UNIVERSITÀ POLITECNICA DELLE MARCHE

Dipartimento di Scienze Agrarie, Alimentari ed Ambientali

Ph.D. program in Agriculture, Food and Environmental Sciences

XXXIV Cycle

Development of new organic functional preserves, sauces and spices from sea fennel (*Crithmum maritimum* L.) and other horticultural products

Ph.D. program coordinator:

Prof. Bruno Mezzetti

Supervisor:

Prof. Lucia Aquilanti

Ph.D. candidate:

Antonietta Maoloni

Academic year 2020/2021

Table of contents

Sommario

Il finocchio marino (*Crithmum maritimum* L.) è un'erba spontanea alofita facoltativa, caratterizzata dalla presenza di numerosi composti bioattivi, ampiamente diffusa nei paesi del Mediterraneo, il cui consumo è fortemente legato alla tradizione culinaria della regione Marche. Il progetto di questa tesi di dottorato è stato finalizzato alla valorizzazione di finocchio marino biologico coltivato nell'area del Parco del Conero, per la produzione di conserve, salse e spezie dall'elevato valore nutrizionale.

Il finocchio marino fresco è stato sottoposto a uno studio fitochimico per identificare nuovi composti con potenziale attività farmacologica. Successivamente, è stato impiegato, da solo o mescolato con olive verdi, per la produzione di conserve fermentate in salamoia, con l'inoculo di uno starter selezionato. Mentre, le dinamiche microbiche che si verificano nel corso di una fermentazione spontanea sono state studiate nel kimchi. Il finocchio marino è stato valutato come veicolo per la somministrazione di batteri probiotici, inoculando *Lactiplantibacillus plantarum* IMC 509 e SYNBIO® in una conserva acidificata a base di finocchio marino e valutandone la vitalità durante una conservazione refrigerata. Sono state inoltre prodotte due salse a base di finocchio marino e sono state sottoposte a test di shelf-life accelerata e microbial challenge test, per valutare la stabilità microbiologica delle salse e il rischio microbiologico associato alla potenziale sopravvivenza o crescita di *Staphylococcus aureus* e *Bacillus cereus*, dopo l'applicazione di pastorizzazioni convenzionali o blande, rispettivamente. Infine, sono stati valutati quattro differenti metodi di essiccazione per la produzione di spezie essiccate.

I risultati complessivamente ottenuti hanno confermato le grandi potenzialità del finocchio marino per la produzione di conserve, salse e spezie dall'elevato valore nutrizionale legate al territorio, con potenziali ricadute positive sul settore produttivo.

Abstract

Sea fennel (*Crithmum maritimum* L.) is a facultative halophyte wild herb, characterized by the presence of several bioactive compounds, widely diffused in Mediterranean countries, whose consumption is strongly linked to the culinary tradition of the Marche region. This Ph.D. thesis project was aimed at the exploitation of organic sea fennel cultivated in the Conero Natural Park area, for the production of high nutritional value preserves, sauces and spices.

Fresh sea fennel was subjected to a phytochemical study to identify new compounds with potential pharmacological activity. Subsequently, sea fennel sprouts, either alone or mixed with green olives, were exploited for the production of fermented preserves in brine, with the inoculation of a selected starter. Whereas, the microbial dynamics occurring during a spontaneous fermentation were studied in kimchi. Sea fennel was evaluated as a carrier for probiotic bacteria delivery, inoculating *Lactiplantibacillus plantarum* IMC 509 and SYNBIO® in an acidified sea fennel-based preserve and assessing their viability during a refrigerated storage. Furthermore, two sea fennel-based sauces were produced and have been subjected to accelerated shelf-life and microbial challenge tests to evaluate the microbial stability of the sauces and the microbial risk associated with the potential survival or growth of *Staphylococcus aureus* and *Bacillus cereus*, after the application of conventional or mild pasteurizations, respectively. Finally, four different drying methods for the production of dried spices were evaluated.

The overall collected results confirmed the great potential of sea fennel for the production of high nutritional value preserves, sauces and spices linked to the territory, with potential impact on the manufacturing sector.

1. Introduction

1.1. Sea fennel (*Crithmum maritimum* L.)

Crithmum maritimum L. is a perennial facultative halophyte wild herb widely diffused along the Mediterranean, Black Sea, European Atlantic coastal areas, growing mainly on rocky maritime cliffs, breakwaters and piers and occasionally on sandy shoreline, at 0 - 150 m above sea level (Cunsolo et al., 1993; Pateira et al., 1999; Renna, 2018; Tardío et al., 2016). The plant derives its name from the Greek "krithe (κριθη)", which means barley, for the similarity between its fruits and barleycorn, and from the Latin "mare", which means sea, for its habitat (Atia et al., 2011; Zafeiropoulou et al., 2021). *C. maritimum* L. is the only species ascribed to the genus *Crithmum* and belongs to the Apiaceae family (Amoruso et al., 2022; Atia et al., 2011). The plant is commonly known as sea fennel, rock samphire or crest marine and is locally named in Italy as finocchio marino, spaccasassi, erba di San Pietro, cretamo, critmo, salissia, basiggia and bacicci (Generalić Mekinić et al., 2018; Pavela et al., 2017).

C. maritimum L. is a highly branched herb, reaching a height of up to 30 to 60 cm, and characterized by a thick and gnarled root. The leaves are fleshy, glabrous, succulent and pinnate, with lanceolate leaflets between 2 and 5 cm long and 0.6 cm wide, usually acute. The flowers are mainly hermaphrodite, of greenish-white or yellowish color and grouped to form a compound umbel. The fruits are between 3 and 6 mm long, glabrous, ovoid-oblong, with 10 ridges. The flowering is between June and September, while the fruits ripening is between October and December (Atia et al., 2011; Renna, 2018; Tardío et al., 2016).

1.1.1. Medicinal and food use

C. maritimum has been used from long times both for culinary and medicinal purposes, being already mentioned in the first century AD in Naturalis historia of Pliny the Elder and in De materia medica of Dioscorides for its positive health effect against diseases of the urinary tract (Aliotta and Pollio, 1994; Touwaide et al., 1997).

The use of sea fennel in folk medicine is documented in several ethnopharmacology studies. Pickled aerial parts of the plant are reported to exhibit antiscorbutic, diuretic and digestive properties (Carrió and Vallès, 2012), while plant decoction is described as a remedy against whooping cough (Savo et al., 2011). Furthermore, effects in lever purification an against colic are reported for leaf decoction, while positive effect against inflammations of the urinary tract and prostate are described in relation to the consumption of decoction of aerial parts harvested before fructification (Cornara et al., 2009).

As the use in culinary is concerned, sea fennel aerial parts have been generally consumed in salads as fresh vegetable, sometimes blanched to soften, and traditionally pickled in vinegar or in brine in many Mediterranean countries (Cornara et al., 2009; Gras et al., 2021; Tardío et al., 2016). Della et al. (2006) reported the use of sea fennel preserved in vinegar as appetizer with different foods in Cyprus. In Italy *C. maritimum* is also used in the preparation of sauces (Guarrera, 2006), and eaten as condiment for fish recipes (Picchi and Pieroni, 2005), or boiled in water and vinegar and preserved in olive oil (Lucchetti et al., 2019) while, in Spain it is used for seasoning olives or homemade anchovies in brine (Tardío et al., 2016). In recent years, sea fennel has been exploited for the production of fermented preserves in brine both with (Özcan, 2000) and without (Özcan et al., 2019) the inoculation of lactic acid bacteria as starter cultures, and used in the preparation of dried spices (Giungato et al., 2019; Renna et al., 2017) as flavoring and/or coloring agent for different foods like “green tagliatelle in marinara style”, “spiced dome on puree of apple and purple carrot” and “gourmet beef in sea fennel dressing” (Renna and Gonnella, 2012).

1.1.2. Nutritional composition and phytochemistry

Sea fennel proximate composition and mineral content is reported in Table 1.1 from Tardío et al., (2016), moreover the plant is characterized by the presence of several bioactive compounds like vitamin C, ω-3 an ω-6 fatty acids, polyphenols and essential oils, which are responsible for a wide range of positive effect on human health (Generalić Mekinić et al., 2016).

Table1.1. Proximate composition and mineral content per 100 g of fresh leaves of *C. maritimum* L. (Tardío et al., 2016).

	Units	Average	Range	References
Energy (calculated value)	kcal	30	23 - 38	–
Moisture	g	86.8	47.7 - 88.6	1, 2, 3
Available carbohydrates	g	2.08	1.90 - 2.7	1
Dietary fibre	g	4.68	3.04 - 5.60	1
Proteins	g	2.23	1.7 - 2.8	1
Lipids	g	0.43	0.28 - 0.58	1, 4, 5
Ash	g	2.90	2.54 - 3.48	1
K	mg	252	198 - 343	1, 3, 6
Na	mg	464	185 - 636	1, 3, 6
Ca	mg	224	85 - 414	1, 3, 6
Mg	mg	76.6	57.4 - 97.0	1, 3, 6, 7
P	mg	21.5	16.0 - 24.0	3, 7
Fe	mg	2.29	1.09 - 3.70	1, 3, 6, 7
Cu	µg	120	95.0 - 151	1, 3
Mn	µg	990	432 - 1080	1, 3
Zn	µg	665	334 - 870	1, 3, 6

References: 1: Guil-Guerrero et al. (1996a); 2: Guil-Guerrero et al. (1997); 3: Guil-Guerrero et al. (1998); 4: Guil-Guerrero et al. (1996b); 5: Guil-Guerrero and Rodríguez García (1999); 6: Romojaro et al (2013); 7: Guil-Guerrero and Torija-Isasa (2002).

The concentration of polyphenols in sea fennel aerial parts is strongly affected by the life cycle stage and the growing condition of the plant, being present in higher amount before and at the beginning of flowering and when subjected to stress factors (Generalić Mekinić et al., 2018; Maleš et al., 2003; Martins-Noguerol et al., 2022a). Sea fennel polyphenolic fraction is mainly constituted by phenolic

acids and for the most part by chlorogenic acid, a compound displaying a broad range of beneficial health behaving as antioxidant, anti-inflammatory, antibacterial, neuroprotective, cardioprotective, and hepatoprotective agent (Meot-Duros and Magné, 2009; Naveed et al., 2018; Souid et al., 2021).

Sea fennel essential oils are generally characterized by the prevalence terpene hydrocarbons and oxygenated terpenes, while the exact composition within each class of compounds is related to geographic and environmental factors. Some of the compounds commonly detected are γ-terpinene, sabinene, β-phellandrene and thymol methyl ether (Alves-Silva et al., 2020; Burczyk et al., 2002; Jallali et al., 2014; Zafeiropoulou et al., 2021). Sea fennel essential oils exhibited antimicrobial, antioxidant, and insecticide properties (Houta et al., 2015; Polatoğlu et al., 2016).

Furthermore, the plant is characterized by high concentration of vitamin C, which determined its consumption as a traditional remedy against scurvy by sailors and fishermen (Franke, 1982), and by high concentration in ω-3 and ω-6 fatty acids, mainly linoleic acid (C18 : 2 ω6) and α-linolenic acid (C18 : 3 ω3) (Guil-Guerrero and Rodríguez-García, 1999). Clinical trials and epidemiological studies suggested a positive health effect of α-linolenic acid in the prevention of coronary heart disease (Bemelmans et al., 2000; De Lorgeril and Salen, 2004).

In addition, Burczyk et al. (2002) identified the coumarins scopoletin and scoparone in sea fennel. Several biological effects have been attributed to coumarins including antimicrobial, anti-coagulant and photosensibilizing properties, spasmolytic action and anticarcinogenic activity.

Finally, falcarindiol were isolated in the apolar extract of sea fennel leaves. This compound displays different biological effect including antimutagenic, anti-inflammatory activities (Meot-Duros et al., 2010).

1.1.3. Physiological response to abiotic stress and cultivation

C. maritimum L. is generally defined as a facultative halophyte herb, because of its capability in growing under salinity stress preserving tissue hydration, as halophyte wild greens, without requiring salt to reach its maximum growth (Ben Amor et al., 2005; Ben Hamed et al., 2004).

Despite the widespread diffusion of the plant along coastal areas in proximity of the sea, seed germination is reported to be adversely effected by salinity levels in a salt-specific way, with $MgCl_2$ exerting the higher adverse action followed by $MgSO_4$, Na_2SO_4 and NaCl. More in detail, magnesium salts exert osmotic and ionic effects, while sodium salts display an osmotic effect; NaCl concentrations higher then 50 mM inhibit seed germination. Given these premises, seeds could possibly remain viable in the presence of high salt concentrations, and start to germinate in spring, when a lower salinity level is present in the soil thanks to the winter precipitations. During the vegetative stage, NaCl concentration affects the plant growth, exerting stimulatory or inhibitory effects for lower (50 mM) and higher (300 mM) concentrations, respectively. Sea fennel salt tolerance is attributed to the capability in accumulating Na^+ and Cl^- ions inside its vacuoles, preserving tissue hydration, and to the activity of antioxidant systems in protecting the plant against Na^+ and Cl^- toxicity. Sea fennel morphological characteristics are also adapted to growth in saline environments, while guaranteeing a good hydration of tissues and the prevention of water loss (Atia et al., 2011; Renna, 2018).

In the current scenario, characterized by fresh water scarcity and soil salinization, halophyte wild greens have been suggested as a “cash crop” in the “saline agriculture” (Martins-Noguerol et al., 2022b), and gained great interest in the food industry, both for their capability to grow with high salt concentrations and low water requirement, and their nutritional value (Agudelo et al., 2021; Koyro et al., 2011). Moreover, halophytes have a remarkable economic potential for their capability in soil remediation, by enhancing nutrient availability, reducing sodium salt concentration, and establishing

environmental conditions suitable for the fodder glycophytes growth (Arya et al., 2019). *C. maritimum* is a native plant in Mediterranean coastal areas well adapted to the local agroecosystems, whose cultivation requires lower inputs in terms of water, agrochemicals and fertilizers, promoting the implementation of a sustainable agriculture system. This plant naturally grows in shallow soils characterized by low organic matter content, and under water stress, high solar irradiation and high temperatures (Corrêa et al., 2020; Zenobi et al., 2021).

1.2. Aim of the thesis

This Ph.D. thesis project was aimed at the exploitation of organic sea fennel (*Crithmum maritimum* L.), cultivated in a plot of land situated in the Conero Natural Park (Marche, Central Italy) area, for the production of high value preserves, sauces and spices. This research was funded by the Marche Region under the project "POR Marche FSE 2014/2020 Progetto "Dottorato Innovativo"- Borse di studio per Dottorato di ricerca per l'innovazione del sistema regionale", and aimed at the application of innovative technologies for the valorization of sea fennel, whose consumption is strongly linked to the Regional territory (Lucchetti et al., 2019).

A phytochemical study on sea fennel was performed aiming at identifying new compounds with potential pharmacological activity. Methanolic extracts from the whole sprouts, leaves and stems were analyzed and characterized through High Performance Thin Layer Chromatography (HPLTC), High Performance Liquid Chromatography (HPLC) and Liquid Chromatography coupled with Mass Spectrometry (LC-MS), to identify the whole range of phenolic constituents.

Therefore, the objective of this thesis was accomplished by the application of four main technologies: i) starter-induced fermentation; ii) inoculation of probiotic lactic acid bacteria iii) mild in-container pasteurization for the production of shelf-stable sauces; iv) drying.

Sea fennel sprouts, either alone or mixed with green olives (*Olea europaea* L. cv. Ascolana tenera) were exploited, at the beginning, for the production of fermented preserves in brine, with the inoculation of lactic acid bacteria selected according to pro-technological and sensory criteria. Prior to the sea fennel fermentation, a preliminary study on microbial diversity and dynamics on a traditional fermented vegetal food, kimchi, was performed (Jung et al., 2014).

Subsequently, the viability of two commercially available probiotic formulations, namely *Lactiplantibacillus plantarum* IMC 509 and SYNBIO® combination (1:1) of *Lacticaseibacillus rhamnosus* IMC 501® and *Lacticaseibacillus paracasei* IMC 502®, was assessed during a prolonged

refrigerate storage in a sea fennel-based preserve, to evaluate the suitability of sea fennel as a carrier for probiotic bacteria delivery.

Furthermore, two innovative shelf-stable sea fennel-based sauces were produced and have been subjected to accelerated shelf-life (ASLT) and microbial challenge (MCT), using *Staphylococcus aureus* and *Bacillus cereus* as test microorganisms, to evaluate: i) the microbial stability of the sauces after the application of conventional in-container pasteurizations ($F_{85}^{7} = 2\ min$ or $F_{95}^{7} = 5\ min$); ii) the microbial risk associated to the potential survival or growth of the test microorganisms, after the application of mild in-container pasteurizations ($F_{75}^{7} = 1 - 2\ min$).

Finally, four different drying methods, namely room temperature drying, oven drying, microwave drying and freeze-drying, were assessed for the production of sea fennel dried spices.

This project was conducted with the collaboration of Rinci S.r.l. (Castelfidardo, Ancona, Italy), Synbiotec S.r.l. (Camerino, Italy) and Prof. Rudolf Bauer of Karl-Franzens-Universität (Graz, Austria).

2. The microbial diversity of non-Korean kimchi as revealed by viable counting and metataxonomic sequencing

The content of this section was redrafted from the published paper: Maoloni, A., Ferrocino, I., Milanović, V., Cocolin, L., Corvaglia, M. R., Ottaviani, D., Bartolini, C., Talevi, G., Belleggia, L., Cardinali, F., Marabini, R., Aquilanti, L., Osimani, A., 2020. The microbial diversity of non-Korean kimchi as revealed by viable counting and metataxonomic sequencing. Foods. 9(11), 1568, available online at https://doi.org/10.3390/foods9111568, and licensed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). Publisher' s note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

The author contribution is subdivided as follows: Conceptualization, A.O. and L.A.; software, I.F.; formal analysis, A.M., I.F., V.M., M.R.C., D.O., C.B., G.T., L.B., F.C. and R.M.; investigation, A.O.; resources, A.O. and L.A.; data curation, A.M.; writing - original draft preparation, L.A., A.O. and A.M.; writing - review and editing, A.O. and L.A.; funding, L.A.; visualization, L.C. All authors have read and agreed to the published version of the manuscript. The author names are abbreviated as follows: Maoloni, A. (A.M.), Ferrocino, I. (I.F.), Milanović, V. (V.M.), Cocolin, L. (L.C.), Corvaglia, M. R. (M.R.C.), Ottaviani, D. (D.O.), Bartolini, C. (C.B.), Talevi, G. (G.T.), Belleggia, L. (L.B.), Cardinali, F. (F.C.), Marabini, R. (R.M.), Aquilanti, L. (L.A.), Osimani, A. (A.O.).
The complete content of this paper was included in this section.

2.1. Introduction

Vegetable-based traditional fermented foods are manufactured worldwide but in some countries, they represent an important part of the daily diet. The most popular fermented vegetables include mudhika produced in Africa using cassava (*Manihot esculenta*) (Misihairabgwi and Cheikhyoussef, 2017); caxiri, tarubà, and yakupa produced in Latin America through cassava fermentation (Ramos and Schwan, 2017); and sauerkraut, mainly produced in Central and Eastern European countries with shredded and salted cabbage (Majcherczyk and Surówka, 2019). Moreover, among the fermented vegetables that are recognized worldwide as unique kickshaws, paocai, jiangshui, miso, natto, tempeh, and kimchi represent further well-known masterpieces of Asian traditions (Jaehae et al., 2015).

Among vegetable-based fermented foods typically produced in Asia, kimchi is recognized as the flagship food of Korea (Patra et al., 2016). Such a preparation, based on the fermentation of cabbage and/or other vegetables, was probably invented around 4000 years ago (Jang et al., 2015). Moreover, during Confucianism, kimchi constituted the core of the ancestral worship table,

together with rice and soup (Jaehae et al., 2015). In 2013, the United Nations Educational, Scientific and Cultural Organization (UNESCO) recognized the cultural importance of kimchi and inscribed this Korean traditional preparation of vegetables in the Representative List of The Intangible Cultural Heritage of Humanity.

Kimchi is usually produced using cabbage, radish, and cucumber as main ingredients; moreover, different seasonings, including salts, garlic, leek, red pepper powder, and ginger are used in accordance with local traditions (Jung et al., 2014). Actually, more than 200 varieties of kimchi are produced, each characterized by peculiar biochemical, nutritional, and sensory features, which are greatly affected by the raw ingredients (e.g., onion, Korean lettuce, sesame leaves, sweet potato vines, *Allium hookeri* Thwaites, deodeok, and water dropwort), the preparation method, and even region, seasonality, and cultural traditions (Jung et al., 2014; Lee et al., 2017a). Baechu (Chinese cabbage) kimchi is the most popular variety. Traditionally, the secret of making kimchi passed through generations from mothers to daughters (Chung et al., 2017). However, as an effect of globalization, individuals and companies worldwide can now access the recipe for kimchi production. It is estimated that the worldwide market for kimchi will reach USD 3850 million in 2024, from USD 3000 million in 2019. Though in Italy kimchi is still far from being popular, various kimchi manufacturers and kimchi suppliers have been operating since the late 2010s.

Mature kimchi possesses unique and flavorful sensory traits, consisting of a combination of fresh, sour, spicy, hot, and sweet tastes (Lee et al., 2017b). It is generally low in calories and rich in vitamins (A, C, and B complex), various phytochemicals, minerals (calcium, iron, potassium), and dietary fiber (Cheigh, 1999).

From a microbiological point of view, mature kimchi is a closed ecosystem where complex microbial interactions occur. Kimchi fermentation is an anaerobic process during which the interactions between the microbiota and the raw materials lead to the production of microbial

metabolites (e.g., mannitol, lactate, acetate, ethanol, exopolysaccharides, etc.) that strongly characterize the sensory traits of the product (Jung et al., 2011). Of note, the production of organic acids from the carbohydrates' metabolism results in a pH drop to 4.2-4.0 (Lee et al., 2015a), which in turn contributes to the palatability and safety of this fermented vegetable-based food.

To the authors' knowledge, most of the microbiological studies to date carried out on kimchi deal with the analysis of Korean manufactures, and no scientific data are currently available on non-Asian produced kimchi. A past taxonomic study carried out on Korean kimchi reported the prevalence of lactic acid bacteria as the main fermenting microorganisms, including *Leuconostoc*, *Lactobacillus*, *Lactococcus*, *Pediococcus,* and *Weissella* species (Jung et al., 2011). More recently, a large-scale targeted metagenomic investigation focusing on the bacterial ecology of kimchi sampled during its natural fermentation showed the occurrence of a more complex and dynamic ecosystem, with the most adapted species prevailing at the end of the fermentation process (Lee et al., 2017b).

Kimchi also represents an important source of candidate bacterial probiotics and protechnological strains that, due to their robustness, are very well adapted to the unfavorable conditions occurring in this food product (Torres et al., 2020). A few lactic acid bacteria strains so far isolated from kimchi, such as *Lactobacillus acidophilus* KFRI342, *Lactococcus lactis* KC24, *L. plantarum* LPpnu, and *Leuconosctoc mesenteroides* LMpnu showed a potential anticancer activity (Chang et al., 2010; Lee et al., 2015b, 2016), thus suggesting a possible beneficial effect of the consumption of kimchi on human health.

Unlike bacteria, there is a paucity of available data about the fungal dynamics occurring during kimchi fermentation. As recently reported by Kim et al. (2020), in the early stages of fermentation low loads of yeasts can colonize the fermenting vegetable substrate, with a positive impact on the sensory traits of kimchi due to the production of fruity aromatic compounds and a mitigation of

kimchi's acidic and moldy flavors (Kim et al., 2020). However, as reported by the same authors, high loads of yeasts (e.g., yeasts forming white colonies) occurring on kimchi*'s* surface, especially during the late phase of fermentation, can negatively affect the quality of the end product (Kim et al., 2020).

Given the above premises, the main aim of the present study was to explore the bacterial and fungal dynamics occurring during the natural fermentation of kimchi handcrafted by an artisan Italian manufacturer through conventional microbiological analyses (viable counting) and metataxonomic sequencing.

2.2. Materials and methods

2.2.1. Kimchi production

Two independent production batches of kimchi, referred to as batch 1 and batch 2, were prepared by a Korean staff at a small artisan producer located in Santa Maria Nuova (Ancona, Italy) using the following ingredients: Chinese cabbage 60.5 (g 100 g^{-1}), turnip 13.5 (g 100 g^{-1}), water 12.0 (g 100 g^{-1}),onion 4.0 (g 100 g^{-1}), pepper chili powder 2.0 (g 100 g^{-1}), red pepper 2.0 (g 100 g^{-1}), garlic leaves 2.0 (g 100 g^{-1}), spring onion 1.0 (g 100 g^{-1}), carrot 1.0 (g 100 g^{-1}), ginger 0.5 (g 100 g^{-1}), sucrose 1.0 (g 100 g^{-1}), and salt 0.5 (g 100 g^{-1}). All the vegetables were purchased from a local grocer. Kimchi was produced according to the traditional Korean process described as follows. Briefly, the outer leaves were removed from the Chinese cabbage, trimmed, and then steeped in 10% (w v^{-1}) NaCl for approximately 16-18 h at room temperature. The sauce used for cabbage dressing was obtained by mixing chopped vegetables (turnip, onion, carrot, ginger, spring onion, red peppers, and garlic leaves) with chili pepper powder, water, sugar, and salt. Thereafter, the sauce was storedunder refrigerated conditions (5 °C) for approximately 16-18 h. The cabbage was rinsed, and the excess water drained. The sauce was carefully spread over the cabbage leaves. Kimchi was fermented at approximately 5 ± 1 °C for 57 days, up until a fixed pH of 4.2 was

reached. Kimchi fermentation was carried out in a plastic box hermetically sealed with a lid. Samples of kimchi (Figure 2.1) were collected with sterile spoons immediately after preparation (t_0) and after 2, 5, 15, 36, 43, 50, and 57 days of fermentation. The samples were transported to the laboratory under refrigerated conditions (4 °C) and processed immediately after arrival.

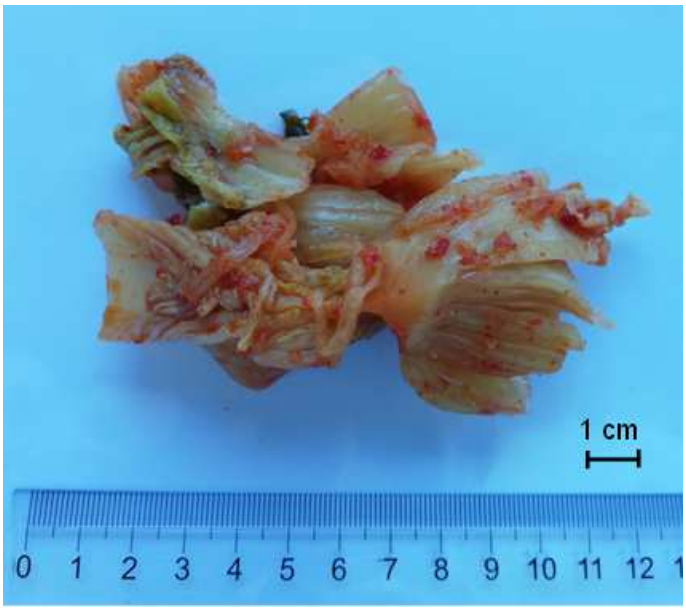


Figure 2.1. Ready-to-eat cabbage-origin kimchi.

2.2.2. pH determination

pH measurements were performed at the core of each kimchi sample as previously described by Belleggia et al. (2020).

2.2.3. Microbial counts

Ninety mL of sterile water containing 1 g L^{-1} bacteriological peptone were added to 10 g aliquots of each sample. The suspensions were homogenized for 2 min at 230 rpm in a Stomacher machine (400 Circulator, International PBI, Milan, Italy). Tenfold serial dilutions were prepared for the enumeration of: (i) mesophilic aerobic bacteria on Plate Count Agar incubated at 30 °C for 48 h; (ii) total mesophilic halophilic aerobic bacteria on Plate Count Agar added with 8% NaCl and incubated at 30 °C for 7 days; (iii) presumptive mesophilic lactobacilli on De Man,

Rogosa, and Sharpe (MRS) agar incubated at 30 °C for 48 h; (iv) presumptive mesophilic lactococci on M17 agar incubated at 22 °C for 72 h; (v) halophilic lactobacilli on MRS agar added with 8% NaCl and incubated at 30 °C for 7 days; (vi) halophilic lactococci on M17 agar added with 8% NaCl and incubated at 22 °C for 10 days; (vii) Enterobacteriaceae on Violet Red Bile Glucose Agar (VRBGA) incubated at 37 °C for 24 h; (viii) Pseudomonadaceae enumerated on Pseudomonas Agar Base (PAB) supplemented with Cetrimide-Fucidin-Cephalosporin (CFC) selective supplement (VWR International, Milan, Italy) incubated at 30 °C for 24-48 h; (ix) yeasts counted on Rose Bengal Chloramphenicol Agar (RBCA) incubated at 25 °C for 72-96 h; (x) halophilic yeasts counted on RBCA agar added with 8% NaCl and incubated at 25 °C for 72 h. MRS agar was supplemented with cycloheximide (250 mg L-1) to inhibit the growth of eumycetes.

The results of viable counts were reported as mean value of two biological and three technical replicates expressed as the Log of colony-forming units (cfu) per gram of sample ± standard deviation. Finally, the presence/absence of *Listeria monocytogenes* and *Salmonella* spp. was determined using a MINI VIDAS (Vitek Immunodiagnostic Assay System) apparatus (Biomerieux, Marcy l'Etoile, France) as previously described (Belleggia et al., 2020; Haouet et al., 2017).

2.2.4. RNA extraction and cDNA synthesis

The 1.5 mL sample homogenates (10^{-1} dilution), prepared as described in Section 2.2.3, were centrifuged at 16,000 rpm for 10 min to obtain cell pellets subsequently covered with RNAlater Stabilization Solution (Ambion, Foster City, CA, USA) and stored at -80 °C until the extraction of RNA using E.Z.N.A. Bacterial RNA Kit (Omega Bio-tek, Norcross, GA, USA). The quantity, purity, and integrity of the extracted RNAs were checked as previously described by Garofalo et al. (2017). cDNA synthesis was performed using SensiFAST cDNA Synthesis Kit for

RT-qPCR (Bioline, London, UK).

2.2.5. Metataxonomic sequencing and bioinformatics analysis

cDNA was used for the amplification of bacterial 16S rRNA (V3/V4 region) (Klindworth et al., 2013) and fungal 26S rRNA genes (D1 domain) (Mota-Gutierrez et al., 2019). The sequencing of the purified PCR amplicons was performed in a MiSeq instrument in a 2 x 250 bp configuration, while QIIME v. 1.9 (Caporaso et al., 2010) was used for the analysisof the obtained reads. For bacteria, after Operational Taxonomic Units (OTUs) clustering at 99% of similarity, centroid sequences were mapped against the Greengenes 16S rRNA gene database, while for eumycetes the in-house database from Mota-Gutierrez et al. (2019) was used. Taxonomic assignments were double-checked using BLAST suite tools. Chloroplast and mitochondria sequences were removed from the data sets. The OTUs table was rarefied at the lowest number of sequence/samples displaying the highest taxonomic resolution.

2.2.6. Data analysis

The alpha diversity index was calculated by the VEGAN package of R. Diversity index, and OTUs table were used in R to find statistically significant differences in the samples as a function of the fermentation time. Principal Component Analysis (PCA), aimed at exploring relationships between experimental variables and detecting possible sample clusters, and one-way ANOVA analysis, used to analyze the effect of ripening time on the dependent variables for each batch separately, were performed as described by Belleggia et al. (2020).

2.3. Results

2.3.1. pH determination

The results of pH measurements are shown in Figure 2.2. The values detected in the two analyzed batches had a similar trend, although kimchi produced in batch 1 was characterized by a faster

pH reduction than batch 2. In more detail, at t_0, the pH values were 5.44 ± 0.01 and 5.22 ± 0.06 for batch 1 and batch 2, respectively. A significant drop in pH was observed between t_{30} and t_{36} for batch 1, whereas a more progressive reduction was observed in batch 2, where a drop in pH was detected between t_{36} and t_{43}. At t_{57} no significant differences were detected between the pH values of both batches, which tested at 3.99 ± 0.01 and 4.17 ± 0.01 for batch 1 and batch 2, respectively.

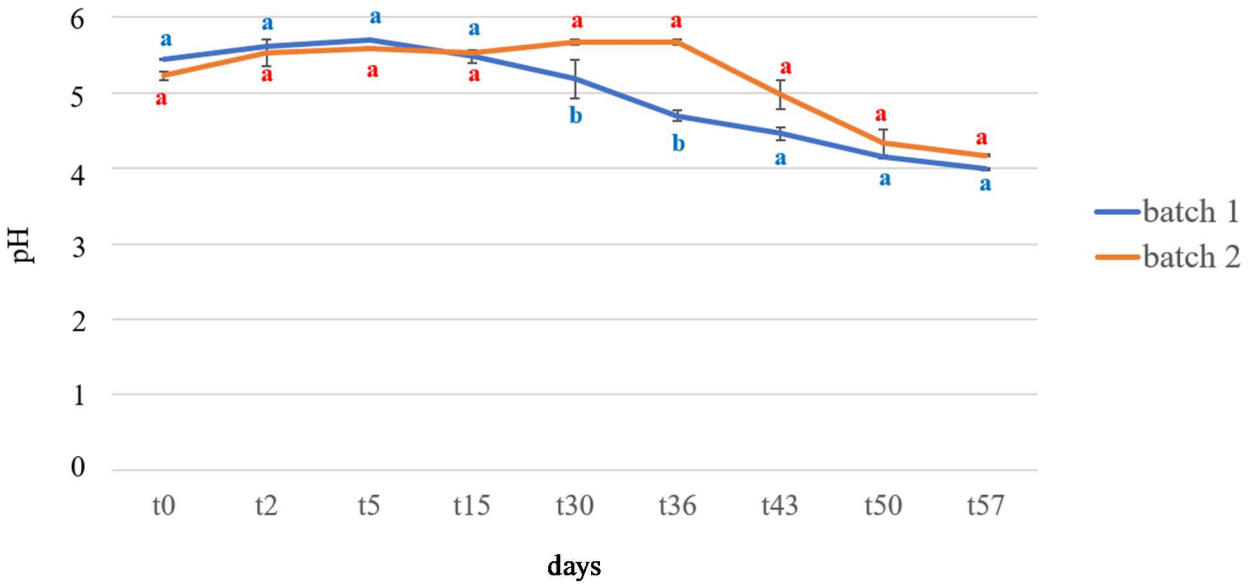


Figure 2.2. Results of pH measurements of two kimchi manufactures (batch 1 and batch 2) during fermentation. Values are expressed as means ± standard deviation. For each sampling time, means with different letters are significantly different ($p < 0.05$).

2.3.2. Microbiological analyses

The results of viable counts performed on the two batches of kimchi are reported in Table 2.1. In more detail, the counts of mesophilic aerobic bacteria showed the same trend in both batches, with no significant differences between them. For halophilic mesophilic aerobic bacteria, as well, at t_{57} no significant differences in both the analyzed batches were observed. Regarding presumptive lactobacilli and presumptive halophilic lactobacilli, a progressive increase in the load of these microorganisms was seen during fermentation; the highest counts were observed from t_{36} to t_{50} in batch 1 and from t_{43} to t_{57} in batch 2. Regarding presumptive lactococci and presumptive halophilic

.

Table 2.1. Results of viable counting (Log cfu g^{-1}) of bacteria and eumycetes in kimchi during fermentation.

Batch	Sampling time (days)	Mesophilic aerobic bacteria	Halophilic mesophilic aerobic bacteria	Presumptive lactobacilli	Presumptive halophilic lactobacilli	Presumptive lactococci	Presumptive halophilic lactococci	Enterobacteriaceae	Yeasts	Halophilic yeasts	Pseudomonadaceae
1	t0	5.2±0.1 [a]	5.3±0.1 [a]	3.5±0.1 [b]	3.5±0.1 [b]	5.0±0.1 [a]	4.9±0.0 [a]	1.7±0.3 [b]	1.3±0.0 [a]	< 1.0 [b]	4.5±0.2 [a]
	t2	5.3±0.0 [a]	5.3±0.1 [a]	3.6±0.5 [b]	3.5±0.5 [b]	5.0±0.1 [a]	5.1±0.1 [a]	2.3±0.6 [b]	1.4±0.1 [a]	1.4±0.1 [a]	5.2±0.1 [a]
	t5	5.2±0.0 [a]	5.2±0.0 [a]	4.2±0.0 [b]	4.2±0.0 [b]	5.0±0.1 [a]	5.1±0.2 [a]	3.5±0.5 [a]	1.1±0.1 [a]	1.2±0.3 [a]	4.8±0.3 [a]
	t15	5.3±0.1 [a]	5.3±0.0 [a]	4.8±0.2 [b]	4.8±0.1 [b]	5.2±0.0 [a]	4.8±0.1 [a]	3.4±0.2 [a]	< 1.0 [b]	< 1.0 [b]	5.1±0.1 [a]
	t36	6.3±0.1 [a]	5.9±0.2 [a]	6.3±0.0 [a]	6.2±0.2 [a]	5.0±0.2 [a]	4.9±0.2 [a]	< 1.0 [c]	< 1.0 [b]	< 1.0 [b]	< 1.0 [b]
	t43	5.6±0.0 [a]	5.3±0.0 [a]	6.3±0.1 [a]	6.4±0.1 [a]	5.0±0.2 [a]	5.1±0.1 [a]	< 1.0 [c]	< 1.0 [b]	< 1.0 [b]	< 1.0 [b]
	t50	5.5±0.0 [a]	5.4±0.0 [a]	6.2±0.2 [a]	5.6±0.1 [a]	4.9±0.3 [a]	5.0±0.1 [a]	< 1.0 [c]	< 1.0 [b]	< 1.0 [b]	< 1.0 [b]
	t57	5.4±0.0 [a]	5.3±0.0 [a]	4.6±0.1 [b]	4.7±0.1 [b]	5.0±0.2 [a]	5.0±0.1 [a]	< 1.0 [c]	< 1.0 [b]	< 1.0 [b]	< 1.0 [b]
2	t0	5.7±0.3 [a]	5.6±0.2 [a]	2.1±0.2 [c]	2.4±0.0 [b]	5.5±0.3 [a]	5.4±0.1 [a]	2.8±0.2 [a]	2.5±0.1 [a]	1.9±0.0 [a]	4.9±0.1 [a]
	t2	5.3±0.1 [a]	5.3±0.0 [a]	1.5±0.1 [c]	1.4±0.2 [b]	5.2±0.1 [a]	5.1±0.2 [a]	2.0±0.2 [a]	2.1±0.0 [a]	1.7±0.0 [a]	4.2±0.0 [a]
	t5	5.2±0.1 [a]	5.2±0.1 [a]	1.2±0.0 [c]	1.2±0.1 [b]	5.0±0.1 [a]	4.8±0.1 [a]	1.8±0.1 [a]	1.5±0.2 [b]	1.0±0.2 [b]	4.9±0.0 [a]
	t15	5.1±0.1 [a]	4.8±0.1 [a]	2.0±0.1 [c]	1.5±0.1 [b]	4.9±0.0 [a]	4.4±0.0 [a]	2.4±0.3 [a]	1.0±0.0 [b]	< 1.0 [b]	4.9±0.0 [a]
	t36	5.1±0.1 [a]	5.1±0.2 [a]	4.8±0.7 [b]	2.5±0.8 [b]	4.8±0.0 [a]	4.3±0.4 [a]	1.6±0.6 [a]	< 1.0 [c]	< 1.0 [b]	< 1.0 [b]
	t43	4.9±0.0 [a]	4.8±0.2 [a]	7.3±0.2 [a]	4.2±0.3 [a]	4.5±0.3 [a]	4.4±0.3 [a]	< 1.0 [c]	< 1.0 [c]	< 1.0 [b]	< 1.0 [b]
	t50	4.9±0.0 [a]	4.7±0.0 [a]	7.3±0.0 [a]	5.0±0.4 [a]	4.6±0.1 [a]	4.6±0.0 [a]	< 1.0 [c]	< 1.0 [c]	< 1.0 [b]	< 1.0 [b]
	t57	4.9±0.1 [a]	4.9±0.1 [a]	6.4±0.5 [a]	4.3±0.1 [a]	4.2±0.1 [a]	4.2±0.1 [a]	< 1.0 [c]	< 1.0 [c]	< 1.0 [b]	< 1.0 [b]

Note: cfu, colony-forming units; Values are expressed as means standard deviation; For each batch, within each column, means with different superscript letters are significantly different ($p < 0.05$).

lactococci, the counts in samples of batches 1 and 2 showed no significant differences at t_{57}. Low Enterobacteriaceae counts were detected, with no significant differences between the two batches from t_{43} to t_{57}. As for Pseudomonadaceae, from t_{36} to t_{57}, they were <1.0 Log cfu g^{-1} in both batches. Regarding yeasts and halophilic yeasts, counts < 1.0 Log cfu g^{-1} were detected in both batches at t_{57}. Finally, *L. monocytogenes* or *Salmonella* spp. were never detected in 25 g of product, irrespective of the sampling time and the production batch.

2.3.3. Microbiota diversity

The total number of sequences used reach 94,353 reads, with an average value of 5585 reads/sample and a mean sequence length of 460 bp. Sample coverage calculation showed a satisfactory coverage for all the samples (>96%), whereas alpha diversity index did not show any significant difference as a function of the batch. Principal component analysis based on OTU table revealed a clear separation of the samples as a function of the batch (Figure 2.3, panel A). Samples analyzed at the beginning of the fermentation clustered together and were well separated from the samples at the end of this process (Figure 2.3, panel C). The microbiota composition at the highest taxonomic level (Figure 2.4) showed that, especially in batch 1, *Leuconostoc kimchii* was the dominant OTU after five days of fermentation (reaching almost 80% of the relative abundance up until the end of fermentation). Moreover, in batch 2, *Leuconostoc kimchii* was the dominant OTU only in the last two sampling points (reaching 90% of the relative abundance). Several spoilage microorganisms were identified in both the batches.

In more detail, the presence of *Erwinia* spp. tohether with *Pseudomonas veronii*, *Pseudomonas viridiflava*, *Rahnella aquatilis,* and *Sphingomonas* spp. was observed during the first 15 days of fermentation. *Erwinia* spp. was predominant in batch 1 after 15 days of fermentation attesting at 28% of the relative abundance, whereas *P. veronii* was predominant in batch 1 in the first 15 days of fermentation (attesting at 25% and 16% of the relative abundance in batch 1 and 2, respectively). *P.*

viridiflava, *Sphingomonas* spp., and *R. aquatilis* were predominant in batch 2 during the first 15 days of fermentation (with a relative abundance of 5%, 6% and 9%, respectively) (Figure 2.4). In addition, a high presence of *Weissella* soli was observed in batch 2 after 36 and 46 days of fermentation, reaching 90% of the relative abundance. Figure 2.5 shows the relative abundance of the sole lactic acid bacteria OTUs detected by sequencing in the two analyzed batches of kimchi.

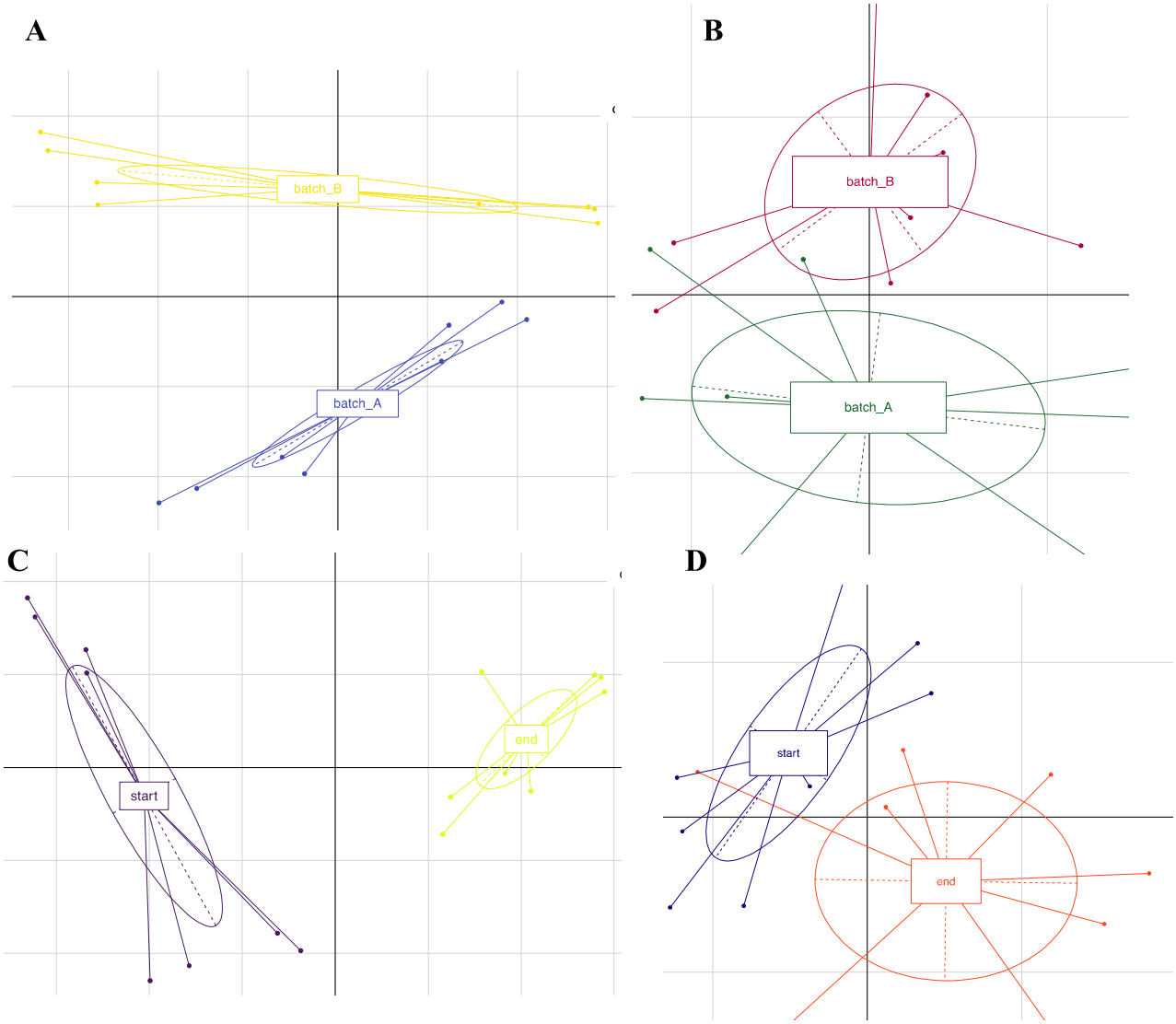


Figure 2.3. Principal Component Analysis (PCA) based on Operational Taxonomic Units (OTUs) relative abundance of kimchi samples for bacteria (**panel A**) and fungi (**panel B**) grouped according to the batch, or according to: (i) the fermentation period; (ii) beginning of fermentation: t0, t2, t5, t15; (iii) end of fermentation: t36, t43, t50, t57, for bacteria (**panel C**) and fungi (**panel D**).

