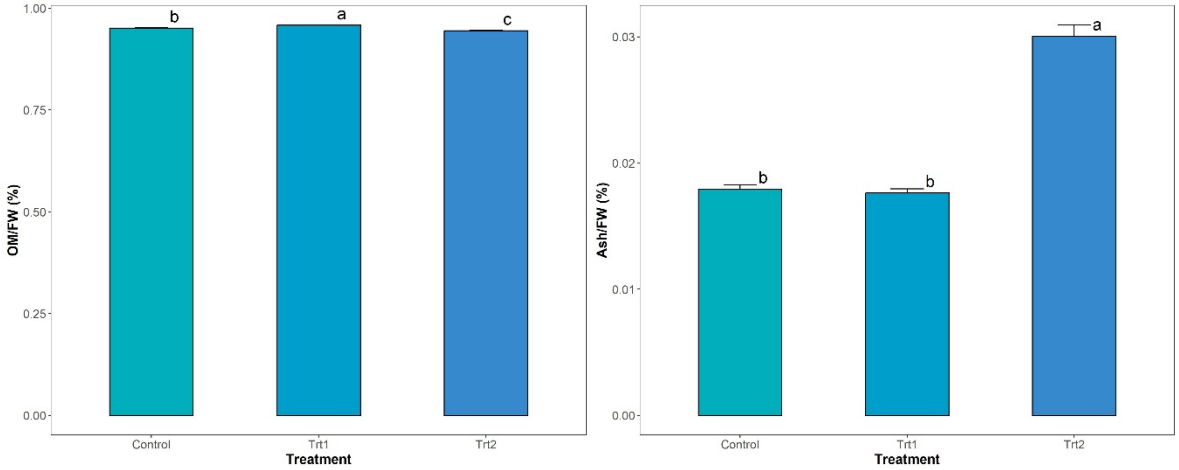
Sensory analysis

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%).

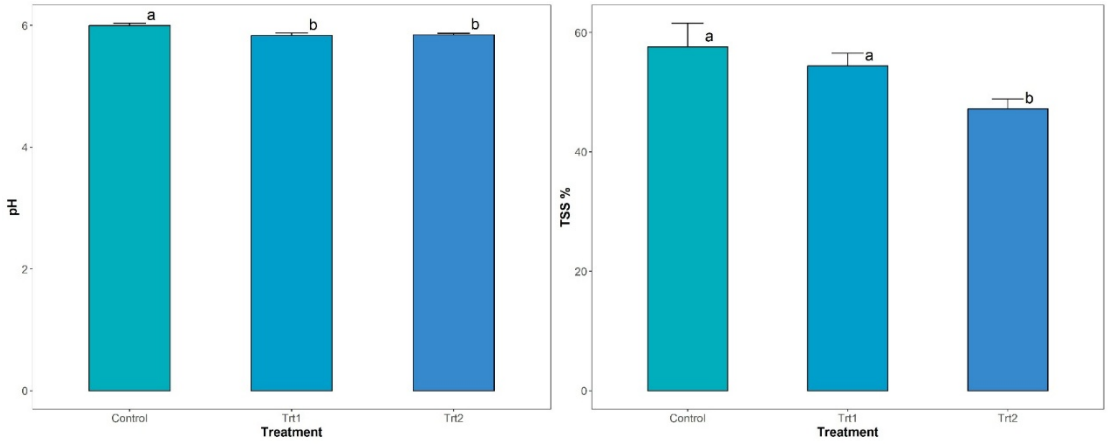


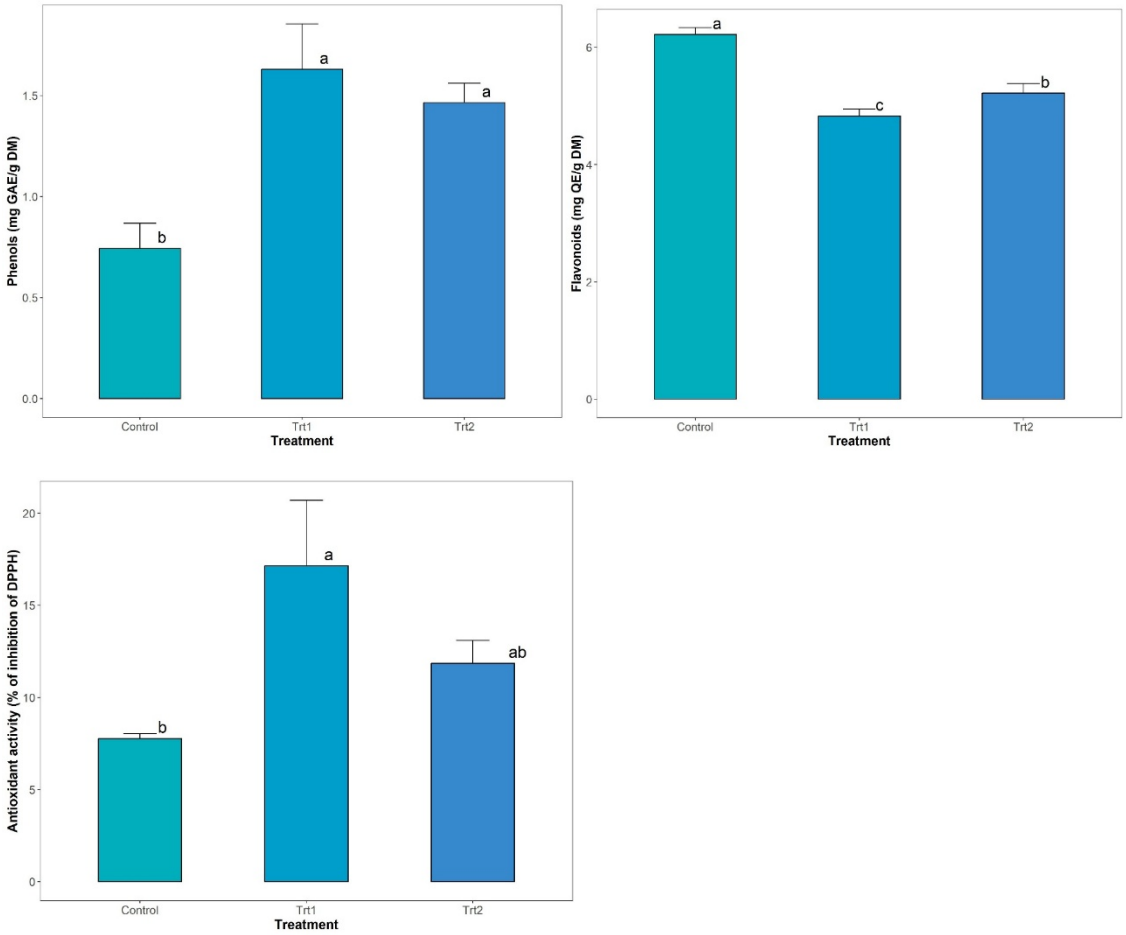
Biochemical analysis

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.