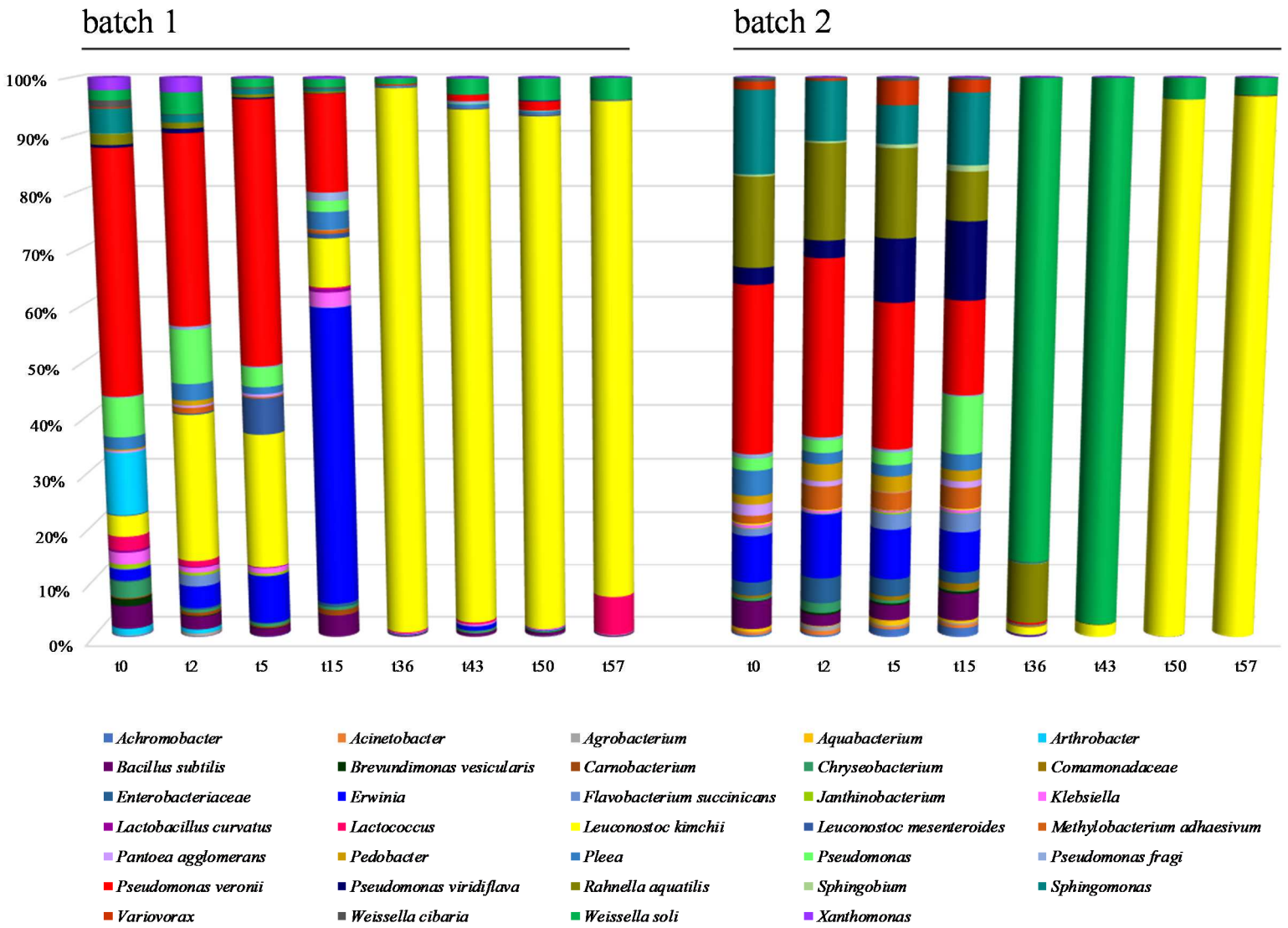


Figure 2.4. Relative abundance of bacterial Operational Taxonomic Units (OTUs) detected by sequencing in the analyzed kimchi batches. Samples are grouped according to batch (1 and 2) and labeled according to fermentation time.

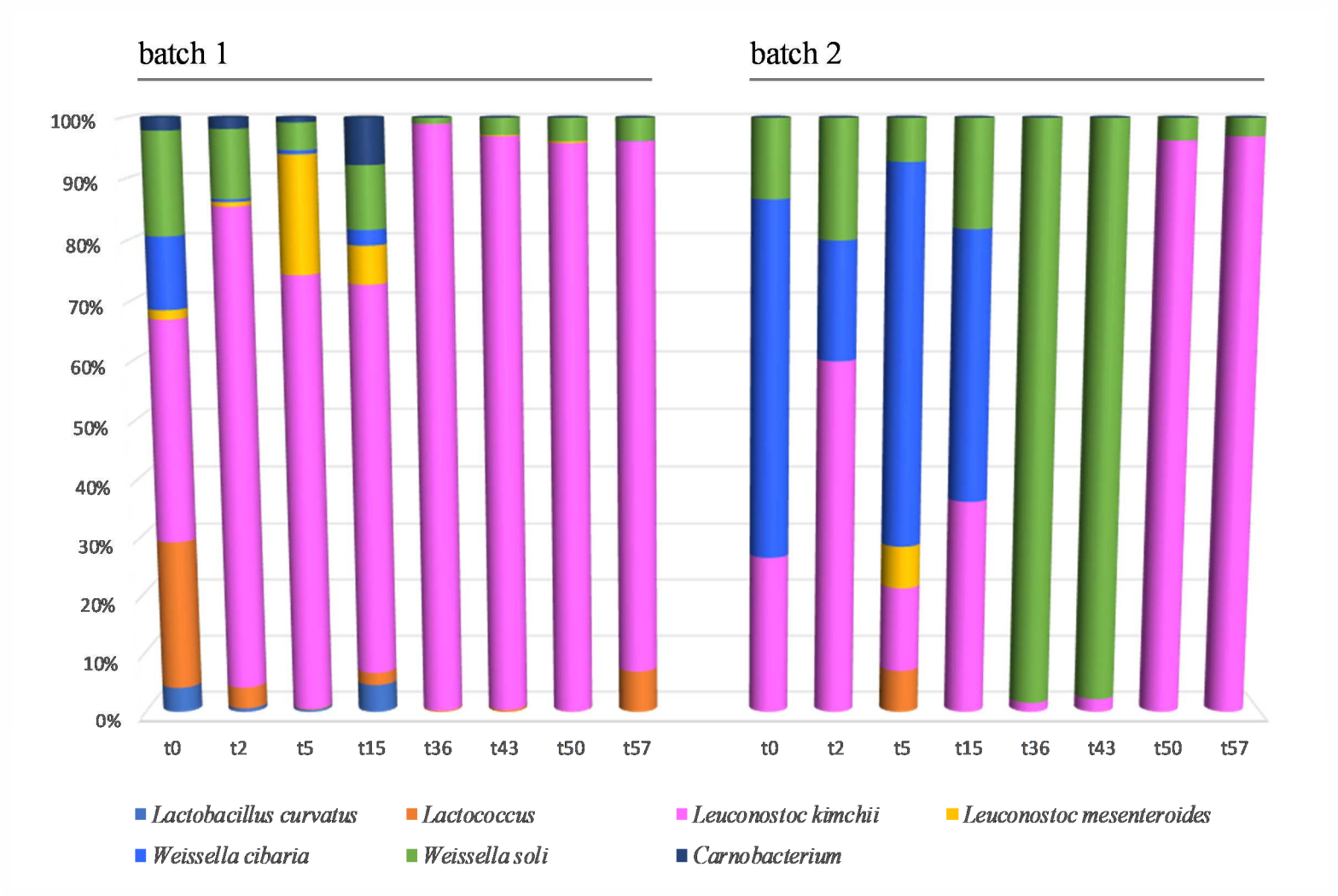


Figure 2.5. Relative abundance of lactic acid bacteria Operational Taxonomic Units (OTUs) detected by sequencing in the analyzed kimchi batches. Samples are grouped according to batch (1 and 2) and labeled according to fermentation time.

By comparing the relative abundance of the OTUs between the beginning and the end of fermentation, only *Weissella soli* and *Leuconostoc kimchii* appeared to be associated with the end of fermentation (FDR < 0.05), whereas the other OTUs (including the minor fraction with <1% of the relative species abundance) were associated with the early step of fermentation (Figure 2.6).

2.3.4. Mycobiota diversity

The total number of sequences used for the downstream analysis reached 634,252 reads, with an average value of 40,910 reads/sample and a mean sequence length of 395 bp. Overall, a satisfactory sample coverage was showed by all the samples (>96%). Alpha diversity indexes were not significantly different according to time or batch.

PCA based on OTUs relative abundance of fungal taxa revealed two clusters separated according to batch and fermentation time (Figure 2.3, panel B and D, respectively).

The mycobiota composition at the highest taxonomic level (Figure 2.7) showed an evolution across time, in both the analyzed batches, with significant differences between them. In batch 1, *Rhizoplaca* and *Pichia orientalis* were the dominant OTUs in the first 15 days of fermentation (with approximately 65% and 17% of the relative abundance, respectively), whereas, at the end of fermentation, *Penicillium*, *Candida sake, Malassezia*, and *Saccharomyces cerevisiae* were the most abundant taxa, reaching 67, 8, 7, and 3% of the relative species abundance, respectively.

In batch 2, a stable occurrence of *Penicillium* spp. was detected, especially at the end of the fermentation (when it reached 40% of the relative abundance), together with *Rhizoplaca* spp. (58% and 11% of the relative abundance, after 50 and 57 days, respectively) (Figure 2.7). *Protomyces inundatus* was predominant after 2 days of fermentation, with 30% relative abundance, whereas *Glomus hyderabadensis* and *Debaromyces hansenii* were predominant after 50 and 57 days of fermentation, reaching 24% and 31% of the relative abundance, respectively. *Malassezia* and

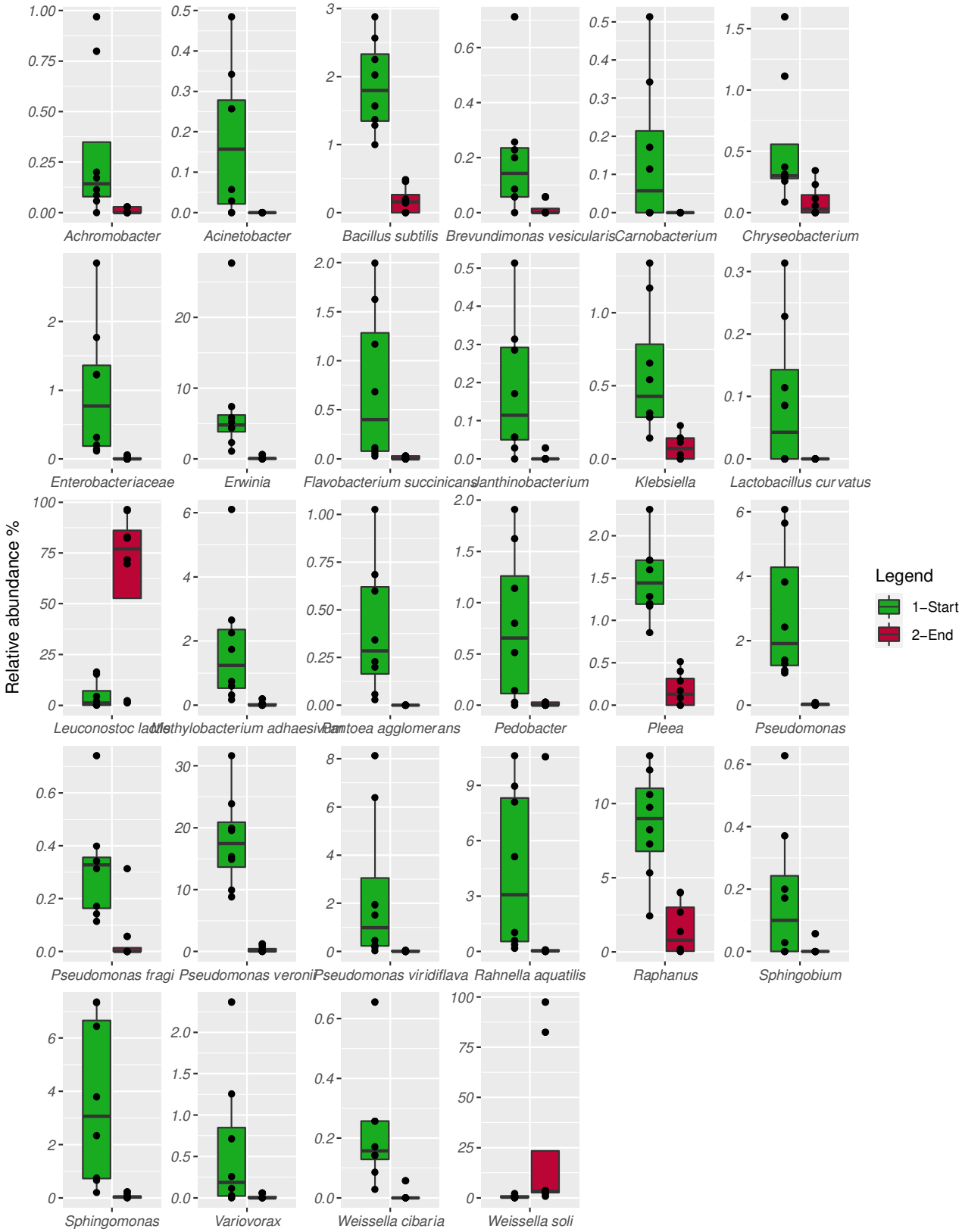


Figure 2.6. Boxplots showing the relative abundance of bacterial Operational Taxonomic Units (OTUs) between the beginning of fermentation: t_0, t_2, t_5, t_{15}, and the end of fermentation: t_{36}, t_{43}, t_{50}, t_{57}. Boxes represent the interquartile range (IQR) between the first and third quartiles, and the line inside represents the median (2nd quartile).

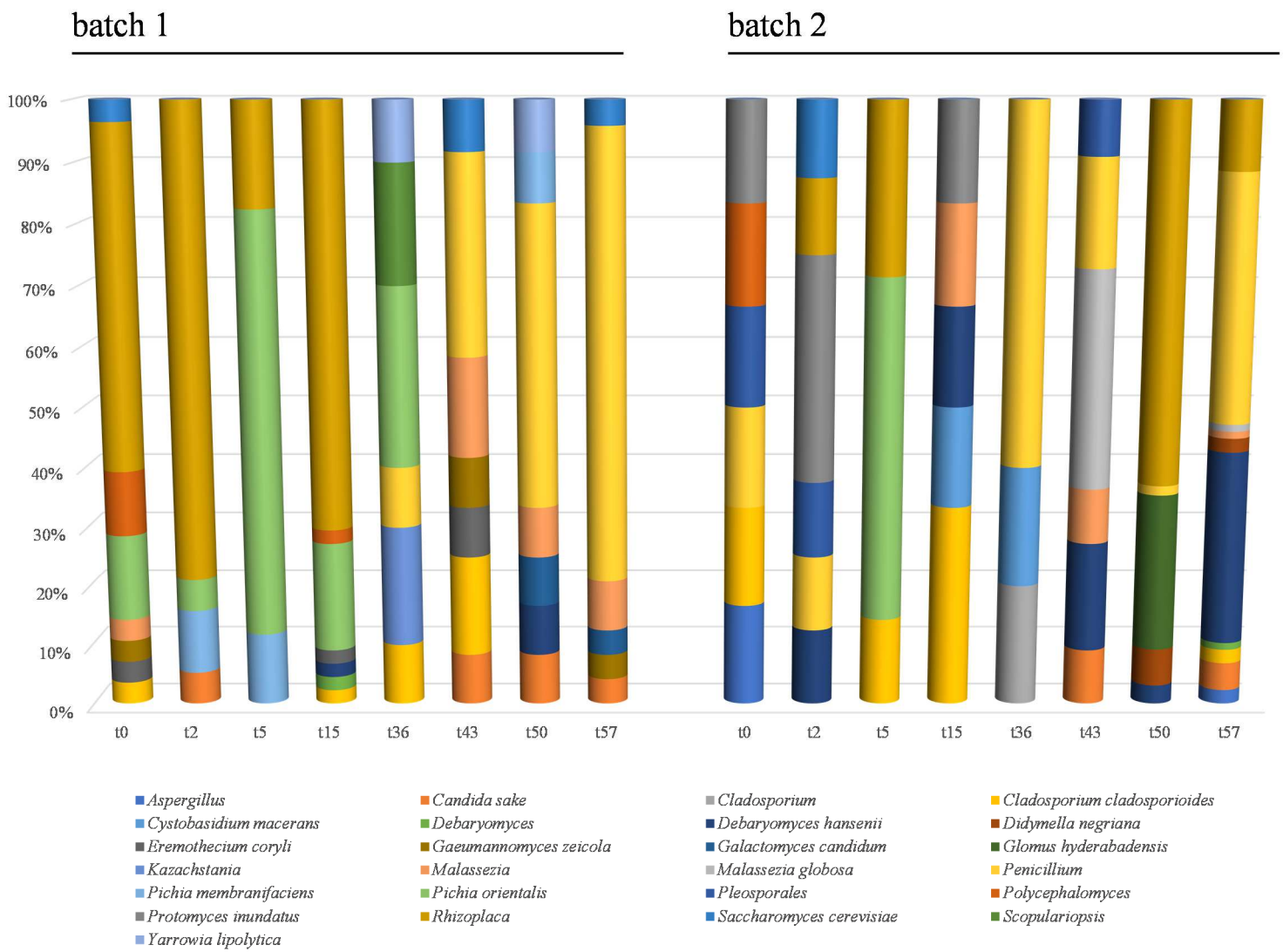


Figure 2.7. Relative abundance of fungal Operational Taxonomic Units (OTUs) detected by sequencing in the analyzed kimchi batches. Samples are grouped according to batch (1 and 2) and labeled according to fermentation time.

Candida sake were also detected as minority taxa (<10% of the relative abundance) at the end of fermentation.

When the relative abundance of the OTUs at the beginning and the end of the fermentation were compared, only *Penicillium* and *Candida sake* appeared to be associated with the end of fermentation (FDR < 0.05) (Figure 2.8).

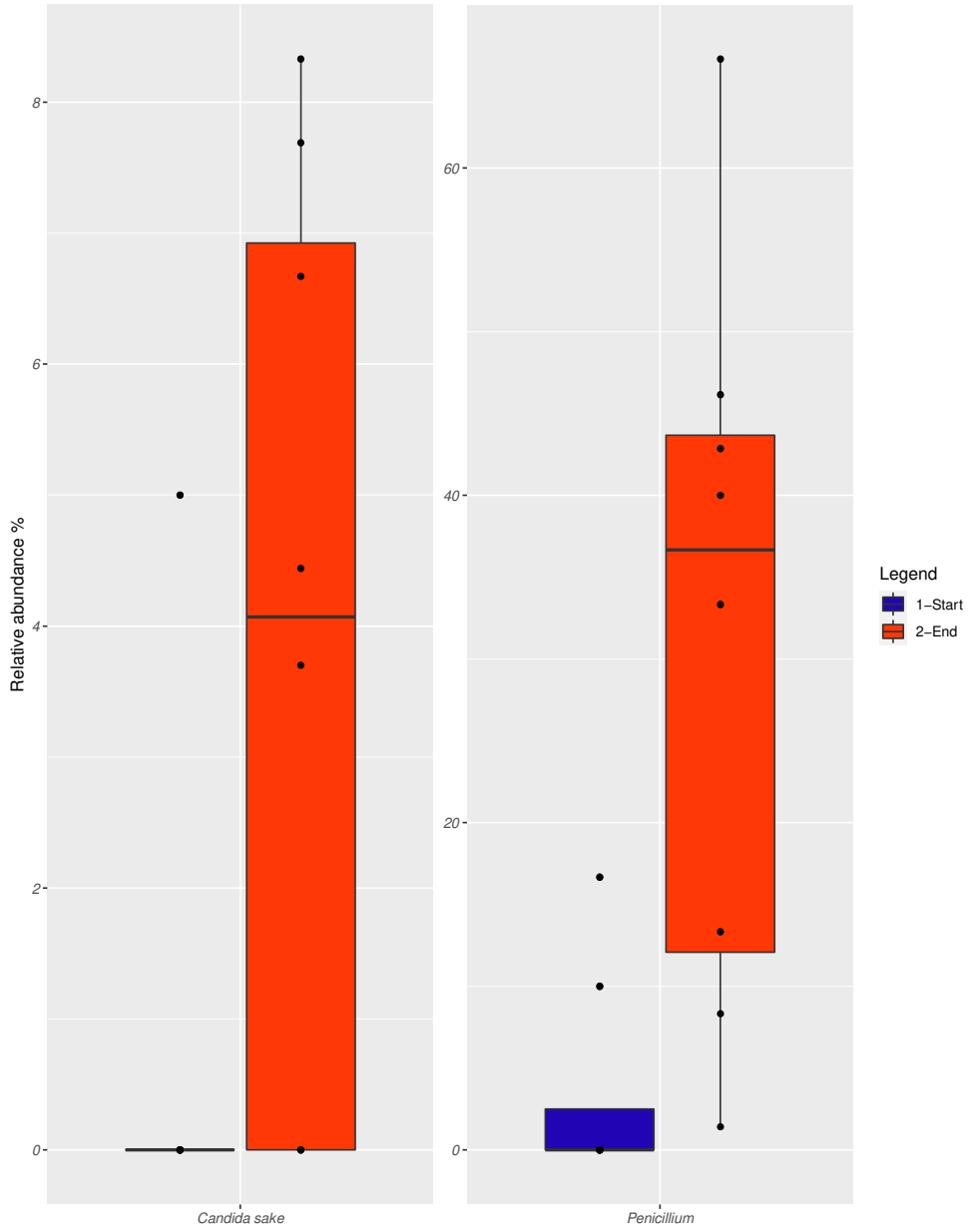


Figure 2.8. Boxplots showing the relative abundance of fungal Operational Taxonomic Units (OTUs) between the beginning of fermentation: t_0, t_2, t_5, t_{15}, and the end of fermentation: t_{36}, t_{43}, t_{50}, t_{57}. Boxes represent the interquartile range (IQR) between the first and third quartiles, and the line inside represents the median (2nd quartile).

2.4. Discussion

As reviewed by Chang (2018), kimchi is produced through a spontaneous fermentation, generally lasting from a few weeks (e.g., four) to months (e.g., three); this fermentation process is mainly mediated by lactic acid bacteria naturally occurring in the raw materials and the production environment.

Regarding the samples of kimchi analyzed in the present study, pH values detected in both batches at t_0 were in accordance with those reported for Korean kimchi at the beginning of fermentation, attesting between 5.0 and 5.4, depending on the raw material used (Jung et al., 2011; Lee et al., 2017b). However, a different evolution of this parameter was seen during the fermentation with respect to Korean kimchi, which reaches a pH of ~ 4.0 as early as 4 weeks after fermentation (Jeong et al., 2013; Lee et al., 2017b). Indeed, in the samples herein analyzed, a pH value of about 4.0 was reached after 57 days of fermentation. It is noteworthy that the type of raw material and its natural contamination can strongly affect the evolution of pH during kimchi fermentation; in this regard, Song et al. (2020) have recently reported different pH values for mature cabbage-, garlic-, ginger-, and red pepper-origin kimchi. Interestingly, the pH values detected in the kimchi manufacture under study were in accordance with those reported for cabbage-kimchi.

As a general trend, viable counts suggested the establishment of an active microbial community composed mainly of mesophilic aerobes and lactic acid bacteria; in contrast, progressively decreasing counts of Enterobacteriaceae, Pseudomonadaceae, and yeasts were seen during kimchi fermentation.

Regarding viable counts of total mesophilic aerobes, enumerated on growth media with or without the addition of 8% (w v^{-1}) NaCl, similar trends were observed between the two analyzed batches. In more detail, almost stable loads attesting at 5 Log cfu g^{-1} were seen during the whole fermentation period. The values detected at t_0 were in accordance with those reported by Lee et

al. (2017b) in the raw materials used for kimchi manufactured in Gyeong-gi province of Korea. By contrast, the counts of total mesophilic aerobes were lower than those reported in Korean kimchi by Hong et al. (2013), attesting at >8 Log cfu g^{-1} after 9 days of fermentation. Interestingly, Hong et al. (2013) reported a linear association between the fermentation temperature and loads of total mesophilic aerobes, with a slowdown of the microbial growth at 4 °C.

Regarding lactic acid bacteria counts, two different trends were seen in the two production batches, feasibly due to the differences occurring in the microbiota composition of the vegetables used as ingredients or the microbial interactions established during fermentation. In more detail, in batch 1 the load of lactobacilli progressively increased up until day 36, when it reached a maximum mean value of 6 Log cfu g^{-1}. In batch 2, the load of lactobacilli remained almost stable during the early stage of fermentation (first 15 days), which was followed by a rapid increase. Markedly higher viable cell counts of lactobacilli, attesting at about 9 Log cfu g^{-1} were reported by Hong et al. (2013) in a previous research dealing with the microbial dynamics of kimchi during its natural fermentation. Lactic acid bacteria counts detected in the present study were also lower that those reported by Lee et al. (2017b) in 88 kimchi manufactures sampled from six provinces (Chungcheong, Gangwon, Gyeonggi, Gyeongsang, Jeolla, and Jeju) in South Korea.

In accordance with lactic acid bacteria increase, in both production batches a progressive decrease of Enterobacteriaceae counts was seen, with a complete disappearance of the latter microorganisms at days 36 and 50 in batches 1 and 2, respectively. As previously found in numerous fermented vegetables, the acidity due to accumulation of organic acids produced by lactic acid bacteria was mainly responsible for the inhibition and death of Enterobacteriaceae (Hurtado et al., 2008; Tamang et al., 2010; Wouters et al., 2013a; Yamani, 1993). Members of this family are acknowledged as indicators of process hygiene, hence, their load in foods should be carefully assessed (Osimani et al., 2018a).

Relatively high counts of Pseudomonadaceae were detected at early stages of fermentation (from day 0 to 15). This finding agrees well with the results reported by Park et al. (2012) about the possible dominance of pseudomonads during the first stages of kimchi fermentation. The counts of Pseudomonadaceae were also in accordance with those reported by Wouters et al. (2013b) in raw materials used for the production of spontaneously fermented leek, attesting at about 5 Log cfu g^{-1} and progressively disappearing during the first week of fermentation.

Finally, viable counts of yeasts on Rose Bengal Agar without or with 8% (w v^{-1}) NaCl showed a similar decreasing trend in both production batches, with values below the limit of detection at the late stage of fermentation. Data herein obtained are in accordance with the occurrence of yeasts in kimchi already reported by Kim et al. (2020), whereas a quite different picture emerged from the study of Jeong et al. (2013), who reported an increase in the load of *Saccharomyces* spp. during fermentation of kimchi, from day 19 up until day 45. Moreover, Chang et al. (2008) reported the isolation of yeasts such as *Pichia* spp. from mature kimchi. Since yeasts are halotolerant and acidophilic microorganisms that are frequently isolated from acidic fermented vegetables and brine (Torres et al., 2020), further research is needed to better elucidate the scarce occurrence of yeasts in the kimchi manufacture herein analyzed.

The results of metataxonomic analyses highlighted the presence of vegetable-associated bacteria with a progressive reduction of spoilage taxa counterbalanced by a progressive increase of pro-technological taxa mainly ascribed to the lactic acid bacteria group, which dominated from t_{36} to t_{57}.

Regarding OTUs that initially characterized the analyzed kimchi, *Pseudomonas* species were detected in samples collected from both batches from t_0 to t_{15}. *Pseudomonas* species had already been detected by Lee et al. (2017a) in samples of Korean kimchi; moreover, as reported by Song et al. (2020), pseudomonads were among the dominating taxa during the early fermentation of cabbage-

origin Korean kimchi. Interestingly, in the present study, the decrease of the relative abundance of *Pseudomonas* spp. during fermentation was in agreement with the progressive decrease of viable counts of pseudomonads, thus confirming the profitability of combining culture-dependent with culture-independent methods. Regarding the occurrence of *P. veronii*, this species was first isolated from natural mineral waters (Elomari et al., 1996) and more recently found as part of the core microbiota of fresh-cut produce processing facilities, together with *P. viridiflava* (Gu et al., 2019). Moreover, both *P. veronii* and *P. viridiflava* were reported to be the causative agents of soft rot in carrot (Kahala et al., 2012), thus suggesting carrots as a potential source of contamination by these two species in the analyzed kimchi.

During the early stage of kimchi fermentation (from t_0 to t_{15}), *R. aquatilis* was abundantly detected in samples from batch 2. This epiphytic bacterium can be frequently isolated from water, plant leaves and fruit, soil, foods as well as non-environmental samples such as blood, bronchial washings, wounds, and urine (Calvo et al., 2007). *Rahnella* species have already been detected in the microbiota of 25 cabbage-origin kimchi samples from traditional Korean temples that produce traditional temple style food (Lee et al., 2019). These microorganisms were also detected in a large-scale metagenomics study carried out on 88 Korean kimchi sampled during fermentation (Lee et al., 2017b), thus confirming the adaptation of this bacterial genus to the kimchi environment.

Regarding the presence of *Erwinia* spp., this bacterial genus, which is included in the Enterobacteriaceae family, was detected until t_{15}. *Erwinia* encompasses species with cellulolytic and pectolytic activities that produce the soft rot of potato tubers (Ragaert et al., 2017). The occurrence of *Erwinia* spp. in the early stage of fermentation of Korean white kimchi was already reported by Park et al. (2016), whereas the same genus was also found by Park et al. (2012) in 10 industrial-scale batches of Korean kimchi.

As for the presence of *Sphingomonas* spp., more than 100 species belonging to this bacterial genus

have already been isolated from various environmental sources, including phyllosphere, rhizosphere, and plant roots (Menon et al., 2019). Moreover, this genus was detected by Jung et al. (2018) during the early stage of Korean kimchi fermentation (Jung et al. 2018).

Between t_{15} and t_{36}, a clear shift in the relative abundance of the microbial species emerged, with an almost complete replacement of spoilage species with lactic acid bacteria, mainly represented by *W. soli* and *L. kimchi*. This finding suggests the establishment during fermentation of unfavorable environmental conditions for the survival of undesired bacteria.

Regarding *W. soli*, principally detected in samples from batch 2, this lactic acid bacteria species belonging to the Leuconostoccaceae family was first isolated from the soil environment by Magnusson et al. (2002). *W. soli* possesses a heterofermentative metabolism that, from glucose fermentation, produces lactic acid, CO_2, ethanol, and/or acetate. Moreover, some *Weissella* strains, including *Weissella cibaria*, also detected in this study, were found to produce bacteriocins, such as weissellin, weissellicin 110, and weissellicin D, L., M and Y (Kim et al., 2017). Although several *Weissella* species were abundantly detected in Korean kimchi (Lee et al., 2017b), to the authors' knowledge *W. soli* has rarely been isolated from this food product, where it was found as a minor species by Jeong et al. (2013). Although there is a lack of knowledge on the role of *W. soli* in kimchi, it is noteworthy that *Weissella* strains isolated from this fermented vegetable have recently shown potential prophylactic properties and probiotic features, being able to tolerate artificial gastric juice and bile salts and showing a high binding capacity for intestinal epithelial cells (Yu et al., 2019).

Leuconostoc kimchii was found to dominate in both analyzed batches at the end of fermentation (from t_{50} to t_{57}). This microorganism was first isolated from kimchi manufactured in Korea, thus suggesting its strong adaptation to this fermented vegetable (Jung et al., 2019; Lee et al., 2011; Oh et al., 2010). The use of *L. kimchi* strains as starter cultures for kimchi production was previously

evaluated thanks to the potential health benefits for the consumers (e.g., lipid metabolism regulation) (Jung et al., 2019). Interestingly, a recent study carried out on diet-induced obese mice reported that in animals administered with *L. kimchii* GJ2 isolated from Korean kimchi a significant decrease in hepatic triglyceride and fatty acid content was observed (Choi et al., 2018). Moreover, Jo et al. (2015) reported that kimchi fermented with the strain *L. kimchii* GJ2 provided efficient cholesterol-lowering effects in rats fed with a high-fat and high-cholesterol diet, thus suggesting the suitable use of this strain for the production of kimchi with functional features. Consistent with the well-known exopolysaccharides (EPS)-producing capabilities of leuconostocs, the production of dextran and levan with potential prebiotic activity by *L. kimchi* was reported by Schleifer (2019) and Torres-Rodríguez et al. (2014). More recently, Rizzello et al. (2019) have characterized the EPS production ability and other technological features of a *L. kimchi* strain for its exploitation as a starter culture for legumes fermentation, thus suggesting the potential suitability of this species for the manufacturing of novel vegetable-based fermented foods.

The metataxonomic analyses applied directly to the food matrix have allowed major and minor fungal taxa to be also detected, thus representing a further step toward the knowledge of the mycobiota of kimchi. As a general trend, *Rhizoplaca* spp. and *P. orientalis* were found to dominate the early stage of kimchi fermentation.

Rhizoplaca is a genus of foliose lichenized fungi (e.g., *Rhizoplaca melanophthalma*) in the family Lecanoraceae, including several morphologically distinct species that are geographically and ecologically widespread (Leavitt et al., 2011).

As for the presence of *P. orientalis* (Syn. *Issatchenkia orientalis*), yeasts in the genus *Pichia* were already isolated from mature kimchi characterized by low pH values (Chang et al., 2008). In this regard, *Pichia kluyveri* was detected by Chang et al. (2008) in Korean kimchi, whereas the osmotolerant yeast *Pichia guilliermondii* was detected in waste brine generated from kimchi

production (Choi et al., 1999). As reported by Chang et al. (2008), the growth of *P. orientalis* is generally suppressed during kimchi fermentation, thus explaining its dominance in the sole early stage fermentation in both batches herein analyzed.

In the present study, *Penicillium* spp., *S. cerevisiae*, and *C. sake* were associated with the late stage of fermentation. However, the low load of eumycetes (<1 Log cfu g^{-1}) found in both analyzed batches at the end of fermentation suggests the occurrence of the detected taxa at very low levels or in the state of viable but not culturable cells.

Interestingly, the occurrence of both *Saccharomyces* and *Candida* species has already been reported by Jeong et al. (2013) during the late stage of fermentation of dongchimi, the traditional Korean watery kimchi. As suggested by Chang et al. (2008), these yeasts can grow in kimchi at pH values between 4.0 and 7.0; moreover, white colony-forming yeasts, such as *C. sake* and *Debaryomyces* spp., were also detected in packed kimchi stored in refrigerated conditions (Kim et al., 2020), thus confirming their adaptation to cold environments.

2.5. Conclusions

Kimchi is one of the food products that best represent the tradition of the Korean cuisine and possess functional and health-promoting features related mainly to the metabolic activity of lactic acid bacteria. Based on the overall results herein collected, the non-Korean kimchi manufacture analyzed was characterized by microbial populations and dynamics that greatly overlapped those of more well-known Korean "relatives." Indeed, typical kimchi-associated lactic acid bacteria species were surprisingly detected as dominant taxa in both batches analyzed, thus confirming their high adaptation to kimchi raw materials and production process. As already revealed by the available scientific literature, lactic acid bacteria isolated from kimchi can likely be used as starter cultures to produce novel functional fermented vegetables. As a matter of fact, lactic acid bacteria occurring in kimchi are well adapted to salty environments, and this feature can

be extremely advantageous for theirexploitation as starters for the fermentation of vegetables in brine. Given this premise, the knowledge gained in the present study represents a step forward in the description of the microbial dynamics of kimchi produced outside the region of origin using local ingredients. At this regard, the results collected compared to those available in the scientific literature for Korean kimchi manufactures seem tosuggest a neatly higher effect of process parameters rather than the adventitious microbial populations on the shaping of mature kimchi microbiota. The evidences emerged from the present study will alsoserve as a starting point for further isolation of vegetable-adapted lactic acid bacteria to be assayed as potential starters for the manufacturing of novel vegetable preserves with high quality and functional traits. The present study also highlighted the presence of fungal taxa, whose contribution to the sensory traits of kimchi must be further investigated.

3. New constituents and potential pharmacological activity of sea fennel (*Crithmum maritimum* L.) cultivated in the Conero Natural Park (Marche region, Central Italy) area

3.1. Introduction

Sea fennel (*Crithmum maritimum* L.) is an aromatic plant, belonging to the Apiaceae family, rich in bioactive substances, with nutritional and medicinal value, growing spontaneously in coastal areas of Mediterranean and Black Sea and Atlantic Europe (Alves-Silva et al., 2020). The fleshy and succulent leaves of this plant are used for the preparation of cooked meals, salads, and pickles (Generalić Mekinić et al., 2016; Meot-Duros and Magné, 2009). In the folk medicine they are applied as carminative, digestive, vermifuge, diuretic, depurative, anti-inflammatory, tonic, and antiscorbutic drug, as well as in the treatment of wounds and common cold (Atia et al., 2011; Zafeiropoulou et al., 2020). Sea fennel is characterized by the presence of several bioactive constituents like vitamin C, essential fatty acids, essential oils and polyphenols. More in detail, previous phytochemical studies performed on sea fennel revealed a high content of phenolic acids, mainly represented by chlorogenic acids, and a high antioxidant activity (Boutellaa et al., 2019; Cunsolo et al., 1993; Franke, 1982; Generalić Mekinić et al., 2016, 2018; Meot-Duros and Magné, 2009; Najjaa et al., 2020; Pereira et al., 2017).

Polyphenols represent an ubiquitous and large group of plant metabolites displaying key functions along their entire life cycle, which exhibit, in humans, important physiological activities and are discussed in the prevention of diabetes, cancer, cardiovascular diseases and neurological ailments, mainly counteracting oxidative stress (Han et al., 2007; Rodrigues et al., 2015; Vrhovsek et al., 2012).

Most of the available studies, dealing with sea fennel polyphenols characterization, relies on classical room temperature extraction (Boutellaa et al., 2019; Nabet et al., 2017; Souid et al., 2021;

Zafeiropoulou et al., 2020), eventually enhanced by the application of ultrasonic waves (ultrasound-assisted extraction, UAE) (Kumar et al., 2021; Martins-Noguerol et al., 2022a), using methanol/water (Martins-Noguerol et al., 2022a) or ethanol/water (Souid et al., 2021) mixtures as solvents. Accelerated solvent extraction (ASE), is an advanced technique allowing a more efficient extraction of phenolic compounds than classical (Li et al., 2019) or ultrasound assisted methods (Pietrzak et al., 2014; Repajić et al., 2020), through the use of pressurized solvent, at a pressure comprised between 10 and 15 MPa, and high temperatures generally comprised between 50 and 200 °C (Wang and Weller, 2006).

Given these premises, the present study aimed at the characterization of the bioactive compounds of sea fennel with a focus on the polyphenolic fraction. Methanolic extracts from the whole sprouts, sole leaves and sole stems, obtained by accelerated solvent extraction, were analyzed through high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and liquid chromatography coupled with mass spectrometry (LC-MS) in order to identify the whole range of phenolic constituents.

3.2. Materials and methods

3.2.1. Test samples

Organic sea fennel sprouts cultivated in the Conero Natural Park area, Ancona, Italy, was kindly supplied by a producer of sea fennel-based preserves (Rinci S.r.l., Castelfidardo, Ancona, Italy). Fresh sea fennel (approximately 1 kg) was transported to the laboratory under cooled conditions (4 ± 2 °C), dried in a dehydrator (Captain Jerky 110, Klarstein, Berlin, Germany) at 30°C, and stored in plastic bags under vacuum condition at room temperature (~18-20°C), prior to the analysis. Eight samples were prepared from the whole sprouts (S1, S2, S3, S4, S5, S6, S7, S8), one only from the stems (S9), and another one only with leaves (S10).

3.2.2. Chemicals and reagents

Ethanol (denatured ≥ 96 %), methanol (ROTIPURAN® ≥ 99.9 %, p.a., ACS, ISO), chloroform (≥ 99 %, DAB, BP, pure), ethyl acetate (≥ 99.5 %, Ph.Eur., pure), polyethylene glycol 4000, vanillin (≥ 99 % for biochemistry), natural product reagent A (≥ 98 %, p.a.), formic acid (ROTIPURAN® ≥ 98 %, p.a., ACS), sulphuric acid (95-98 %, Ph.Eur), ortho-phosphoric acid (85 % ROTIPURAN®, p.a., ISO), luteolin (ROTICHROM® 90 %), hyperoside (ROTICHROM® TLC), chlorogenic acid ROTICHROM® TLC), aesculetin (purum CHR), (+) catechin (pract CHR), (-) quinic acid (purum CHR), (-) epicatechin (~ 95 %), ferulic acid (≥ 99 %), gallic acid (≥ 98 %, p.a., ACS), hesperidin (CHR), protocatecuic acid (purum CHR), rutin (purum), syringic acid (purum CHR), kaempferol (ROTICHROM® CHR) and quercetin (puriss. CHR) were purchased from Carl Roth GmbH (Karlsruhe, Germany).

Neochlorogenic acid (≥ 98.0 %), cryptochlorogenic acid (≥ 98.0 %), isochlorogenic acid A (≥ 98.0 %) and isochlorogenic acid B (≥ 98.0 %) were purchased from Chengdu Push Bio-technology Co., Ltd (Wuhou Science Park, Chengdu, China).

Methanol (HiPerSolv, CHROMANORM®), acetonitrile (HiPerSolv, CHROMANORM®), and water (HiPerSolv, CHROMANORM®) were purchased from VWR International SAS (Fontenay Sous Bois, France), while glacial acetic acid (100% anhydrous for analysis) was bought from Merck KGaA (Darmstadt, Germany).

The reference compounds rosmarinic acid, p-coumaric acid (purum ≥ 98 %, HPLC) and quercetin-3-O-glucopyranoside were purchased from PhytoLab GmbH and Co. KG (Vestenbergsgreuth, Germany), Thermo Fisher Scientific, (Waltham, MA, USA) and Extrasynthese (Genay, France), respectively. Quercitrin CRS (European Pharmacopoeia Reference Standard) was purchased from EDQM (Strasbourg, France).

3.2.3. Accelerated solvent extraction

Dried sea fennel samples were ground by using an analytical mill (A 11 basic, IKA®-Werke GmbH & Co. KG Staufen, Germany), mixed 4:1 (w w^{-1}) ratio with diatomaceous earth (Thermo Fisher Scientific, Waltham, MA, USA), and successively extracted with methanol by means of an accelerated solvent extractor (Dionex™ ASE™ 150, Thermo Fisher Scientific, Waltham, MA, USA). The extraction was performed setting the parameters as follows: heat time: 5 min; static time: 5 min; rinse volume: 40%; purge time: 60 s; cycles: 3; temperature: 68 °C. The extracts were dried under nitrogen flow and then stored at -20°C until use.

3.2.4. Sample preparation

The dried extracts were dissolved in methanol at a concentration of 10 mg mL^{-1}, sonicated for 5 min at room temperature in an ultrasonic bath (Transsonic T 460/H, Elma Schmidbauer GmbH, Singen, Germany) and centrifuged for 15 min at 13.000 rpm with a centrifuge (Biofuge® pico, Heraeus, Hanau, Germany), to obtain a clear sample for further analyses. The reference compounds were dissolved in methanol at a concentration of 1 mg mL^{-1}. For high-performance thin-layer chromatography analysis, three separate solutions with a mix of reference compounds were prepared as follows: mix 1: esculetin, protocatechuic acid, gallic acid, and hyperoside; mix 2: ferulic acid, quercitrin, rosmarinic acid, quercetin-3-O-glucoside, and rutin; mix 3: kaempferol, quercetin, and chlorogenic acid.

3.2.5. High-performance thin-layer chromatography

High-performance thin-layer chromatographic (HPTLC) analyses were performed using a CAMAG-HPTLC system (CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG, Muttenz, Switzerland) operated with winCATS software (CAMAG). Aliquots of samples (10 µL) and mixed reference compound solutions (5 µL) were applied to HPTLC glass plates coated with silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany) by a CAMAG Automatic TLC Sampler ATS 4. Two HPTLC

separations were performed using two different mobile phase systems. In the first analysis, the application length for all the samples was set at 7 mm and a mobile phase consisting of chloroform-glacial acetic acid-methanol-water (64:32:12:8) was employed (Wagner and Bladt, 1996). While, in the second analysis, the application length for all the samples was set at 8 mm and a mobile phase consisting of ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) was employed (Wagner and Bladt, 1996). HPTLC plates were developed in a CAMAG Automatic Developing Chamber ADC2 after 20 min equilibration with saturation pad and 5 min plate preconditioning to a final migration distance of 75 mm. After drying, the plates were derivatized with natural products-polyethylene glycol reagent (NP/PEG). The plates were visualised and photographed with a CAMAG TLC visualizer 2 after development and after derivatization at UV 254 and 366 nm, and at white light.

3.2.6. High-performance liquid chromatography-diode array detection-mass spectrometry

3.2.6.1. Fingerprint analyses and annotation of major compounds

High-performance liquid chromatography-diode array detector-high-resolution mass spectrometry (HPLC-DAD-HRMS) analyses was performed on an Ultimate 3000 HPLC hyphenated with a Q Exactive™ hybrid quadrupole Orbitrap mass spectrometer (Thermo Fisher Scientific) in both HESI positive and negative mode. The separation was carried out on a Zorbax Extend-C18 column (3.5 µm, 4.6 mm × 150 mm, Agilent). The mobile phase consisted of water +0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). A flow rate of 1.0 ml min^{-1} was applied and the gradient was set as follows: 0–4 min, 12% B in A; 4–5 min, 12%–20% B in A; 5–15 min, 20% B in A; 15–16 min, 20%–25% B in A; 16–20 min, 25% B in A; 20–22 min, 25%–50% B in A, 22–25 min, 50% B in A; 25–27 min, 50%–95% B in A; 27–29 min, 95% B in A; 29–30 min, 95%–12% B in A; 30–35 min, 12% B in A. The column temperature was set to 30°C. The diode array detector was set in a range from 200 to 400 nm. The mass spectrometer was run in both, HESI positive and negative modes using the following parameters: probe heater temperature 350°C; capillary temperature 330°C; spray

voltage 3.5 kV for positive and 3.1 kV for negative ion mode; sheath gas flow 65 arbitrary units; auxiliary gas flow 20 arbitrary units; resolution: 70.000 (full MS) and 17.500 (data-dependent MS^2). A volume of 5 µL was injected for the samples, reference and blank solutions. Data evaluation was performed with Thermo Xcalibur 2.2.44 (Thermo Fisher Scientific) for LC-MS. Compounds were annotated by comparing retention time, precursor monoisotopic mass, and MS/MS fragment ion masses with authentic references, or by comparing MS/MS fragmentation patterns with existing data from databases and literature data as well as molecular formulas calculated from the exact mass.

3.2.6.2. Annotation of flavonoid aglycone moieties

Identification of flavonoid aglycones was performed by an Ultimate 3000 HPLC hyphenated LTQ-XL linear ion trap mass spectrometer with HESI interface (Thermo Fisher Scientific) operated in negative mode. The HPLC separation was carried out on a Zorbax Extend-C18 column (3.5 µm, 4.6 mm × 150 mm, Agilent). The mobile phase consisted of water + 0.1% formic acid (A) and acetonitrile +0.1% formic acid (B). The gradient system described in section 3.2.6.1 was used for separation. The mass spectrometer was run in the HESI negative mode using the following parameters: source heater temperature 350°C; capillary temperature 330°C; spray voltage 3.0 kV, sheath gas flow 50 arbitrary units; auxiliary gas flow 10 arbitrary units. The volume of 5 µL was injected for the samples, for reference and blank solutions. Flavonoid aglycone moieties were identified by comparison of their fragmentation pattern to authentic reference compounds.

3.2.7. Semiquantitative determination of dihydroxycinnamic acid derivatives and flavonoids by high performance liquid chromatography with diode array detection

High performance liquid chromatography (HPLC) analyses were performed on a 1260 Infinity HPLC-DAD (Agilent Technologies, Inc., Santa Clara, CA, USA). The separation was carried out on a Zorbax Extend-C18 column (3.5 µm, 4.6 mm × 150 mm, Agilent). The mobile phase consisted of water + 0.1% ortho-phosphoric acid (A) and acetonitrile (B), and the separation was performed with

the gradient system described in section 3.2.6.1. The column temperature was set to 30°C. Aliquots of 5 µL were injected of the samples, and of reference and blank solutions. Detection was carried out setting the DAD signal at 220, 254, 280, 320 and 360 nm. UV spectra were acquired in a range of wavelengths comprised between 210 and 400 nm. Chlorogenic acid and dihydroxycinnamic acid derivatives were quantified using chlorogenic acid as external standard, while flavonoids were quantified using rutin as an external standard. Chlorogenic acid calibration curve was prepared using six different concentration of the reference compound dissolved in methanol (1, 10, 50, 100, 500, 1000 µg mL^{-1}) injected in the same condition as the samples, and detected setting the DAD signal at 320 nm. For quantification, the peak area of each dihydroxycinnamic acid derivative was recorded at the same wavelength as of the reference compound (320 nm). For establishing the rutin calibration curve, the same six concentrations as for chlorogenic acid were used. Detection was carried out setting the DAD signal at 360 nm. For quantification, the peak area of flavonoids was determined at 360 nm. The results were expressed as g $100g^{-1}$ dry weight (DW) of sea fennel, as mean value of two replicates ± standard deviation. Data evaluation was performed with Agilent ChemStation.

3.2.8. Statistical analysis

The results of the quantification of chlorogenic acid, dihydroxycinnamic acid derivatives and flavonoids were subjected to one-way analysis of variance (ANOVA) through the Tukey-Kramer honest significant difference (HSD) test ($P \leq 0.05$), to evaluate differences between the samples. The software JMP Version 11.0.0 (SAS Institute Inc., Cary, NC, USA) was used for the analysis.

3.3. Results and discussion

Starting from the assumption that sea fennel is rich in several bioactive compounds and taking into consideration the increasing interest in polyphenols for their health benefit (Vrhovsek et al., 2012), this study aimed in a holistic characterization of sea fennel phenolic compounds. Accelerated solvent extraction using pure methanol as solvent was performed, in order to recover the phenolic fraction

(Alonso-Salces et al., 2001; Sun et al., 2012) from the whole sprouts, leaves, and stems, to be subjected to further characterization analyses.

The extraction yields of each sample are listed in Table 3.1, as absolute (g) and relative (%) values. Stems showed a higher extraction yield than leaves, highlighting how this parameter is influenced either by the extraction solvent and method and by the vegetable matrices itself (Pferschy-Wenzig and Bauer, 2015).

Table 3.1. Absolute and relative extract yields obtained by accelerated solvent extraction of (ASE) of sea fennel whole sprouts (sample S1, S2, S2, S3, S4, S5, S6, S7, S8), stems (S9) and leaves (S10).

Sample	Initial weight (g)	Yield MeOH extract (g)	Yield MeOH extract (%)
S1	8.79	1.37	15.55
S2	7.70	1.46	18.98
S3	9.30	1.70	18.25
S4	7.81	1.47	18.80
S5	10.08	1.76	17.51
S6	9.88	1.62	16.35
S7	11.28	1.89	16.79
S8	10.98	1.65	15.07
S9	8.99	1.71	19.01
S10	11.23	1.61	14.30

The combination of two HPTLC analyses, performed using two different mobile phases allowed a good separation for all the reference compounds, characterized by different polarity (Jesionek et al., 2015). More in detail, the use of a mobile phase generally employed for the analysis of polyphenols, namely ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26), allowed a proper chromatographic separation for rutin, chlorogenic acid, hyperoside, quercetin-3-O-glucoside, quercitrin, and rosmarinic acid. While, the use of a mobile phase constituted by chloroform-glacial acetic acid-methanol-water (64:32:12:8), allowed the separation of gallic acid, protocatechuic acid, esculetin, quercetin, kaempferol and ferulic acid. The derivatization of the plates with natural products-polyethylene glycol reagent (NP/PEG), generally employed for polyphenol analysis, and the observation at UV 366 nm returned structural dependent typical fluorescence (Wagner and Bladt, 1996). The HPTLC analyses of sea fennel extracts highlighted similar chromatographic profiles among the tested samples. The use of corresponding reference compounds, the mobile phase ethyl

acetate-formic acid-glacial acetic acid-water (100:11:11:26), and derivatization with natural products-polyethylene glycol reagent (NP/PEG) (Figure 3.1) allowed the identification of rutin (R_f =0.34) and chlorogenic acid (R_f =0.42). Three more compounds, namely hyperoside, quercetin-3-O-glucoside and quercitrin could also be present, but the bands in the samples are not well separated for a proper identification. On the contrary, ferulic acid, kaempferol, quercetin, esculetin, protocatechuic acid, gallic acid and rosmarinic acid could not be detected in the extracts by HPTLC (Figure 3.2). Furthermore, when the intensity of the bands was taken into consideration, chlorogenic acid was the most represented compound in all the samples.

Preliminary HPLC analyses, performed as described in section 3.2.7, confirmed the presence of the same compounds in all the test samples, therefore the sample S1, constituted by sea fennel whole sprouts, was chosen for LC-MS analyses.

The base peak chromatogram in HESI negative mode and DAD chromatogram are depicted in Figure 3.3. The chromatogram in HESI positive mode was excluded because all the annotated compounds, except for chlorogenic acid, were detected exclusively in the negative mode. In sea fennel methanolic extracts, 26 compounds were annotated, belonging to the following classes: organic acids, hydroxycinnamic acid derivatives, flavones, flavonols, triterpene saponins, and hydroxylated fatty acids (Table 3.2). Nine of these compounds were unambiguously identified by comparing their retention times, precursor monoisotopic mass and MS/MS fragmentation patterns with that of authentic reference substances, and 21 compounds were tentatively annotated by comparing precursor monoisotopic mass, MS/MS fragmentation patterns and the molecular formulas calculated from the exact mass with existing data from databases and literature. The aglycone moieties of flavonoid glycosides No. 11, 19, 20, 21 and 22 (Table 3.2) were annotated by comparing their MS^3 fragmentation patterns generated in a linear ion trap mass spectrometer with the MS^2 fragmentation patterns of authentic flavonoid aglycone references.

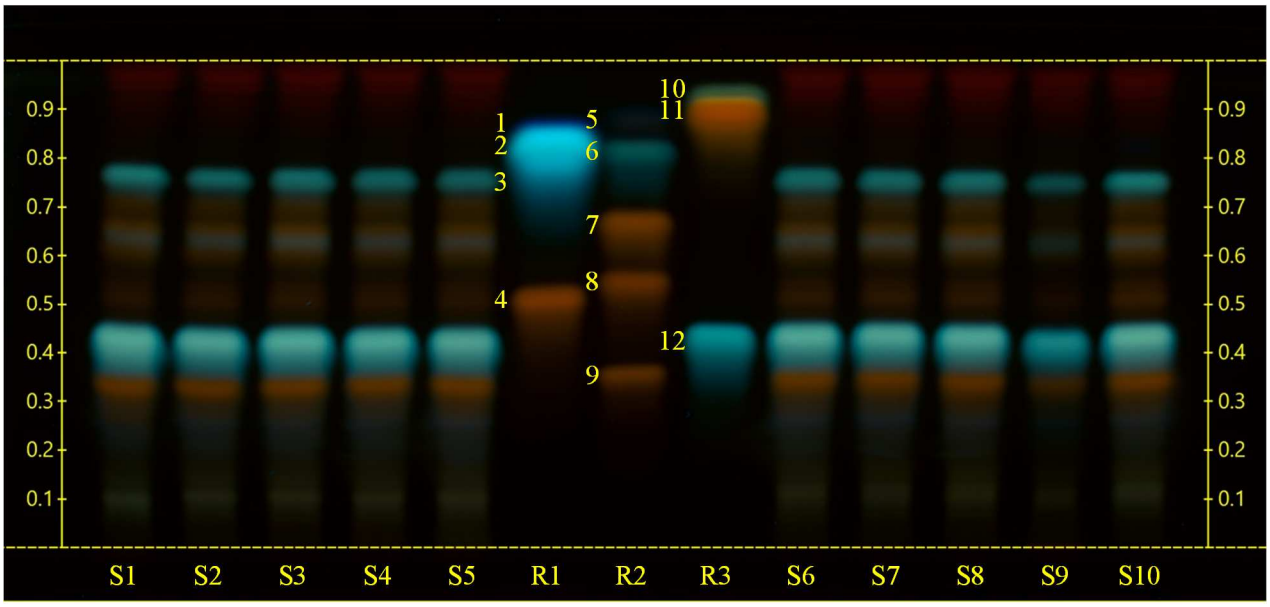


Figure 3.1. High-performance thin-layer chromatography (HPTLC) carried out using a mobile phase consisting of ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26), derivatized with natural products-polyethylene glycol reagent (NP/PEG) and observed at UV 366 nm. S1, S2, S3, S4, S5, S6, S7, S8: sea fennel whole sprouts; S9 sea fennel stems; S10: sea fennel leaves. R1: reference compounds mix 1 (1: protocatechuic acid; 2: esculetin; 3: gallic acid; 4: hyperoside); R2: reference compounds mix 2 (5: ferulic acid; 6: rosmarinic acid; 7: quercitrin; 8: quercetin-3-O-glucoside; 9: rutin); R3: reference compounds mix 3 (10: kaempferol; 11: quercetin; 12: chlorogenic acid).

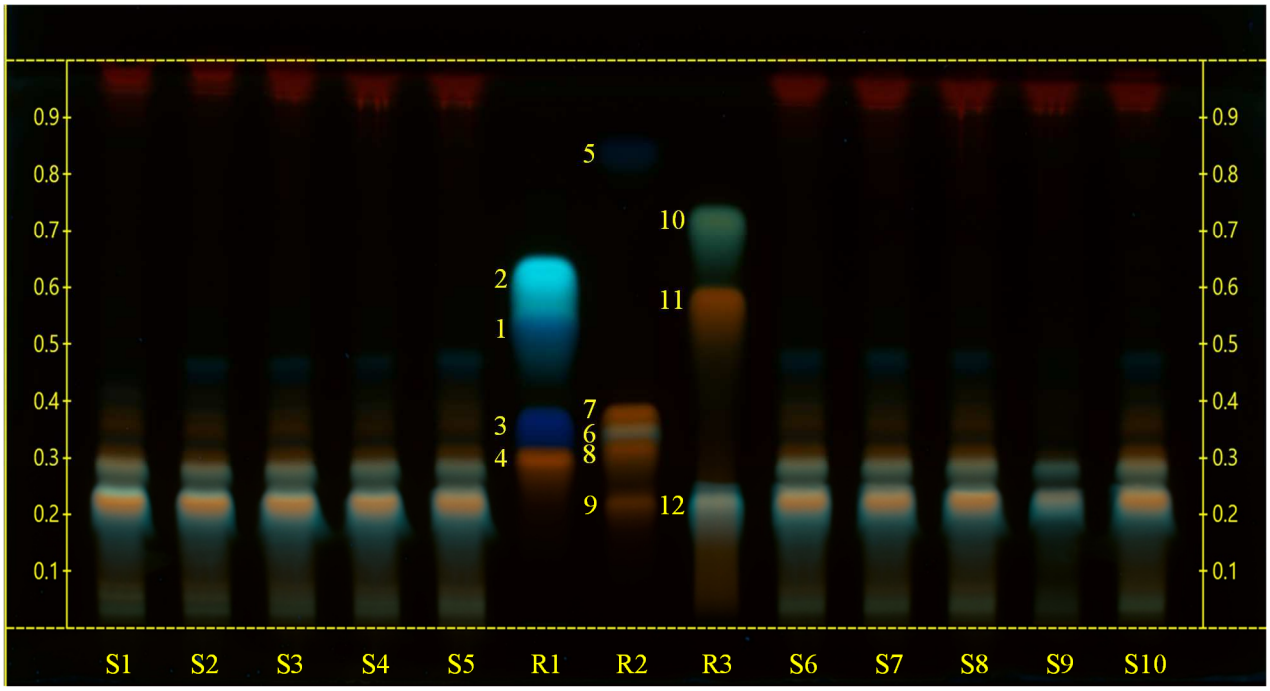
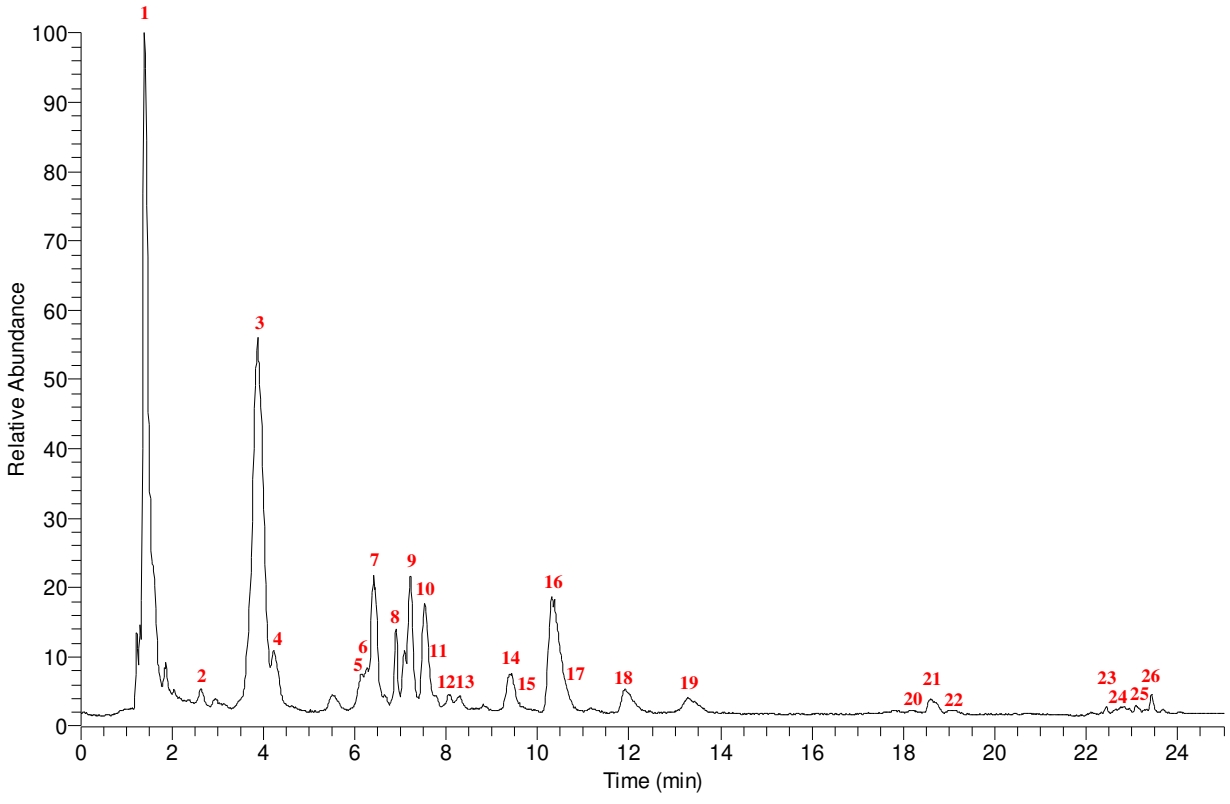
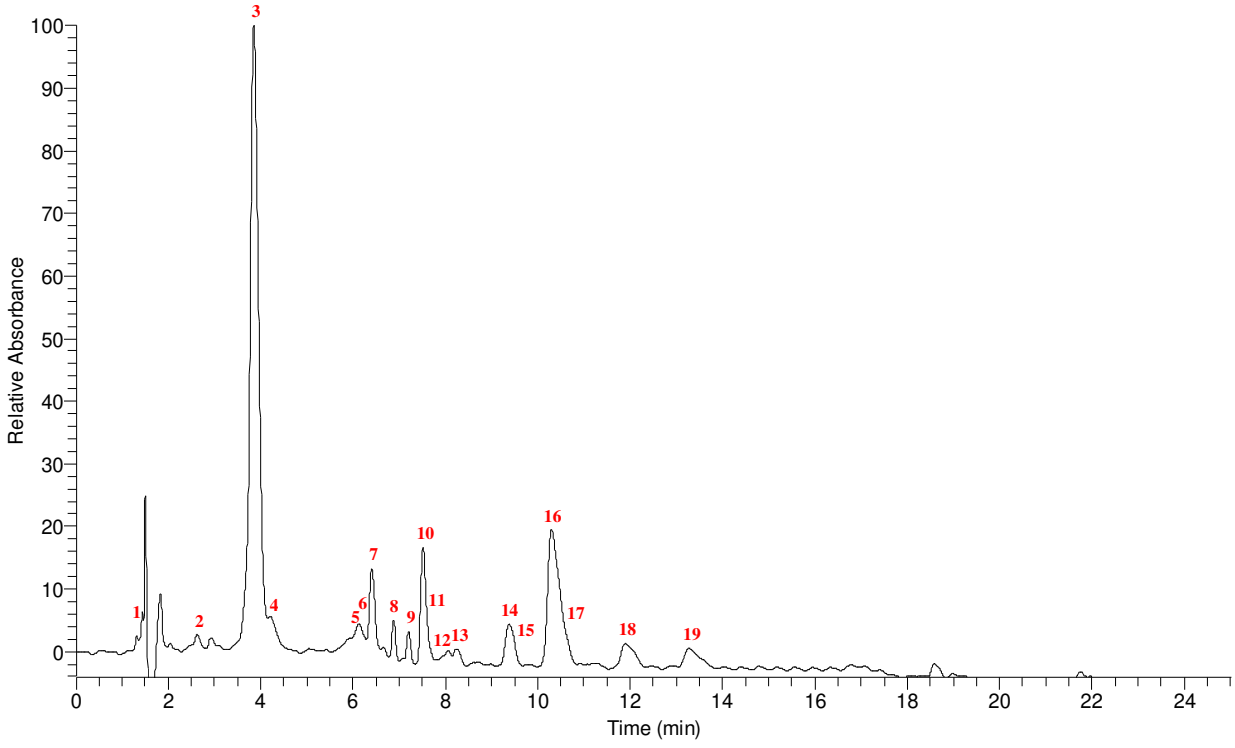


Figure 3.2. High-performance thin-layer chromatography (HPTLC) carried out using a mobile phase consisting of chloroform-glacial acetic acid-methanol-water (64:32:12:8), derivatized with natural products-polyethylene glycol reagent (NP/PEG) and observed at UV 366 nm. S1, S2, S3, S4, S5, S6, S7, S8: sea fennel whole sprouts; S9 sea fennel stems; S10: sea fennel leaves. R1: reference compounds mix 1 (1: protocatechuic acid; 2: esculetin; 3: gallic acid; 4: hyperoside); R2: reference compounds mix 2 (5: ferulic acid; 6: rosmarinic acid; 7: quercitrin; 8: quercetin-3-O-glucoside; 9: rutin); R3: reference compounds mix 3 (10: kaempferol; 11: quercetin; 12: chlorogenic acid).



a)



b)

Figure 3.3. Base peak chromatogram in HESI negative mode (panel **a**) and DAD chromatogram (panel **b**) of sea fennel methanolic extract.

Table 3.2. Compounds annotated in sea fennel methanolic extract by HPLC-DAD-HRMS/MS.

Compound No.	RT(min)	Monoisotopic mass (Da)	Molecular formula	Delta (ppm)	DAD λ_{max} (nm)	Negative ion mode		Annotation	Compound class	Literature reference
						Parent ion	Fragment ions			
1	1.41	192.0634	$C_7H_{12}O_6$	4.582		191.0559 [M-H]-	191	quinic acid*	Organic acids	
2	2.64	354.0951	$C_{16}H_{18}O_9$	2.751	215, 325	353.0877 [M-H]-	135, 179, 191	neochlorogenic acid*	Hydroxycinnamic acids	
3	3.88	354.0951	$C_{16}H_{18}O_9$	2.043	215, 240, 325	353.0874 [M-H]-	191	chlorogenic acid*	Hydroxycinnamic acids	
4	4.24	354.0951	$C_{16}H_{18}O_9$	2.383	220, 240, 325	353.0876 [M-H]-	135, 173, 179, 191	cryptochlorogenic acid*	Hydroxycinnamic acids	
5	6.15	594.1585	$C_{27}H_{30}O_{15}$	1.945	210, 275, 325	593.1513 [M-H]-	353, 383, 473	vicenin-2	Flavones	Gouvea et al., 2017; Silva et al., 2014
6	6.18	354.0951	$C_{16}H_{18}O_9$	2.383	215, 275, 325	353.0876 [M-H]-	191	chlorogenic acid isomer	Hydroxycinnamic acids	Farag et al., 2015; Granato et al., 2016; Jaiswal et al., 2014; Pacifico et al., 2018; Sánchez-Faure et al., 2020
7	6.42	338.1002	$C_{16}H_{18}O_8$	2.718	225, 310	337.0927 [M-H]-	191	coumaroylquinic acid isomer	Hydroxycinnamic acids	Granato et al., 2016; Jaiswal et al., 2014; Sánchez-Faure et al., 2020
8	6.90	368.1107	$C_{17}H_{20}O_9$	2.074	240, 325	367.1031 [M-H]-	93, 173, 191, 193	feruoylquinic acid isomer	Hydroxycinnamic acids	Jaiswal et al., 2014; Sánchez-Faure et al., 2020
9	7.23	338.1002	$C_{16}H_{18}O_8$	2.629	235, 305	337.0927 [M-H]-	191	coumaroylquinic acid isomer	Hydroxycinnamic acids	Granato et al., 2016; Jaiswal et al., 2014; Sánchez-Faure et al., 2020
10	7.53	610.1534	$C_{27}H_{30}O_{16}$	1.722	205, 255, 355	609.1461 [M-H]-	300, 301	rutin*	Flavones	
11	7.68	594.1585	$C_{27}H_{30}O_{15}$	2.147	255, 350	593.1514 [M-H]-	285	luteolin-O-dihexoside	Flavones	Kumar et al., 2015
12	8.09	464.0955	$C_{21}H_{20}O_{12}$	2.090	255, 350	463.0881 [M-H]-	300, 301	hyperoside*	Flavonols	
13	8.26	464.0955	$C_{21}H_{20}O_{12}$	2.305	255, 350	463.0882 [M-H]-	300, 301	quercetin-3-O-glucoside*	Flavonols	
14	9.40	434.0849	$C_{20}H_{18}O_{11}$	1.829	250, 330	433.0773 [M-H]-	300, 301	quercetin-O-pentoside	Flavonols	Sánchez-Faure et al., 2020

Table 3.2. (Continued).

Compound No.	RT(min)	Monoisotopic mass (Da)	Molecular formula	Delta (ppm)	DAD λ_{max} (nm)	Negative ion mode: Parent ion	Fragment ions	Annotation	Compound class	Literature reference
15	9.50	516.1268	$C_{25}H_{24}O_{12}$	1.548	250, 325	515.1192 [M-H]-	173, 179, 191, 353	isochlorogenic acid B*	Hydroxycinnamic acids	
16	10.33	516.1268	$C_{25}H_{24}O_{12}$	0.966	240, 325	515.1189 [M-H]-	135, 179, 191, 353	isochlorogenic acid A*	Hydroxycinnamic acids	
17	10.59	608.1741	$C_{28}H_{32}O_{15}$	2.460	250, 335	607.1672 [M-H]-	284, 299, 300	diosmin	Flavones	Adouani et al., 2013; Sánchez-Faure et al., 2020
18	11.93	516.1268	$C_{25}H_{24}O_{12}$	1.432	245, 325	515.1191 [M-H]-	173, 179, 191, 353	dicaffeoylquinic acid isomer	Hydroxycinnamic acids	Farag et al., 2015; Pacifico et al., 2018; Sánchez-Faure et al., 2020
19	13.31	626.1272	$C_{30}H_{26}O_{15}$	2.597	255, 330	625.1204 [M-H]-	300, 463	quercetin-caffeoyl-hexoside	Flavonols	Farag et al., 2015
20	18.16	610.1323	$C_{30}H_{26}O_{14}$	2.739		609.1256 [M-H]-	300, 301, 463, 464, 151, 179	quercetin- coumaroyl-hexoside isomer 1	Flavonols	García-Villalba et al., 2017
21	18.60	610.1323	$C_{30}H_{26}O_{14}$	2.131		609.1252 [M-H]-	300, 463, 301, 151, 179	quercetin- coumaroyl-hexoside isomer 2	Flavonols	García-Villalba et al., 2017
22	19.03	640.1428	$C_{31}H_{28}O_{15}$	3.463		639.1367 [M-H]-	300, 463, 301, 151, 179	quercetin-feruloyl-hexoside	Flavonols	De Andrade Neves et al., 2018
23	22.43	1148.5615	$C_{55}H_{88}O_{25}$	6.384		1147.5604 [M-H]-	221, 521, 553, 986, 625, 824, 910, 967, 451, 486	trihexosyl-monohexuronyl triterpene (aglycone: C31 H50 O4)	Triterpene saponins	
24	22.75	1116.5353	$C_{54}H_{84}O_{24}$	6.652		1115.5343 [M-H]-	221, 909, 521, 935, 454, 491	trihexosyl-monohexuronyl triterpene (aglycone: C30 H46 O3)	Triterpene saponins	
25	23.08	328.2250	$C_{18}H_{32}O_5$	3.482		327.2177 [M-H]-	171, 201, 125, 155, 209, 211	trihydroxy-octadecadienoic acid	Hydroxylated fatty acids	Farag et al., 2015
26	23.43	330.2406	$C_{18}H_{34}O_5$	3.309		329.2333 [M-H]-	171, 211, 229, 125, 209, 211, 155, 167, 183, 193	tianshic acid or isomer	Hydroxylated fatty acids	Kothari et al., 2020

*Identifed by authentic references. RT: retention time, Delta (ppm): between measured m/z and calculated molecular formula.

Nineteen of these compounds were already identified in previous phytochemical studies (Alves-Silva et al., 2020; Nabet et al., 2017; Sánchez-Faure et al., 2020; Souid et al., 2020; Zafeiropoulou et al., 2020), while the remaining seven, namely luteolin-O-dihexoside, quercetin-caffeoyl-hexoside, quercetin-feruoyl-hexoside, trihexosyl-monohexuronyl triterpene (aglycone: $C_{31}H_{50}O_4$), trihexosyl-monohexuronyl triterpene (aglycone: $C_{30}H_{46}O_3$), trihydroxy-octadecadienoic acid, and tianshic acid or isomer were newly found in sea fennel. Saponins are a group of compounds constituted of a triterpene or steroid aglycone and one or more sugar chains. These compounds, traditionally considered as "antinutritional factors" in food, are recognized, nowadays, as the active principles in many herbs used in traditional medicine. Saponins exhibited a wide range of biological activities behaving as hypocholesterolemic, antimutagenic, anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective and neuroprotective agents (Güçlü-Üstündağ and Mazza, 2007; Liu and Henkel, 2002; Sparg et al., 2004). Furthermore, among the tentatively identified compounds new for sea fennel, hydroxylated fatty acids are described in literature as bioactive compounds with antimicrobial, cytotoxic and anti-neuroinflammatory properties (Masoodi et al., 2008; Serag et al., 2020).

The results of the semiquantitative determination of dihydroxycinnamic acid derivatives and flavonoids are reported in Table 3.3.

Table 3.3. Concentration of chlorogenic acid, dihydroxycinnamic acid derivatives and flavonoids in sea fennel.

Sample	Chlorogenic acid (g 100g^{-1} DW)	Dihydroxycinnamic acid derivatives (g 100g^{-1} DW)	Flavonoids (g 100g^{-1} DW)
S1	0.81 ± 0.02^{d}	1.29 ± 0.03^{f}	0.29 ± 0.00^{f}
S2	1.03 ± 0.01^{b}	1.59 ± 0.02^{b}	0.44 ± 0.00^{bc}
S3	1.19 ± 0.00^{a}	1.87 ± 0.01^{a}	0.46 ± 0.00^{a}
S4	1.02 ± 0.00^{b}	1.54 ± 0.00^{bc}	0.38 ± 0.00^{e}
S5	0.93 ± 0.00^{c}	1.46 ± 0.00^{de}	0.46 ± 0.00^{ab}
S6	1.01 ± 0.00^{b}	1.57 ± 0.01^{b}	0.39 ± 0.01^{de}
S7	0.95 ± 0.01^{c}	1.51 ± 0.02^{cd}	0.43 ± 0.00^{c}
S8	0.83 ± 0.01^{d}	1.34 ± 0.01^{f}	0.40 ± 0.01^{d}
S9	0.43 ± 0.00^{e}	0.77 ± 0.00^{g}	0.15 ± 0.00^{g}
S10	0.93 ± 0.01^{c}	1.43 ± 0.01^{e}	0.44 ± 0.00^{c}

Values are expressed as mean value ± standard deviation as g 100g^{-1} DW sea fennel. Values labelled with different letters in the same column are significantly different ($P < 0.05$).

One-way analysis of variance (ANOVA) highlighted significant differences in the concentration of the quantified classes of compounds among the whole sprouts samples. All the compounds resulted to be less concentrated in stems (S9) than in leafs (S10), in agreement with the results previously obtained by Pereira et al. (2017). Whereas, chlorogenic acid resulted to be the most concentrated polyphenol with contents ranging from 0.81 ± 0.02 (S1) to 1.19 ± 0.00 (S3) g $100g^{-1}$ DW sea fennel in the whole sprouts, again in accordance with previously phytochemical studies on sea fennel (Meot-Duros and Magné, 2009). Chlorogenic acid is a hydroxycinnamic acid described in literature as one of the most widely distributed and functional polyphenols in the human diet, displaying health beneficial effects behaving as antioxidant, anti-inflammatory, antimicrobial, antimutagenic, cardiovascular protective, neuroprotective, renoprotective, gastrointestinal protective and hepatoprotective agent, and modulating lipid and glucose metabolism in the treatment or prevention of the metaboli□syndrome (Lu et al., 2020; Naveed et al., 2018).

3.4. Conclusions

The characterization of constituents with potential pharmacological activity performed on sea fennel (*Crithmum maritimum* L.), cultivated in the Conero Natural Park, highlighted similar polyphenolic profiles among different sprouts despite slight differences in the concentration of the single compounds, and confirmed the predominance of chlorogenic acid in the phenolic fraction. Moreover, the use of accelerated solvent extraction allowed the annotation of a wide range of phenolic constituents, some of them new for sea fennel, and triterpene saponins and hydroxylated fatty acids, again newly detected in this plant.

4. Exploitation of sea fennel (*Crithmum maritimum* L.) for manufacturing of novel high-value fermented preserves

The content of this section was redrafted from the published paper: Maoloni, A., Milanović, V., Osimani, A., Cardinali, F., Garofalo, C., Belleggia, L., Foligni, R., Mannozzi, C., Mozzon, M., Cirlini, M., Spaggiari, M., Reale, A., Boscaino, F., Di Renzo, T., Haouet, M. N., Staccini, B., Di Bella, S., Aquilanti, L., 2021. Exploitation of sea fennel (*Crithmum maritimum* L.) for manufacturing of novel high-value fermented preserves. Food Bioprod. Process. 127, 174-197. Available online at https://doi.org/10.1016/j.fbp.2021.03.001. The complete content of this paper was included in this section with slight modifications to follow the logic course of this thesis. As first author, Maoloni, A., contributed to microbiological, physiochemical and sensory formal analysis, to data curation, and to writing-original draft preparation.

4.1. Introduction

The world is presently overdependent on a few plant species for the supply of staple foods. Diversification of production and consumption habits to include a broader range of undervalued and underused plant species could significantly contribute to improving human health and nutrition while increasing livelihoods and environmental sustainability. Edible wild herbs growing naturally on uncultivated lands or as weeds in farms have traditionally played an important role in supplementing staple foods, and they might do so again in the future. Indeed, given their innate resilience to rapid climate change, they could play an increasingly important role in buffering against food shortages due to periods of low agricultural productivity associated with climate events. Among edible wild herbs, halophytes include salt-resistant plants equipped with well-defined adaptive mechanisms that enable them not only to withstand periodic high salinity but also to complete their entire life cycles at high salinity (Koyro et al., 2011; Romojaro et al., 2013; Ventura et al., 2011). Wild or partially domesticated halophytes already exist in virtually all regional ecosystems, especially in the Mediterranean basin. Among halophytes, *C. maritimum* L., commonly known as sea fennel, rock samphire or St. Peter's herb, grows spontaneously along coastlines and is particularly abundant in Mediterranean countries, including Italy, North Africa, Croatia, Turkey, and Greece. This plant has several usage areas, such as culinary, medicine, and cosmetics, because of its nutrient and phytochemical contents (Ashaolu and Reale, 2020). Due to the acknowledged high content of vitamin

C, in the past, the consumption of sea fennel was particularly common among seafarers as a valuable aid for the prevention of scurvy (Generalić Mekinić et al., 2016). In addition to vitamin C, the succulent leaves of this spontaneous halophyte contain high levels of numerous bioactive compounds, such as flavonoids, carotenoids, polyphenols, and antioxidant compounds (Maleš et al., 2003; Meot-Duros and Magné, 2009). To date, approximately thirty substances have been identified in sea fennel essential oils (Jallali et al., 2014; Özcan et al., 2006). The trade of this spontaneous herb was very popular all over Europe until the early 1900s; however, its indiscriminate harvest determined its disappearance in various European coastal areas. Forgotten for a long time, this highly aromatic herb has recently been rediscovered, having been defined as a "cash crop" due to its high economic potential (Atia et al., 2011; Yensen, 2008) or, more recently, as an "emerging vegetable crop" (Renna, 2018). As far as the food sector is considered, to date, only three available studies have explored the use of sea fennel for the manufacturing of either fermented preserves (Özcan, 2000; Özcan et al., 2019) or dried spices (Renna and Gonnella, 2012). In a few Mediterranean countries, such as Italy, Portugal, Greece and France, *C. maritimum* is consumed fresh in salads or as unfermented preserves in brine or olive oil.

Given these premises, the present study aimed to explore the lactic acid fermentation of sea fennel sprouts for the manufacturing of novel high-value vegetable preserves. To this end, a pool of lactic acid bacteria (LAB) previously isolated from various vegetable sources was first typed at the molecular level and further assayed as monocultures in sea fennel-based mini-batches for the evaluation of key pro-technological traits. Hence, 10 selected strains were exploited for the formulation of five multiple strain starters destined for the manufacture of laboratory-scale prototypes of fermented sea fennel preserves. Once the laboratory-scale prototypes were analyzed for their physico-chemical, nutritional, microbiological, and sensory traits, one multiple strain starter culture was finally exploited for a pilot-scale manufacture of fermented sea fennel-based preserves at an industrial plant routinely producing unfermented sea fennel preserves and sauces.

4.2. Materials and methods

4.2.1. Experimental design

The research work was articulated in three subsequent steps: (i) step 1 aimed at the formulation of five multiple strain starters for the fermentation of sea fennel sprouts; (ii) step 2 aimed at the manufacture and further analysis of laboratory-scale prototypes of sea fennel-based fermented preserves; (iii) step 3 aimed at the manufacture and further analysis of a pilot-scale prototype. The whole experimental design is depicted in Figure 4.1. All details referring to each experimental step are given in the sections below.

4.2.2. Microorganisms

Twenty-seven pure cultures of lactic acid bacteria, belonging to the genera *Lactiplantibacillus, Levilactobacillus, Companilactobacillus* (*Lactobacillus* old classification), *Leuconostoc*, *Pediococcus* and *Weissella,* previously isolated from food sources of vegetal origin (Table 4.1), were assayed for the fermentation of sea fennel (*Crithmum maritimum* L.) sprouts. All the cultures, belonging to the Culture Collection of the Department of Agricultural, Food, and Environmental Sciences (D3A, Università Politecnica delle Marche) were stored at -80 °C in a mixture of de Man Rogosa and Sharpe (MRS) broth (VWR, International, Radnor, Pennsylvania, USA) and glycerol at a 3:2 ratio. They were subcultured on MRS agar (VWR) at 30°C for 48-72 h prior to use.

4.2.3. Sea fennel sprouts purchase and pretreatment

Approximately 31.8 Kg of fresh sea fennel sprouts were purchased from a local industrial producer (Rinci S.r.l., Castelfidardo, Ancona, Italy) of unfermented sea fennel-based preserves and sauces. Sea fennel sprouts were manually harvested in April 2018 (1 Kg, harvest 1), July 2018 (0.8 Kg, harvest 2) and October 2019 (30 Kg, harvest 3). Immediately after harvesting, the woody or damaged parts were manually removed.

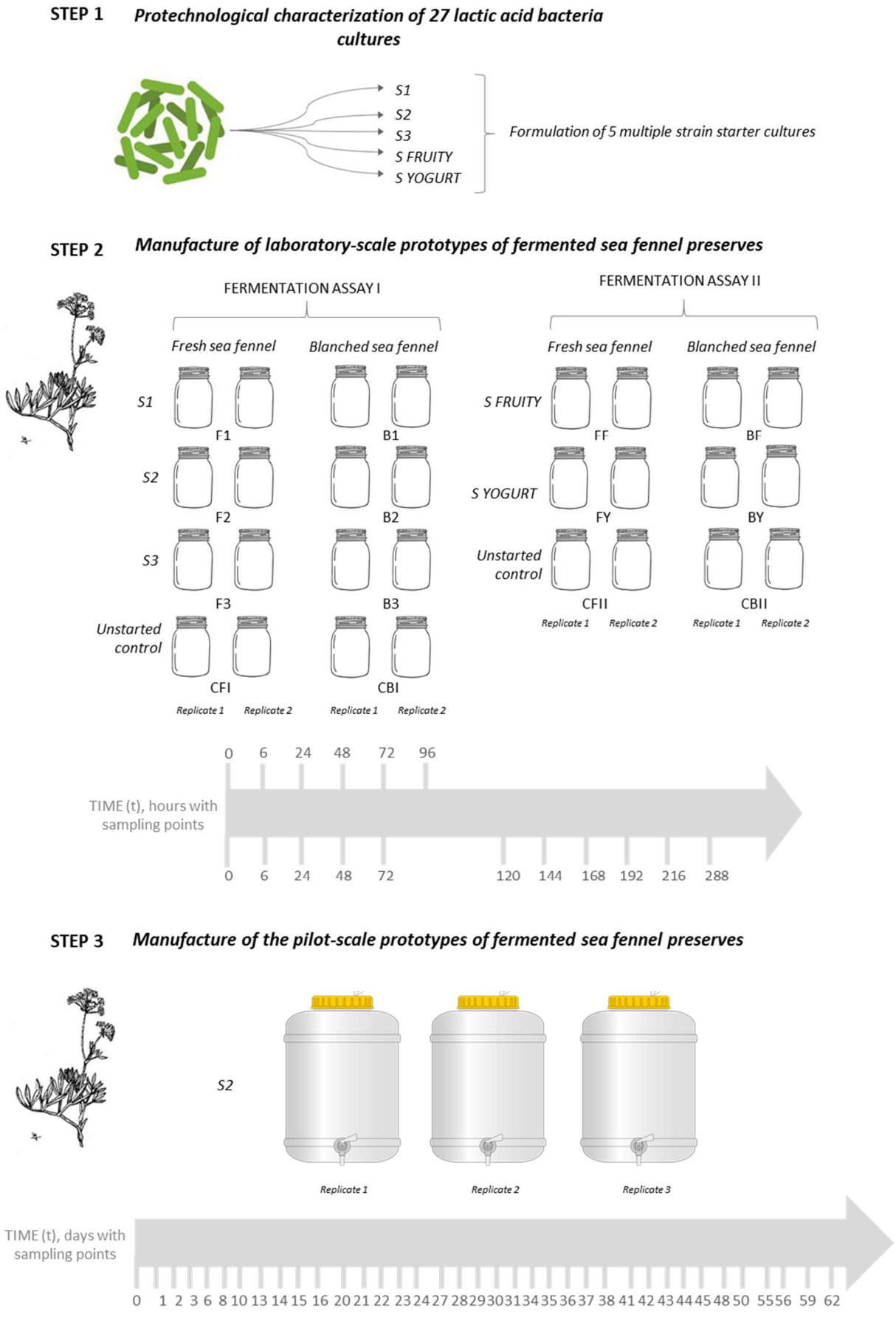


Figure 4.1. Experimental design of the study.

Table 4.1. Lactic acid bacteria from the Culture Collection of the Department of Agricultural, Food and Environmental Sciences (D3A, Università Politecnica delle Marche) screened for their selection as starter strains.

Species	**Isolate**	**Reference**
Lactiplantibacillus plantarum (basonym *Lactobacillus plantarum*)	PB11; PB97; PB98; PB104; PB151; PB193; PB242; PB257; PB268; PB278; PB296; PB297; PB305; PB306; PB307; PB308	Osimani et al., 2009
	FO2	Milanović et al., 2020
Levilactobacillus brevis (basonym *Lactobacillus brevis*)	LM9	Taccari ei al., 2016
Companilactobacillus paralimentarius (basonym *Lactobacillus paralimentarius*)	PB126	Osimani et al., 2009
Leuconostoc pseudomesenteroides	PB288; PB295	Osimani et al., 2009
Pediococcus pentosaceus	FF71; FF78 FO40; FO41	Milanović et al., 2020
Weissella confusa	PB321	Osimani et al., 2009
Weissella kimchii	PB337	Osimani et al., 2009

Selected sprouts from harvest 1 and 2 were transported to the laboratory in sterile plastic bags under refrigerated conditions (+ 4 °C). They were carefully washed under tap water, gently drained, left to dry on blotting paper at room temperature for about 6-8 h, and hence divided in two equal portions (~ 0.5 and 0.4 Kg each, for harvest 1 and 2, respectively), with one portion being blanched in hot water at 95 °C for 30 s and the second portion remaining untreated. Both the aliquots of blanched (B) and untreated (fresh, F) sprouts were stored at -20 °C in hermetically sealed sterile plastic bags until use. Briefly, sprouts from harvest 1 were used for the assay of lactic acid bacteria pure cultures and production of some laboratory scale prototypes (first fermentation assay) whereas sprouts from harvest 2 were used for production of further laboratory scale prototypes (second fermentation assay).

Sprouts from harvest 3 were washed in a hypoclorite solution (60 mg L^{-1}), rinsed in tap water, blanched at 95 °C for 30 s, and hence used to produce a pilot-scale prototype at Rinci S.r.l.

4.2.4. Lactic acid bacteria molecular typing

The isolates ascribed to a species including at least two cultures were subjected to molecular typing. Accordingly, 23 isolates were subjected to DNA extraction using the method suggested by Hynes et al. (1992) with some slight modifications, as previously described by Osimani et al. (2015). The DNA extracts were quantified by optical reading at 260 nm using a UV-Vis Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) and diluted with sterile molecular grade water to reach a final concentration of 25 ng μL^{-1} of DNA. Aliquots (5 µl) of the extracts (containing approximately 125 ng of DNA) were amplified in a 20 µL reaction volume containing 1 U of Taq DNA polymerase (SibEnzyme Ltd, Novosibirsk, Russia), 1× reaction buffer, 0.2 mM dNTPs and 0.2 µM of primer M13 (5' -GAGGGTGGCGGTTCT- 3'). The amplification was performed in a thermal cycler (My Cycler, Bio-Rad Laboratories, Hercules, USA) using the following cycling program: initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min, and extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. Aliquots (10 µL) of each PCR product were subjected to electrophoresis in 2% (w v^{-1}) agarose gel in 0.5 × TBE buffer (Tris Borate-EDTA) containing 1X GelRed™ reagent (Biotium, Fremont, California). The electrophoretic run was carried out at 100 V for 2 h, using a molecular weight standard (HyperLadder™ 100bp Mix, BioLine, London, UK). The amplicons were visualized under UV light and photographed with the Complete Photo XT101 system (Explera, Jesi, Italy).

4.2.5. Lactic acid bacteria screening

Lactic acid bacteria were first screened for their pro-technological traits in mini-batch fermentations by adding 2 g of whole fresh sea fennel sprouts to 12 mL of sterile brine in 50-mL tubes, followed by autoclaving at 121 °C for 15 min and bacterial inoculation (in duplicate) to a final load of ~ 7 log colony-forming units (CFU) mL^{-1}. The inocula were prepared by subculturing each lactic acid bacteria stain in MRS broth (VWR) incubated at 30 °C for 24 h, followed by centrifugation at 4,000

rpm for 10 min, washing of the pellets with sterile 0.85% (w v^{-1}) NaCl saline solution and final resuspension in sterile brine containing 7% (w v^{-1}) NaCl and 1% (w v^{-1}) fructose. Cell concentration of the inocula was determined by optical reading at 600 nm using a UV-Vis Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation). The microbial load of the inoculum was verified by viable counting on MRS agar (VWR) incubated at 30 °C for 48-72 h.

After inoculation, the tubes were statically incubated for 120 h at 30 °C. Aliquots (1 mL) of brine were aseptically sampled immediately after inoculation and at regular intervals during the incubation period (6, 24, 48, and 120 h). All the brine samples were subjected to pH measurements, using a pH meter model 300 equipped with an HI2031 solid electrode (Hanna Instruments, Padova, Italy), and the results were expressed as the mean of three technical replicates ± standard deviation. After 120 h incubation, an olfactory analysis was carried by a panel consisting of 12 nonsmokers, made up of 8 females and 4 males aged between 25 and 45. Panelists were preliminarily trained to describe the attributes of sea fennel. Briefly, a variety of sea fennel samples (fresh, dried, in brine, in olive oil, in sauce, etc.) were presented once a week for three consecutive weeks to the group of assessors. During each training session, a discussion was conducted by the panel to find out the most appropriate sensory attributes of sea fennel. Regarding the evaluation of the monocultures, these were presented to the panelists at room temperature and coded with random, three-digit numbers. The olfactory test was divided into sessions, in which each panelist evaluated 4 samples at a time, working in individual booths and using coffee beans to cleanse olfactory palate. For each monoculture, panelists were asked to draft a list of perceptible olfactory notes.

4.2.6. Multiple strain starter formulation

The ten cultures with the best fermentation performance, in terms of (i) acidification rate and extent evaluated based on mean pH values reached after 24 and 120 h of fermentation, respectively, and (ii) production of pleasant odorous notes, were selected for the formulation of five multiple strain starters

(Table 4.2). These latter were conceived according to two main criteria: (i) inclusion of 1 facultative heterofermentative, 1 obligate heterofermentative, and 1 obligate homofermentative species per starter; and (ii) inclusion of 1 strain per species. The five multiple strain starters, labeled S1, S2, S3, S FRUITY, and S YOGURT, were assayed for the manufacture of laboratory-scale prototypes of fermented sea fennel preserves, as described in section 4.2.7.

Table 4.2. Multiple strain starters.

Multiple strain starter	Strain	Species	Acidifying activity	Perceived aromatic note[#]
S1	PB11	*Lactiplantibacillus plantarum*	++	vegetable, fruity
	PB288	*Leuconostoc pseudomesenteroides*	++	caper, sour
	FF78	*Pediococcus pentosaceus*	+	fruity, pungent
	PB321	*Weissella confusa*	-	butter, almond
S2	PB257	*Lactiplantibacillus plantarum*	+++	vegetable, artichoke
	PB288	*Leuconostoc pseudomesenteroides*	++	caper, sour
	FF78	*Pediococcus pentosaceus*	+	fruity, pungent
	PB321	*Weissella confusa*	-	butter, almond
S3	PB278	*Lactiplantibacillus plantarum*	+++	banana, olive pomace
	PB288	*Leuconostoc pseudomesenteroides*	++	caper, sour
	FF78	*Pediococcus pentosaceus*	+	fruity, pungent
	PB321	*Weissella confusa*	-	butter, almond
S FRUITY	PB278	*Lactiplantibacillus plantarum*	+++	banana, olive pomace
	PB295	*Leuconostoc pseudomesenteroides*	++	vegetable, fruity, bitter
	FO40	*Pediococcus pentosaceus*	+	fruity, hay
S YOGURT	PB126	*Companilactobacillus*	++	yogurt, olive pomace,
	PB297	*paralimentarius*	++	butter
	PB321	*Lactiplantibacillus plantarum*	-	yogurt, vegetable,
		Weissella confusa		fruity
				butter, almond

aromatic notes perceived by the trained panel in the mini-batch fermentation trials
+++ pH < 3.5 after 24 h of fermentation
++ pH < 4 after 24 h of fermentation
+ pH < 4.5 after 24 h of fermentation
- pH > 4.5 after 24 h of fermentation

4.2.7. Manufacture of laboratory-scale prototypes of fermented sea fennel preserves

Each multiple strain starter was separately inoculated in 150-mL glass jars containing 105 mL of sterile brine (prepared as described in section 4.2.5) and 35 g of fresh (F) or blanched (B) sea fennel sprouts to reach a final load of ~ 7 Log CFU mL^{-1}. The microbial load of the inoculum was verified as described in section 4.2.5. The jars were incubated statically at 30 °C until a fixed pH value of

approximately 3.8 was reached. The first (I) fermentation assay performed (in duplicate) with starters S1, S2 and S3 using fresh (F) or blanched (B) sea fennel sprouts lasted 5 days and led to the production of six prototypes (F1, F2, F3, B1, B2, and B3, respectively). The second (II) fermentation assay performed (in duplicate) with the starters S FRUITY or S YOGURT and fresh (F) or blanched (B) sea fennel sprouts lasted 13 days and produced four prototypes (FF, FY, BF and BY, respectively). In both assays (I and II), the control samples, consisting of uninoculated preserves made with fresh (CFI and CFII) or blanched (CBI and CBII) sea fennel sprouts (CBI and CBII), were also incubated under the same conditions as the inoculated preserves.

4.2.8. Analysis of the laboratory-scale prototypes

Aliquots of brine were regularly sampled under sterile conditions from the experimental and control prototypes immediately after inoculation and during the fermentation process (at 6, 24, 48, 72 and 96 h for the first assay; at 6, 24, 48, 72, 120, 144, 168, 192, 216 and 288 h for the second assay, Figure 4.1) and subjected to pH measurement (section 4.2.8.1) and microbiological analysis (section 4.2.8.2). At the end of the monitoring period, aliquots of brine and/or sea fennel sprouts collected from both the experimental and control prototypes underwent: (i) the quantification of organic acids (section 4.2.8.1), fatty acids (section 4.2.8.3), and volatile components (section 4.2.8.4); (iii) the olfactory analysis (section 4.2.8.5).

4.2.8.1. pH measurement and organic acid quantification

Brine samples (1 mL) underwent pH analysis as previously described in section 4.2.5.

Organic acid determination was carried out on deproteinized and decolored brines. Deproteinization was accomplished using (i) Carrez I solution, prepared by dissolving 3.60 g of potassium hexacyanoferrate (II) {$K_4[Fe(CN)_6]$ x $3H_2O$} (Sigma Aldrich, Milan, Italy) in 100 mL of distilled water; (ii) Carrez II solution, prepared by dissolving 7.20 g of zinc sulphate ($ZnSO_4.7H_2O$) (Sigma) in 100 mL of distilled water; and (iii) sodium hydroxide solution (NaOH, 100 mM), prepared by

dissolving 4 g of NaOH in 1 L of distilled water. Once clarified, brine samples were decolored with 2% (w v^{-1}) polyvinylpolypyrrolidone. Lactic acid was quantified using the commercial K-DLATE 08/18 kit (Megazyme, USA), whereas acetic acid was quantified with the K-ACETRM 06/18 kit (Megazyme, USA) following the manufacturers' instructions. The results of organic acid quantification (expressed as mM) were expressed as the mean of three technical replicates ± standard deviation.

4.2.8.2. Microbiological analysis

Brine samples (1 mL) were serially diluted in sterile 0.1% (w v^{-1}) peptone water and subjected to the enumeration of (i) mesophilic lactic acid bacteria on MRS agar (VWR) supplemented with cycloheximide (VWR) (100 mg L^{-1}) incubated at 37 °C for 48-72 h; (ii) yeasts on Rose Bengal chloramphenicol agar (VWR) incubated at 25 °C for 5 days; and (iii) Enterobacteriaceae on violet red bile agar (VWR) incubated at 37 °C for 24 h. The results were expressed as the mean log CFU mL^{-1} of brine of two replicates ± standard deviation.

4.2.8.3. Fatty acids analysis

Sampled aliquots (25 g) of sea fennel sprouts were freeze-dried and subjected to raw lipid extraction with an automated Soxhlet apparatus using petroleum ether as a solvent. Fatty acid methyl esters (FAMEs) were prepared by acid-catalyzed transesterification (Ahmed et al., 2020) of lipid extracts and were analyzed by gas chromatography, according to the conditions previously described by Tavoletti et al. (2018).

4.2.8.4. Volatile components analysis

Volatile sampling was carried out by solid phase microextraction (SPME) in 10-mL screw cap septum vials filled with 3 mL of brine and 0.5 g of sea fennel sprouts according to Mozzon et al. (2020a). Sample equilibration was carried out at 38 °C for 15 min in a thermostatic bath, and then the extraction was performed for 5 min using a 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane

(DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA). Volatiles were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Varian 3900 gas chromatograph coupled with a Saturn 2100T ion trap mass detector (Varian Analytical Instruments, Walnut Creek, CA) according to the operative parameters described by Belleggia et al. (2020). Identification of chromatographic peaks was accomplished by comparison with Kovats Retention Indexes and MS data published in the NIST/EPA/NIH Mass Spectral Library (Mozzon et al., 2015).

4.2.8.5. Olfactory analysis

The olfactory analysis was carried out as detailed in section 4.2.5. The panelists were asked to evaluate the presence and intensity of 13 odorous notes (vegetable, fruity, pungent, sour, bitter, caper, artichoke, butter, almond, banana, olive pomace, hay, and yogurt) selected from among those previously perceived in the monocultures. For each olfactory descriptor, the panelists were asked to assign a score ranging between 1 and 5, with 1 expressing the lowest and 5 the highest intensity. The panelists were also invited to express their degree of liking by using a 9-point hedonic scale, where 1 expressed the lowest (dislike extremely) and 9 the highest (like extremely) degree of liking (Peryam and Pilgrim, 1957).

4.2.9. Manufacture of the pilot-scale prototypes of fermented sea fennel preserves

Multiple strain starter S2 was selected for the manufacture of pilot-scale prototypes of fermented sea fennel sprouts at a local industrial producer of vegetable preserves (Rinci S.r.l.). One production batch, consisting of three biological replicates, was manufactured. For each replicate, 10 Kg of blanched sea fennel sprouts was supplemented with 30 L of brine prepared as described in section 4.2.5 in 30 L steel casks and inoculated with the multiple strain starter culture to reach a final load of ~ 7 Log CFU mL^{-1}. The casks were maintained at 18 ± 2 °C until a fixed pH value of 3.80 was reached (approximately after 9 weeks of fermentation). During the incubation period, brine and sea fennel sprouts were regularly mixed by using a sterilized steel tool.

4.2.10. Analysis of the pilot-scale prototype

Aliquots of brine were sampled under aseptic conditions from each replicate of the pilot-scale prototype immediately after inoculation (t_0) and during the fermentation process for pH determination (Figure 4.1, all sampling points). Additional brine aliquots (sampled at t_0 and days 1, 3, 6, 10, 15, 21, 29, 42, 62) underwent total titratable acidity (TTA) determination (section 4.2.10.1) and viable counting (section 4.2.10.2) together with organic acid quantification (t_0 and days 29, 42, 62). Aliquots of sea fennel sprouts sampled at t_0 and at days 29, 42 and 62 underwent quantification of vitamin C and dietary fiber (section 4.2.10.3), antioxidant capacity (section 4.2.10.4), total polyphenols (section 4.2.10.5), fatty acids (section 4.2.10.6) and volatile organic compounds (VOCs) (section 4.2.10.7). At day 29, aliquots of a mixture of brine and sea fennel sprouts at a 1:1 ratio were also analyzed (in duplicate) for the absence of *Clostridium botulinum* and coagulase-positive staphylococci (section 4.2.10.2). Finally, at the end of fermentation (day 62), aliquots of sea fennel sprouts were subjected to sensory analysis (section 4.2.10.8).

4.2.10.1. Physico-chemical analyses

pH measurement and organic acid quantification were performed as previously described in sections 4.2.5 and 4.2.8.1, respectively.

For TTA determination, aliquots (10 mL) of brine samples were diluted with distilled water (90 mL), and then the resulting solution underwent titration with NaOH (0.1 N). The results were expressed as mL of NaOH solution added to reach a fixed pH value of 8.3. All analyses were performed in triplicate, and the results were expressed as the mean of three technical replicates ± standard deviation.

4.2.10.2. Microbiological analysis

Ten mL of each brine sample was serially diluted in sterile 0.1% (w v^{-1}) peptone water for the enumeration of (i) mesophilic lactic acid bacteria; (ii) yeasts; and (iii) Enterobacteriaceae as described

in section 4.2.8.2. Total mesophilic aerobes were counted on plate count agar (PCA) (VWR) incubated at 30 °C for 48 h. The results of the viable counts are expressed as the mean log CFU mL^{-1} of brine of three replicates ± standard deviation. The occurrence of the genes coding for botulinic toxins, *bont/A, bont/B, bont/E, bont/F*, and *4 gyrB (CP)*, was determined according to the multiplex real-time PCR method of the Italian National Reference Centre for botulism (http://old.iss.it/binary/cnrb/cont/CNRB31.010.pdf). Coagulase-positive staphylococci were counted in accordance with the TEMPO:AFNOR BIO 12/28-04/10 standard method.

4.2.10.3. Vitamin C and dietary fiber determination

For each sample, 10 g of sea fennel sprouts and 100 mL of 5% metaphosphoric acid were mechanically mixed for 15 min, centrifuged at 4000 rpm for 10 min and filtered using filter paper. The filtered solution was purified on a SEP-PACK C18 column using a vacuum manifold. The extract was filtered through a 0.45-µm syringe filter and analyzed by HPLC with a UV-DAD detector (SHIMADZU Nexera X2, Milano, Italy) using an RP-C18 column (250 x 3.0 mm i.d., 5 µm), a run time of 15 min and 245 nm absorbance. Chromatographic separation was performed at an isocratic mobile phase of 0.04 mol L^{-1} phosphate buffer, pH 2.8, with a 0.34 mL min^{-1} flow rate and an injection volume of 20 µL. Ascorbic acid identification was performed based on retention time and the UV absorption spectrum of the standard. The results were performed on a standard calibration curve.

Crude fiber was determined by the fritted glass crucible method (AOAC, 978.10), as previously described (Roncolini et al., 2020).

4.2.10.4. Antioxidant capacity

For each sample, an aliquot (5 g) of sea fennel sprouts was accurately minced to produce a sea fennel paste using the A-11 basic analytical mill (IKA-Werke, Staufen, Germany) along with liquid nitrogen and were stored at -20 °C prior to analysis. The extraction was performed by adding 10 mL of a methanol and water solution (at a 70:30 ratio, v v^{-1}) to 0.1 mg of sea fennel paste. The samples were

mixed for 30 min on a shaker at 200 strokes min^{-1} and then centrifuged at 4,000 rpm for 10 min. The supernatants were recovered, filtered through a filter with a pore size of 0.40 µM, and the extracts were stored at -20 °C under dark conditions until analysis.

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids contained in the samples was investigated following the method previously described by Brand-Williams et al. (1995).

The reducing potential was assessed by using a simple test measuring the ferric-reducing ability of plasma, referred to as the FRAP test. This latter was performed following the procedure reported by Pulido et al. (2000).

Total antioxidant capacity (T-AOC), corresponding to the cumulative effect of both the enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px)] and nonenzymatic (uric acid, vitamin C, vitamin E, glutathione, bilirubin, α-lipoic acid, carotenoid) antioxidant systems, was also evaluated using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay as previously described by Re et al. (1999). Briefly, a 2.45 mM K_2SO_4 aqueous solution was added to a 7 mM ABTS aqueous solution and kept under dark conditions for 12-16 h to generate the ABTS radical cation (ABTS·+). The resulting mixture was diluted with ethanol at a 1:70 ratio to reach an absorbance at 734 nm of approximately 0.70 and, hence, was used as a control. Ten microliters of the extract was added to 990 µL of the ABTS + aqueous solution. The absorbance was measured at 734 nm 5, 10, 20 and 30 min after the initial mixing. Ten microliters of the extraction solvent (made of a methanol and water solution at a 70:30 ratio) was added to 990 µL of ABTS·+. aqueous solution, which was then used as a control. The inhibition percentage (%IP) was calculated by the equation:

$$[(Ac - As)/Ac] * 100$$

where *Ac* and *As* are the absorbance levels of the control and the test samples, respectively.

A regression equation was prepared using Trolox solutions in methanol with increasing molarity (from 0.1 to 1 mM). The results were expressed as mmol Trolox equivalent (TEAC) g^{-1} wet basis of sea fennel sprouts of three replicates ± standard deviation.

4.2.10.5. Total polyphenols determination

The total polyphenol content of sea fennel sprouts was determined according to the Folin-Ciocalteu method, as described by Mozzon et al. (2020b). A set of gallic acid solutions in methanol/water 80:20 v v^{-1} (100, 300, 500, and 700 ppm) was used as an external standard.

4.2.10.6. Analysis of fatty acids

For each sample, an aliquot (50 g) of sea fennel sprouts underwent fatty acid analysis as previously described (section 4.2.8.3).

4.2.10.7. Volatile compound determination by headspace-solid phase microextraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS)

The volatile fraction of each sea fennel sample was analyzed by headspace sampling using the solid-phase microextraction technique (SPME) according to Reale et al. (2016) with some modifications. In detail, a 500-mg aliquot of a mixture of sea fennel sprouts and brine (at 1:1 ratio) was placed in a 20 mL headspace vial. The sample was equilibrated at 40 °C for 2 min at 250 rpm using a Gerstel MPS2 automatic sampling system (Gerstel GmbH & Co., Mülheim, Germany). The analysis was conducted by a GC/MS system (Agilent 7890/5975 Inert, Agilent, Santa Clara, CA, United States) equipped with the abovementioned autosampler with helium as the carrier gas (1 mL min^{-1}). A coated divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Sigma Aldrich S.r.l., Milan, Italy) was exposed to the headspace of the sample for 15 min, maintaining the sample at 40 °C. The fiber was desorbed for 5 min at 240 °C in the injection unit in split mode (split ratio 50:1). The separation was carried out in a capillary column (HP-Innowax, Agilent Technologies, USA) (30 m × 0.25 mm i.d. × 0.50 μm film thickness). The GC oven temperature program started at 35 °C for

5 min, then was ramped to 150 °C at 5 °C min^{-1} and to 240 °C at 15 °C min^{-1} and maintained the final temperature for 1 min. The mass spectrometer operated with an ion source of 230 °C, a quadrupole temperature of 150 °C, 70 eV electron energy and acquired in TIC mode from m z^{-1} 30 to 300 uma. Identification of volatile compounds was achieved by comparing mass spectra with the Wiley library (Wiley7, NIST 05). The proportion of each compound was estimated by dividing its mean area by the total area of the chromatogram and was expressed as a percentage. Blank experiments were conducted in two different modalities: blank of the fiber and blank of the empty vial. These types of controls were carried out after every 4 analyses. All the analyses were performed in duplicate.

4.2.10.8. Sensory analysis

The three replicates of the pilot-scale prototype were evaluated by the trained panelists for (i) two olfactory descriptors, selected from among those prevailing in the laboratory-scale prototypes, being vegetable and fruity; (ii) three new olfactory descriptors suggested by the local producer of sea fennel-based foods and food ingredients, being woody, spicy, and kerosene-like; (iii) four flavor descriptors, being vegetable, woody, spicy, and kerosene-like; (iii) and four taste descriptors, being sour, bitter, salty, and sweet; (iv) three textural descriptors, being hardness, fibrousness, and crunchiness; (v) global acceptance. Aliquots of drained sea fennel sprouts (~ 10 g per panelist) were placed in white plastic cups, blindly labeled, and presented to the panelists as previously described in section 4.2.5. This time each booth was equipped with both still bottled water for oral rinsing and coffee beans for olfactory cleansing before and between the evaluations (Resurreccion, 1998). The 9-point hedonic scale described in section 4.2.8.5 was used by the panelists to assign a score to the intensity of descriptors. Final data were reported as mean value scores ± standard deviation.

4.2.11. Statistical analyses

The RAPD profiles of the selected lactic acid bacteria isolates were compared using CLIQS 1D Pro Version 1.1 software (TotalLab Ltd, Newcastle upon Tyne, UK). An unweighted pair-group method

using arithmetic average (UPGMA) (Sokal & Sneath, 1963) dendrogram was constructed based on the number and position of RAPD bands in the different RAPD profiles.

Data overall collected during the pro-technological characterization of the 27 lactic acid bacteria and the analysis of the laboratory and pilot-scale prototypes were subjected to one-way analysis of variance (ANOVA): multiple means comparisons were carried out through the Tukey-Kramer honest significant difference (HSD) test at the level of significance 0.05. A multivariate approach (principal component analysis, PCA) was used to explore the structure of experimental data (microbial counts, organic acids, pH, fatty acids, volatile components, sensory descriptors) collected from laboratory-scale prototypes. A normalized data matrix was used to optimally describe the orientations of the scores and loadings. JMP Version 11.0.0 software (SAS Institute Inc., Cary, NC) was used for ANOVA and PCA tests.

Pearson correlation analysis was performed to measure the relationship between the antioxidant activity tests using SPSS Statistics 21.0 software (SPSS Inc., Chicago, IL).

Statistical analyses of VOCs were performed using XLSTAT 2009 (www.xlstat.com). Data were expressed as the mean ± standard deviation using ANOVA. Differences at $P < 0.05$ were considered statistically significant by Duncan's test.

4.3. Results

4.3.1. Lactic acid bacteria molecular typing

The lactic acid bacteria listed in Table 4.1, except for LM9, PB126, PB321, and PB337, were subjected to RAPD analysis. Hence, the obtained RAPD profiles were used for the construction of a UPGMA dendrogram (Supplementary Figure 4.1). Considering an arbitrarily selected similarity cutoff equal to 75%, 6 clusters were identified. In detail, for *Lacp. plantarum*, 5 clusters and 6 independent strains were found. For *P. pentosaceus*, the 4 isolates produced RAPD profiles with less

than 75% similarity. Analogously, RAPD profiles with more than 75% similarity were shown by the two *L. pseudomesenteroides* isolates.

4.3.2. Screening of lactic acid bacteria

The results of ΔpH reached by the 27 monocultures after 6, 24, 48, and 120 hours of fermentation are reported in Supplementary Table 4.1. As early as 24 hours, all the assayed cultures, except for FF71, FO40, FO41, LM9, PB104, PB151, and PB337, reached pH values lower than 3.80. After 120 hours, even the latter cultures, with the sole exception of FF71, reduced the pH to values below this threshold.

Regarding the sensory analysis, the perceived aromatic notes identified by the trained panel are listed in Supplementary Table 4.2.

As detailed in section 4.2.6, ten strains with the best performance in terms of acidification and/or production of peculiar/pleasant aromatic notes were selected for the formulation of five multiple strain starters (Table 4.2), which were exploited for the manufacture of laboratory-scale prototypes in two successive fermentation assays. The results of the analyses carried out on these prototypes are detailed in section 4.3.3.

4.3.3. Laboratory-scale fermented preserves

4.3.3.1. Determination of pH and organic acids quantification

The results of pH determination are shown in Table 4.3. At t_0, the pH values ranged from 5.28 ± 0.06 (F2) to 5.78 ± 0.06 (CBI) (first fermentation assay) and from 5.64 ± 0.00 (FF) to 6.41 ± 0.01 (CBII) (second fermentation assay). In both assays, the pH progressively decreased, reaching final values ranging from 3.73 ± 0.08 (F2) to 3.81 ± 0.08 (B2) (first assay) and from 3.83 ± 0.06 (FY) to 3.94 ± 0.04 (BY) (second assay). For the uninoculated controls, the following pH values were reached: 4.74

± 0.03 (CFI) and 4.92 ± 0.06 (CBI) (first assay) and 5.18 ± 0.01 (CFII) and 4.97 ± 0.10 (CBII) (second assay). A progressive pH reduction was seen in all the prototypes, including the uninoculated controls.

The results of lactic and acetic acid quantification are shown in Table 4.4. In more detail, acetic acid ranged from 0.40 ± 0.02 (F2) to 3.39 ± 0.58 mM (CFII), whereas total lactic acid ranged from 2.48 ± 0.02 (CFII) to 59.81 ± 8.13 mM (F3). As all the prototypes (from assays I and II) were comparatively evaluated, lactic acid was the primary metabolite produced at the highest level. In almost half of the prototypes, the isomer L-lactic acid was significantly more abundant than D-lactic acid. No trends associated with the initial treatment of sea fennel sprouts subjected to fermentation emerged. Finally, in all the uninoculated controls, organic acids were produced, with CFII and CBI being among the prototypes showing the highest level of acetic acid (3.39 and 2.14, respectively).

4.3.3.2. Microbiological analysis

The results of viable counting carried out on the laboratory-scale prototypes are reported in Table 4.5.

For mesophilic lactic acid bacteria, no significant differences were seen at t_0 in the load of this microbial group if the prototypes produced with fresh and blanched sea fennel were compared. In almost all the inoculated prototypes, except for B1, from t_0 to t_{48} (for assay I) or t_{168} (for assay II), the loads of these microorganisms showed reductions followed by a subsequent increase up to values comparable to those recorded at t_0. A different picture emerged from the controls, where the load of mesophilic lactic acid bacteria gradually increased from < 1.0 Log CFU mL^{-1} to values higher than the detection limit.

Regarding yeasts and Enterobacteriaceae, two different pictures emerged from the prototypes produced with fresh or blanched sea fennel; in the latter, mean counts < 1.0 Log CFU mL^{-1} were observed in the early fermentation stage, followed by counts progressively increasing in almost all the prototypes. In contrast, in the first prototypes, progressive decreases in the loads of both microbial groups were observed.

Table 4.3. Results of the pH determination of the laboratory-scale prototypes made with fresh (F) and blanched (B) sea fennel sprouts.

Fermentation assay	Sampling time (t, hours)	Prototype							
		CFI	F1	F2	F3	CBI	B1	B2	B3
I	t_0	$5.44 \pm 0.06^{cd\ A}$	$5.34 \pm 0.01^{d\ A}$	$5.28 \pm 0.06^{d\ A}$	$5.34 \pm 0.03^{d\ A}$	$5.78 \pm 0.06^{a\ A}$	$5.67 \pm 0.01^{ab\ A}$	$5.55 \pm 0.10^{bc\ A}$	$5.64 \pm 0.02^{abc\ A}$
	t_6	$5.31 \pm 0.03^{bcd\ AB}$	$5.24 \pm 0.01^{cd\ AB}$	$5.16 \pm 0.07^{d\ A}$	$5.24 \pm 0.01^{cd\ AB}$	$5.71 \pm 0.03^{a\ A}$	$5.50 \pm 0.08^{abc\ A}$	$5.39 \pm 0.15^{bcd\ A}$	$5.52 \pm 0.06^{ab\ A}$
	t_{24}	$5.31 \pm 0.01^{bcd\ AB}$	$5.19 \pm 0.04^{bcd\ B}$	$5.09 \pm 0.06^{d\ A}$	$5.17 \pm 0.04^{cd\ B}$	$5.73 \pm 0.04^{a\ A}$	$5.49 \pm 0.21^{abc\ A}$	$5.45 \pm 0.08^{abcd\ A}$	$5.56 \pm 0.11^{ab\ A}$
	t_{48}	$5.09 \pm 0.06^{a\ C}$	$4.07 \pm 0.05^{b\ C}$	$3.89 \pm 0.08^{b\ B}$	$3.97 \pm 0.03^{b\ C}$	$5.35 \pm 0.07^{a\ B}$	$4.10 \pm 0.06^{b\ B}$	$4.00 \pm 0.10^{b\ B}$	$4.20 \pm 0.17^{b\ B}$
	t_{72}	$5.15 \pm 0.08^{a\ BC}$	$3.71 \pm 0.04^{b\ D}$	$3.72 \pm 0.11^{b\ B}$	$3.70 \pm 0.04^{b\ D}$	$5.02 \pm 0.03^{a\ C}$	$3.91 \pm 0.21^{b\ B}$	$3.86 \pm 0.13^{b\ B}$	$3.82 \pm 0.09^{b\ C}$
	t_{96}	$4.74 \pm 0.03^{a\ D}$	$3.73 \pm 0.01^{b\ D}$	$3.73 \pm 0.08^{b\ B}$	$3.73 \pm 0.04^{b\ D}$	$4.92 \pm 0.06^{a\ C}$	$3.78 \pm 0.08^{b\ B}$	$3.81 \pm 0.08^{b\ B}$	$3.77 \pm 0.03^{b\ C}$
		CFII	FF	FY		CBII	BF	BY	
II	t_0	$6.11 \pm 0.04^{b\ A}$	$5.64 \pm 0.00^{c\ A}$	$5.81 \pm 0.03^{c\ A}$		$6.41 \pm 0.01^{a\ A}$	$6.08 \pm 0.15^{b\ A}$	$6.17 \pm 0.03^{ab\ A}$	
	t_6	$5.64 \pm 0.04^{bc\ B}$	$5.28 \pm 0.01^{d\ AB}$	$5.46 \pm 0.05^{cd\ B}$		$6.00 \pm 0.00^{a\ AB}$	$5.68 \pm 0.20^{abc\ AB}$	$5.89 \pm 0.01^{ab\ AB}$	
	t_{24}	$5.27 \pm 0.03^{cd\ DEF}$	$5.10 \pm 0.01^{d\ B}$	$5.16 \pm 0.02^{d\ BC}$		$5.66 \pm 0.01^{a\ BCD}$	$5.42 \pm 0.13^{bc\ BC}$	$5.57 \pm 0.01^{ab\ B}$	
	t_{48}	$5.31 \pm 0.02^{bc\ DEF}$	$5.07 \pm 0.16^{c\ B}$	$5.05 \pm 0.09^{c\ C}$		$5.94 \pm 0.16^{a\ BC}$	$5.45 \pm 0.18^{bc\ BC}$	$5.75 \pm 0.00^{ab\ AB}$	
	t_{72}	$5.24 \pm 0.04^{b\ EF}$	$4.93 \pm 0.13^{b\ B}$	$4.94 \pm 0.16^{b\ C}$		$5.95 \pm 0.10^{a\ BC}$	$4.93 \pm 0.19^{b\ CD}$	$5.84 \pm 0.06^{a\ AB}$	
	t_{120}	$5.45 \pm 0.06^{ab\ C}$	$4.15 \pm 0.23^{c\ C}$	$4.14 \pm 0.16^{c\ D}$		$5.65 \pm 0.11^{a\ BCD}$	$4.45 \pm 0.18^{c\ DE}$	$4.79 \pm 0.27^{bc\ C}$	
	t_{144}	$5.40 \pm 0.00^{a\ CD}$	$4.04 \pm 0.18^{bc\ C}$	$3.88 \pm 0.03^{c\ DE}$		$5.66 \pm 0.07^{a\ BCD}$	$4.24 \pm 0.05^{bc\ EF}$	$4.47 \pm 0.29^{b\ CD}$	
	t_{168}	$5.36 \pm 0.04^{a\ CDE}$	$4.01 \pm 0.15^{b\ C}$	$3.84 \pm 0.03^{b\ DE}$		$5.45 \pm 0.04^{a\ D}$	$4.16 \pm 0.02^{b\ EF}$	$4.26 \pm 0.22^{b\ CD}$	
	t_{192}	$5.25 \pm 0.01^{a\ EF}$	$4.00 \pm 0.16^{b\ C}$	$3.83 \pm 0.04^{b\ DE}$		$5.55 \pm 0.18^{a\ CD}$	$4.13 \pm 0.01^{b\ EF}$	$4.11 \pm 0.13^{b\ D}$	
	t_{216}	$5.27 \pm 0.01^{a\ EF}$	$3.99 \pm 0.16^{b\ C}$	$3.81 \pm 0.04^{b\ E}$		$5.52 \pm 0.14^{a\ D}$	$4.09 \pm 0.01^{b\ EF}$	$4.01 \pm 0.09^{b\ D}$	
	t_{288}	$5.18 \pm 0.01^{a\ F}$	$3.90 \pm 0.01^{b\ C}$	$3.83 \pm 0.06^{b\ DE}$		$4.97 \pm 0.10^{a\ E}$	$3.87 \pm 0.20^{b\ F}$	$3.94 \pm 0.04^{b\ D}$	

The results are expressed as means of two biological and three technical replicates ± standard deviations. For each fermentation assay (I or II), values labelled with different small letters in the same row are significantly different ($P < 0.05$), whereas values labelled with different capital letters in the same column are significantly different ($P < 0.05$).

CFI Uninoculated control prototype produced with fresh sea fennel in fermentation assay I
F1 Prototype produced with fresh sea fennel and multiple strain starter S1, in fermentation assay I
F2 Prototype produced with fresh sea fennel and multiple strain starter S2, in fermentation assay I
F3 Prototype produced with fresh sea fennel and multiple strain starter S3, in fermentation assay I
CBI Uninoculated laboratory-scale control prototype produced with blanched sea fennel in fermentation assay I
B1 Prototype produced with blanched sea fennel and multiple strain starter S1, in fermentation assay I
B2 Prototype produced with blanched sea fennel and multiple strain starter S2, in fermentation assay I
B3 Prototype produced with blanched sea fennel and multiple strain starter S3, in fermentation assay I
CFII Uninoculated laboratory-scale control prototype produced with fresh sea fennel in fermentation assay II
FF Prototype produced with fresh sea fennel and multiple strain starter S FRUITY, in fermentation assay II
FY Prototype produced with fresh sea fennel and multiple strain starter S YOGURT, in fermentation assay II
CBII Uninoculated laboratory-scale control prototype produced with blanched sea fennel in fermentation assay II
BF Prototype produced with blanched sea fennel and multiple strain starter S FRUITY, in fermentation assay II
BY Prototype produced with blanched sea fennel and multiple strain starter S YOGURT, in fermentation assay II

4.3.3.3. Fatty acids analysis

The fatty acid composition of sea fennel sprouts sampled from the laboratory-scale preserves at the end of the monitoring period is shown in Table 4.6. Overall, no significant differences in the fatty acid compositions of the samples were seen, with some exceptions: C16:1 and C20:1 in the first fermentation assay and C18:0 plus C18:1Δ9t in the second fermentation assay. The most abundant detected fatty acids over all the samples were C18:2Δ9,12 and C18:3Δ9,12,15, whereas conjugated fatty acids derived from hydrogenation of C:18:2 and C18:3 were never detected.

4.3.3.4. Volatile components analysis

The results of the analysis of the volatile compounds of the laboratory-scale prototypes at the end of the first and second fermentation assays are reported in Table 4.7. As a general trend, monoterpene hydrocarbons were the major aromatic constituents, accounting for 64 to 83% of the total volatiles. In more detail, γ-terpinene (30-51%), p-cimene (16-32%) and thymol methyl ether were the most represented volatiles, accounting for 14 to 27%, while only low amounts of acetic acid were detected.

In the first fermentation assay, for both the controls (CFI, CBI), blanching treatment seemed not to affect the volatiles; however, for the inoculated prototypes, significantly higher mean levels of γ-terpinene (51.08% in B1 vs 30.26% in F1) and myrcene (1.31% in B1 vs 0.55% in F1 and 1.39% in B2 vs 0.79% in F2) and lower levels of p-cymene (17.94% in B3 vs 24.97% in F3) were found in the prototypes prepared with blanched sea fennel than in those prepared with fresh sea fennel. In the second fermentation assay, blanching had apparently no effect on the volatiles of either the controls (CFII, CBII) or the inoculated samples. The sole exception was represented by BY, where a significantly higher mean % level of sabinene (0.12%) was found with respect to FY (0.04%).

Table 4.4. Results of the organic acid quantification of the laboratory scale prototypes realized (in duplicate) using fresh (F) and blanched (B) sea fennel sprouts, at the end of the fermentation period.

Fermentation assay	Prototype	Lactic acid			Acetic acid
		D-lactic acid	L-lactic acid	Total lactic acid	
I	CFI	4.90 ± 0.14^{b} C	9.68 ± 0.05^{b} B	14.58 ± 0.09^{b} A	0.45 ± 0.02^{bc} D
	F1	18.44 ± 0.88^{a} B	31.25 ± 5.28^{a} B	49.69 ± 6.16^{a} A	1.52 ± 0.78^{abc} C
	F2	23.65 ± 5.23^{a} B	34.75 ± 3.07^{a} B	58.40 ± 8.30^{a} A	0.40 ± 0.02^{c} C
	F3	22.83 ± 2.06^{a} B	36.97 ± 6.06^{a} B	59.81 ± 8.13^{a} A	1.31 ± 0.25^{abc} C
II	CFII	0.62 ± 0.34^{a} C	1.85 ± 0.32^{d} BC	2.48 ± 0.02^{c} AB	3.39 ± 0.58^{a} A
	FF	17.84 ± 0.03^{a} B	18.64 ± 1.24^{ab} B	36.48 ± 1.22^{a} A	2.44 ± 1.29^{ab} C
	FY	13.46 ± 7.07^{a} AB	17.87 ± 1.22^{b} AB	31.33 ± 5.85^{ab} A	1.26 ± 0.03^{ab} B
I	CBI	6.58 ± 0.02^{b} C	8.74 ± 0.10^{b} B	15.32 ± 0.12^{b} A	2.14 ± 0.03^{a} D
	B1	22.44 ± 1.26^{a} C	28.86 ± 1.09^{a} B	51.31 ± 2.35^{a} A	1.49 ± 0.24^{abc} D
	B2	19.67 ± 5.83^{a} BC	31.70 ± 2.82^{a} AB	51.37 ± 8.65^{a} A	0.69 ± 0.08^{abc} C
	B3	19.30 ± 0.83^{a} C	28.11 ± 1.34^{a} B	47.41 ± 2.17^{a} A	1.99 ± 0.70^{ab} D
II	CBII	2.85 ± 0.16^{a} B	12.97 ± 1.28^{c} A	15.82 ± 1.12^{bc} A	0.84 ± 0.42^{b} B
	BF	17.93 ± 6.94^{a} AB	19.97 ± 0.28^{ab} AB	37.89 ± 7.22^{a} A	0.70 ± 0.34^{b} B
	BY	17.94 ± 6.41^{a} BC	22.82 ± 1.73^{a} B	40.76 ± 4.69^{a} A	2.68 ± 0.30^{ab} C

The results (expressed as mM) are expressed as mean of two biological and three technical replicates ± standard deviation.
For each type of raw material (fresh or blanched sea fennel), values with different small letters in the same column are significantly different ($P < 0.05$), whereas values with different capital letters in the same row are significantly different ($P < 0.05$).
For legend of prototypes see Table 4.3.

Table 4.5. Microbial viable counts of mesophilic lactic acid bacteria, yeasts and Enterobacteriaceae on the laboratory-scale prototypes made with fresh (F) and blanched (B) sea fennel sprouts.

Microbial group	Fermentation assay	Sampling time (t, hours)	Prototypes							
Mesophilic lactic acid bacteria (log CFU mL^{-1})	I		CFI	F1	F2	F3	CBI	B1	B2	B3
		t_0	$< 1.0^{b}$ D	7.2 ± 0.1^{a} A	7.4 ± 0.1^{a} A	7.2 ± 0.2^{a} A	$< 1.0^{b}$ D	7.2 ± 0.3^{a} A	7.5 ± 0.2^{a} AB	7.2 ± 0.2^{a} AB
		t_6	$< 1.0^{b}$ D	6.2 ± 0.2^{a} BC	6.3 ± 0.3^{a} B	6.3 ± 0.0^{a} B	$< 1.0^{b}$ D	6.3 ± 0.8^{a} A	6.8 ± 0.1^{a} C	6.5 ± 0.5^{a} AB
		t_{24}	$< 1.0^{b}$ D	6.1 ± 0.2^{a} C	6.3 ± 0.1^{a} B	5.9 ± 0.0^{a} B	$< 1.0^{b}$ D	6.1 ± 0.9^{a} A	7.0 ± 0.1^{a} BC	6.1 ± 0.6^{a} B
		t_{48}	3.9 ± 0.1^{b} C	7.5 ± 0.2^{a} A	7.5 ± 0.1^{a} A	7.6 ± 0.1^{a} A	3.1 ± 0.2^{b} B	7.7 ± 0.7^{a} A	7.8 ± 0.2^{a} A	7.8 ± 0.1^{a} A
		t_{72}	5.2 ± 0.3^{b} B	7.3 ± 0.3^{a} A	7.3 ± 0.0^{a} A	7.5 ± 0.2^{a} A	3.7 ± 0.1^{c} A	7.2 ± 0.2^{a} A	7.4 ± 0.0^{a} AB	7.4 ± 0.0^{a} A
		t_{96}	7.0 ± 0.1^{a} A	6.9 ± 0.2^{a} AB	7.0 ± 0.1^{a} A	7.1 ± 0.2^{a} A	1.5 ± 0.1^{b} C	7.1 ± 0.1^{a} A	7.3 ± 0.2^{a} ABC	7.0 ± 0.1^{a} AB
Yeasts (log CFU ml^{-1})			CFI	F1	F2	F3	CBI	B1	B2	B3
		t_0	2.8 ± 0.0^{a} E	3.5 ± 0.2^{a} B	3.4 ± 0.6^{a} A	3.6 ± 1.3^{a} AB	$< 1.0^{b}$ D	$< 1.0^{b}$ D	$< 1.0^{b}$ D	$< 1.0^{b}$ C
		t_6	4.0 ± 0.0^{a} D	3.0 ± 0.0^{ab} BC	2.2 ± 0.5^{b} AB	2.8 ± 0.1^{ab} B	3.1 ± 0.0^{ab} BC	2.9 ± 0.4^{ab} A	2.2 ± 0.3^{b} BC	2.0 ± 1.0^{b} BC
		t_{24}	4.6 ± 0.1^{a} D	3.8 ± 0.2^{abc} B	3.4 ± 0.2^{cd} A	4.4 ± 0.1^{ab} AB	2.7 ± 0.3^{d} C	1.3 ± 0.4^{e} C	1.4 ± 0.3^{e} C	3.6 ± 0.0^{bc} AB

Table 4.5. (Continued).

Microbial group	Fermentation assay	Sampling time (t, hours)	Prototypes							
Yeasts (log CFU ml^{-1})			CFI	F1	F2	F3	CBI	B1	B2	B3
		t_{48}	7.0 ± 0.3^{a} B	5.0 ± 0.0^{b} A	3.2 ± 0.4^{c} A	3.9 ± 0.2^{bc} AB	4.9 ± 0.1^{b} A	1.7 ± 0.0^{d} BC	1.9 ± 0.3^{d} BC	4.2 ± 0.3^{bc} A
		t_{72}	8.5 ± 0.1^{a} A	2.1 ± 0.4^{e} CD	3.9 ± 0.0^{cd} A	5.4 ± 0.3^{b} A	4.8 ± 0.1^{bc} A	2.8 ± 0.1^{de} AB	2.9 ± 0.3^{de} B	3.6 ± 0.6^{cd} AB
		t_{96}	5.5 ± 0.2^{a} C	1.2 ± 0.3^{c} D	1.0 ± 0.5^{c} B	4.1 ± 0.2^{b} AB	3.6 ± 0.2^{b} B	3.4 ± 0.4^{b} A	3.9 ± 0.1^{b} A	4.1 ± 0.4^{b} AB
Enterobacteriaceae (log CFU mL^{-1})			CFI	F1	F2	F3	CBI	B1	B2	B3
		t_{0}	3.0 ± 0.1^{a} AB	2.3 ± 0.1^{b} BC	2.5 ± 0.0^{b} AB	3.1 ± 0.3^{a} A	$< 1.0^{c}$ D	$< 1.0^{c}$ A	$< 1.0^{c}$ B	$< 1.0^{c}$ C
		t_{6}	2.8 ± 0.4^{a} AB	2.4 ± 0.4^{a} BC	2.1 ± 0.1^{a} B	3.2 ± 1.2^{a} A	$< 1.0^{b}$ D	$< 1.0^{b}$ A	$< 1.0^{b}$ B	$< 1.0^{b}$ C
		t_{24}	2.6 ± 0.1^{b} B	3.2 ± 0.1^{b} B	3.1 ± 0.2^{b} A	4.5 ± 0.1^{a} A	2.7 ± 0.1^{b} B	$< 1.0^{d}$ A	1.1 ± 0.6^{c} AB	3.4 ± 0.1^{b} B
		t_{48}	3.5 ± 0.1^{bc} A	4.4 ± 0.3^{b} A	$< 1.0^{e}$ C	3.2 ± 0.4^{c} A	3.6 ± 0.0^{bc} A	$< 1.0^{e}$ A	1.8 ± 0.4^{d} A	6.9 ± 0.0^{a} A
		t_{72}	2.3 ± 0.0^{b} B	1.9 ± 0.4^{b} C	$< 1.0^{c}$ C	$< 1.0^{c}$ B	2.2 ± 0.1^{b} C	$< 1.0^{c}$ A	$< 1.0^{c}$ B	4.1 ± 0.2^{a} B
		t_{96}	2.6 ± 0.2^{a} B	$< 1.0^{b}$ D	$< 1.0^{b}$ C	$< 1.0^{b}$ B	3.4 ± 0.1^{a} A	$< 1.0^{b}$ A	$< 1.0^{b}$ B	3.0 ± 0.8^{a} B
Mesophilic lactic acid bacteria (log CFU mL^{-1})	II		CFII	FF	FY		CBII	BF	BY	
		t_{0}	$< 1.0^{b}$ A	7.4 ± 0.0^{a} A	7.3 ± 0.4^{a} A		$< 1.0^{b}$ B	7.4 ± 0.0^{a} A	7.1 ± 0.1^{a} A	
		t_{6}	$< 1.0^{b}$ A	5.3 ± 0.7^{a} AB	4.1 ± 0.1^{a} B		$< 1.0^{b}$ B	6.1 ± 0.2^{a} AB	4.2 ± 0.4^{a} C	
		t_{24}	1.3 ± 1.9^{ab} A	3.7 ± 1.0^{ab} B	3.4 ± 0.1^{ab} B		$< 1.0^{b}$ B	4.9 ± 0.3^{a} B	4.9 ± 0.1^{a} C	
		t_{48}	1.7 ± 2.4^{bc} A	5.3 ± 0.7^{ab} AB	4.0 ± 0.2^{abc} B		$< 1.0^{c}$ B	6.4 ± 0.0^{a} AB	5.6 ± 0.1^{ab} B	
		t_{168}	1.8 ± 2.5^{b} A	6.7 ± 0.1^{a} A	6.3 ± 0.7^{ab} A		4.5 ± 0.2^{ab} A	6.1 ± 1.1^{ab} AB	7.1 ± 0.1^{a} A	
		t_{288}	4.5 ± 0.1^{c} A	7.1 ± 0.1^{a} A	6.3 ± 0.1^{b} A		6.1 ± 0.2^{b} A	7.3 ± 0.3^{a} A	6.4 ± 0.1^{b} A	
Yeasts (log CFU ml^{-1})			CFII	FF	FY		CBII	BF	BY	
		t_{0}	3.1 ± 0.3^{ab} D	2.6 ± 0.2^{b} C	3.3 ± 0.0^{a} D		$< 1.0^{c}$ D	$< 1.0^{c}$ C	$< 1.0^{c}$ C	
		t_{6}	3.2 ± 0.3^{a} D	2.6 ± 1.0^{a} C	3.5 ± 0.3^{a} CD		$< 1.0^{b}$ D	$< 1.0^{b}$ C	$< 1.0^{b}$ C	
		t_{24}	5.3 ± 0.1^{a} C	4.8 ± 0.9^{a} AB	5.6 ± 0.4^{a} AB		$< 1.0^{b}$ CD	2.0 ± 0.5^{b} B	$< 1.0^{b}$ C	
		t_{48}	6.9 ± 0.0^{a} A	6.1 ± 0.1^{b} A	6.5 ± 0.1^{ab} A		1.2 ± 0.0^{d} C	4.0 ± 0.2^{c} A	$< 1.0^{dB}$ C	
		t_{168}	6.2 ± 0.1^{ab} AB	3.7 ± 0.1^{c} BC	3.6 ± 0.6^{c} CD		7.5 ± 0.0^{a} A	3.2 ± 0.2^{c} A	4.6 ± 1.4^{bc} A	
		t_{288}	6.2 ± 0.1^{a} B	4.3 ± 0.1^{bc} ABC	4.9 ± 0.5^{ab} BC		5.2 ± 0.2^{ab} B	$< 1.0^{d}$ C	3.3 ± 0.7^{c} AB	
Enterobacteriaceae (log CFU mL^{-1})			CFII	FF	FY		CBII	BF	BY	
		t_{0}	2.8 ± 0.2^{a} C	2.8 ± 0.0^{a} C	3.0 ± 0.4^{a} BC		$< 1.0^{b}$ C	$< 1.0^{b}$ C	$< 1.0^{b}$ B	
		t_{6}	3.2 ± 0.2^{b} BC	2.9 ± 0.2^{b} C	3.6 ± 0.0^{a} BC		$< 1.0^{c}$ C	$< 1.0^{c}$ C	$< 1.0^{c}$ B	
		t_{24}	4.3 ± 0.7^{ab} B	1.4 ± 0.2^{bc} D	4.7 ± 1.4^{a} AB		$< 1.0^{c}$ C	1.4 ± 1.0^{bc} BC	$< 1.0^{c}$ B	
		t_{48}	6.7 ± 0.2^{a} A	5.3 ± 0.0^{b} A	6.7 ± 0.2^{a} A		$< 1.0^{d}$ C	3.3 ± 0.3^{c} A	$< 1.0^{d}$ B	
		t_{168}	6.8 ± 0.3^{a} A	4.2 ± 0.6^{b} B	3.0 ± 0.0^{b} BC		6.8 ± 0.3^{a} A	3.1 ± 0.2^{b} AB	4.8 ± 1.2^{ab} A	
		t_{288}	5.8 ± 0.4^{a} A	2.9 ± 0.2^{b} C	1.9 ± 0.5^{b} C		4.7 ± 0.3^{a} B	$< 1.0^{c}$ C	2.7 ± 0.6^{b} A	

The results are expressed as mean Log CFU mL^{-1} of brine of two replicates ± standard deviation. For each fermentation batch, values labelled with different small letters in the same row are significantly different ($P < 0.05$), whereas values labelled with different capital letters in the same column are significantly different ($P < 0.05$).
For legend of prototypes see Table 4.3.

Table 4.6. Fatty acid composition (ww-1 %, as methyl esters) of the total lipids extracted from the laboratory-scale prototypes produced with fresh (F) or blanched (B) sea fennel sprouts. Mean values ± standard deviation of two replicates are reported.

Fermentation assay	Fatty acid	Prototypes						
I		CFI	F1	F2	F3	CFII	FF	YF
	C14:0	0.83 ± 0.04^{a}	0.96 ± 0.23^{a}	0.93 ± 0.28^{a}	1.21 ± 0.14^{a}	1.30 ± 0.12^{a}	1.12 ± 0.09^{a}	1.04 ± 0.18^{a}
	C15:0	0.18 ± 0.02^{a}	0.22 ± 0.02^{a}	0.18 ± 0.06^{a}	0.18 ± 0.03^{a}	0.18 ± 0.02^{a}	0.20 ± 0.01^{a}	0.19 ± 0.01^{a}
	C16:0	20.92 ± 1.22^{a}	20.76 ± 1.21^{a}	21.37 ± 0.47^{a}	19.92 ± 1.63^{a}	21.98 ± 1.99^{a}	22.36 ± 1.07^{a}	20.07 ± 1.13^{a}
	C16:1	2.83 ± 0.28^{abc}	3.36 ± 0.35^{ab}	3.09 ± 0.58^{abc}	3.83 ± 0.42^{a}	3.01 ± 0.24^{a}	3.24 ± 0.16^{a}	2.96 ± 0.33^{a}
	C17:0	0.21 ± 0.02^{a}	0.22 ± 0.04^{a}	0.22 ± 0.04^{a}	0.23 ± 0.01^{a}	0.22 ± 0.01^{a}	0.24 ± 0.04^{a}	0.22 ± 0.03^{a}
	C18:0	2.18 ± 0.72^{a}	1.93 ± 1.44^{a}	2.61 ± 0.29^{a}	2.54 ± 0.68^{a}	4.75 ± 1.10^{ab}	1.02 ± 0.16^{b}	1.05 ± 0.06^{b}
	C18:1Δ9t	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}	0.06 ± 0.00^{a}	0.07 ± 0.02^{a}	0.01 ± 0.00^{b}	0.05 ± 0.01^{ab}	0.04 ± 0.01^{ab}
	C18:1Δ9	4.65 ± 0.71^{a}	4.59 ± 0.92^{a}	3.97 ± 1.02^{a}	3.33 ± 0.99^{a}	4.08 ± 0.43^{a}	4.77 ± 0.20^{a}	4.53 ± 0.42^{a}
	C18:2Δ9t,Δ12t	0.09 ± 0.01^{a}	0.14 ± 0.06^{a}	0.14 ± 0.01^{a}	0.16 ± 0.02^{a}	0.10 ± 0.00^{a}	0.07 ± 0.02^{a}	0.09 ± 0.01^{a}
	C18:2Δ9,12	39.60 ± 2.76^{a}	40.32 ± 1.56^{a}	36.84 ± 2.83^{a}	37.71 ± 2.17^{a}	37.32 ± 3.86^{a}	41.20 ± 3.97^{a}	40.58 ± 2.32^{a}
	C20:0	0.48 ± 0.04^{a}	0.77 ± 0.04^{a}	0.72 ± 0.13^{a}	0.66 ± 0.08^{a}	0.73 ± 0.06^{a}	0.89 ± 0.07^{a}	1.20 ± 0.11^{a}
	C18:3Δ9,12,15	26.93 ± 4.26^{a}	25.45 ± 2.76^{a}	28.72 ± 6.05^{a}	28.95 ± 2.94^{a}	25.26 ± 1.96^{a}	23.53 ± 2.79^{a}	26.63 ± 2.98^{a}
	C20:1Δ11	0.21 ± 0.03^{ab}	0.21 ± 0.01^{ab}	0.18 ± 0.01^{ab}	0.14 ± 0.03^{b}	0.18 ± 0.02^{a}	0.21 ± 0.04^{a}	0.23 ± 0.11^{a}
	C20:2Δ11,14	0.16 ± 0.02^{a}	0.18 ± 0.02^{a}	0.14 ± 0.03^{a}	0.16 ± 0.02^{a}	0.11 ± 0.03^{a}	0.19 ± 0.03^{a}	0.19 ± 0.02^{a}
	C20:4Δ5,8,11,14	0.69 ± 0.08^{a}	0.87 ± 0.08^{a}	0.86 ± 0.32^{a}	0.94 ± 0.13^{a}	0.80 ± 0.08^{a}	0.93 ± 0.07^{a}	1.00 ± 0.12^{a}
II		CBI	B1	B2	B3	CBII	BF	BY
	C14:0	1.02 ± 0.41^{a}	1.78 ± 0.66^{a}	2.35 ± 0.86^{a}	2.31 ± 0.42^{a}	1.58 ± 0.08^{a}	2.14 ± 0.75^{a}	1.44 ± 0.53^{a}
	C15:0	0.24 ± 0.03^{a}	0.22 ± 0.08^{a}	0.45 ± 0.06^{a}	0.20 ± 0.01^{a}	0.18 ± 0.01^{a}	0.28 ± 0.04^{a}	0.18 ± 0.06^{a}
	C16:0	18.03 ± 3.12^{a}	15.79 ± 1.72^{a}	17.71 ± 2.40^{a}	17.19 ± 3.01^{a}	19.72 ± 1.17^{a}	19.74 ± 1.32^{a}	20.62 ± 1.33^{a}
	C16:1	1.86 ± 0.63^{bc}	1.61 ± 0.22^{c}	1.67 ± 0.44^{c}	1.79 ± 0.28^{bc}	2.84 ± 1.17^{a}	2.57 ± 1.65^{a}	2.62 ± 0.14^{a}
	C17:0	0.19 ± 0.04^{a}	0.20 ± 0.08^{a}	0.24 ± 0.01^{a}	0.23 ± 0.03^{a}	0.20 ± 0.03^{a}	0.21 ± 0.08^{a}	0.21 ± 0.01^{a}
	C18:0	2.10 ± 0.38^{a}	1.98 ± 0.57^{a}	2.82 ± 0.28^{a}	2.31 ± 0.42^{a}	5.43 ± 1.46^{a}	2.70 ± 1.37^{ab}	2.07 ± 1.03^{ab}
	C18:1Δ9t	0.08 ± 0.03^{a}	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.05 ± 0.00^{a}	0.06 ± 0.01^{a}	0.07 ± 0.02^{a}	0.07 ± 0.01^{a}
	C18:1Δ9	3.68 ± 0.91^{a}	4.90 ± 1.90^{a}	3.35 ± 0.57^{a}	4.24 ± 0.28^{a}	6.56 ± 1.65^{a}	4.26 ± 0.44^{a}	6.75 ± 0.16^{a}
	C18:2Δ9t,Δ12t	0.10 ± 0.00^{a}	0.20 ± 0.08^{a}	0.22 ± 0.01^{a}	0.21 ± 0.05^{a}	0.12 ± 0.01^{a}	0.15 ± 0.02^{a}	0.10 ± 0.04^{a}
	C18:2Δ9,12	36.63 ± 2.72^{a}	38.41 ± 4.75^{a}	32.82 ± 4.12^{a}	33.53 ± 2.24^{a}	38.02 ± 3.92^{a}	38.71 ± 3.78^{a}	39.17 ± 0.86^{a}
	C20:0	0.72 ± 0.16^{a}	0.92 ± 0.37^{a}	0.84 ± 0.14^{a}	0.79 ± 0.14^{a}	0.87 ± 0.10^{a}	0.99 ± 0.11^{a}	0.86 ± 0.47^{a}
	C18:3Δ9,12,15	34.38 ± 2.93^{a}	32.85 ± 2.88^{a}	36.30 ± 3.45^{a}	36.00 ± 4.00^{a}	23.05 ± 1.94^{a}	26.90 ± 1.94^{a}	24.83 ± 0.31^{a}
	C20:1Δ11	0.21 ± 0.03^{ab}	0.29 ± 0.08^{a}	0.18 ± 0.01^{ab}	0.23 ± 0.04^{ab}	0.25 ± 0.06^{a}	0.21 ± 0.08^{a}	0.20 ± 0.08^{a}
	C20:2Δ11,14	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	0.17 ± 0.06^{a}	0.13 ± 0.04^{a}	0.20 ± 0.02^{a}	0.18 ± 0.08^{a}	0.17 ± 0.05^{a}
	C20:4Δ5,8,11,14	0.69 ± 0.11^{a}	0.76 ± 0.06^{a}	0.84 ± 0.28^{a}	0.80 ± 0.14^{a}	0.93 ± 0.14^{a}	0.90 ± 0.07^{a}	0.73 ± 0.27^{a}

Cm:n Δx, m = number of carbon atoms; n, number of double bonds; x, position of double bonds; t, *trans* double bond.
Means labelled with different letters in the same row are significantly different ($P < 0.05$).
For legend of prototypes see Table 4.3.

Table 4.7. Volatile compounds identified in the headspace of the laboratory-scale prototypes by SPME-GC/MS. Quantitative data are relative % of total ion counts. Mean values ± standard deviation of two replicates are reported.

Ferm. Assay	RI	Compound	CAS no.	Type	Prototype							
I					CFI	F1	F2	F3	CBI	B1	B2	B3
	600	acetic acid	64-19-7	OTH	0.00 ± 0.00^{a}	0.25 ± 0.33^{a}	0.04 ± 0.04^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.01^{a}	0.00 ± 0.00^{a}	0.02 ± 0.02^{a}
	929	oxime. methoxy-phenyl-	na	OTH	0.02 ± 0.00^{a}	0.02 ± 0.01^{a}	0.22 ± 0.29^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.00^{a}
	932	α-thujene	2867-05-2	MTH	0.97 ± 0.11^{a}	0.62 ± 0.52^{a}	0.29 ± 0.03^{a}	0.32 ± 0.00^{a}	0.93 ± 0.05^{a}	0.41 ± 0.19^{a}	0.50 ± 0.33^{a}	0.43 ± 0.09^{a}
	938	α-pinene	80-56-8	MTH	3.11 ± 0.25^{a}	1.05 ± 0.72^{b}	0.94 ± 0.00^{b}	1.12 ± 0.41^{b}	3.20 ± 0.12^{a}	1.55 ± 0.28^{b}	1.86 ± 0.44^{ab}	1.80 ± 0.09^{ab}
	974	unidentified		MTH	0.02 ± 0.00^{a}	0.14 ± 0.17^{a}	0.09 ± 0.11^{a}	0.04 ± 0.04^{a}	0.02 ± 0.00^{a}	0.04 ± 0.04^{a}	0.04 ± 0.02^{a}	0.03 ± 0.01^{a}
	977	sabinene	3387-41-5	MTH	0.76 ± 0.06^{a}	0.12 ± 0.05^{b}	0.10 ± 0.05^{b}	0.13 ± 0.02^{b}	0.78 ± 0.03^{a}	0.33 ± 0.31^{ab}	0.29 ± 0.28^{ab}	0.18 ± 0.05^{ab}
	979	β-pinene	127-91-3	MTH	0.63 ± 0.07^{a}	0.17 ± 0.02^{b}	0.15 ± 0.04^{b}	0.17 ± 0.04^{b}	0.61 ± 0.03^{a}	0.19 ± 0.03^{b}	0.22 ± 0.07^{b}	0.26 ± 0.05^{b}
	987	3-p-menthene	500-00-5	MTH	0.05 ± 0.00^{a}	0.06 ± 0.01^{a}	0.08 ± 0.07^{a}	0.05 ± 0.04^{a}	0.05 ± 0.00^{a}	0.07 ± 0.05^{a}	0.05 ± 0.01^{a}	0.05 ± 0.02^{a}
	995	myrcene	123-35-3	MTH	1.10 ± 0.12^{ab}	0.55 ± 0.06^{c}	0.79 ± 0.16^{c}	1.06 ± 0.08^{ab}	1.06 ± 0.06^{ab}	1.31 ± 0.34^{ab}	1.39 ± 0.25^{ab}	1.49 ± 0.06^{a}
	1006	α-phellandrene	99-83-2	MTH	0.56 ± 0.05^{a}	0.99 ± 0.66^{a}	0.61 ± 0.75^{a}	0.67 ± 0.14^{a}	0.58 ± 0.02^{a}	0.76 ± 0.00^{a}	0.90 ± 0.53^{a}	1.00 ± 0.26^{a}
	1021	α-terpinene	99-86-5	MTH	0.44 ± 0.05^{a}	1.00 ± 0.42^{a}	1.46 ± 0.30^{a}	0.75 ± 0.26^{a}	0.43 ± 0.02^{a}	0.96 ± 0.10^{a}	0.78 ± 0.51^{a}	0.86 ± 0.38^{a}
	1037	p-cymene	99-87-6	MTH	31.32 ± 2.54^{ab}	23.29 ± 1.34^{cd}	25.96 ± 1.63^{abc}	24.97 ± 2.20^{bc}	32.22 ± 1.25^{a}	16.90 ± 2.23^{d}	19.28 ± 0.03^{cd}	17.94 ± 1.39^{d}
	1039	b-phellandrene	555-10-2	MTH	2.26 ± 0.25^{a}	4.15 ± 0.00^{a}	4.34 ± 0.84^{a}	3.81 ± 2.66^{a}	2.17 ± 0.12^{a}	4.38 ± 2.43^{a}	5.89 ± 4.75^{a}	6.46 ± 4.01^{a}
	1039	(Z)-b-ocimene	3338-55-4	MTH	0.40 ± 0.03^{a}	0.90 ± 0.39^{a}	0.94 ± 0.08^{a}	1.05 ± 0.18^{a}	0.42 ± 0.02^{a}	0.71 ± 0.39^{a}	0.76 ± 0.14^{a}	1.04 ± 0.52^{a}
	1074	γ-terpinene	99-85-4	MTH	36.30 ± 4.00^{bc}	30.26 ± 1.81^{c}	40.23 ± 0.36^{abc}	43.64 ± 7.05^{ab}	34.87 ±1.98^{bc}	51.08 ± 0.94^{a}	48.91 ± 0.60^{a}	51.51 ± 2.46^{a}
	1090	terpinolene	586-62-9	MTH	0.04 ± 0.00^{a}	0.04 ± 0.04^{a}	0.04 ± 0.04^{a}	0.03 ± 0.03^{a}	0.04 ± 0.00^{a}	0.03 ± 0.02^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}
	1092	unidentified		MTH	0.17 ± 0.02^{a}	0.21 ± 0.05^{a}	0.32 ± 0.14^{a}	0.22 ± 0.08^{a}	0.16 ± 0.01^{a}	0.19 ± 0.07^{a}	0.17 ± 0.17^{a}	0.19 ± 0.19^{a}
	1134	Cyclohexadiene. tetramethyl- isomer		MTH	0.07 ± 0.01^{a}	0.12 ± 0.10^{a}	0.12 ± 0.07^{a}	0.16 ± 0.10^{a}	0.07 ± 0.00^{a}	0.09 ± 0.03^{a}	0.14 ± 0.13^{a}	0.11 ± 0.00^{a}
	1146	Cyclohexadiene. tetramethyl- isomer		MTH	0.07 ± 0.01^{a}	0.09 ± 0.02^{a}	0.08 ± 0.02^{a}	0.08 ± 0.03^{a}	0.06 ± 0.00^{a}	0.07 ± 0.03^{a}	0.09 ± 0.07^{a}	0.05 ± 0.05^{a}
	1183	1-terpinen-4-ol	562-74-3	OMT	0.56 ± 0.05^{b}	3.99 ± 0.06^{a}	2.79 ± 1.46^{ab}	1.74 ± 1.11^{ab}	0.57 ± 0.02^{b}	1.53 ± 0.65^{ab}	1.29 ± 0.03^{ab}	1.19 ± 0.23^{b}
	1194	α-terpineol	98-55-5	OMT	0.05 ± 0.01^{b}	0.18 ± 0.00^{a}	0.11 ± 0.05^{ab}	0.09 ± 0.05^{ab}	0.05 ± 0.00^{b}	0.06 ± 0.03^{ab}	0.07 ± 0.03^{ab}	0.05 ± 0.01^{b}
	1200	octanoic acid. ethyl ester	106-32-1	EST	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.04 ± 0.05^{a}	0.02 ± 0.02^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
	1235	2-isopropil-1-methoxy-4-	31574-44-	OTH	0.20 ± 0.02^{b}	0.60 ± 0.12^{a}	0.36 ± 0.06^{ab}	0.28 ± 0.15^{ab}	0.20 ± 0.01^{b}	0.24 ± 0.14^{ab}	0.18 ± 0.06^{b}	0.20 ± 0.11^{b}
	1244	thymol. methyl ether	1076-56-8	OTH	19.09 ± 1.55^{ab}	27.18 ± 4.58^{ab}	17.62 ± 0.93^{ab}	17.85 ± 0.53^{b}	19.64 ± 0.76^{ab}	17.54 ± 1.46^{a}	15.58 ± 6.85^{ab}	13.97 ±
	1398	decanoic acid. ethyl ester	110-38-3	EST	0.04 ± 0.00^{a}	0.01 ± 0.02^{a}	0.06 ± 0.07^{a}	0.02 ± 0.03^{a}	0.04 ± 0.00^{a}	0.02 ± 0.01^{a}	0.01 ± 0.02^{a}	0.01 ± 0.01^{a}
		dillapiol	484-31-1	OTH	1.64 ± 0.13^{ab}	3.97 ± 1.71^{ab}	2.09 ± 0.73^{ab}	1.61 ± 0.47^{b}	1.68 ± 0.07^{ab}	1.43 ± 0.24^{a}	1.46 ± 0.60^{ab}	1.07 ± 0.11^{ab}
		apiol	523-80-8	OTH	0.11 ± 0.01^{a}	0.05 ± 0.06^{a}	0.12 ± 0.14^{a}	0.08 ± 0.10^{a}	0.11 ± 0.01^{a}	0.07 ± 0.08^{a}	0.09 ± 0.12^{a}	0.06 ± 0.08^{a}
II					CFII	FF	FY		CBII	BF	BY	
	600	acetic acid	64-19-7	OTH	0.00 ± 0.00^{b}	0.03 ± 0.02^{ab}	0.04 ± 0.02^{a}		0.00 ± 0.00^{b}	0.01 ± 0.01^{ab}	0.01 ± 0.00^{ab}	
	929	oxime. methoxy-phenyl-	na	OTH	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.13 ± 0.17^{a}		0.01 ± 0.00^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	
	932	α-thujene	2867-05-2	MTH	0.77 ± 0.03^{ab}	0.29 ± 0.08^{b}	0.39 ± 0.06^{ab}		0.89 ± 0.19^{a}	0.27 ± 0.13^{b}	0.31 ± 0.17^{b}	
	938	α-pinene	80-56-8	MTH	0.49 ± 0.18^{a}	0.32 ± 0.10^{a}	0.60 ± 0.49^{a}		0.47 ± 0.15^{a}	0.30 ± 0.07^{a}	0.30 ± 0.04^{a}	
	974	unidentified		MTH	0.03 ± 0.01^{a}	0.03 ± 0.02^{a}	0.04 ± 0.04^{a}		0.02 ± 0.01^{a}	0.03 ± 0.03^{a}	0.03 ± 0.02^{a}	
	977	sabinene	3387-41-5	MTH	1.20 ± 0.53^{ab}	0.07 ± 0.04^{c}	0.04 ± 0.03^{c}		1.30 ± 0.38^{a}	0.06 ± 0.04^{c}	0.12 ± 0.12^{b}	
	979	β-pinene	127-91-3	MTH	0.37 ± 0.51^{a}	0.10 ± 0.03^{a}	0.15 ± 0.08^{a}		0.29 ± 0.41^{a}	0.10 ± 0.00^{a}	0.09 ± 0.00^{a}	

Table 4.7. (Continued).

Ferm. Assay	RI	Compound	CAS no.	Type	Prototype					
II					CFII	FF	FY	CBII	BF	BY
	987	3-p-menthene	500-00-5	MTH	0.03 ± 0.01[a]	0.05 ± 0.03[a]	0.06 ± 0.05[a]	0.03 ± 0.00[a]	0.05 ± 0.04[a]	0.04 ± 0.02[a]
	995	myrcene	123-35-3	MTH	1.03 ± 0.43[a]	0.91 ± 0.29[a]	1.08 ± 0.40[a]	1.41 ± 0.11[a]	0.93 ± 0.37[a]	0.92 ± 0.45[a]
	1006	α-phellandrene	99-83-2	MTH	0.76 ± 0.10[a]	0.61 ± 0.10[a]	0.88 ± 0.56[a]	0.81 ± 0.17[a]	0.58 ± 0.25[a]	0.56 ± 0.06[a]
	1021	α-terpinene	99-86-5	MTH	1.11 ± 0.21[a]	0.54 ± 0.62[a]	0.77 ± 0.07[a]	1.15 ± 0.15[a]	0.67 ± 0.10[a]	0.51 ± 0.22[a]
	1037	p-cymene	99-87-6	MTH	22.66 ± 4.68[a]	26.15 ± 8.25[a]	29.03 ± 9.28[a]	19.35 ± 0.00[a]	31.74 ± 5.95[a]	25.49 ± 8.65[a]
	1039	b-phellandrene	555-10-2	MTH	4.01 ± 0.42[a]	4.09 ± 2.26[a]	4.24 ± 0.75[a]	5.65 ± 1.90[a]	4.43 ± 1.36[a]	4.03 ± 1.22[a]
	1039	(Z)-b-ocimene	3338-55-4	MTH	0.90 ± 0.51[a]	1.04 ± 0.62[a]	0.71 ± 0.51[a]	1.36 ± 0.15[a]	1.00 ± 0.70[a]	0.85 ± 0.56[a]
	1074	γ-terpinene	99-85-4	MTH	45.59 ± 2.08[a]	43.36 ± 1.30[a]	41.38 ± 10.32[a]	47.41 ± 0.49[a]	37.11 ± 2.28[a]	40.98 ± 0.02[a]
	1090	terpinolene	586-62-9	MTH	0.03 ± 0.01[a]	0.03 ± 0.02[a]	0.03 ± 0.02[a]	0.02 ± 0.00[a]	0.03 ± 0.02[a]	0.03 ± 0.02[a]
	1092	unidentified		MTH	0.19 ± 0.05[a]	0.15 ± 0.09[a]	0.11 ± 0.03[a]	0.21 ± 0.03[a]	0.11 ± 0.05[a]	0.11 ± 0.02[a]
	1134	Cyclohexadiene. tetramethyl- isomer		MTH	0.18 ± 0.03[a]	0.11 ± 0.02[ab]	0.07 ± 0.01[b]	0.13 ± 0.04[ab]	0.10 ± 0.02[ab]	0.09 ± 0.02[ab]
	1146	Cyclohexadiene. tetramethyl- isomer		MTH	0.08 ± 0.06[a]	0.08 ± 0.02[a]	0.08 ± 0.02[a]	0.03 ± 0.01[a]	0.08 ± 0.03[a]	0.08 ± 0.01[a]
	1183	1-terpinen-4-ol	562-74-3	OMT	1.27 ± 1.72[a]	3.14 ± 2.04[a]	2.50 ± 0.59[a]	1.86 ± 0.90[a]	2.63 ± 1.88[a]	2.52 ± 1.54[a]
	1194	α-terpineol	98-55-5	OMT	0.19 ± 0.12[a]	0.18 ± 0.13[a]	0.18 ± 0.07[a]	0.16 ± 0.15[a]	0.11 ± 0.06[a]	0.12 ± 0.08[a]
	1200	octanoic acid. ethyl ester	106-32-1	EST	0.01 ± 0.01[a]	0.02 ± 0.00[a]	0.01 ± 0.01[a]	0.00 ± 0.00[a]	0.02 ± 0.02[a]	0.01 ± 0.00[a]
	1235	2-isopropil-1-methoxy-4-	31574-44-	OTH	0.32 ± 0.19[a]	0.32 ± 0.26[a]	0.36 ± 0.10[a]	0.44 ± 0.02[a]	0.35 ± 0.25[a]	0.30 ± 0.20[a]
	1244	thymol. methyl ether	1076-56-8	OTH	18.34 ± 1.14[a]	17.62 ± 0.18[a]	16.58 ± 0.63[a]	16.36 ± 1.67[a]	18.45 ± 2.68[a]	21.82 ± 3.77[a]
	1398	decanoic acid. ethyl ester	110-38-3	EST	0.01 ± 0.02[a]	0.04 ± 0.04[a]	0.01 ± 0.01[a]	0.00 ± 0.00[a]	0.05 ± 0.06[a]	0.02 ± 0.03[a]
		dillapiol	484-31-1	OTH	0.39 ± 0.53[a]	0.69 ± 0.44[a]	0.50 ± 0.13[a]	0.62 ± 0.21[a]	0.77 ± 0.60[a]	0.63 ± 0.34[a]
		apiol	523-80-8	OTH	0.03 ± 0.03[a]	0.02 ± 0.02[a]	0.03 ± 0.04[a]	0.01 ± 0.01[a]	0.01 ± 0.02[a]	0.02 ± 0.02[a]

RI = Kovats retention index experimentally determined on a DB-5 type column; OTH = others; MTH = monoterpene hydrocarbons; OMT = oxygenated monoterpenes; EST = esters; na = not available. Means labelled with different letters in the same row are significantly different ($P < 0.05$).

For legend of prototypes see Table 4.3.

4.3.3.5. Sensory analysis

The results of the sensory analysis are shown in Supplementary Figure 4.2. In the laboratory-scale prototypes fermented with the multiple strain starters S1, S2, and S3, plant-based, fruity, and pungent were the aromatic notes most intensely perceived, whereas as expected, the two prototypes fermented by S YOGURT had a strong yogurt note, with BY receiving a significantly higher mean score than FY for this descriptor. Regarding global liking, the first group of prototypes (S1, S2 and S3) received a significantly greater mean appreciation than those from the second fermentation assay, attesting to 5.18 ± 0.55 and 4.80 ± 0.25, respectively.

4.3.3.6. Principal component analysis (PCA)

Figure 4.2 shows the results of the PCA carried out on the prototypes from the first fermentation assay. The first two PCs globally explained 47.8% of the total variability. PC1 was mainly affected by lactic acid, vegetable and global liking descriptors, β-ocimene, and lactic acid bacteria, with positive loadings, and by pH and a cluster of monoterpene hydrocarbons (p-cymene, α-pinene, α-thujene), with negative loadings. The first PC was able to discriminate between spontaneously (CBI, CFI) occurring and starter-induced fermentations, with the latter driven towards positive loadings on PC1 by higher levels of lactic acid, better global liking scores, and stronger "vegetable" flavor, which could be ascribed to higher amounts of β-ocimene. Oxygenated volatiles (dillapiole, 1-terpinen-4-ol, thymol, methyl ether) had the highest positive loadings on PC2, while myrcene and total yeast count had the highest negative loadings on PC2. The composition of the volatile fraction differentiated the fresh (F1, F2, F3) and blanched (B1, B2, B3) fermentation substrates along PC2, with the latter having higher scores of the flavor descriptor "banana", while the fresh sea fennel preserves had higher scores of the flavor descriptors "pungent", "artichoke", and "olive pomace".

In the second fermentation assay, higher amounts of lactic acid and lactic acid bacteria loads and lower pH values and α-thujene contents characterized the preserves starting with the selected cultures

with respect to the spontaneously fermented cultures (CBII, CFII) along PC1 (Figure 4.3). However, the effect of the blanching treatment was not as clear as in the first fermentation, since blanched (BY, BF) and fresh (FY, FF) samples were not fully separated along PC2. The unexpected distance among the replicates of the controls (CBII and CFII) might have affected the comparison between the preserves made up of blanched and fresh sea fennel.

4.3.4. Pilot-scale prototype of fermented sea fennel preserves

Based on the overall results collected for the laboratory-scale prototypes, specifically acidification rate and extent, sensory traits and global liking, the multiple strain starter S2 was selected for the scaling up of the fermentation process at a local industrial plant producing vegetable preserves. The results of the analyses performed on the pilot-scale prototype are reported in the following subsections.

4.3.4.1. Physical-chemical and chemical analyses

Supplementary Figure 4.3 reports the pH and TTA values measured at regular intervals during the fermentation of the pilot-scale prototype. A progressive pH decrease was observed, from 7.75 ± 0.00 at t_0 to 3.80 ± 0.02 at the end of the monitoring period (t_{62}). In parallel, TTA increased from 0.36 ± 0.07 (t_0) to 4.05 ± 0.43 (t_{62}) mL NaOH [0.1 N].

Regarding the organic acid quantification, D-lactic acid, L-lactic acid, total lactic acid, and acetic acid were significantly more concentrated during the fermentation process than prior to its start. Moreover, at all sampling times, total lactic acid was significantly more concentrated than acetic acid (Figure 4.4a).

The results of the antioxidant capacity (AOC) analysis are also shown in Figure 4.4b. Overall, a significantly higher antioxidant potential was observed in the prototype immediately after inoculation (t_0) than at the end of fermentation. When the samples collected from day 29 to day 62 were compared, no significant differences were seen in their AOC. Moreover, a Pearson correlation coefficient > 0.99

was observed between the three different assays applied for each sample, thus indicating that DPPH, ABTS, and FRAP showed strong positive correlations.

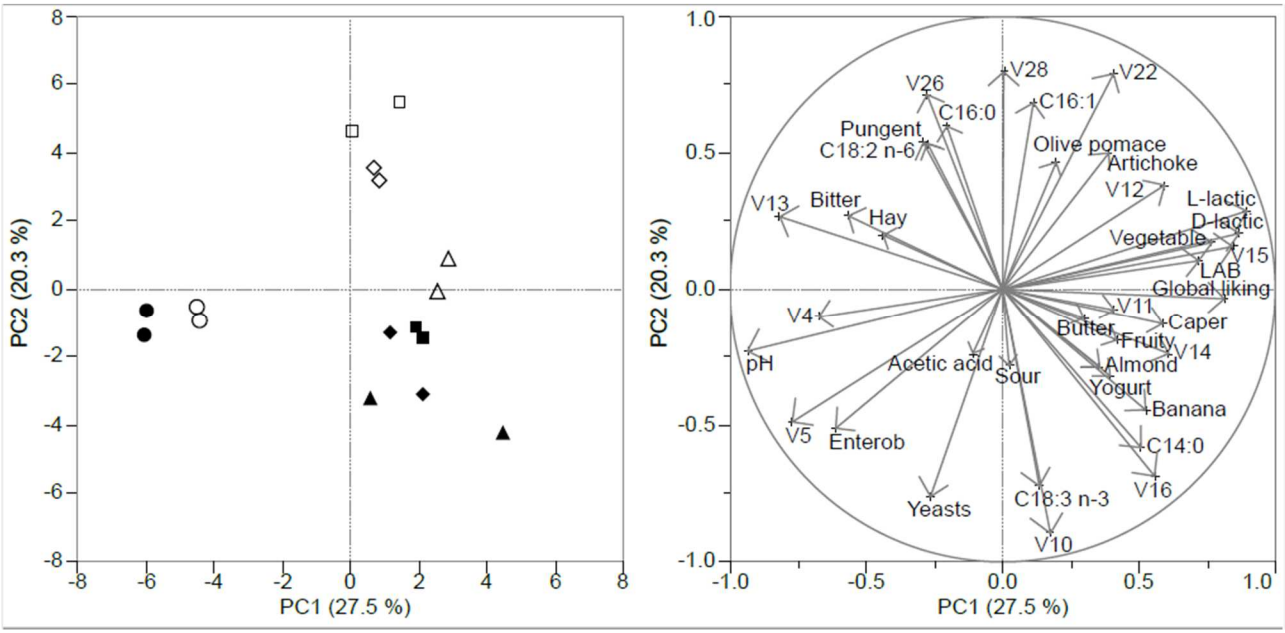


Figure 4.2. Left: PCA scores plot of the laboratory-scale prototypes realized (in duplicate) during the first fermentation assay. Sample identifiers are as in Table 4.3: ○ CFI, □ F1, ◇ F2, △ F3, ● CBI, ■ B1, ◆ B2, ▲ B3. Right: PCA loadings plot of variables. Identifiers of variables are as in Tables 4.3-4.7.

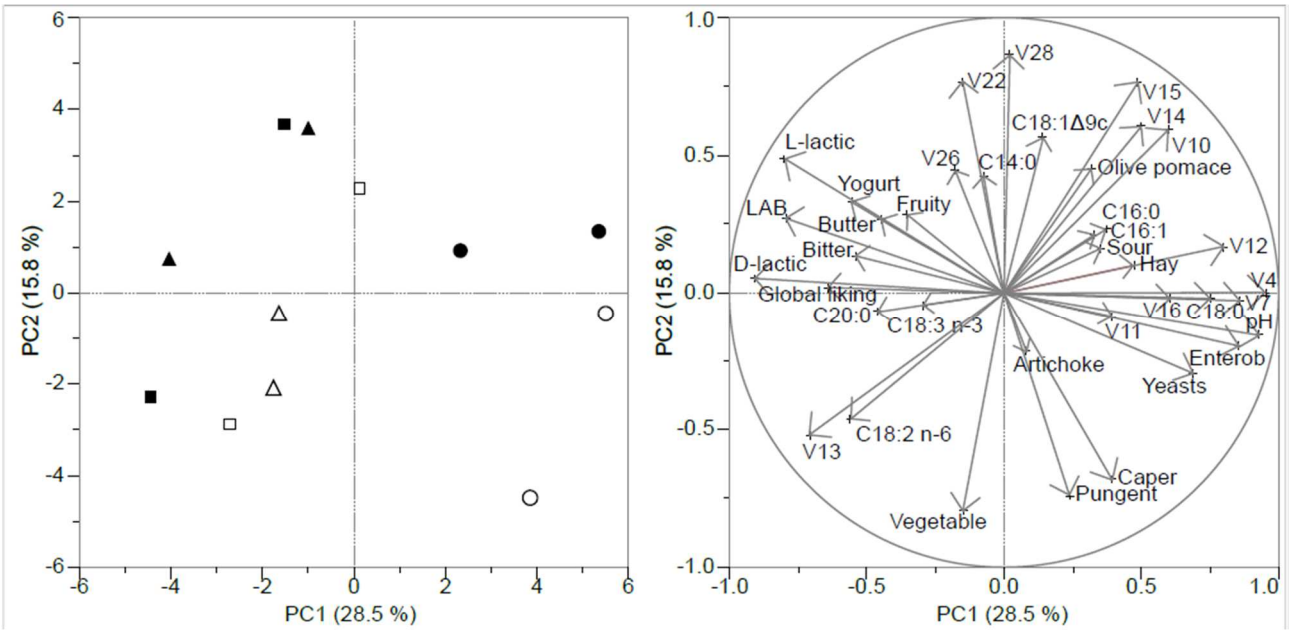
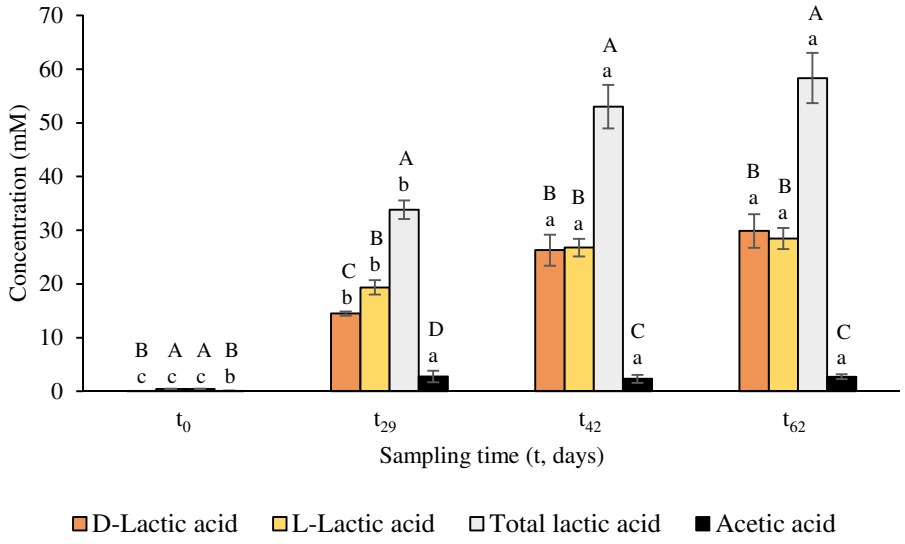


Figure 4.3. Left: PCA scores plot of the laboratory-scale prototypes realized (in duplicate) during the second fermentation assay. Sample identifiers are as in Table 4.3: ○ CFII, □ FF, △ FY, ● CBII, ■ BF, ▲ BY. Right: PCA loadings plot of variables. Identifiers of variables are as in Tables 4.3-4.7. For legend of prototypes see Figure 4.2.

a)



b)

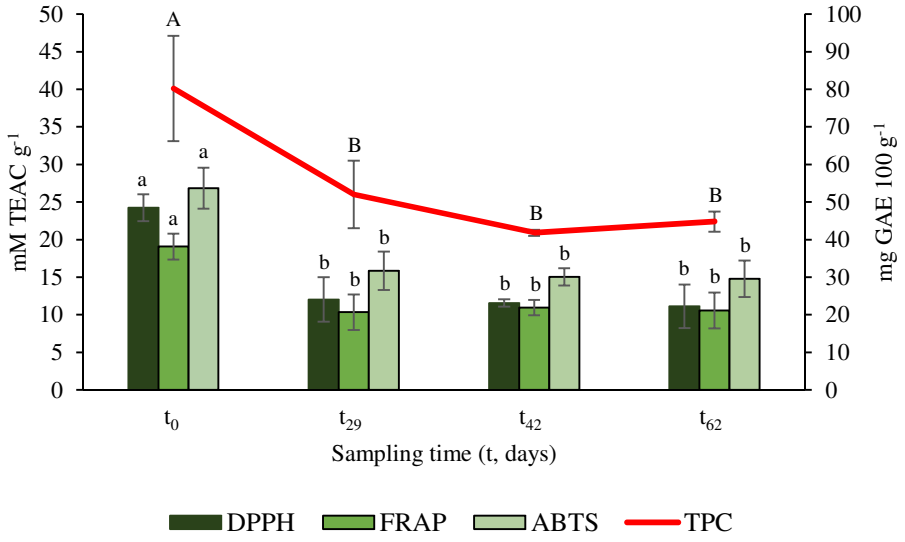


Figure 4.4. a) Organic acids content assessed on the pilot-scale prototype at t_0 and after 29 (t_{29}), 42 (t_{42}) and 62 (t_{62}) days of fermentation; the results (expressed as mM) are expressed as mean of three biological and three technical replicates ± standard deviation. For each compound assayed, columns labelled with different small letters are significantly different ($P < 0.05$) while, for each sampling time, columns labelled with different capital letters are significantly different ($P < 0.05$). **b)** Antioxidant capacity (AOC) and total polyphenols content (TPC) assessed on the pilot scale prototype at t_0 and after 29 (t_{29}), 42 (t_{42}) and 62 (t_{62}) days of fermentation. For AOC, determined using the DPPH, FRAP and ABTS assays, results were expressed as mM TEAC g^{-1} on wet basis; mean of measurements were carried out in triplicate; mean values ± standard deviation (n=18) were reported. For each assay, columns labelled with different small letters are significantly different (<alpha> = 0.05). For TPC the results (expressed as mg GAE per 100 g of dry matter) are expressed as mean ± standard deviation of three replicates. Values labelled with different capital letters are significantly different ($P < 0.05$).

4.3.4.2. Microbiological analysis

Viable counts are reported in Figure 4.5. After the first 24 h of fermentation, the inoculated lactic acid bacteria showed a strong reduction from 7.0 ± 0.0 (t_0) to 3.7 ± 1.0 (t_1) Log CFU mL^{-1}; this was followed by a further progressive increase up to the initial load, which was reached at 29 days. From this time point until the end of the fermentation period, the load of lactic acid bacteria remained almost stable. Yeasts gradually increased during fermentation from < 1.0 (t_0) to 6.2 ± 0.2 (t_{62}) Log CFU mL^{-1}. Similarly, Enterobacteriaceae, which were under the detection limit at the time of inoculation, reached 5.8 ± 0.1 Log CFU mL^{-1} at day 6 and then decreased again to < 1.0 Log CFU mL^{-1} at day 21, remaining stable until the end of fermentation. Mesophilic aerobic bacteria showed variable trends, with counts ranging from 5.0 ± 0.3 (t_1) to 6.9 ± 0.0 (t_0) Log CFU mL^{-1}. Finally, in all the analyzed samples, coagulase-positive staphylococci were < 1.0 Log CFU g^{-1}, while botulinum toxin-producing clostridia were never detected in 25 g.

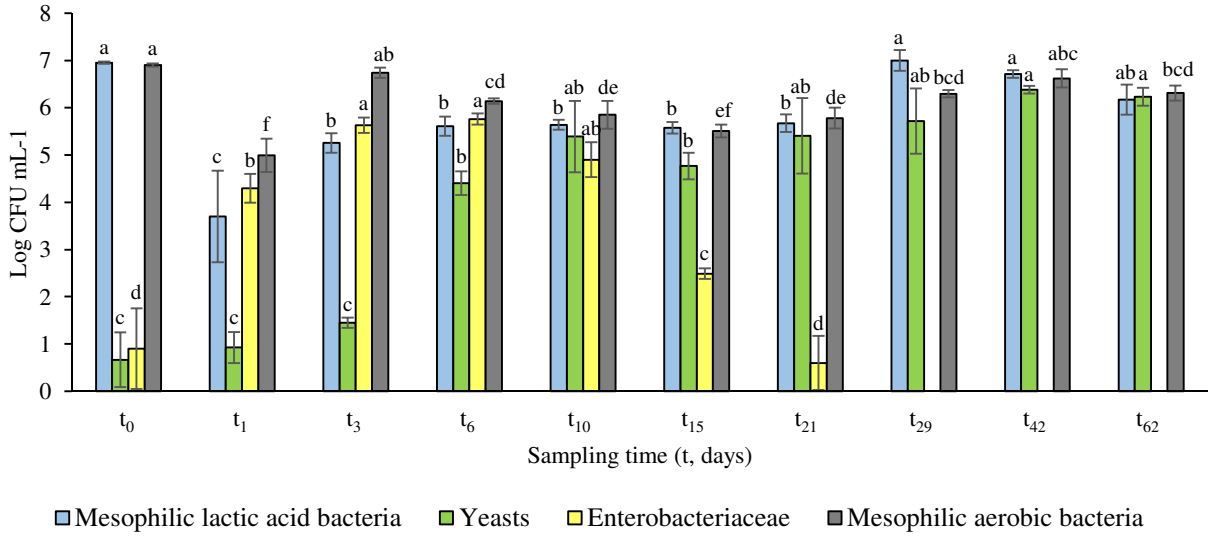


Figure 4.5. Viable counting of mesophilic lactic acid bacteria, yeasts, Enterobacteriaceae and mesophilic aerobic bacteria in the pilot scale prototype. The results are expressed as mean Log CFU mL^{-1} of brine of three replicates ± standard deviation. For each microbial group columns labelled with different letters are significantly different ($P < 0.05$).

4.3.4.3. Vitamin C and dietary fiber content determinations

The contents of vitamin C (expressed as ascorbic acid plus ascorbates) in fresh sea fennel was 51.0 ± 4.2 mg 100 g^{-1} whereas immediately after blanching it was 22.5 ± 7.8 mg 100g^{-1}. The content of dietary fiber at t_0 corresponded to 6.21 ±0.37 g 100 g^{-1}. At t_{29}, in all three replicates, vitamin C was under the detection limit (1.00 mg 100 g^{-1}), whereas dietaryfiber was detected at 4.94 ± 0.19 g 100 g^{-1}, remaining almoststable after 42 and 62 days of fermentation, where it was detected at 4.95 ± 0.15 and 4.99 ± 0.16 g 100 g^{-1}, respectively.

4.3.4.4. Fatty acid composition and total polyphenols content

A significant reduction in the total polyphenol content (TPC) was observed during the first four weeks of fermentation, while no changes were detected between 29 and 62 days (Figure 4.4b). The results of fatty acids determination are shown in Table 4.8.

Table 4.8. Fatty acids (ww^{-1} percentage, as methyl esters) of the total lipids extracted from the pilot scale prototype of fermented sea fennel preserves at t_0 and after 29, 42, and 62 days of fermentation. Mean values ± standard deviation of three replicates are reported.

Analyte	Sampling time (t, days)			
	t_0	t_{29}	t_{42}	t_{62}
C14:0	2.92 ± 0.38[a]	3.01 ± 0.05[a]	1.88 ± 0.03[b]	1.95 ± 0.14[b]
C15:0	0.46 ± 0.00[a]	0.55 ± 0.18[a]	0.35 ± 0.03[a]	0.45 ± 0.12[a]
C16:0	17.34 ± 0.99[b]	19.81 ± 0.28[a]	18.50 ± 0.77[ab]	18.88 ± 0.30[ab]
C16:1	0.66 ± 0.14[a]	2.86 ± 0.35[a]	2.79 ± 0.17[a]	2.76 ± 0.27[a]
C17:0	0.33 ± 0.05[a]	0.46 ± 0.12[a]	0.33 ± 0.05[a]	0.40 ± 0.10[a]
C18:0	3.75 ± 1.10[a]	3.67 ± 0.56[a]	2.84 ± 0.53[a]	3.01 ± 0.34[a]
C18:1Δ9t	0.08 ± 0.03[a]	0.08 ± 0.01[a]	0.12 ± 0.02[a]	0.09 ± 0.02[a]
C18:1Δ9	4.36 ± 1.18[a]	4.72 ± 0.88[a]	4.94 ± 1.57[a]	5.44 ± 0.26[a]
C18:2Δ9t,Δ12t	0.09 ± 0.04[b]	0.25 ± 0.05[a]	0.13 ± 0.01[b]	0.13 ± 0.03[b]
C18:2Δ9,12	40.99 ± 3.34[a]	38.89 ± 1.01[a]	38.42 ± 2.48[a]	41.34 ± 3,06[a]
C20:0	1.55 ± 0.19[a]	1.61 ± 0.19[a]	1.07 ± 0.36[a]	1.30 ± 0.21a
C18:3Δ9,12,15	26.04 ± 2.28[a]	22.56 ± 1.95[a]	27.30 ± 2.21[a]	22.82 ± 3.89[a]
C20:1Δ11	0.14 ± 0.01[a]	0.13 ± 0.02[a]	0.14 ± 0.05[a]	0.14 ± 0.02[a]
C20:2Δ11,14	0.14 ± 0.07[a]	0.14 ± 0.01[a]	0.10 ± 0.01[a]	0.12 ± 0.02[a]
C20:4Δ5,8,11,14	1.14 ± 0.12[a]	1.24 ± 0.08[a]	1.07 ± 0.26[a]	1.15 ± 0.18[a]

Cm:n Δx, m = number of carbon atoms; n, number of double bonds; x, position of double bonds; t, trans double bond; Values labelled with different letters in the same row are significantly different ($P < 0.05$).

Regarding fatty acids of the omega-3 and omega-6 series, mean contents of 118.0 mg and 83.0 mg, 0.5 mg, and 1.9 mg per 100 g of fermented preserve were calculated for alpha-linolenic acid

(C18:3Δ9,12,15), linoleic acid (C18:2Δ9,12), eicosadienoic acid (C20:2Δ11,14), and arachidonic acid (C20:4Δ5,8,11,14), respectively.

4.3.4.5. Volatile compounds determination

HS-SPME-GC/MS analysis revealed the presence of 46 VOCs (Table 4.9) classified into six classes: terpene hydrocarbons (19 compounds); oxygenated terpenes (14 compounds); sesquiterpene hydrocarbons (7 compounds); phenyl propanoids (1 compound); alcohols (2 compounds) and other compounds (3 compounds). Terpene hydrocarbons were the most abundant volatile compounds at t_0. In fact, by summing the peak areas of the volatiles at t_0, terpene hydrocarbons represented 71.3% of the peak areas, followed by oxygenated terpenes (28.2%) and other volatiles occurring at very low levels (~1%). Among the terpene hydrocarbons, γ-terpinene had the highest chromatographic peak area (53.77%), followed by p-cymene (6.72%), sabinene (3.99%), limonene (3.35%), and β–mircene (1.04%). Among the oxygenated terpenes, thymol methyl ether was the most abundant (28%), with the remaining compounds from the same class showing a peak area < 1%. Finally, sesquiterpene hydrocarbons, phenyl propanoids and other substances (ethanol, phenethyl alcohol, ethyl acetate, and acetic acid) were found in trace amounts (< 1%).

During fermentation, γ-terpinene, carvacrol methyl ether, and thymol methyl ether contents significantly decreased, reaching chromatographic peak areas of 35.73, 0.38, and 14.93%, respectively, at 62 days. In contrast, α-pinene, α-terpinene, limonene, β-phellandrene, α-ocimene, 4-terpinol, and 1-terpineol contents increased significantly, reaching chromatographic peak areas of 0.48, 0.95, 22.84, 3.29, 3.23, 6.04, and 0.11%, respectively. Additionally, volatile compounds originating from microbial metabolism, such as ethanol, ethyl acetate, acetic acid, and cyclooctane, increased during the fermentation process, reaching chromatographic peak areas of 0.40, 1.09, 0.07, and 0.14% after 62 days, respectively.

Table 4.9. Volatile organic compounds identified in the pilot scale prototypes of fermented sea fennel preserves at t_0 and after 29 (t_{29}), 42 (t_{42}) and 62 (t_{62}) days of fermentation. nd, not detected. The values are expressed as mean value ± standard deviation of three biological and two technical replicates. Lowercase letters in the same row indicate significant differences ($P < 0.05$) in relative volatile compounds among different samples.

Compound	**Sampling time (t, days)**			
	t_0	**t_{29}**	**t_{42}**	**t_{62}**
Terpene hydrocarbons				
alpha pinene	0.09 ± 0.02^{a}	0.57 ± 0.18^{b}	0.41 ± 0.02^{b}	0.48 ± 0.08^{b}
alpha thujene	0.37 ± 0.16^{abcd}	0.40 ± 0.04^{b}	0.30 ± 0.02^{c}	0.20 ± 0.03^{d}
camphene	0.01 ± 0.00^{a}	0.02 ± 0.00^{b}	0.01 ± 0.00^{a}	0.01 ± 0.01^{ab}
beta pinene	0.04 ± 0.02^{a}	0.07 ± 0.01^{a}	0.06 ± 0.00^{a}	0.06 ± 0.01^{a}
sabinene	3.99 ± 0.85^{a}	6.53 ± 0.46^{b}	1.76 ± 1.71^{c}	0.11 ± 0.05^{d}
delta 3-carene	0.05 ± 0.02^{a}	0.04 ± 0.00^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}
alpha phellandrene	0.01 ± 0.00^{a}	0.06 ± 0.01^{b}	0.12 ± 0.02^{c}	0.15 ± 0.02^{c}
beta myrcene	1.04 ± 0.35^{a}	1.35 ± 0.07^{a}	1.26 ± 0.09^{a}	1.24 ± 0.07^{a}
alpha terpinene	0.30 ± 0.08^{a}	0.94 ± 0.18^{b}	1.44 ± 0.23^{c}	0.95 ± 0.20^{b}
limonene	3.35 ± 0.85^{a}	22.10 ± 3.19^{b}	21.51 ± 3.46^{b}	22.84 ± 2.31^{b}
beta phellandrene	0.49 ± 0.20^{a}	3.05 ± 0.52^{b}	3.48 ± 0.46^{b}	3.29 ± 0.46^{b}
alpha ocimene	nd	2.98 ± 0.18^{a}	3.21 ± 0.39^{ab}	3.23 ± 0.04^{b}
gamma terpinene	53.77 ± 3.94^{a}	33.17 ± 2.34^{b}	36.00 ± 2.79^{b}	35.73 ± 1.81^{b}
beta ocimene	0.35 ± 0.06^{a}	0.27 ± 0.02^{a}	0.32 ± 0.03^{a}	0.29 ± 0.05^{a}
p-cymene	6.72 ± 1.22^{a}	8.01 ± 0.49^{a}	5.85 ± 0.58^{b}	6.31 ± 0.43^{ab}
alpha terpinolene	0.70 ± 0.15^{a}	0.78 ± 0.19^{a}	0.99 ± 0.14^{a}	0.78 ± 0.18^{a}
allocimene	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}
alpha longipinene	0.01 ± 0.00^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}
beta bisabolene	0.02 ± 0.01^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}
Total	**71.33 ± 4.32^{a}**	**80.42 ± 2.51^{b}**	**76.85 ± 4.23^{ab}**	**75.79 ± 3.52^{ab}**
Oxygenated terpenes				
trans sabinene hydrate	0.02 ± 0.01^{a}	0.04 ± 0.00^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}
cis-sabinene hydrate	0.01 ± 0.00^{a}	0.04 ± 0.01^{b}	0.05 ± 0.06^{b}	0.02 ± 0.00^{c}
carvacrol methyl ether	0.89 ± 0.16^{a}	0.36 ± 0.04^{b}	0.36 ± 0.06^{b}	0.38 ± 0.06^{b}
thymol methyl ether	26.92 ± 4.87^{a}	14.42 ± 2.24^{b}	14.99 ± 2.91^{b}	14.93 ± 2.51^{b}
4-terpineol	0.26 ± 0.03^{a}	3.34 ± 0.54^{b}	5.49 ± 1.37^{c}	6.04 ± 1.14^{c}
1-terpineol	nd	0.08 ± 0.02^{a}	0.11 ± 0.03^{a}	0.11 ± 0.02^{a}
alpha terpineol	0.01 ± 0.00^{a}	0.05 ± 0.00^{b}	0.09 ± 0.02^{c}	0.10 ± 0.01^{c}
p-menth-2-en-1-ol	0.01 ± 0.00^{a}	0.09 ± 0.01^{b}	0.11 ± 0.04^{b}	0.10 ± 0.02^{b}
thymol	0.01 ± 0.00^{a}	0.02 ± 0.01^{b}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}
piperitol isomer (trans)	0.01 ± 0.00^{a}	0.02 ± 0.00^{b}	0.03 ± 0.00^{c}	0.03 ± 0.01^{bc}
1.8-menthadien-4-ol	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.04 ± 0.01^{b}	0.06 ± 0.01^{b}
carvacrol	0.01 ± 0.01^{a}	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}
piperitol isomer (cis)	0.01 ± 0.00^{a}	0.03 ± 0.01^{b}	0.05 ± 0.01^{b}	0.05 ± 0.01^{b}
ascaridole	nd	0.06 ± 0.02^{a}	0.10 ± 0.02^{a}	0.16 ± 0.02^{b}
Total	**28.17 ± 4.31^{a}**	**18.39 ± 2.19^{b}**	**21.49 ± 3.91^{ab}**	**22.04 ± 3.31^{ab}**
Sesquiterpen hydrocarbons				
trans alpha bergamotene	0.02 ± 0.00^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}
zingiberene	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.01^{ab}	0.04 ± 0.01^{b}
beta sesquiphellandrene	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.02^{a}	0.03 ± 0.01^{a}
ar-curcumene	0.02 ± 0.03^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}
germacrene B	0.10 ± 0.00^{a}	0.02 ± 0.02^{b}	0.05 ± 0.03^{b}	0.04 ± 0.00^{b}
bicyclogermacrene	0.01 ± 0.00^{a}	0.03 ± 0.02^{ab}	0.03 ± 0.00^{b}	0.01 ± 0.00^{a}
gamma elemene	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}
Total	**0.21 ± 0.05^{a}**	**0.19 ± 0.03^{a}**	**0.20 ± 0.04^{a}**	**0.18 ± 0.01^{a}**
Phenyl propanoids				
dill apiol /apiol	0.07 ± 0.00^{a}	0.52 ± 0.09^{b}	0.58 ± 0.22^{b}	0.55 ± 0.17^{b}
Total	**0.07 ± 0.00^{a}**	**0.52 ± 0.09^{b}**	**0.58 ± 0.22^{b}**	**0.55 ± 0.17^{b}**
Alcohols				
ethanol	0.06 ± 0.01^{a}	0.06 ± 0.03^{a}	0.20 ± 0.07^{b}	0.40 ± 0.07^{c}
phenethyl alcohol	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
Total	**0.08 ± 0.01^{a}**	**0.09 ± 0.03^{a}**	**0.20 ± 0.06^{b}**	**0.40 ± 0.06^{c}**
Others				
ethyl acetate	nd	0.28 ± 0.03^{a}	0.60 ± 0.02^{b}	1.09 ± 0.45^{c}
acetic acid	0.02 ± 0.00^{a}	0.02 ± 0.01^{a}	0.04 ± 0.02^{ab}	0.07 ± 0.02^{b}
cycloctane	nd	0.06 ± 0.02^{a}	0.09 ± 0.02^{ab}	0.14 ± 0.01^{b}
Total	**0.02 ± 0.01^{a}**	**0.37 ± 0.02^{b}**	**0.73 ± 0.02^{c}**	**1.30 ± 0.40^{d}**

4.3.4.6. Sensory analysis

The results of the sensory analysis performed on the pilot-scale prototypes are shown in Figure 4.6. Vegetable and kerosene-like odorous notes were the most intensely perceived by the assessors, followed by fruity, spicy, and woody. For the taste analysis, the descriptor salty had the highest mean score (6.31 ± 0.10), followed by sour (4.97 ± 0.05) and bitter (2.06 ± 0.25). Among descriptors of consistency, crunchy prevailed over hard and fibrous. Overall, good appreciation was expressed by the assessors towards the innovative fermented sea fennel-based preserves, with a mean score of 6.72 ± 0.38 for global liking.

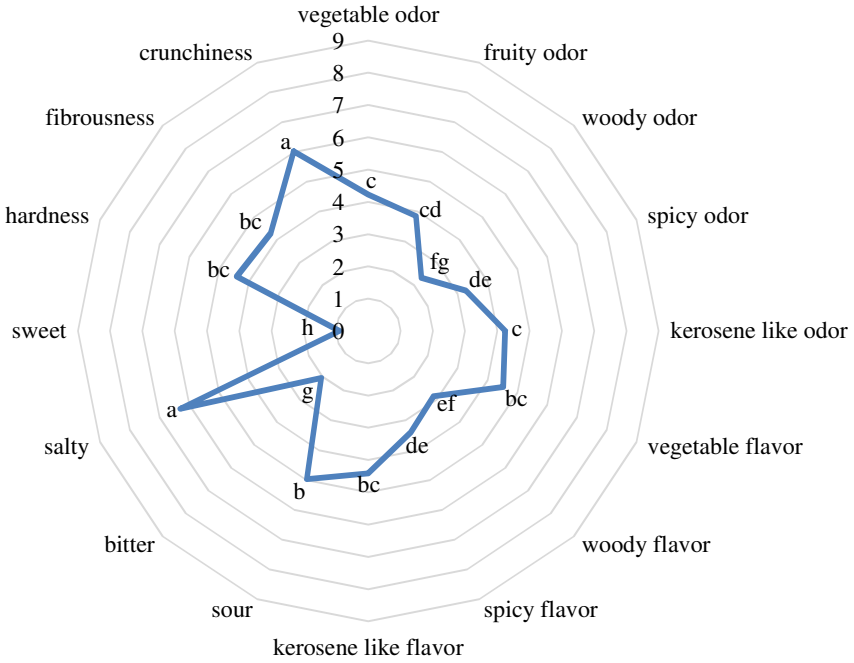


Figure 4.6. Results of sensory analysis performed on the pilot-scale prototypes of fermented sea fennel preserves. Each sample was evaluated, by a trained panel, consisting of 12 non-smoker tasters aged between 25 and 45, for the presence and intensity of (i) five olfactory descriptors (vegetable, fruity, woody, spicy, and kerosene-like odor); (ii) four flavor descriptors (vegetable, woody, spicy, and kerosene-like flavor); (iii) four taste descriptors (sour, bitter, salty, and sweet); (iv) three oral-tactile texture descriptors (hardness, fibrousness, and crunchiness). Each descriptor was evaluated by assigning 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. Values labelled with different letters are significantly different ($P < 0.05$).

4.4. Discussion

The present study started from the assumption that sea fennel could be an ideal candidate for the manufacturing of fermented vegetable preserves with high-value nutritional and sensory traits. This assumption was supported by the available literature describing this aromatic herb as a source of numerous bioactive compounds, such as polyphenols, carotenoids, vitamin C, essential oils, and essential ω-3 and ω-6 fatty acids (Generalić Mekinić et al., 2016).

At the industrial scale, the manufacturing of fermented preserves mainly relies on the exploitation of carefully selected lactic acid bacteria starter cultures capable of rapidly adapting to the specific vegetable substrate used (Rodríguez et al., 2009; Zaika et al., 1983). Given this premise, fermentation of sea fennel sprouts with well-adapted strains started from the molecular typing of a pool of candidate starters followed by mini-batch fermentation tests for the assessment of key pro-technological traits. In this regard, it is widely accepted that a polyphasic approach, investigating both the genetic diversity and technological properties of lactic acid bacteria, is required to widen the detectable levels of strain heterogeneities among candidate starters (Aquilanti et al., 2007).

Regarding the mini-batch fermentation tests used for the technological characterization of the monocultures, young sea fennel leaves were sterilized in brine supplemented with 1% fructose. This monosaccharide acts as an external acceptor of electrons from the intracellular NADH coenzyme and is reduced to mannitol. In heterofermentative lactic acid bacteria, the regeneration of NAD^+ carried out by mannitol dehydrogenase promotes the activation of the acetate kinase pathway, which in turn leads to the synthesis of acetic acid from acyl phosphate. In vegetable preserves, mannitol is a key taste compound (Liu and Narbad, 2018), whereas acetic acid positively contributes to the shelf-life and sensory attributes of the product (Gardner et al., 2001). The brine used for the mini-batch fermentations contained 7% sodium chloride, in agreement with the available literature indicating 5 - 10% as the optimal salt concentration range for the promotion of lactic acid bacteria fermentation

in brine (Barrett, 2003). Brining is undoubtedly one of the most ancient methods of food preservation against undesirable growth of pathogens (Xiong et al., 2014). In fermented vegetables characterized by moderate-high crunchiness, such as typical table olives and cucumbers, salt contained in brine also improves the organoleptic characteristics of the final product (Anagnostopoulos et al., 2020).

Concerning the fermentation performance of the lactic acid bacteria monocultures, differences in the acidification rate and extent were seen, as a feasible consequence of the different adaptation of the assayed strains to the peculiar substrate and to their fermentative metabolisms. Regarding this latter aspect, the obligate homofermentative species *Coml. paralimentarius* and *P. pentosaceus* metabolize 1 mole of glucose, yielding 2 moles of lactate through the Embden-Meyerhof-Parnas (EMP) pathway; the obligate heterofermentative species *Levl. brevis*, *Leuc. pseudomesenteroides*, and *W. confusa* oxidize 1 mole of glucose into 1 mole of lactate, CO_2, and acetic acid/ethanol through the 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway; finally, the facultative heterofermentative species *Lacp. plantarum* switches from the two metabolic pathways depending on the type of substrate occurring in the growth medium (Ray and Joshi, 2015). Considering that lactic acid (pKa 3.86 at 25 °C) is a stronger acid than acetic acid (pKa 4.76 at 25 °C), the fermentation of carbohydrates by homofermentative species leads to a faster pH drop than that of heterofermentative species (Muck, 2010).

Ten lactic acid bacteria strains were selected for the formulation of the multiple strain starters. The pool of selected cultures included 3 obligate homofermentants (PB 126, FO 40, FF 78), 4 facultative heterofermentants (PB 11, PB 257, PB 278, PB 297) and 3 obligate heterofermentants (PB 288, PB 295, PB 321). The inclusion of one strain per fermentative pathway in each multiple strain starter was driven by the aim of reproducing the microbial dynamics typically occurring in spontaneously fermented vegetables. As an example, in kimchi, a traditional Korean vegetable preserve, microbial succession occurs during fermentation, with a dominance, in the early phase, of obligate heterofermentative species (e.g., *Leuc. mesenteroides*), followed by a progressive multiplication of

acid-tolerant (e.g., *Levl. brevis*, *Latilactibacillus sakei*) and, later on, facultative heterofermentative species (e.g., *Lacp. plantarum*), the latter contributing to a further pH drop (Breidt et al., 2013).

Regarding the performance of the multiple strain starters in the laboratory-scale prototypes, longer fermentation times were required to reach a fixed pH of 3.80 with respect to the monocultures. This finding might be feasibly ascribed to the different thermal treatments applied to sea fennel prior to fermentation, namely, sterilization and blanching. In the first case, the heat treatment at 120 °C for 15 min eliminated any interference due to the competition of the autochthonous microbiota while favoring the solubilization of nutrients and reducing the antimicrobial activity of the phenolic compounds and essential oils contained in sea fennel. Far from sterilization, blanching, which consists of a fast immersion of vegetables or fruits in boiling water for a short time followed by plunging into ice water, is extremely useful to inactivate the enzymes responsible for the loss of flavor, color and texture and to retard loss of vitamins, but it does not determine a complete removal of the autochthonous microbiota of vegetables (Linares-Morales et al., 2018).

As the two batches of laboratory-scale prototypes are comparatively evaluated, those started with S1, S2, and S3 reached a fixed pH in a notably shorter time than S FRUITY and S YOGURT. This finding might be feasibly explained by the moderate acidifying extent of the strains included in the starters S FRUITY and S YOGURT, as observed in the preliminary monoculture assay. A contribution of the differences potentially occurring in the chemical composition of the two batches of sea fennel sprouts used for the preparation of the prototypes might also be hypothesized. In this regard, it has previously been demonstrated that sea fennel aerial parts harvested in summer, such as those assayed in the second fermentation batch, are characterized by a higher content of total polyphenols than those harvested in the other three seasons (Meot-Duros and Magné, 2009). As previously elucidated, a high content of polyphenols correlates positively with more intense antimicrobial activity against lactic acid bacteria and, hence, with slower acidification.

Concerning the analysis of organic acids, both lactic and acetic acids were stably detected in all the laboratory-scale prototypes. As a general trend, lactic acid was generally more concentrated than acetic acid, thus suggesting a greater contribution of the homofermentative metabolic pathway than of the heterofermentative pathway, as previously elucidated in other fermented vegetables (Blana et al., 2014).

Regarding the microbiological analyses performed on the laboratory-scale prototypes, viable counts of lactic acid bacteria assessed during the fermentation process agree well with the pH trends discussed above. In more detail, the inoculated starters were subjected to an initial inhibition, which was clearly stronger in the second fermentation assay than in the first one. This finding might be ascribed to either competition of the inoculated strains with the resident microbiota or the documented antimicrobial activity towards lactic acid bacteria of phenolic compounds and essential oils contained at high levels in sea fennel aerial parts (Gann, 2013; Rodríguez et al., 2009). After the first fermentation stage, increases in the load of lactic acid bacteria were observed in both batches, in accordance with the findings of Zaika et al. (1983). In the latter study, essential oils exerted an initial bacteriostatic action against lactic acid bacteria, followed by stimulation of their growth. Finally, a gradual increase in the viable counts of this microbial group was seen in the controls, with a load < 1 Log CFU mL^{-1} in fresh sea fennel at t_0; this trend was in accordance with previous reports on spontaneously fermented vegetables (Blana et al., 2014; Xiong et al., 2012).

As the effects of blanching on viable counts are considered, as expected, a strong reduction in the load of yeasts and Enterobacteriaceae was seen. Indeed, this treatment is acknowledged as a unit operation allowing a decrease in the microbial load of microorganisms, potentially causing food spoilage or poisoning (Xiao et al., 2017). At the end of fermentation, yeasts occurred in all the samples, irrespective of the initial blanching or starter inoculation. This finding agrees well with previous reports, such as those for fermented green olives (Blana et al., 2014; Leal-Sánchez et al.,

2003), while as a general trend, Enterobacteriaceae reached lower loads in the inoculated samples than in the controls, as an effect of the low pH values reached by the prototypes started.

For fatty acids, the results overall collected onto the laboratory-scale prototypes agree well with those very recently reported by Sánchez-Faure et al. (2020) on fresh sea fennel leaves. More specifically, relatively high amounts of linoleic and α-linolenic acids were detected, with both compounds being essential polyunsaturated fatty acids. Moreover, α-linolenic acid is proven to have significant bioactive properties as a precursor of eicosapentaenoic and docosahexaenoic acids, the latter displaying a broad range of functional positive effects on human health (i.e., prevention from cardiovascular disease) (Simopoulos, 1999). This evidence confirms the potential of sea fennel as a functional food.

Regarding the volatilome of the laboratory-scale prototypes, monoterpene hydrocarbons were the major constituents. Terpens are reported as the major class of hydrocarbons in essential oils. Among terpenes, the main volatiles detected herein were among those previously found in essential oils of sea fennel (*Crithmum maritimum* L.) by Özcan et al. (2006).

For the olfactory analysis, vegetal, fruity, pungent and yogurt were the most perceived odorous notes. In fermented food, the development of flavors is related to the accumulation of low-molecular-weight and highly volatile compounds belonging to different classes of compounds, such as alcohols, aldehydes, ketones, acids, esters and sulfur compounds, which are mainly derived from the microbial metabolism of citrate, lipids, proteins and polyphenols (Smid and Kleerebezem, 2014). Based on the available literature, the most perceived odors in fermented sea fennel are associated with linear aldehydes (vegetal odor), esters (fruity odor), organic acids such as acetic acid (pungent odor), and ketones such as diacetyl and acetoin (yogurt odor) (Cheng, 2010; Engels et al., 1997).

Going into the analysis of the pilot-scale prototypes produced with starter S2, a much slower acidification rate than that of the laboratory-scale prototype manufactured with the same starter was

recorded. This behavior could be due to the scaling-up process itself but is mostly due to the different incubation temperatures maintained for the laboratory- and pilot-scale fermentations, namely, 30 and 20 °C, respectively. Temperature is undoubtedly one of the extrinsic food parameters largely affecting the growth kinetics of lactic acid bacteria (Adamberg et al. 2003); accordingly, in a past study focusing on the fermentation of kimchi at different temperatures, it was highlighted how the acidification rate was faster at 20 °C than at 15 or 10 °C (Mheen and Kwon, 1984). Although 20 °C was expected to slow the fermentation process compared with 30 °C, the first temperature was preferred to produce sea fennel preserves at industrial plants due to the lower ecological and economic impacts.

For the organic acid quantification, a slightly higher mean content was found in the pilot scale preserve with respect to the corresponding laboratory scale preserve; however, lactic acid was again more concentrated than acetic acid, thus confirming the assumption that, in starter S2, homofermentative lactic acid bacteria were metabolically more active than heterofermentative bacteria. For TTA, the trend observed was comparable to those of other fermented vegetables, such as sauerkraut (Xiong et al., 2012) and green olives (Leal-Sánchez et al., 2003).

Regarding the bacterial and yeast dynamics, the results collected herein were in accordance with those of the laboratory-scale prototype started with S2, except for some differences, such as the longer time required to achieve complete elimination of *Enterobacteriaceae*. For yeasts, at the end of the fermentation process, counts two orders of magnitude higher with respect to the corresponding laboratory scale prototype (B2) were observed, whereas the load of mesophilic aerobic bacteria was one order of magnitude lower. Neither coagulase-positive staphylococci nor botulinum toxin-producing clostridia were detected. Staphylococci have previously been found in fermented olives (Heperkan, 2013; Pino et al., 2018) and are able to grow in a wide range of pH values and temperatures and in the presence of high sodium chloride concentrations (up to 15%). On its side,

Clostridium botulinum is a food-borne pathogen typically associated with vegetable preserves, thanks to its acknowledged capacity of growing without oxygen and at pH values > 4.6 (Heperkan, 2013).

For the nutritional evaluation, an approximately one order of magnitude reduction in the vitamin C content was seen in blanched sea fennel at t_0 with respect to the content of fresh sea fennel leaves reported in the available literature (Franke, 1982). This finding was expected since vitamin C is a water-soluble vitamin that may be easily destroyed by heat treatments, including blanching (Selman, 1994). For fresh sea fennel, the data collected were comparable to those retrieved from the available literature, reporting vitamin C contents between 39.0 and 76.6 mg 100 g^{-1} (Tardío et al., 2016).

Intriguingly, at the end of fermentation, vitamin C was below the limit of detection (< 1.00 mg 100 g^{-1}) in all three replicates. This drastic drop might be mostly explained by the progressive degradation operated by the residual oxygen occurring in the steel casks during fermentation. In a past study on fermented French beans, carrots, and marrows, decreases in the vitamin C content were seen at the end of the monitoring period in both the inoculated samples and the uninoculated controls (Di Cagno et al., 2008), thus suggesting a mere effect of the environmental conditions rather than starter inoculation on vitamin C degradation. Similarly, a further study evaluating the stability of this vitamin during storage of strawberry juices highlighted a gradual reduction in the vitamin C content during the monitoring period as a feasible consequence of the reaction of ascorbic acid with the dissolved oxygen molecules (Sapei and Hwa, 2014). However, a contribution of the metabolism of lactic acid bacteria might also be hypothesized, given some evidence emerging from the available literature about some auxotrophic lactic acid bacteria isolates from fermented vegetables with respect to vitamins (Ruiz-Barba and Jiménez-Díaz, 1994).

Regarding dietary fiber, the amount measured in blanched sea fennel at t_0 (6.21 ± 0.37 g 100 g^{-1}) was almost comparable to the available data for fresh sea fennel, with contents ranging from 3.04 to 5.60 g 100 g^{-1} (Tardío et al., 2016). Further analyses performed during fermentation revealed a reduction

in the concentration of dietary fiber, again in agreement with previous results collected on fermented green olives (Montaño et al., 2010).

A feasible explanation of dietary fiber reduction during fermentation of sea fennel relies on the metabolic activity of the starter cultures. Indeed, dietary fibers are defined as prebiotics, a class of molecules that are nondigestible by human enzymes but are metabolized by probiotic microorganisms, including lactobacilli, to obtain energy by fermentation (Tomasik and Tomasik, 2003).

Notwithstanding this decrease, the levels of dietary fiber measured at the end of fermentation were above the threshold of 3 g per 100 g established by Regulation (EC) No 1924/2006 to claim that a food is a "source of fiber". Again, this finding agrees well with the dietary fiber content reported by Sánchez-Faure et al. (2020) on fresh sea fennel.

For the analysis of free fatty acids, notable mean contents in the omega-3 alpha-linoleic acid and in some fatty acids of the omega-6 series, namely, linoleic, eicosadienoic, and arachidonic acids, were again measured; alpha-linoleic acid is an essential fatty acid serving as an exclusive source of omega-3 fatty acids in vegetarian diets (Riediger et al., 2008).

For the antioxidant activity, three different assays, each targeting a specific compound, were applied in parallel, namely, ABTS, DPPH and FRAP. In more detail, ABTS and DPPH reveal the presence of hydrogen-donating antioxidants, while FRAP allows the detection of antioxidants capable of reducing Fe^{3+} to Fe^{2+}. Since none of these three methods can give an unequivocal result, a combination of these three assays is generally used to evaluate the antioxidant activities in foods (Carocho and Ferreira, 2013). In the present study, the strong positive correlation revealed by the Pearson correlation coefficient might suggest a major contribution of radical scavenger antioxidants to sea fennel antioxidant activity at t_0, thus supporting the available literature (Generalić Mekinić et al., 2018). Even the reduction in antioxidant activity observed during fermentation agrees well with

what was previously reported for fermented sea fennel (Özcan et al., 2019) and Chétoui olives (Othman et al., 2009). In the latter vegetables, the decrease in antioxidant activity during fermentation was tentatively ascribed by the authors to the loss in phenolic compounds due to their diffusion from the olives to the brine (Othman et al., 2009). This hypothesis might also partially explain the results collected in the present study, given the antioxidant activity displayed by polyphenols (Proteggente et al., 2002).

As the volatilome profile is considered, at t_0, high amounts of terpene hydrocarbons (71%) and oxygenated monoterpenes (28%) were found, with γ-terpinene (53%) and thymol methyl ether (26%) as the most abundant compounds. Other components occurring at noteworthy amounts were sabinene (4%), limonene (3.3%) and p-cymene (6.72%). These findings are in accordance with those reported by Pavela et al. (2017) regarding the dominance of monoterpene hydrocarbons (83.3%) in the central Italian sea fennel volatilome, which were mainly represented by limonene, γ-terpinene and sabinene. Previous phytochemical investigations on *Crithmum maritimum* showed that the composition of this halophyte herb is highly sensitive to the geographic origins of the plants and other factors, such as life-cycle stage and year of harvest, which are characterized by different chemotypes (Kulisic-Bilusic et al., 2010; Pateira et al., 1999).

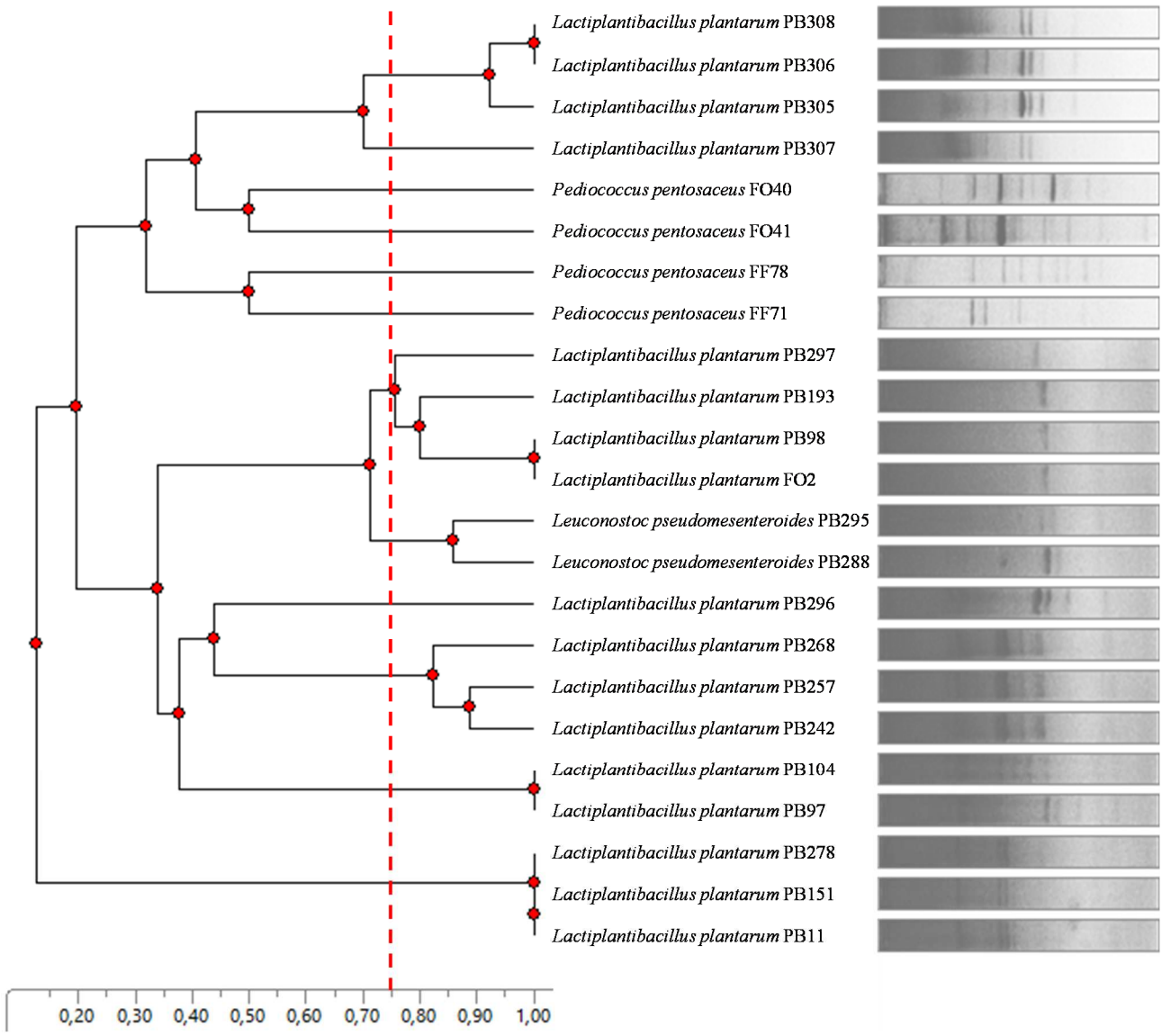
The terpenic compounds found in the prototypes are known to be responsible for various odor notes, such ascelery, common fennel and peel of green citrus characterized by a pungent aftertaste, which explain the numerous culinary applications of sea fennel in many European countries (Giungato et al., 2019; Renna, 2018). Interestingly, during fermentation, the composition of the volatile profile of sea fennel changed, with increases in some compounds and decreases in others. These modifications were probably due to the biochemical activities exerted by microorganisms during fermentation. This hypothesis is also supported by what was previously found by Özcan et al. (2019), who highlighted increases and decreases in some terpenic compounds prior to and after fermentation of sea fennel.

Finally, for the sensory analysis performed on the pilot-scale prototypes, the data were almost comparable to those obtained for the laboratory-scale prototype produced with the same starter. Perceived sensory attributes are the result of the simultaneous stimulation of taste and odor senses by both low- and high-molecular-weight compounds (Ohloff et al., 1985). In the present study, the two most perceived aromatic notes, herbal and kerosene-like, might feasibly be ascribed to α-pinene and p-cymene (Ohloff et al., 1985; Oyama-Okubo and Tsuji, 2013), two compounds both present in fermented sea fennel, as revealed by the analysis of volatile compounds.

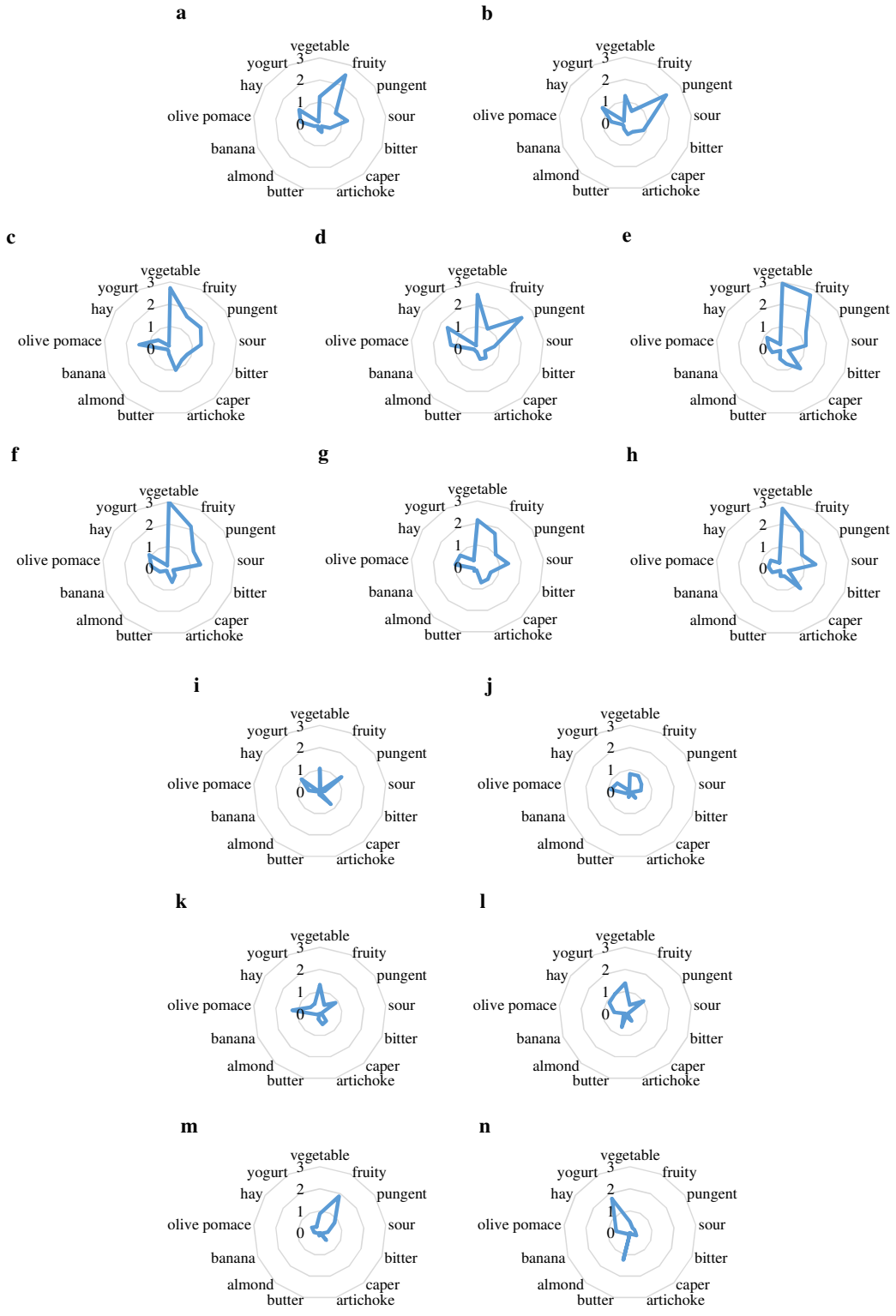
4.5. Conclusions

The pro-technological characterization of a pool of lactic acid bacteria as monocultures and cocultures allowed the formulation of a multiple strain starter culture tailored for the manufacturing of high-value functional sea fennel preserves in brine. On the one hand, the results collected from laboratory-scale prototypes highlighted the importance of the selection and inoculation of a multiple strain starter able to guide and control the fermentation process to produce microbiologically stable and safe preserves. On the one other hand, data overall collected on industrial-scale prototypes corroborate the intuition of previous authors regarding the great potential of sea fennel for the production of foods with high-value sensory traits and beneficial health effects. Finally, through the scale-up of the fermentation process with a selected starter, this study pointed out the possibility of reproducibly manufacturing high-value sea fennel preserves at an industrial level.

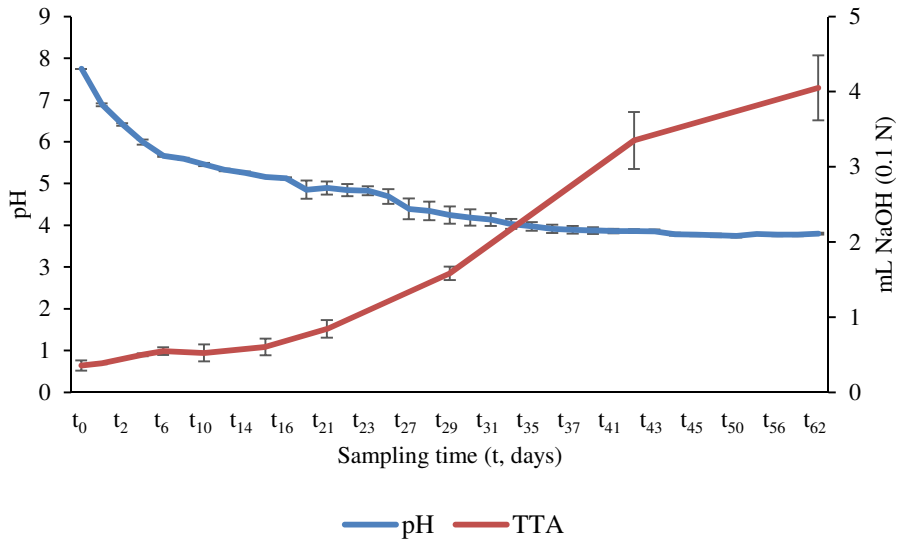
4.6. Supplementary data



Supplementary Figure 4.1. UPGMA dendrogram of Randomly Amplified Polymorphic DNA (RAPD) profiles from the 23 lactic acid bacteria (LAB) isolates subjected to molecular typing.



Supplementary Figure 4.2. Results of sensory analysis performed on the laboratory-scale prototypes fermented with the multiple strain starters S1, S2, S3, S FRUITY and S YOGURT and on the controls: a) CFI; b) CBI; c) F1; d) F2; e) F3; f) B1; g) B2; h) B3; i) CFII; j) CBII; k) FF; l) FY; m) BF; n) BY. Each sample was evaluated, by a trained panel, consisting of 12 non-smoker tasters aged between 25 and 45, for the presence and intensity of 13 olfactory descriptors (vegetable, fruity, pungent, sour, bitter, caper, artichoke, butter, almond, banana, olive pomace, hay, and yogurt), by assigning a score comprised between 1 and 5, with 1 expressing the lowest and 5 the highest intensity. Results are reported as mean values ± standard deviation. For legend of prototypes see Figure 4.1.



Supplementary Figure 4.3. Results of pH measurement and total titratable acidity (TTA) determination performed on the pilot scale prototypes of fermented sea fennel preserves. The results are expressed as means of three biological and three technical replicates ± standard deviations.

Supplementary Table 4.1. ΔpH calculated by measuring pH immediately before inoculation (t_0) and after 6 (t_6), 24 (t_{26}), 48 (t_{48}), and 120 (t_{120}) hours of mini-batch fermentation by the 27 lactic acid bacteria pure cultures assayed.

Culture	$\Delta(t_0-t_6)$		$\Delta(t_0-t_{24})$		$\Delta(t_0-t_{48})$		$\Delta(t_0-t_{120})$	
FF71	0.04 ± 0.01^{bc}	A	-0.38 ± 0.00^{i}	A	0.14 ± 0.00^{gh}	A	0.45 ± 0.59^{e}	A
FF78	-0.04 ± 0.17^{c}	B	1.11 ± 0.26^{def}	A	1.42 ± 0.12^{cde}	A	1.57 ± 0.38^{abcd}	A
FO2	0.01 ± 0.24^{c}	B	1.56 ± 0.11^{abcde}	A	1.70 ± 0.01^{abcd}	A	1.83 ± 0.13^{abc}	A
FO40	0.05 ± 0.03^{bc}	BC	-0.20 ± 0.11^{hi}	C	0.36 ± 0.16^{g}	B	1.05 ± 0.04^{de}	A
FO41	0.02 ± 0.01^{c}	C	-0.21 ± 0.04^{hi}	C	0.88 ± 0.13^{f}	B	1.28 ± 0.08^{bcd}	A
LM9	0.29 ± 0.03^{abc}	C	0.93 ± 0.07^{efg}	B	1.22 ± 0.10^{ef}	AB	1.73 ± 0.23^{abcd}	A
PB11	0.01 ± 0.04^{c}	C	1.41 ± 0.21^{abcdef}	B	1.86 ± 0.01^{abc}	A	1.95 ± 0.01^{abc}	A
PB97	0.03 ± 0.03^{c}	C	0.04 ± 0.09^{hi}	C	1.55 ± 0.01^{bcde}	B	1.92 ± 0.06^{abc}	A
PB98	0.09 ± 0.01^{bc}	C	0.84 ± 0.23^{fg}	B	1.82 ± 0.03^{abc}	A	2.04 ± 0.04^{ab}	A
PB104	0.10 ± 0.04^{bc}	B	-0.06 ± 0.25^{hi}	B	0.38 ± 0.28^{g}	B	1.53 ± 0.40^{abcd}	A
PB126	0.19 ± 0.04^{bc}	D	1.36 ± 0.02^{bcdef}	C	1.69 ± 0.03^{abcde}	B	2.00 ± 0.09^{ab}	A
PB151	-0.04 ± 0.03^{c}	BC	-0.39 ± 0.22^{i}	C	0.29 ± 0.06^{gh}	B	1.20 ± 0.14^{cde}	A
PB193	0.18 ± 0.01^{bc}	C	1.66 ± 0.01^{abcd}	B	1.98 ± 0.05^{ab}	A	2.02 ± 0.01^{ab}	A
PB242	0.46 ± 0.08^{ab}	C	2.02 ± 0.00^{ab}	B	2.15 ± 0.00^{a}	A	2.25 ± 0.03^{a}	A
PB257	0.68 ± 0.31^{a}	B	1.82 ± 0.03^{abc}	A	1.97 ± 0.00^{ab}	A	1.96 ± 0.10^{abc}	A
PB268	0.12 ± 0.04^{bc}	B	-0.01 ± 0.03^{hi}	B	1.48 ± 0.06^{cde}	A	1.49 ± 0.01^{abcd}	A
PB278	0.61 ± 0.13^{a}	B	2.08 ± 0.04^{a}	A	2.16 ± 0.02^{a}	A	2.23 ± 0.05^{a}	A
PB288	0.31 ± 0.07^{abc}	B	1.53 ± 0.11^{abcdef}	A	1.66 ± 0.12^{bcde}	A	1.74 ± 0.17^{abcd}	A
PB295	-0.02 ± 0.16^{c}	B	0.05 ± 0.11^{hi}	B	1.34 ± 0.06^{def}	A	1.34 ± 0.01^{bcd}	A
PB296	0.01 ± 0.00^{c}	B	0.94 ± 0.45^{efg}	AB	1.47 ± 0.18^{cde}	A	1.41 ± 0.05^{bcd}	A
PB297	0.08 ± 0.05^{bc}	B	1.29 ± 0.33^{cdef}	A	1.83 ± 0.18^{abc}	A	1.99 ± 0.08^{ab}	A
PB305	0.07 ± 0.02^{bc}	C	1.32 ± 0.03^{bcdef}	B	1.75 ± 0.10^{abcd}	A	1.94 ± 0.05^{abc}	A
PB 306	0.07 ± 0.11^{bc}	C	1.30 ± 0.17^{cdef}	B	1.70 ± 0.01^{abcd}	AB	1.77 ± 0.01^{abcd}	A
PB307	0.10 ± 0.01^{bc}	B	1.31 ± 0.13^{bcdef}	A	1.60 ± 0.02^{bcde}	A	1.56 ± 0.06^{abcd}	A
PB308	0.04 ± 0.04^{c}	B	1.51 ± 0.13^{abcdef}	A	1.57 ± 0.20^{bcde}	A	1.81 ± 0.04^{abcd}	A
PB 321	0.06 ± 0.12^{bc}	B	0.39 ± 0.26^{gh}	B	1.74 ± 0.22^{abcd}	A	1.75 ± 0.33^{abcd}	A
PB337	0.08 ± 0.02^{bc}	B	-0.08 ± 0.20^{hi}	B	-0.14 ± 0.15^{h}	B	1.42 ± 0.08^{bcd}	A

The results are expressed as means of two biological and three technical replicates ± standard deviations. Values labelled with different small letters in the same column are significantly different ($P < 0.05$). Values labelled with different capital letters in the same row are significantly different ($P < 0.05$).

Supplementary Table 4.2. Perceptible aromatic notes identified during the sensory analysis of the 27 pure cultures of lactic acid bacteria assayed for key pro-technological traits in view of the further selection of candidate starters for the fermentation of sea fennel (*Crithmum maritimum* L.) sprouts.

Culture	Species	Aromatic notes
PB11	*Lactiplantibacillus plantarum*	vegetable, fruity
PB97	*Lacp. Plantarum*	vegetable, olive, bitter
PB98	*Lacp. Plantarum*	vegetable, artichoke, caper
PB104	*Lacp. plantarum*	olive, spicy, pungent
PB151	*Lacp. plantarum*	pungent, hay, hop
PB193	*Lacp. plantarum*	ash, olive, pungent
PB242	*Lacp. plantarum*	vegetable, caper
PB257	*Lacp. plantarum*	vegetable, artichoke
PB268	*Lacp. plantarum*	citrus, fruity
PB278	*Lacp. plantarum*	banana, olive pomace
PB296	*Lacp. plantarum*	vegetable, hay, fennel
PB297	*Lacp. plantarum*	yogurt, vegetable, fruity
PB305	*Lacp. plantarum*	fruity, pungent
PB306	*Lacp. plantarum*	vegetable, pungent
PB307	*Lacp. plantarum*	hay, tobacco
PB308	*Lacp. plantarum*	vegetable, hazelnut
FO2	*Lacp. plantarum*	vegetable, caper
LM9	*Levilactobacillus brevis*	sour, pungent
PB126	*Companilactobacillus paralimentarius*	yogurt, olive pomace, butter
PB288	*Leuconostoc pseudomesenteroides*	caper, sour
PB295	*Leuc. pseudomesenteroides*	vegetable, fruity, bitter
FF71	*Pediococcus pentosaceus*	astringent, sour
FF78	*Pc. pentosaceus*	fruity, pungent
FO40	*Pc. pentosaceus*	fruity, hay
FO41	*Pc. pentosaceus*	hay, coffe, almond
PB321	*Weissella confusa*	butter, almond
PB337	*Weissella kimchii*	fruity, sour

5. Microbial dynamics and sensory traits of innovative high value preserves made by fermentation of started and not started table olives (*Olea europaea* L. cv. Ascolana tenera) with sea fennel (*Crithmum maritimum* L.)

5.1. Introduction

Table olives are among the most important fermented vegetables all over the world, with an average 2016/17 - 2020/21 production attesting at 2.900 thousand tons (CMTD, 2022). The European Union is the main producer of table olives with an amount accounting at about one third of the total world production, followed by Egypt, Turkey, Algeria, and Morocco. Inside the European Union, the main producing country is Spain, followed by Greece and Italy (IOOC, 2022a, 2022b).

According to the International Olive Oil Council (IOOC, 2004), table olives are prepared from the sound fruits of varieties of the cultivated olive tree (*Olea europaea* L.), treated to remove their bitterness and preserved by natural fermentation or, alternatively, heat treatment, with or without the addition of preservatives, and finally packed with or without covering liquid.

Table olives can be classified into three types, depending on the degree of ripeness of the fresh fruits, being: green olives, turning-color olives, and black olives (Rejano et al., 2010). The first type refers to fruits harvested during the ripening cycle prior to coloring, once that they have reached their normal size.

Two main methods are used for industrial-scale production of table olives, the Spanish (or Sevillean) style method and the Greek style method, the latter being also known as the “natural” method. The first method is articulated in the following phases: (i) deamarization of the olives by immersion in an aqueous sodium hydroxide solution (1.5 - 3.0 % NaOH, w v^{-1}) for 8 - 12 hours; (ii) washing to reduce residual alkali; (iii) fermentation and preservation in brine, an aqueous saline solution made with 8 - 10% NaCl w v^{-1}. In the Greek method, deamarization is carried out by placing the olives directly in

brine; the duration of the process, which strictly depends on the degree of ripeness of the olives, requires at least 10 months of fermentation and storage (Corsetti et al., 2012; Rejano et al., 2010).

Lactic acid bacteria (LAB) are undoubtedly the main actors during table olives fermentation, though these microorganisms compete with yeasts for the fermentation, with the latter microbial group potentially contributing to the flavor and aroma of table olives (Hurtado et al., 2012). The main activity of LAB during table olives fermentation is the release of organic acids, especially lactic acid, with a consequent pH drop and free acidity increase. Lactic acid produced by LAB inhibits the multiplication of spoilage and even pathogenic bacteria. Among LAB, leuconostocs, and streptococci are the least acid-producing, whereas homofermentative *lactobacilli* produce the highest amounts of lactic acid, followed by heterofermentative lactobacilli and pediococci (Hurtado et al., 2012).

Though a fast growth of LAB is required for a correct fermentation (Garrido-Fernández et al., 1997), the use of starter cultures is still debated; indeed, alongside authors who recommend the exploitation of inocula to control the fermentation and hence avoid defective products (Corsetti et al., 2012), numerous other authors have reported the successful fermentation of not started olives (Balatsouras et al., 1983; De la Borbolla y Alcalá et al., 1964; Fernández-Díez et al., 1985; Garrido-Fernández et al., 1997; Montaño et al., 1993; Pelagatti and Brighigna, 1981; Sánchez et al., 1995).

Fermented table olives represent a valuable Mediterranean healthy food, as well, thanks to their high content of bioactive compounds, dietary fibers, fatty acids, and antioxidants (Boskou et al., 2015; Campus et al., 2018; Perpetuini et al., 2020). In addition to this, they have been recognized as a source or even a vehicle of probiotic microorganisms (Bautista-Gallego et al., 2013; Bevilacqua et al., 2010a; Blana et al., 2014; De Bellis et al., 2010; Peres et al., 2014).

Hence, they represent an ideal ingredient for the development of innovative vegetable-based foods, characterized by health-beneficial traits, balanced nutritional content, and pleasant sensory traits (Galanakis et al., 2021).

Given these premises, the present study was aimed at exploring the co-fermentation of Spanish and Greek style table olives (*Olea europaea* L.) and sea fennel (*Crithmum maritimum* L.) sprouts to produce started and not started laboratory-scale prototypes of innovative high value preserves. The latter were hence evaluated for their microbial dynamics as well as key sensory traits by a panel of trained assessors.

Green olives of the Ascolana tenera variety were selected taking into consideration the large size of the drupe, the very high flesh-to-stone ratio, and the low flash firmness, together with the acknowledged content of bioactive compounds, like polyphenols, phytosterols, monosaturated fatty acids, and tocopherols, able to reduce the risk of cancer and coronary heart diseases (Kailis and Kiritsakis, 2017; Kiai and Hafidi, 2014; Pannelli et al., 2001). Similarly, sea fennel was selected for its aromatic properties, thanks to the presence of essential oils, and crunchiness, but above all for the high content in bioactive compounds like essential ω-3 and ω-6 fatty acids, polyphenols, carotenoids, and vitamin C, able to prevent the development of chronic degenerative diseases (Generalić Mekinić et al., 2016; Maoloni et al., 2021).

5.2. Materials and methods

5.2.1. Microbial starter formulation

A multiple strain starter of lactic acid bacteria was used; it had previously been formulated with a pool of selected strains screened for key technological traits and exploited for fermentation of sea fennel sprouts in a brine salt solution (Maoloni et al., 2021). Briefly, it included 4 strains ascribed to the following species: *Lactiplantibacillus plantarum* (strain PB257)), *Leuconostoc pseudomesenteroides* (strain PB288), *Pediococcus pentosaceus* (strain FF78), and *Weissella confusa* (strain PB321). All the strains belonged to the Culture Collection of the Department of Agricultural, Food, and Environmental Sciences (D3A, Università Politecnica delle Marche). 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 subcultured in MRS broth (VWR) at 30 °C for 24 h, prior to their use.

5.2.2. Sea fennel and green olives supply and pre-treatment

Organic sea fennel sprouts (~ 6.5 Kg) were kindly supplied by a local farm (Azienda Agricola Paccasassi del Conero di Galeazzi Luca, Velieri Francesco e Babbini Alessandro, Ancona, Italy), which routinely cultivates sea fennel crop destined for the food industry. They were manually harvested in October 2019, washed in an aqueous hypochlorite solution (60 mg L^{-1}), rinsed in tap water, blanched at 95 °C for 30 s, and drained for 5 min using an industrial stainless steel vegetable strainer basket.

Green olives of the Ascolana tenera variety (*Olea europaea* L. cv. Ascolana tenera) were kindly supplied by an industrial producer of table olives (Olive Gregori Società Agricola Semplice, Montalto delle Marche, Ascoli Piceno, Italy). Green olives were divided in two equal portions: one portion (~6 Kg) was subjected to an alkali treatment by submerging the olives in a 1.5 % NaOH solution (w v^{-1}), followed by rinsing with tap water, while the other portion (~6 Kg) was just washed with tap water without any alkali treatment. Both portions were drained 5 min with vegetable strainer baskets, prior to use.

5.2.3. Production of laboratory scale prototypes of fermented preserves

Laboratory-scale prototypes of fermented preserves were produced by mixing blanched sea fennel sprouts with treated or untreated green olives, according to: (i) recipe A (Figure 5.1, panel a), consisting of 10 % drained weight (dw) of sea fennel sprouts and 90 % (dw) of green olives; or (ii) recipe B (Figure 5.1, panel b), consisting of 60 % dw of sea fennel sprouts and 40 % dw of green olives. The fermentation was accomplished according to: (i) the Spanish style (S) method, using the green olives treated with alkali; or (ii) the Greek style (G) method, using the untreated olives, with or without (c, control) the inoculation of the multiple strain starter (s) to reach a final bacterial load of ~

7 Log CFU mL^{-1} of brine. Four started laboratory-scale prototypes (SAs, SBs, GAs, and GBs) and four not started controls (SAc, SBc, GAc, and GBc) were then manufactured.

a) **b)**

Figure 5.1. Laboratory-scale prototypes of fermented preserves produced by mixing blanched sea fennel sprouts with treated or untreated green olives, according to recipe A (panel **a**), consisting of 10 % drained weight (dw) of sea fennel sprouts and 90 % (dw) of green olives, or recipe B (panel **b**), consisting of 60 % dw of sea fennel sprouts and 40 % dw of green olives.

For each recipe (A or B) and each processing method (Spanish or Greek), three replicates were produced for both started and control preserves; each replicate consisted of 1.5 L glass jar filled with 750 mL of sterile brine (8 % w v^{-1} NaCl), previously autoclaved at 121 °C for 15 min, and 750 g of a mixture of sea fennel sprouts and green olives, at a ratio depending on the recipe (A or B). The fermentation was conducted at room temperature (18 ± 2 °C), for 63 (Spanish style method) or 373 (Greek style method) days, respectively.

The overall experimental plan is depicted in Figure 5.2.

5.2.4. pH measurement

Aliquots (1 mL) of brine were aseptically collected immediately after the inoculation (t_0) and during the fermentation process up until the end of the monitoring period, corresponding to 63 and 373 days, depending on the processing method, Spanish or Greek style, respectively (Figure 5.2). The pH measurement was accomplished with a pH meter model 300 (Hanna Instruments, Padova, Italy). The results were expressed as the mean of three replicates ± standard deviation.

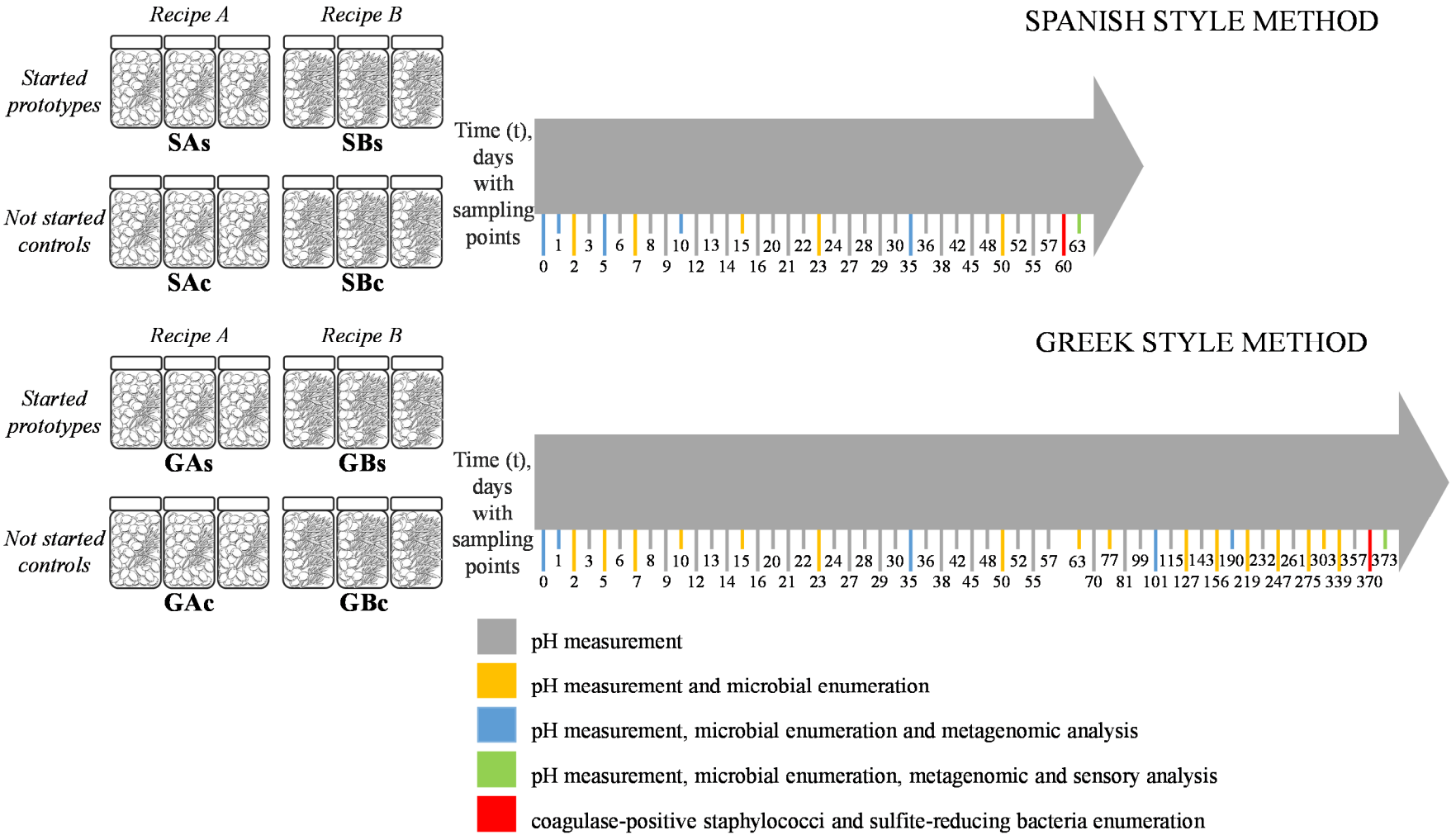


Figure 5.2. Experimental design overview.

5.2.5. Microbial enumeration

For the microbiological analysis, additional aliquots (1 mL) of brine were aseptically collected immediately after the inoculation (t_0) and at selected intervals during the fermentation process (Figure 5.2). Each aliquot was serially ten-fold diluted in sterile 0.1% (w v^{-1}) peptone water for the enumeration of: (i) mesophilic aerobic bacteria on Plate Count Agar (PCA, VWR), by incubating at 30 °C for 48 h; (ii) mesophilic lactic acid bacteria 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) 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 (RBA, VWR), by incubating at 25 °C for 5 days; and (v) Enterobacteriaceae on Violet Red Bile Agar (VRBA, VWR), by incubating at 37 °C for 24 h. The results of viable counting were expressed as the mean Log CFU mL^{-1} of brine of three replicates ± standard deviation.

Aliquots of sea fennel sprouts and olives were aseptically collected from each replicate of the laboratory-scale prototypes 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.

5.2.6. DNA extraction

Aliquots (1 mL) of the first decimal dilutions of selected brine samples (Figure 5.2), prepared as described in section 5.2.5, were used for bulk cells preparations, by mixing the three dilution replicates of each sampling time. The mixtures were centrifuged at 14000 rpm to obtain cell pellets; these latter underwent total DNA extraction by using E.Z.N.A. soil DNA kit (Omega bio-tek, Norcross, GA, USA) according to the manufacturer's instructions.

5.2.7. Metagenomic analysis

Microbiota was analysed by amplifying the V3-V4 region of the bacterial 16S rRNA gene according to Klindworth et al. (2013) (16S Amplicon PCR Forward Primer 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG; 16S Amplicon PCR Reverse Primer 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC. Library preparation and pooling were performed according to the 16S metagenomic sequencing library preparation from Illumina (Illumina, San Diego, CA). Paired end (2x250 bp) sequencing was performed with a MiSeq instrument platform according to manufacturing instruction (Illumina, San Diego, CA). QIIME2 software (Callahan et al., 2016) was used for data analysis by following the quality filtering step of the dada2 denoise-paired plug in (Callahan et al., 2017) to obtain the Amplicon Sequence Variants (ASVs). Sequence variants with less than 10 reads in two samples were excluded from further analysis to increase the confidence of sequence reads and reduce bias by possible sequencing errors. Taxonomy assignment of ASVs was obtained through the qiime feature-classifier against the Greengenes database. Sequences of each ASVs were manually checked by Basic Local Alignment Search Tool (BLAST) to confirm the taxonomic assignment. The ASVs tables, rarefied at lowest number of sequence/sample, displays the higher taxonomy resolution reached when the taxonomy assignment was not able to reach the species level, genus was displayed. The sequencing data were deposited in the NCBI Sequence Read Archive (SRA) and are available under the Bioprojects Accession Number PRJNA811759.

5.2.8. Sensory analysis

At the end of the fermentation period, all the laboratory-scale prototypes were subjected to a sensory analysis carried out by a panel consisting of 10 non-smoker assessors, 5 males and 5 females, aged between 25 and 45, trained as described by Maoloni et al. (2021) with slight modifications. In more

deatil, the panel was subjected to weekly training sessions, for three consecutive weeks, to identify the more suitable sensory attributes of both fermented greed olives of Ascolana tenera variety and sea fennel sprouts. The sensory analysis was performed in individual booths equipped with coffee beans for olfactory cleansing and still bottled water for oral rinsing before and between the evaluations (Resurreccion, 1998). The samples were coded with random, three-digit numbers and aliquots (10 g) of drained green olives and sea fennel sprouts were presented at room temperature to the panel. The panel was asked to separately evaluate sea fennel sprouts and green olives, based on the attributes previously set. In more detail, sea fennel sprouts were evaluated for: i) five olfactory descriptors, being herbal, woody, spicy, kerosene-like, and green olive; and (ii) five flavor descriptors, being herbal, woody, spicy, kerosene-like and green olive. Green olives were evaluated for: i) three olfactory descriptors, being green olive, spicy and kerosene-like and (ii) three flavor descriptors, being green olive, spicy, and kerosene-like. Occurrence of off-odors and off-flavors was also evaluated. Both green olives and sea fennel sprouts were evaluated for: i) four taste descriptors, being sour, bitter, salty, and sweet; and ii) three textural descriptors, being hardness, fibrousness, and crunchiness. For each ingredient (sea fennel or table olives) and each descriptor, panelists assigned a score comprised between 1 and 9, where 1 expressed the lowest and 9 the highest intensity. The panelists were also invited to express their global acceptance by using a 9-point hedonic scale, where 1 expressed the lowest (dislike extremely) and 9 the highest (like extremely) degree of liking (Peryam and Pilgrim, 1957). The results were expressed as means ± standard deviations.

5.2.9. Statistical analysis

The results of pH measurement, microbial enumeration, and sensory analysis were subjected to one-way analysis of variance (ANOVA) using JMP Version 11.0.0 software (SAS Institute Inc., Cary, NC, USA). The Tukey-Kramer honest significant difference (HSD) test ($P \leq 0.05$) was carried out to detect differences through multiple mean comparisons. Alpha diversity index was calculated by the

diversity function of QIIME2 and analyzed by R-Studio software. Variables were compared by the non-parametric Wilcox test and visualized by R-studio as a box plot.

5.3. Results

5.3.1. pH measurement

The pH values measured in the prototypes during their fermentation are reported in Supplementary Table 5.1, whereas the pH trends are shown in Figure 5.3. At t_0, the prototypes prepared according to recipe B showed significantly higher mean pH values (SBs: 6.14 ± 0.03; GBs: 6.82 ± 0.05) than the prototypes prepared according to recipe A (GAs: 5.75 ± 0.04; SAs: 5.89 ± 0.01), irrespective of the processing method applied. During the fermentation period, a progressive pH drop was seen in both the started and control (unstarted) samples.

Regarding the Spanish style method, no significant differences were seen in the final mean pH values reached by the started (SBs: 3.47 ± 0.08) and control (SBc: 3.75 ± 0.27) prototypes made according to recipe B, whereas for the prototypes made according to recipe A, those started with the selected pool of lactic acid bacteria reached significantly lower mean values (SAs: 4.17 ± 0.05) than the controls (SAc: 4.55 ± 0.02).

For the Greek style method, again, no significant differences were seen in the final mean pH values reached by the started (GBs: 3.42 ± 0.0) and control (GBc: 3.40 ± 0.04) prototypes made according to recipe B, whereas for the prototypes made according to recipe A, those started with the selected pool of lactic acid bacteria strains reached significantly lower mean values (GAs: 3.88 ± 0.03) than the controls (GAc: 4.03 ± 0.03).

5.3.2. Microbial enumeration

The dynamic changes in the growth of specific bacterial groups are depicted in Figure 5.3, whereas viable cell counts are reported in Supplementary Table 5.2.

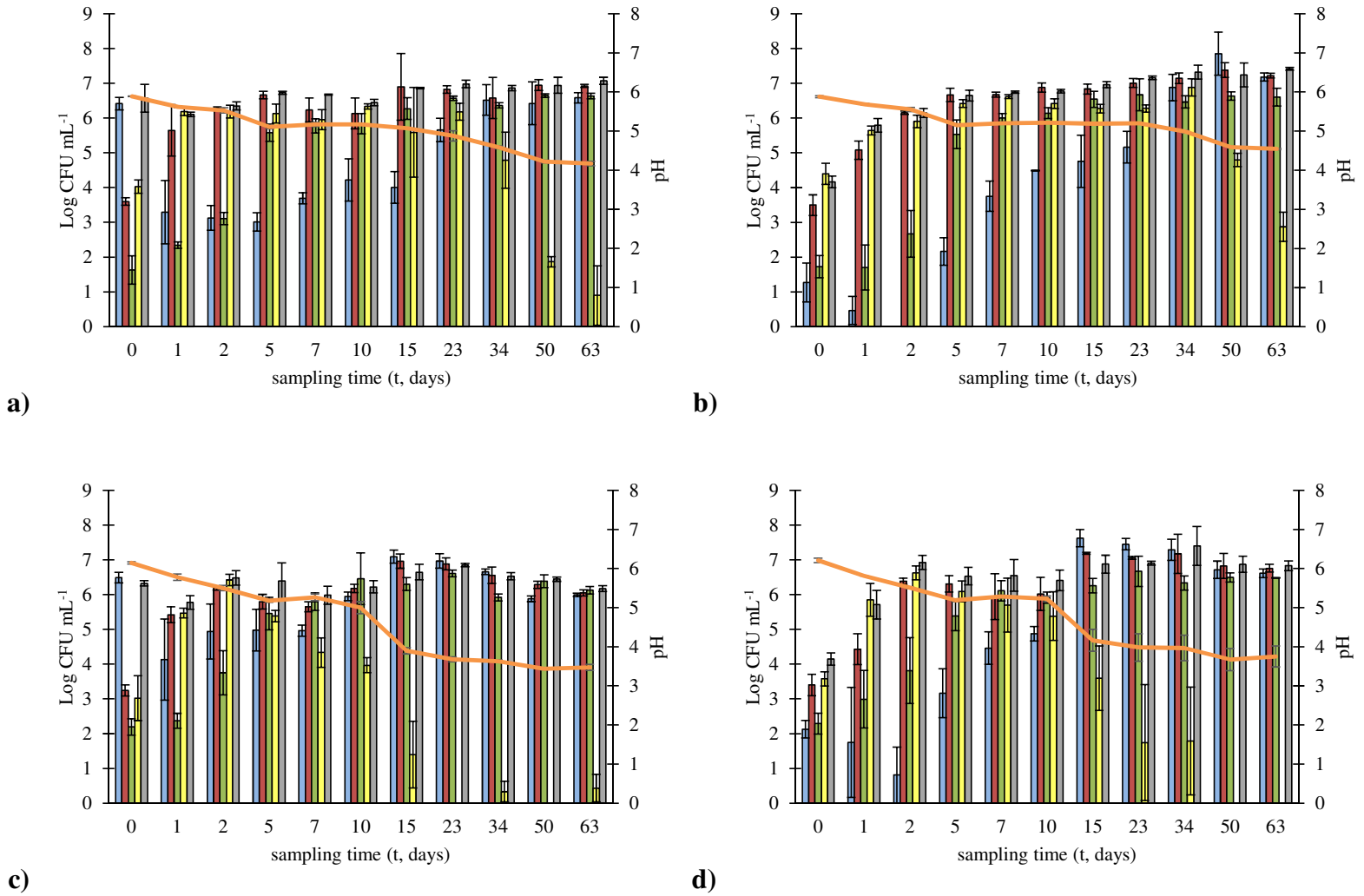
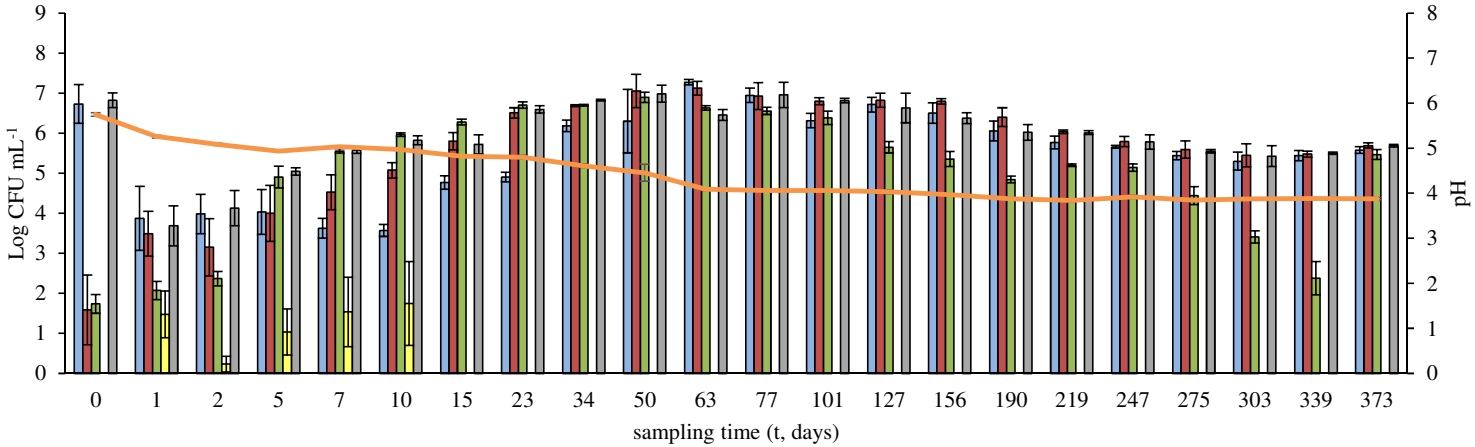
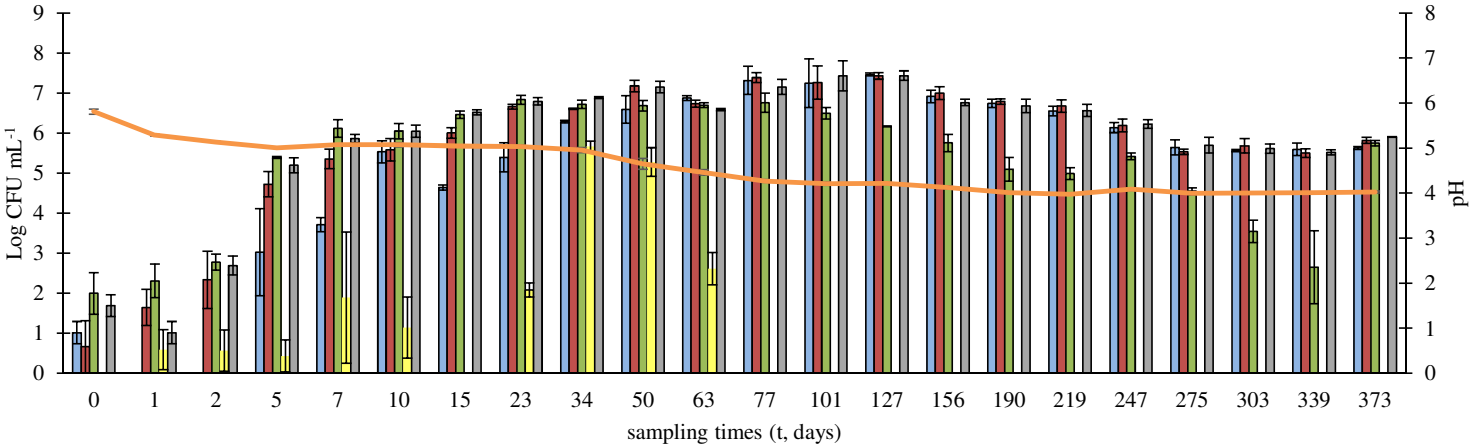


Figure 5.3. pH ( ) and viable counts trend of mesophilic lactobacilli , mesophilic lactococci, yeasts, Enterobacteriaceae and mesophilic aerobic bacteria in the laboratory-scale prototypes made according to recipe A (10% sea fennel sprouts and 60% green olives, dry weight) or B (60% drained weight of sea fennel sprouts and 40% drained weight of green olives), and processed according to the Spanish style (SAs panel **a**, SAc panel **b**, SBs panel **c**, SBc panel **d**) or Greek style (GAs panel **e**, GAc panel **f**, GBs panel **g**, GBc panel **h**) method, with (SAs panel **a**, SBs panel **c**, GAs panel **e**, GBs panel **g**) or without (SBs panel **c**, SBc panel **d**, GBs panel **g**, GBc panel **h**) the inoculation of a multiple strain starter. The results are expressed as means of three replicates ± standard deviations.

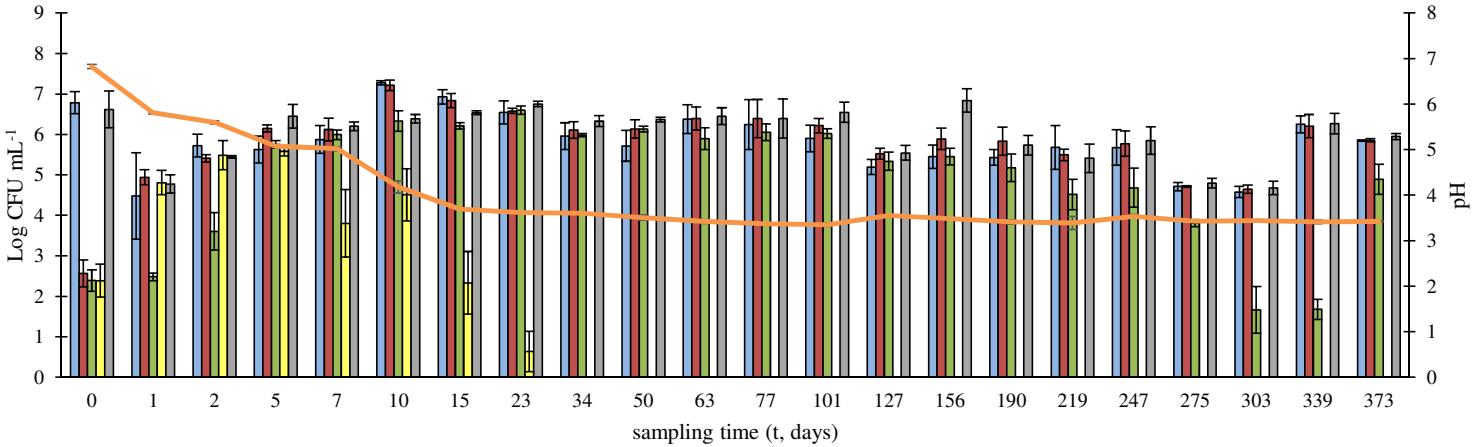


e)

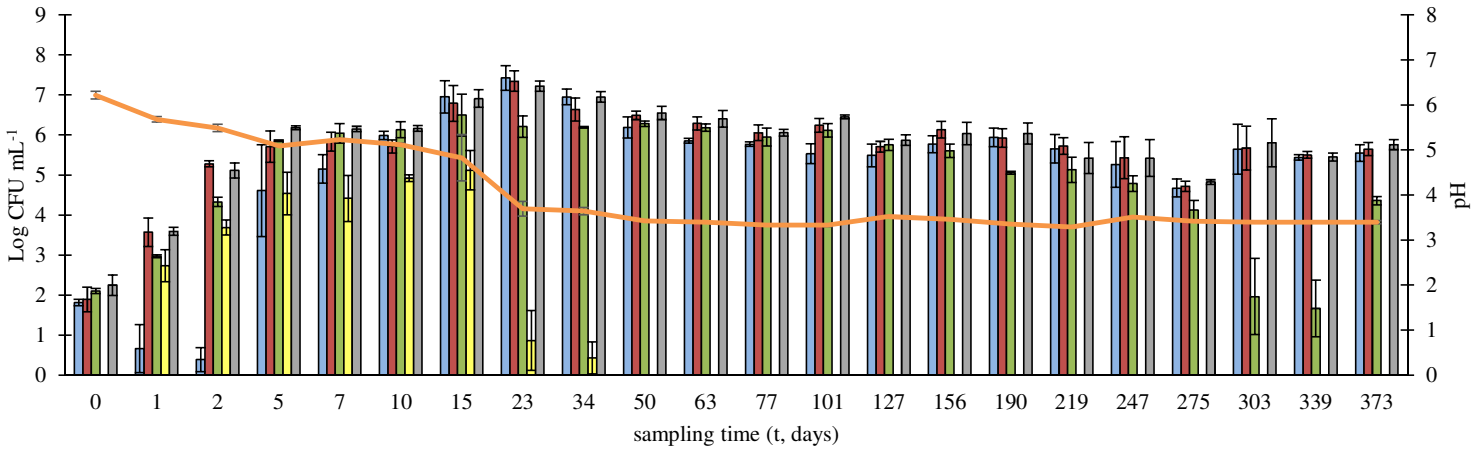


f)

Figure 5.3. (Continued).



g)



h)

Figure 5.3. (Continued). **SAs** Started (s) Spanish style (S) prototype made according to recipe A; **SAc** Control (c) unstarted Spanish style prototype made according to recipe A; **SBs** Started (s) Spanish style (S) prototype made according to recipe B; **SBc** Control (c) Spanish style (S) prototype made according to recipe B; **GAs** Started (s) Greek style (B) prototype made according to recipe A; **GAc** Control (c) Greek style (B) prototypes made according to recipe A; **GBs** Started (s) Greek style (G) prototype made according to recipe B; **GBc** Control (c) Greek style (G) prototype made according to recipe B.

Two similar trends emerged from the analysis of the prototypes processed according to the Spanish and Greek style methods, with some significant differences.

For mesophilic lactobacilli, at t_0 significantly higher values were recorded in the started prototypes than the controls, irrespective of the recipe. However, as early as after 24 h of fermentation, a drastic decrease in the viable counts of this bacterial group was seen in all the started prototypes. At the end of the monitoring period, mesophilic lactobacilli reached mean viable counts ≥ 5.6 Log CFU mL^{-1} in the Greek style prototypes, with no significant differences between the two recipes, whereas in the Spanish style prototypes, they reached mean viable counts ≥ 6.6 Log CFU mL^{-1} in SAs, SAc, and SBc and equal to 6.0 Log CFU mL^{-1} in SBs.

Concerning mesophilic lactococci, at t_0 comparable low counts were recorded in the prototypes prepared according to the same method, with counts two orders of magnitude higher in the Spanish style prototypes than those processed according to the Greek style method; this microbial group showed an increase during fermentation, up to mean counts ≥ 6.1 and 5.7 Log CFU mL^{-1} in the Spanish and Greek style prototypes, respectively.

For yeasts, no differences were seen in the initial mean counts by comparing the two recipes or the two processing methods, with an increase up to mean counts ≥ 6.1 Log CFU mL^{-1} in the Spanish style prototypes and up to 5.5 - 5.7 or to 4.9 - 4.4 Log CFU mL^{-1}, in the Greek style prototypes made according to recipe A and B, respectively.

Viable counts of mesophilic aerobic bacteria revealed a similar behavior as the one depicted for mesophilic lactobacilli. In more detail, for the Spanish style method, at t_0 mean counts were comprised between 4.1 ± 0.2 (SBc) and 6.6 ± 0.4 (SAs) Log CFU mL^{-1}, whereas at the end of the fermentation they ranged from 6.2 ± 0.1 (SBs t_{63}) and 7.4 ± 0.0 (SAc t_{63}) Log CFU mL^{-1}. For the Greek style method, mesophilic aerobic bacteria ranged from 1.7 ± 0.3 (GAc t_0) and 6.8 ± 0.2 (GAs

t_0) Log CFU mL^{-1} at t_0 to 5.7 ± 0.0 (GAs t_{373}) and 5.9 ± 0.0 (GAc t_{373}) Log CFU mL^{-1} at the end of the fermentation, respectively.

Enterobacteriaceae disappeared in all the prototypes at the end of their fermentation, except for SAc, where mean viable counts equal to 2.9 Log CFU mL^{-1} were detected. As a general trend, a faster reduction of this microbial group was observed in the started prototypes in respect with the control prototypes.

Coagulase-positive staphylococci and sulfite-reducing bacteria were under the detection limit (< 1 Log CFU g^{-1}) in all the prototypes.

5.3.3. Metataxonomic analysis

Figure 5.4 shows an overview of the ASVs detected by metataxonomic analysis, irrespective of the sampling time and the recipe. In the Spanish style prototypes of fermented preserves, *Lactiplantibacillus plantarum* (29% of the relative frequency), *Enterobacter cloacae* (30%), *Pediococcus pentosaceus* (7%), *Weissella confusa* (7%) and *Citrobacter freundii* (10%) were the main taxa. Except for *Citrobacter freundii*, the same microorganisms were found in the prototypes processed according to the Greek style method, with some differences; indeed, in the latter prototypes, *Lactiplantibacillus plantarum* was detected with a higher relative frequency (53%), whereas *Enterobacter cloacae* was among the minority taxa (6%). Moreover, in the Greek style prototypes, *Leuconostoc pseudomesenteroides*, added as a starter culture, was also detected, but at a relative frequency lower than 1%.

Figure 5.5 shows the bacterial dynamics assessed during the fermentation process of the Spanish style (panel a) and Greek style (panel b) prototypes, irrespective of their recipe, with *Lactiplantibacillus plantarum* being the dominant ASVs in the prototypes manufactured according to both the processing methods at the end of fermentation (Figure 5.5, panel a and b).

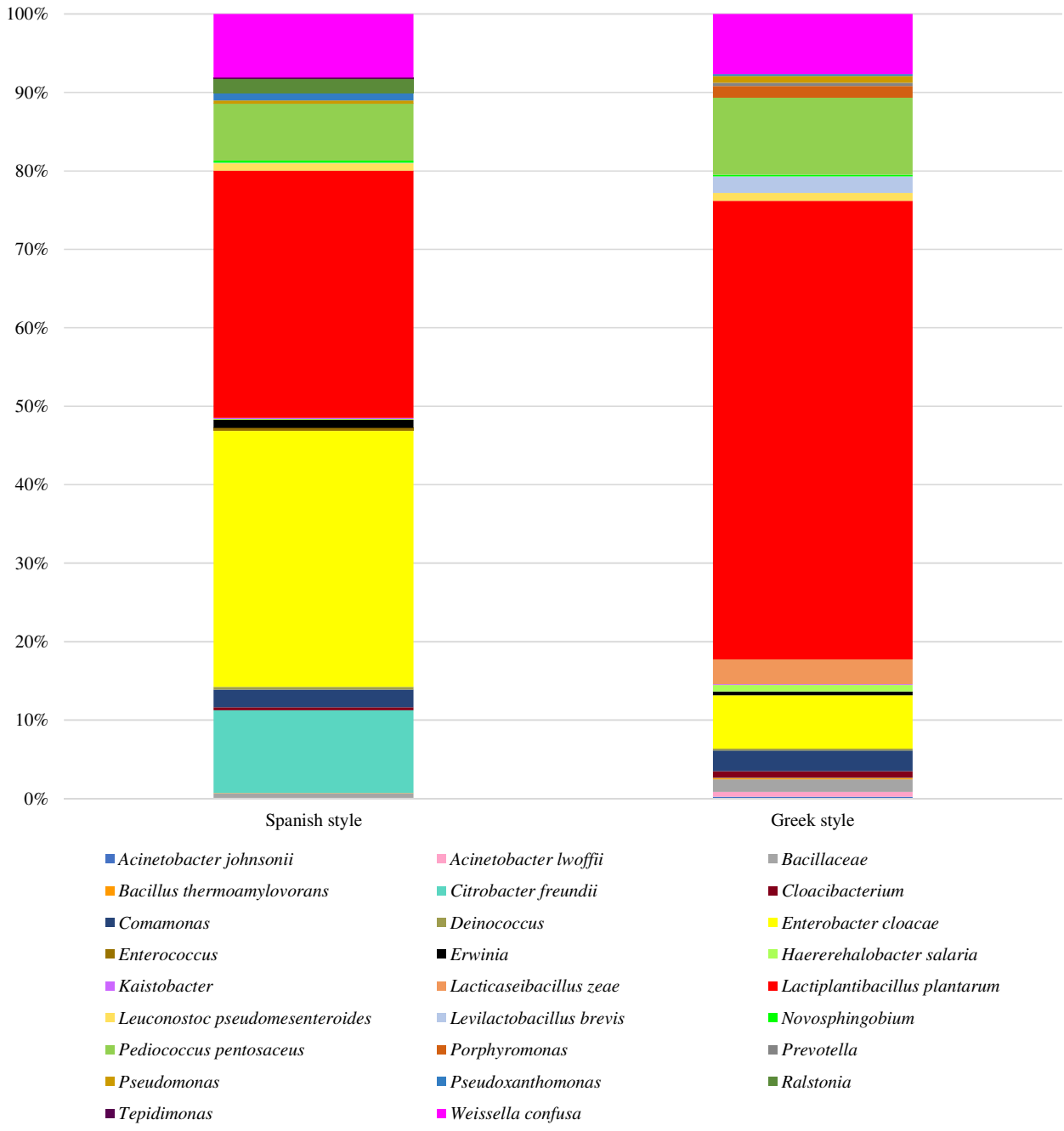


Figure 5.4. Relative frequency of the main bacterial Amplicon Sequencing Variants (ASVs) detected along the whole fermentation processes in the laboratory-scale prototypes made with green olives and sea fennel sprouts according to Spanish or Greek style method.

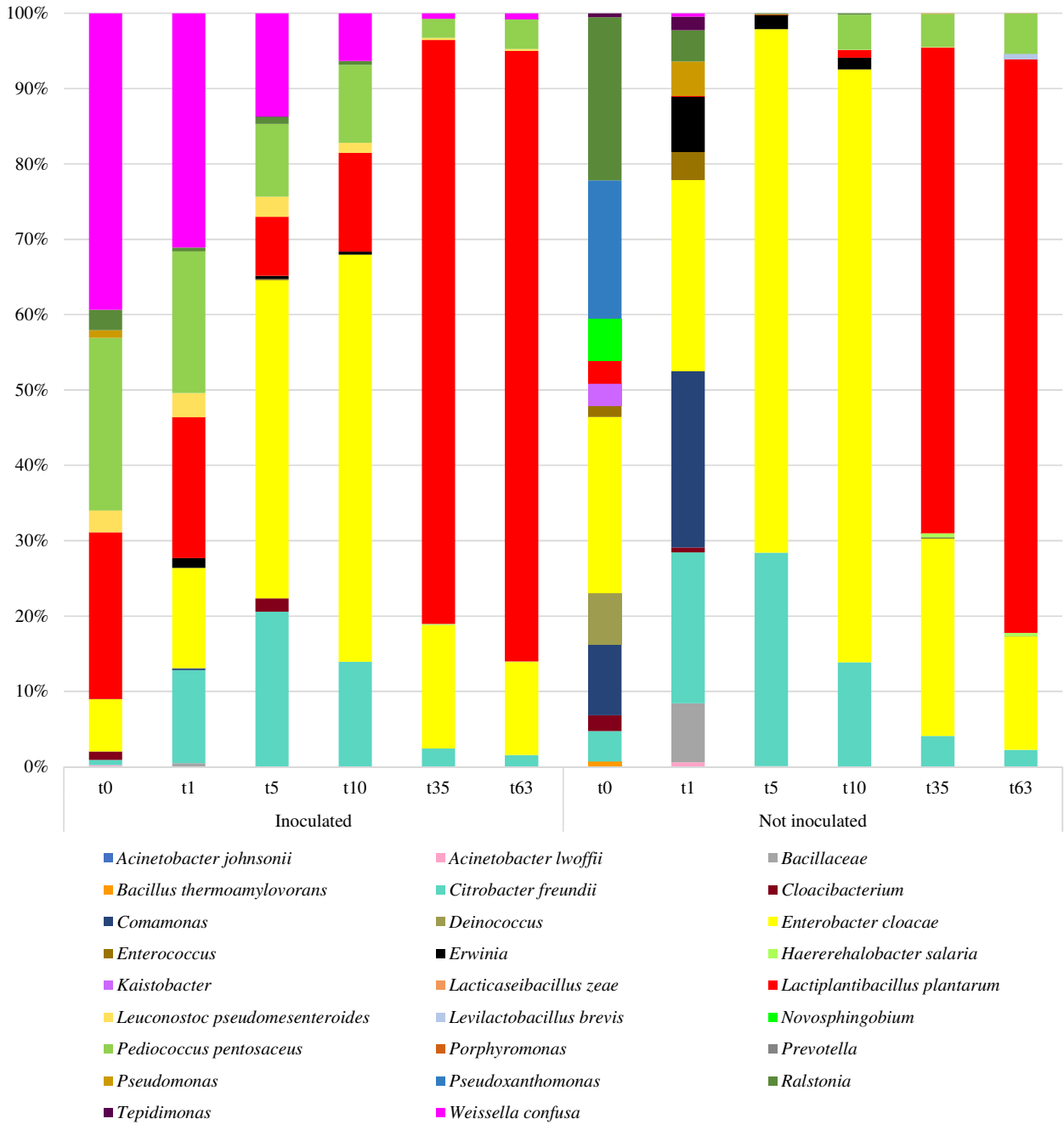
In the prototypes processed according to the Spanish style method, an evolution of the microbiota was seen during the fermentation process, with a stabilization in the late stage (Figure 5.5, panel a). At t_0, the control (uninoculated) prototypes showed a higher heterogeneity of the microbiota than the inoculated prototypes. However, a very similar composition of the bacterial community was seen at

the end of the fermentation process, in both the inoculated and control prototypes (Figure 5.5, panel a). Even evaluation of alpha diversity showed an increase in both the Chao1 index and the number of observations up until t_{10} followed by a stability over time, whereas the Shannon index highlighted an increase in the bacterial diversity from t_0 to t_{10}, followed by a sharp decrease in the last fermentation stage (Supplementary Figure 5.1). As a general trend, *Citrobacter freundii*, *Enterobacter cloacae*, *Lactiplantibacillus plantarum*, *Ralstonia* spp., and *Erwinia* spp., were the most abundant taxa (Figure 5.5, panel a). In more detail, *C. freundii* and *E. cloacae* increased up until t_5 and t_{10} respectively, whereas both decreased at the end of fermentation. *L. plantarum* increase at the end of fermentation (t_{35}, t_{63}), whereas *Ralstonia* spp. decreased during the fermentation process (Figure 5.6, panel a). As far as the recipe is concerned, no differences were seen between the Spanish style prototypes prepared according to recipe A or B (data not shown). Moreover, in the started prototypes, the relative frequencies of *L. plantarum*, *L. pseudomesenteroides*, *P. pentosaceus*, *W. confusa* were higher than those of the control prototypes (Supplementary Figure 5.2).

In the prototypes processed according to the Greek style method, a similar trend to the Spanish style prototypes was seen, with an initial higher bacterial diversity of the control prototypes in respect with the started ones (Figure 5.5, panel b). Again, a stabilization of the bacterial biota was seen in the late stage of fermentation ($t_{101} – t_{373}$), irrespective of the use of the starter. The alpha diversity of the Greek style prototypes showed a decrease of Chao 1 index, number of observations, and Shannon index during fermentation (Supplementary Figure 5.3).

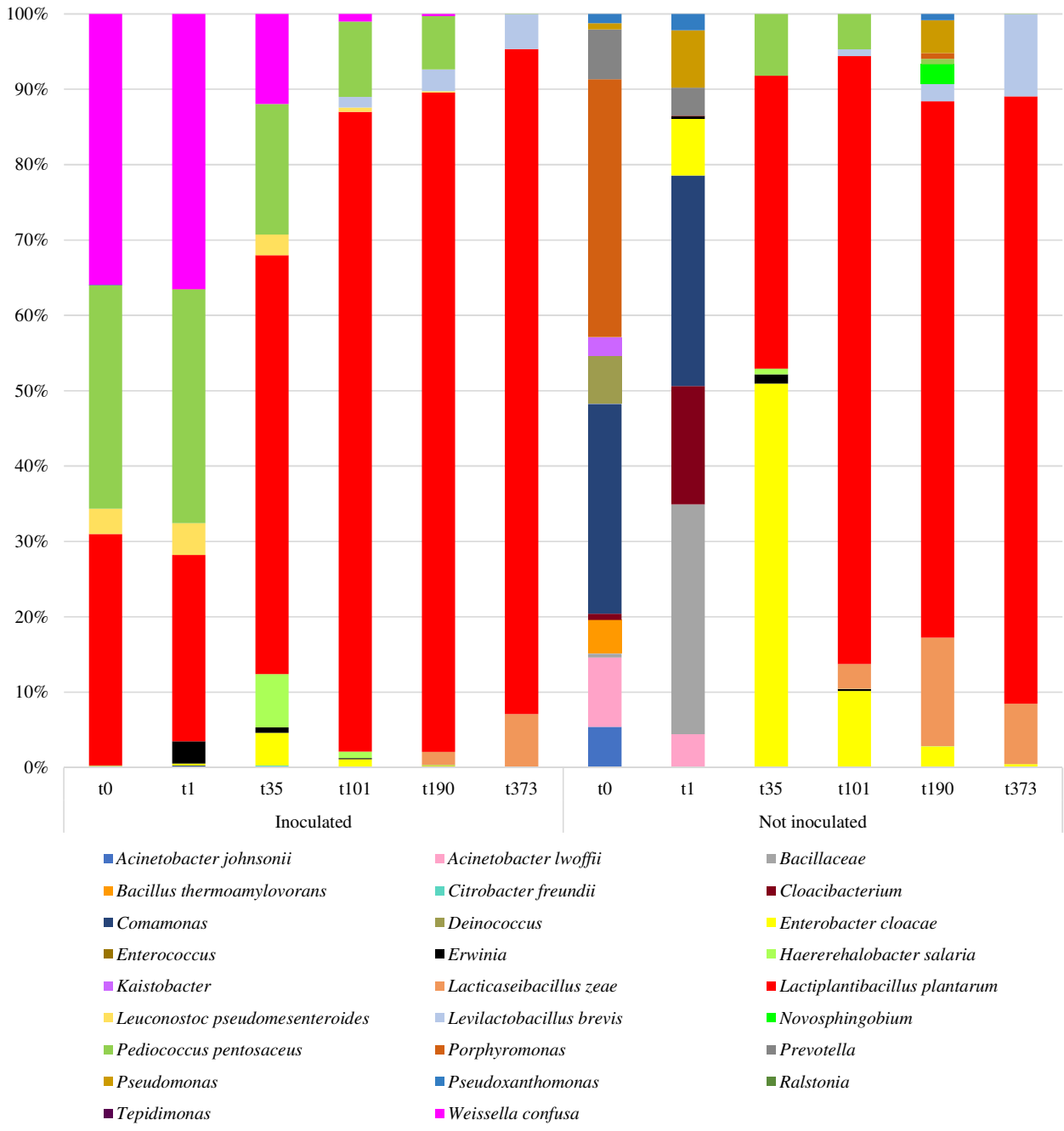
L. plantarum, *Lacticaseibacillus zeae, Levilactobacillus brevis, P. pentosaceus*, and *Erwinia* spp. were the most abundant taxa (Figure 5.5, panel b). In more detail, the relative frequencies of *L. plantarum*, *Lacticaseibacillus zeae* and *Levilactobacillus brevis* increased in the late stage of fermentation (from t_{101} to t_{373}), whereas *P. pentosaceus* decreased over time (Figure 5.6 panel b). As far as the recipe is concerned, ASVs related to *Erwinia* spp. and *L. plantarum* were more abundant in the prototypes containing the highest amount of sea fennel (recipe B) (Supplementary Figure 5.4).

Moreover, significantly higher relative frequencies of *L. pseudomesenteoides*, *P. pentosaceus,* and *W. confusa* were seen in the started prototypes in respect with the control (uninoculated) prototypes ($P < 0.05$) (Supplementary Figure 5.5).



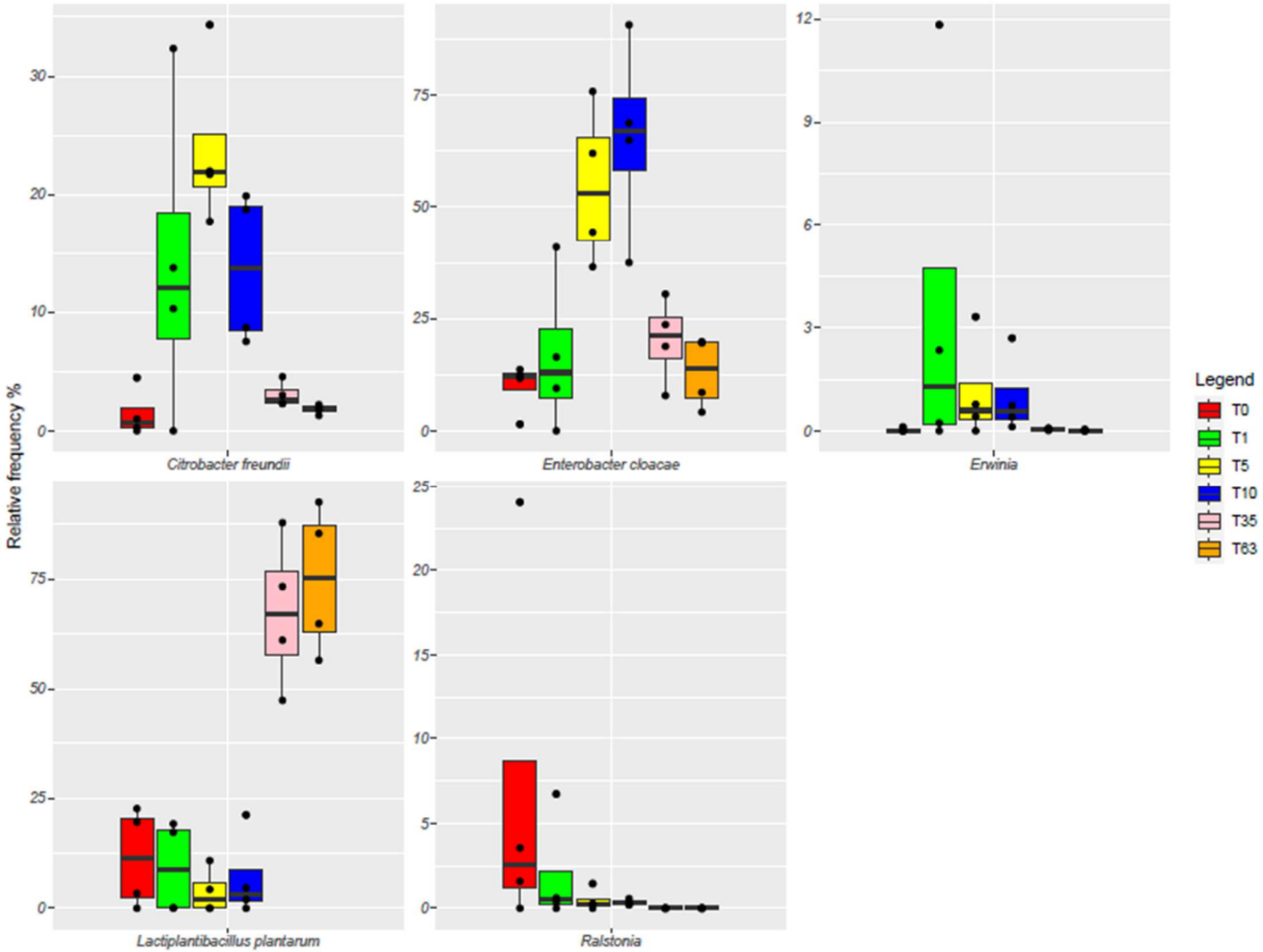
a)

Figure 5.5. Relative frequency of bacterial Amplicon Sequencing Variants (ASVs) detected in the laboratory-scale prototypes made with green olives and sea fennel, according to the Spanish style (panel **a**) and Greek-style (panel **b**) method with and without the inoculation of the multiple strain starter



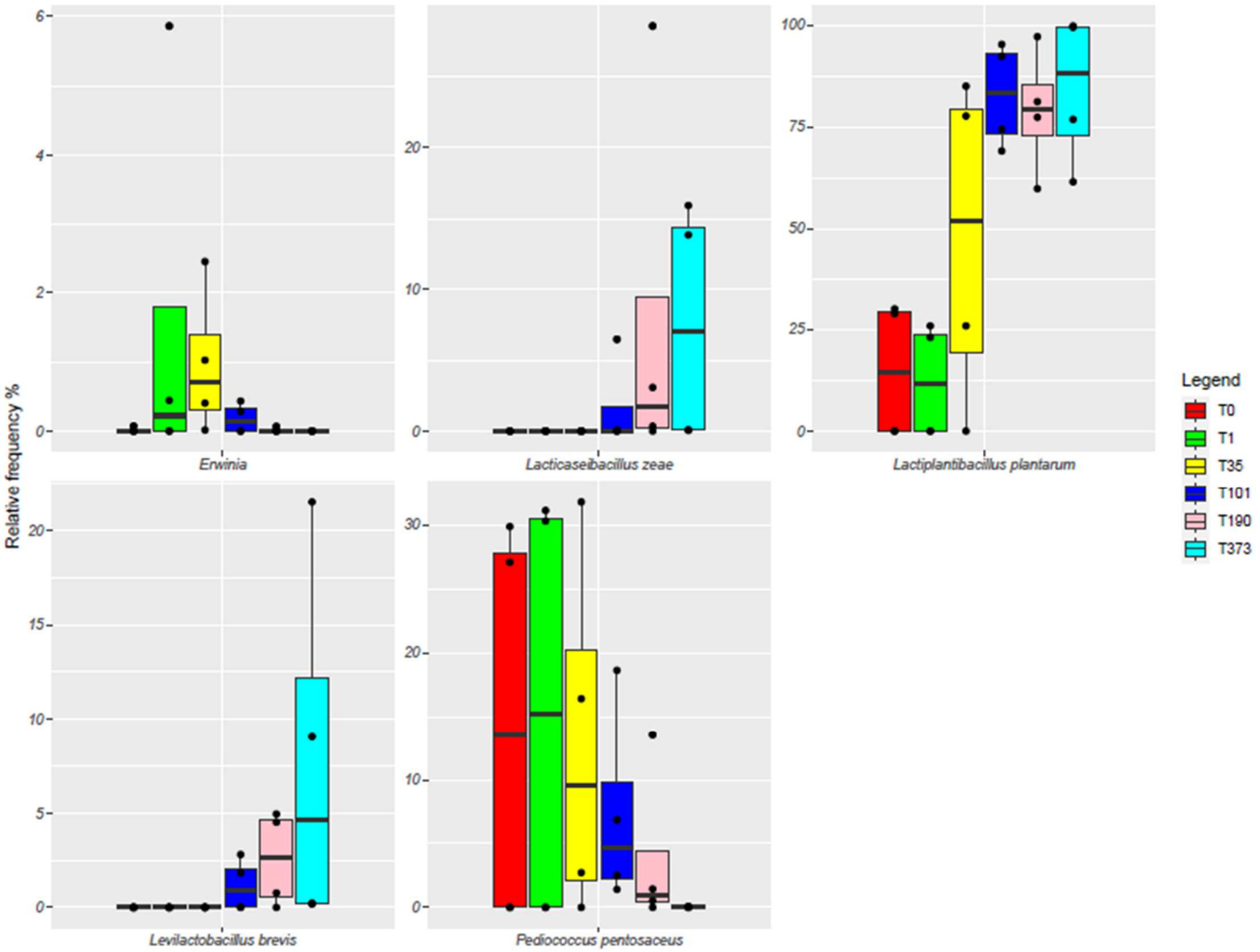
b)

Figure 5.5. (Continued).



a)

Figure 5.6. Boxplots showing the relative abundance of bacterial Amplicon Sequencing Variants (ASVs) along the fermentation period of the prototypes made according to Spanish style (panel **a**) or Greek style method (panel **b**).

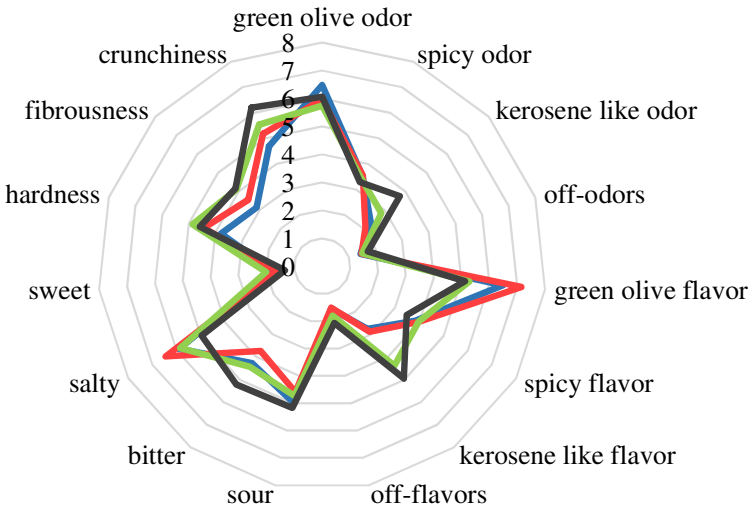


b)

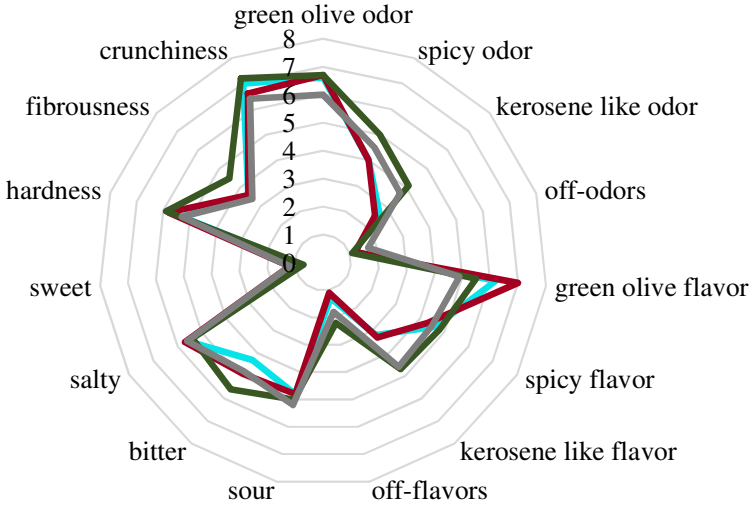
Figure 5.6. (Continued).

5.3.4. Sensory analysis

The results of the sensory analysis are depicted in Figure 5.7.

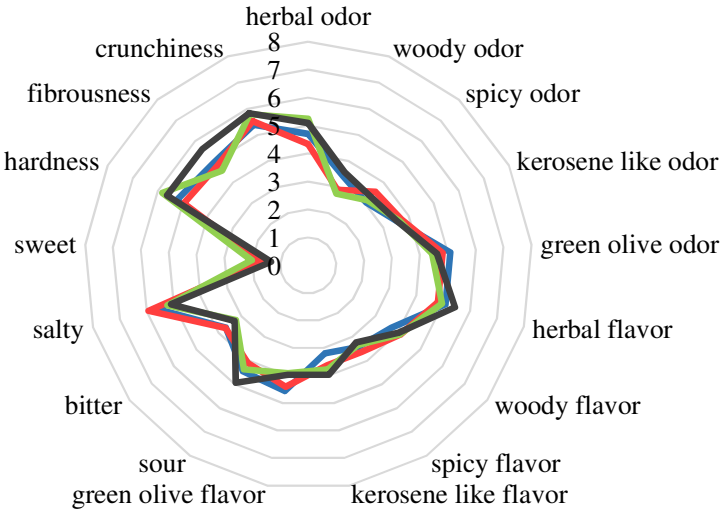


a)

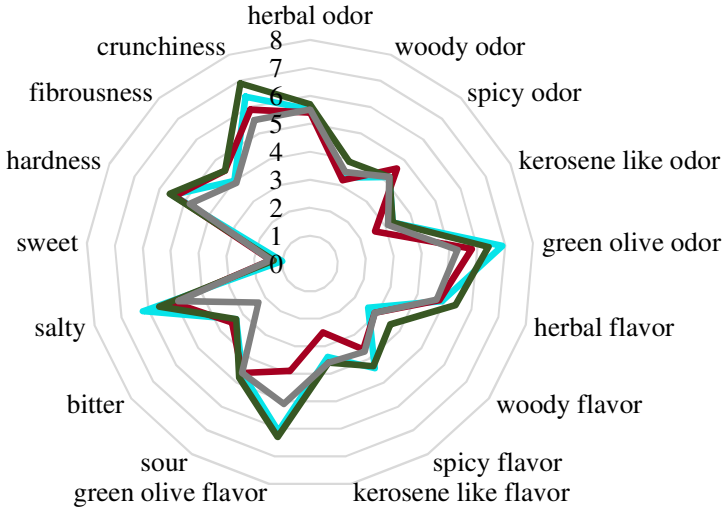


b)

Figure 5.7. Results of the sensory analysis performed onto green olives (panels **a** and **b**) or sea fennel sprouts (panels **c** and **d**) sampled at the end of the fermentation period of the Spanish style (FAS, FAN, FBS and FBN, panels **a** and **c**) and Greek style (GAS, GAN, GBS and GBN, panels **b** and **d**) laboratory-scale prototypes.



c)



d)

Figure 5.7. (Continued). The evaluation was performed by a trained panel, consisting of 10 non-smoker tasters aged between 25 and 45, which analyzed sea fennel sprouts for: i) five olfactory descriptors (herbal, woody, spicy, kerosene-like and green olive); and (ii) five flavor descriptors (herbal, woody, spicy, kerosene-like and green olive), and green olives for: i) three olfactory descriptors (green olive, spicy and kerosene-like) and (ii) three flavor descriptors (green olive, spicy, and kerosene-like); off-odors and off-flavors were also assayed. Both green olives and sea fennel sprouts were evaluated for: i) four taste descriptors (sour, bitter, salty, and sweet); and ii) three textural descriptors (hardness, fibrousness, and crunchiness). Each descriptor was evaluated by assigning 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.
For legend of prototypes see Figure 5.2.

As a general trend, no faint off-odors or off-flavors were perceived in the assayed samples, irrespective of the recipe, the fermentation method, and the use of the starter. The tasting of sea fennel sprouts revealed hints of green olive in all the prototypes, except for SBs, together with herbaceous hints, again perceived in all the prototypes except for GAs and GBs. Going into green olives tasting, the kerosene-like scent was perceived in all the four prototypes (SBs, SBc, GBs and GBc) prepared according to recipe B, containing the highest percentage of sea fennel sprouts (60%). High scores for the salty descriptor and low scores for the sweet descriptor were assigned to both sea fennel sprouts and green olives. Regarding texture, sea fennel sprouts fermented according to the Greek style method were perceived as more crunchy and less fibrous than those fermented according to the Spanish style method. On the other hand, green olives fermented according to the Greek style method were perceived as more crunchy and harder than those manufactured according to the Spanish style method. Looking into the global acceptance, the prototypes prepared according to the Greek style method received a greater appreciation in respect with those manufactured according to the Spanish style method, with GAs and SBs gaining the highest (7.41 ± 0.08) and lowest (5.55 ± 0.09) scores, respectively.

5.4. Discussion

In this study, a multiple strain starter made of strains of lactic acid bacteria selected based on their pro-technological traits (Maoloni et al., 2021) was exploited to guide the co-fermentation of mixtures of green olives and sea fennel sprouts in a brine salt solution. The starter was formulated including strains with different fermentative pathways, namely an obligate homofermentant (*P. pentosaceus* strain FF78), a facultative heterofermentant (*L. plantarum* strain PB257) and two obligate heterofermentants (*L. pseudomesenteroides* strain PB288 and *W. confusa* strain PB321). The composition of the starter was aimed at possibly support the establishment of a microbial succession during the fermentation process, with the homofermentative strains with a higher tolerance for acidic and anaerobic conditions dominating during the late fermentation stage. In fact, in cabbage-based

products (e.g.: sauerkraut and kimchi), optimally fermented at 18°C with ~2% NaCI, a two-stage fermentation is established, with an initial dominance by heterofermentative lactic acid bacteria, including *Leuconostoc* spp. and *Weissella* spp., followed by a homolactic stage, where lactobacilli prevail (Snyder et al., 2020). By contrast, in fermentation of cucumbers, usually conducted at temperatures ≥ 20°C and NaCl content ≥ 6%, homolactic fermentation predominates, with *L. plantarum* being the prevailing species.

In the present study, the fermentation was conducted at 18 °C with 8% NaCl. A temperature range between ~18 and 24°C has previously been reported to stimulate the metabolic activity of lactic acid bacteria, and hence production of organic acids and flavor enhancement (Snyder et al., 2020).

As far as pH evolution is concerned, at the beginning of fermentation, the prototypes prepared according to recipe B, containing 60% drained weight of sea fennel, showed significantly higher pH values than the prototypes containing 10% drained weight of sea fennel; by contrast, an opposite trend was seen at the end of fermentation, with significantly lower pH values measured in the prototypes manufactured according to recipe A in respect with recipe B.

In fermented vegetables, acidification mainly results from the accumulation of organic acids (especially lactic and acetic acid) produced by homo- or hetero-fermentative lactic acid bacteria from free sugars, primarily glucose and fructose (Snyder et al., 2020). In addition to this, during olives fermentation, alkaline hydrolysis of oleuropein results in the production of phenolic compounds, whose further breakdown leads to the release of additional acids, like elenolic acid (Kiai and Hafidi 2014).

In the present study, the more intense acidification observed in the prototypes with the highest sea fennel content might feasibly be related to the faster growth of lactic acid bacteria and hence a more intense production of organic acids in presence of this aromatic herb. A similar behavior has already been described by Zaika et al. (1983), in a study where the effects of different aromatic herbs

(oregano, rosemary, sage, and thyme) on growth and acidification of *Lactiplantibacillus plantarum* and *Pediococcus acidilactici* were evaluated. Intriguingly, after an initial inhibition, and hence a delay in fermentation, at sublethal concentrations, all the assayed herbs exerted a stimulation of the two lactic acid bacteria growth and acids production.

Going into the microbial enumeration, in the started prototypes, as early as after 24 hours of fermentation, mesophilic lactobacilli showed a drastic reduction, followed by a slight but continuous increase up until the late fermentation stage, whereas in the control prototypes a progressive growth of this microbial group was seen. Similar trends were observed by Maoloni et al. (2021), while fermenting sea fennel sprouts started with the starter culture formulation assayed in the present study. The early reduction of viable counts of mesophilic lactobacilli seen in the all the started prototypes irrespective of the processing method might be tentatively ascribed to the low competitiveness and hence adaptation ability of the inoculated strains in the specific substrate assayed. As it has been underlined above, for various aromatic herbs a stimulation of the lactic acid bacteria growth has been observed, after the bacteriostatic activity is overcome, (Zaika et al., 1983). To date, numerous studies have reported the antimicrobial effect of sea fennel essential oils (Senatore et al., 2000), but no data are available on a possible growth stimulation of lactic acid bacteria by this aromatic herb.

Regarding the control prototypes, dynamics of mesophilic lactobacilli were in accordance with the available literature on green olives fermentation (Marsilio et al., 2005; Panagou and Tassou, 2006; Sánchez et al., 2000).

As far as the enumeration of Enterobacteriaceae is concerned, the growth dynamics emerged from the present study were comparable to those previously reported by other authors in either fermented table olives (Hurtado et al., 2008; Panagou and Tassou, 2006) or sea fennel (Maoloni et al., 2021), with an initial multiplication of these microorganisms followed by a decrease up until a complete dye

off at the end of the fermentation period, as a direct consequence of the pH drop due to lactic acid bacteria metabolism (Botta and Cocolin, 2012; Hurtado et al., 2008).

For yeasts, despite initial (t_0) low loads, an overall significant increase of viable counts was seen during fermentation; indeed, at the end of the monitoring period, viable counts of yeasts were comparable to those of mesophilic lactobacilli in all the prototypes, except for GBs and GBc, where yeasts were about one order of magnitude lower than mesophilic lactobacilli. A similar trend has already been described by monitoring the microbial dynamics during sea fennel pilot scale fermentations (Maoloni at al., 2021). Regarding green olives, a higher load of yeasts in respect with mesophilic lactobacilli was seen at the end of fermentation of unstarted Greek style olives (Marsilio et al., 2005). Various detrimental properties have previously been associated with yeasts in olive fermentation, including: (i) antagonistic effect against lactic acid bacteria; (ii) fruit damage by excessive CO_2 production; (iii) degradation of polysaccharides of the olive cell wall through a polysaccharolytic activity; (iv) softening of the fruit during storage through a polygalactunorase activity (Arroyo-López et al., 2008, 2012). However, yeasts can also carry out some beneficial activities, including: (i) production of key aromatic compounds such as glycerol, esters, ethanol, higher alcohols and other volatile compounds; (ii) synthesis of bioactive compounds with antioxidant properties; (iii) synthesis of killer toxins active against spoilage microorganisms and human pathogens; (iv) degradation of phenolic compounds; (v) improvement of lactic acid bacteria growth through the synthesis of vitamins, purines, and amino acids or the breakdown of complex carbohydrates (Arroyo-López et al., 2008, 2012).

The metataxonomic analysis clearly showed the occurrence of *L. plantarum*, *P. pentosaceus*, *W. confusa, E. cloacae*, and *C. freundii.* Members of the *L. plantarum* group*,* including *L. plantarum*, *L. pentosus*, and *L. paraplantarum*, are crucial microorganisms in table olives fermentation, with the first two species being also extensively exploited as starter cultures (Cocolin et al., 2013; Lucena-Padrós et al., 2015; Randazzo et al., 2012). In more detail, *L. plantarum* has been reported to dominate

up until the end of table olive fermentation (Zago et al., 2013). The latter species is known to: (i) boost oleuropein breaking down due to its β-glucosidase activity; (ii) produce antimicrobial molecules against undesirable microorganisms; (iii) grow in a wide range of pH values and temperatures; and (iv) tolerate saline environments (Snyder et al., 2020). Starter cultures belonging to *L. plantarum* are known to improve the microbiological stability and safety of fermented table olives (Randazzo et al., 2012; Zago et al., 2013). In the present study, as expected, the starter species *L. plantarum* was found to quantitatively prevail in the inoculated prototypes, followed by the other inoculated species *L. pseudomesenteroides*, *P. pentosaceus*, and *W. confusa*. However, *L. plantarum* was also predominant in the uninoculated prototypes, thus demonstrating its occurrence on vegetable tissues and its high adaptation to the specific fermentation conditions applied for olive fermentation (Snyder et al., 2020).

Even *W. confusa* and *P. pentosaceus* are vegetable associated microorganisms, being often isolated from fermented vegetables (Hurtado et al., 2012; Quattrini et al., 2020). Though *W. confusa* is not included among the major genera used as starters, it is frequently isolated from spontaneous fermented foods, where it contributes to the characteristics of the products (Fessard and Remize, 2017). More specifically, *W. confusa* is known to produce high amounts of exopolysaccharides with texturizing properties, whereas some *Weissella* strains were found to decarboxylate polymeric phenolic compounds, thus increasing bioavailability of these health beneficial compounds. Despite the above cited properties, the exploitation of *Weissella* spp. as a commercial starter is currently underexplored.

By contrast, *P. pentosaceus* is recognized as a promising lactic acid bacteria species for its application as adjutant or probiotic by the food industry, thanks to (i) the improvement of texture, sourness, and other organoleptic properties of foods; (ii) the antioxidant effects; (iii) the inhibition of pathogenic bacteria and fungi (Jiang et al., 2021).

Enterobacter and *Citrobacter* were both detected with relative high frequencies in the Spanish style prototypes, whereas the first genus was also found with a relative low frequency in the Greek style prototypes. Both these genera include opportunistic human pathogenic species, such as *E. cloacae* and *C. freundii,* whose occurrence in food products might represent a sanitary risk (Bevilacqua et al., 2010b, 2013; Liu et al., 2018; Surowsky et al., 2014).

During the Spanish style fermentation, the plant-pathogenic genus *Erwinia* and the soil associated genus *Ralstonia* (Kado et al., 2006; Penland et al., 2020) were mainly detected in the early stage of fermentation, whereas in the late stage they were completely replaced by *L. plantarum*. This finding agrees well with what reported in the literature about the occurrence of a microbial succession during fermentation of vegetables, with epiphytic aerobic microbiota being replaced by facultatively anaerobic, acid-tolerant lactic acid bacteria (Snyder et al., 2020).

During the Greek style fermentation, the most significant detected species were *L. plantarum*, *Lacticaseibacillus zeae*, *Levilactobacillus brevis,* and *P. pentosaceus*. Among these, both *L. brevis* and *P. pentosaceus* have previously been detected in green table olives (Hurtado et al., 2012), whereas *L. zeae* is generally associated with dairy products (Poltronieri et al., 2008).

Going into the sensory analysis, as a general trend, a higher crunchiness was perceived by panelists by tasting sea fennel processed according to the Greek style in respect with the Spanish style method. Crunchiness undoubtedly represents a quality trait of sour vegetable-based fermented pickles, such as cucumbers and Capparis (Behera et al., 2020). Similarly, olives fermented according to the Greek style method were perceived as harder and crunchier than those processed according to the Spanish style method. These findings agree well with those previously reported by Marsilio et al. (2005) in a study dealing with the fermentation of green olives of the Ascolana tenera variety, processed according to both Spanish and Greek style methods. In fact, as it has previously been elucidated, the

treatment of olives with lye in the Sevillian method leads to a softening of the olive flesh and hence to a modification of its texture (Chranioti at al., 2018).

Furthermore, the greater overall acceptability of the Greek style prototypes in respect with the Spanish style prototypes might be tentatively ascribed to the prolonged fermentation process, with long-term fermentations being known to greatly contribute to the flavor and aroma of fermented products (Sabatini and Marsilio, 2008). In fact, the development of odors and flavors in a fermented food is related to the progressive accumulation of alcohols, acids, aldehydes, ketones, esters, sulfur compounds, terpenes and lactones from lactic acid bacteria and yeasts metabolism of carbohydrates, proteins, lipids, citrate, and polyphenols (Carballo, 2012; Smid and Kleerebezem, 2014).

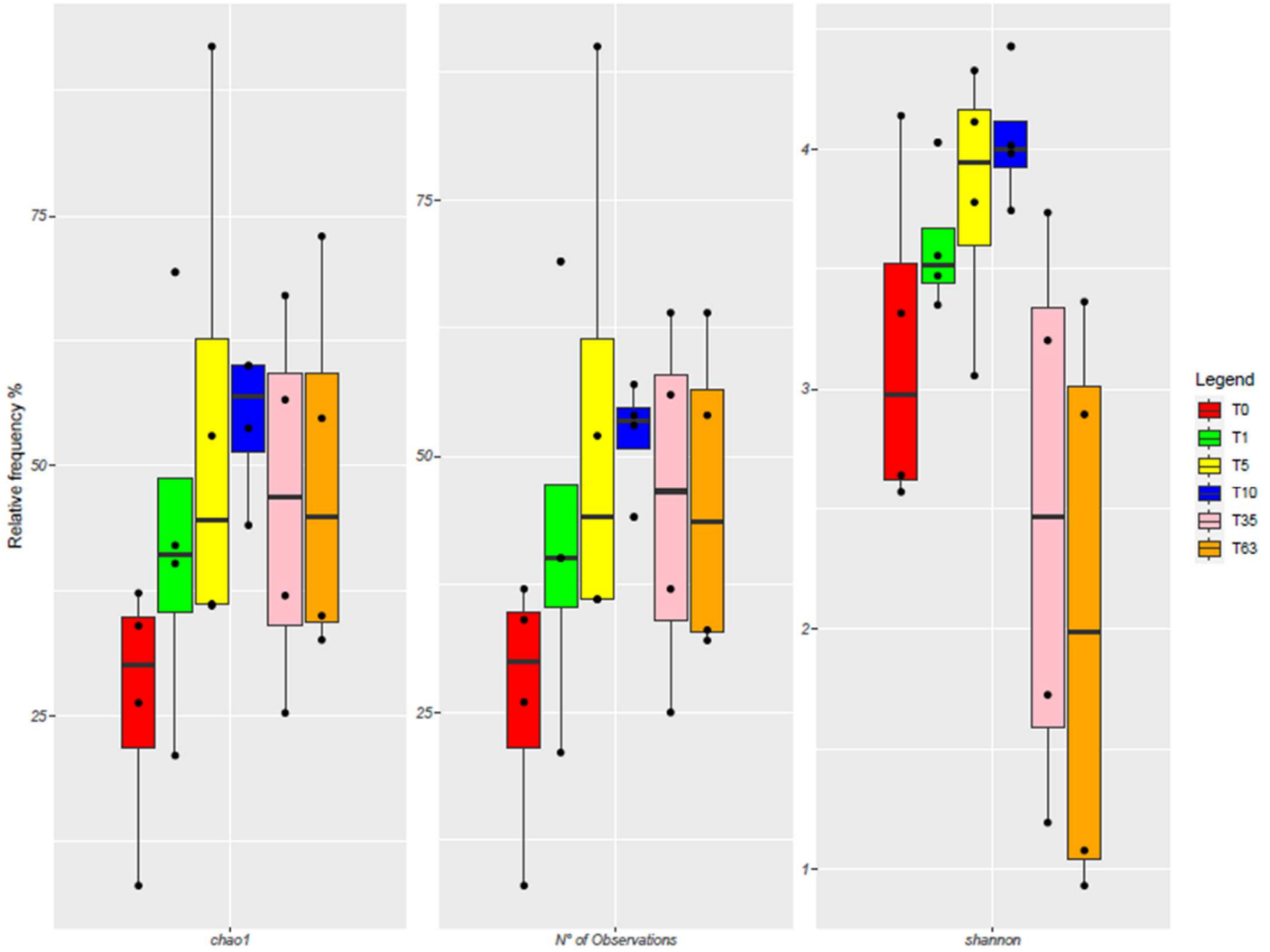
5.5. Conclusions

Based on the results overall collected a few considerations can be made. The pH evolution was apparently affected by the recipe, with initial significantly higher and final significantly lower pH values of the prototypes with the highest sea fennel content. The inoculation of the starter culture had apparently no impact on the load of mesophilic lactobacilli, except for the very early fermentation stage; similarly, in both the started and control prototypes, a complete dominance of the species *L. plantarum* was seen in the late fermentation stage, irrespective of the recipe, the use of the multiple strain starter, and the processing method. By contrast, this latter variable seemed to affect the final loads of mesophilic bacteria and yeasts, with significantly higher counts of both these microbial groups detected in the Spanish style rather than the Greek style prototypes.

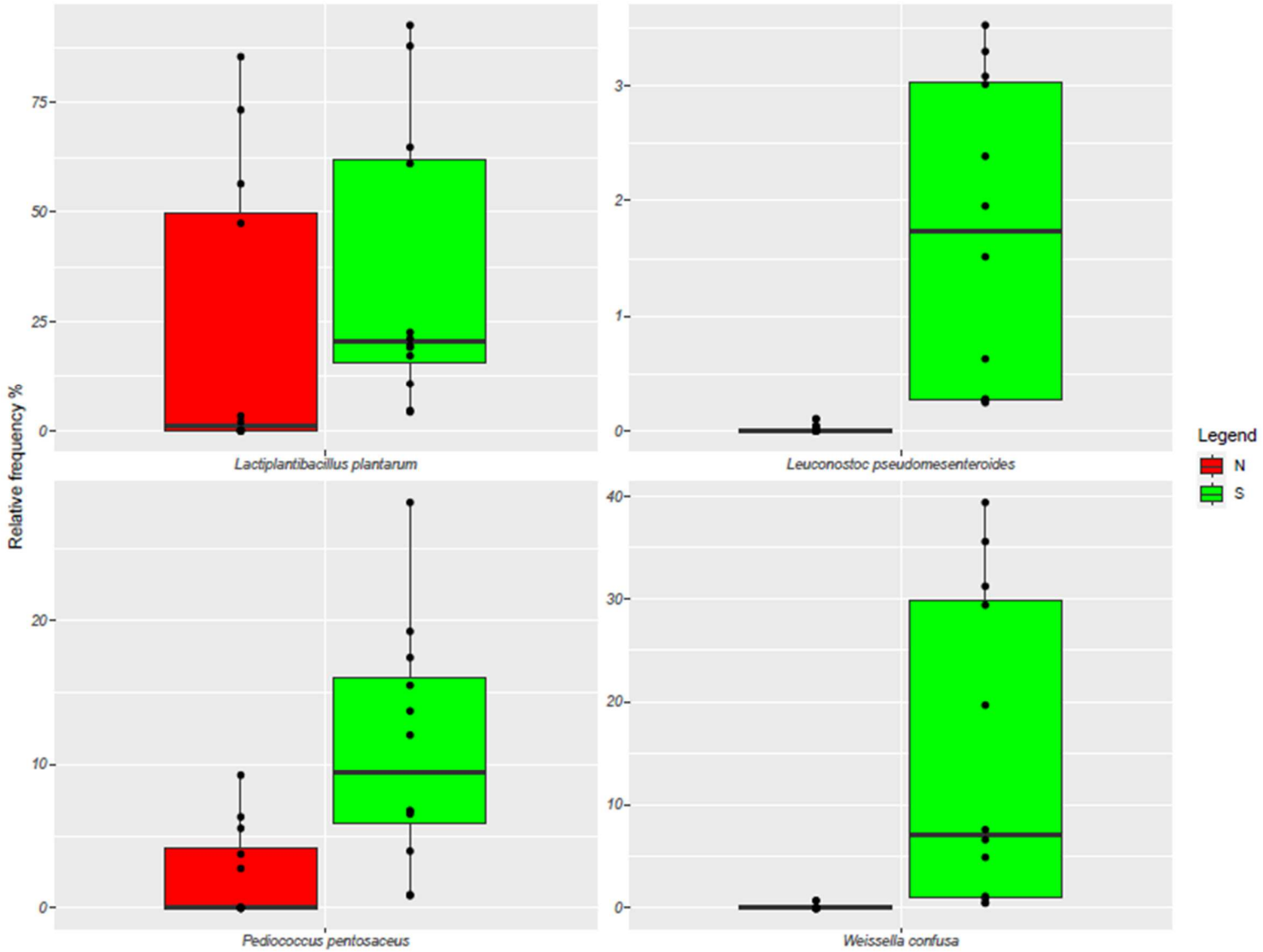
As a general trend, a faster reduction of Enterobacteriaceae was observed in the started prototypes than the controls. In any case, a microbial succession was established during fermentation, irrespective of the recipe and processing method, with species dominating in the very first fermentation stage (e.g.: epiphytic bacteria) been replaced by acid resistant, facultative anaerobic microorganisms in the late stage. Finally, a higher crunchiness, a lower fibrousness, and a greater

overall acceptability were scored in the Greek style prototypes than the Spanish style ones, with recipe A, characterized by the lowest sea fennel content, been preferred in respect with recipe B by the trained panelists.

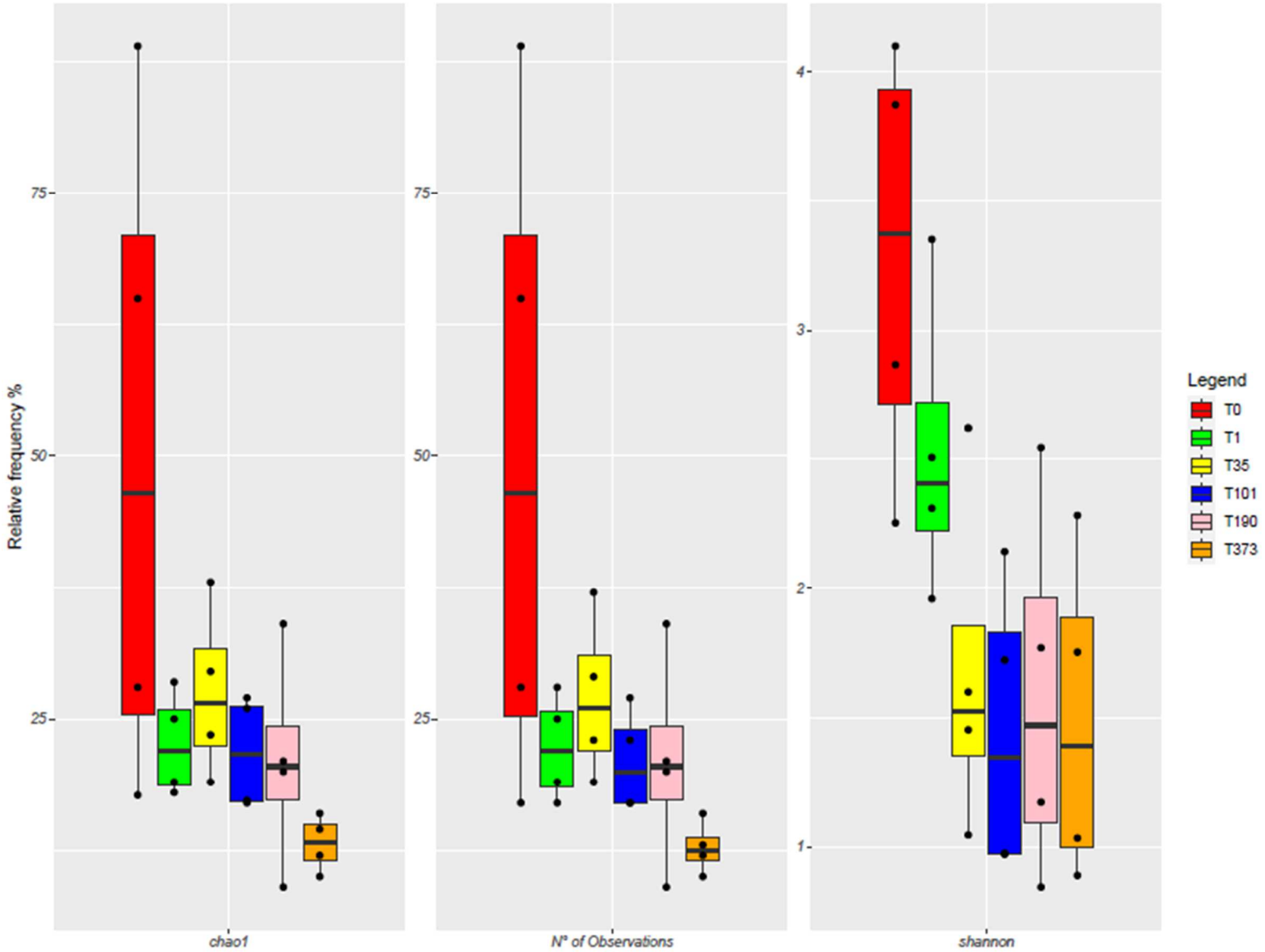
5.6. Supplementary data



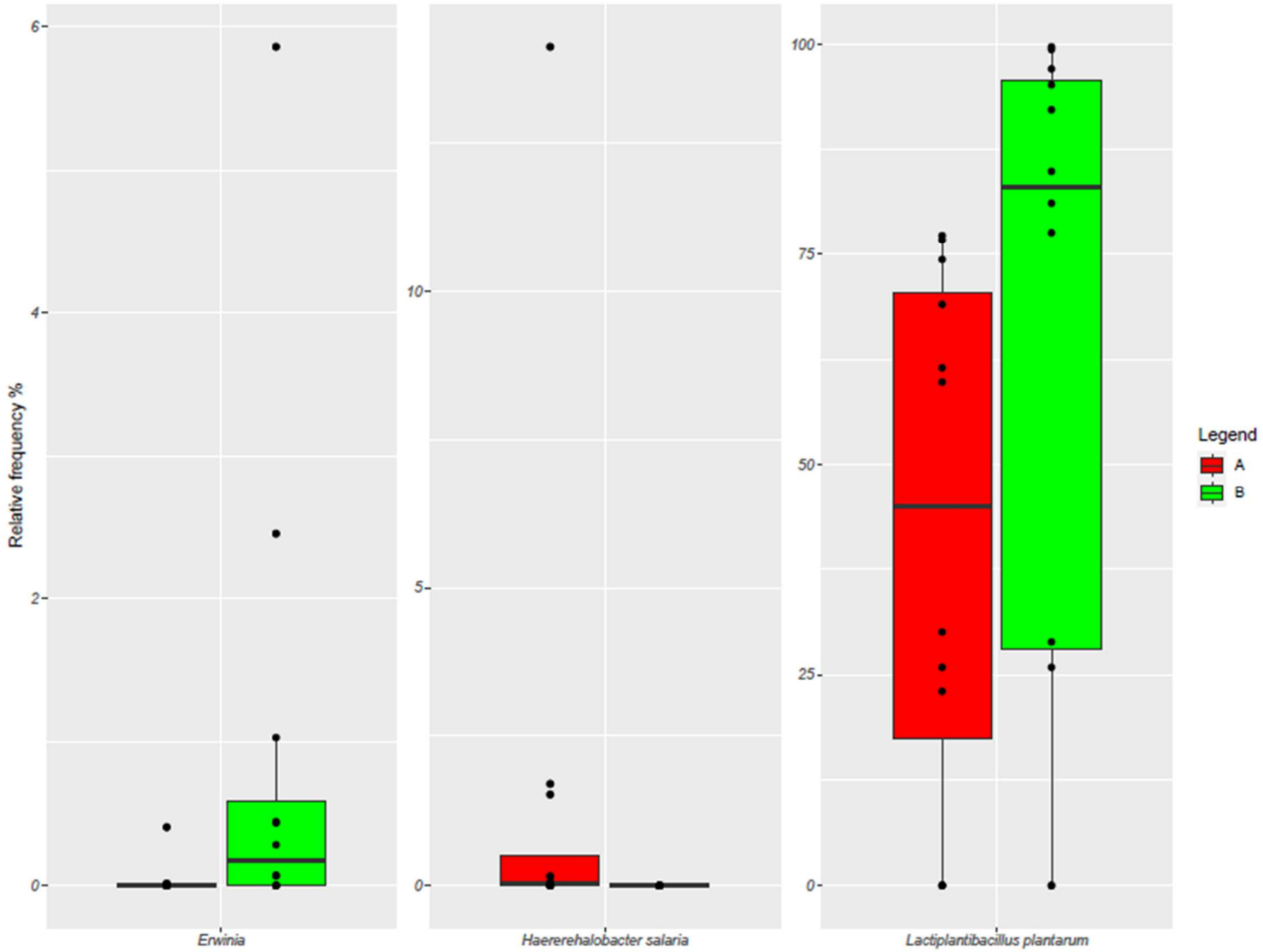
Supplementary Figure 5.1. Chao 1, number of observed ASVs and Shannon index over time, in sample fermented according to the Spanish style method.



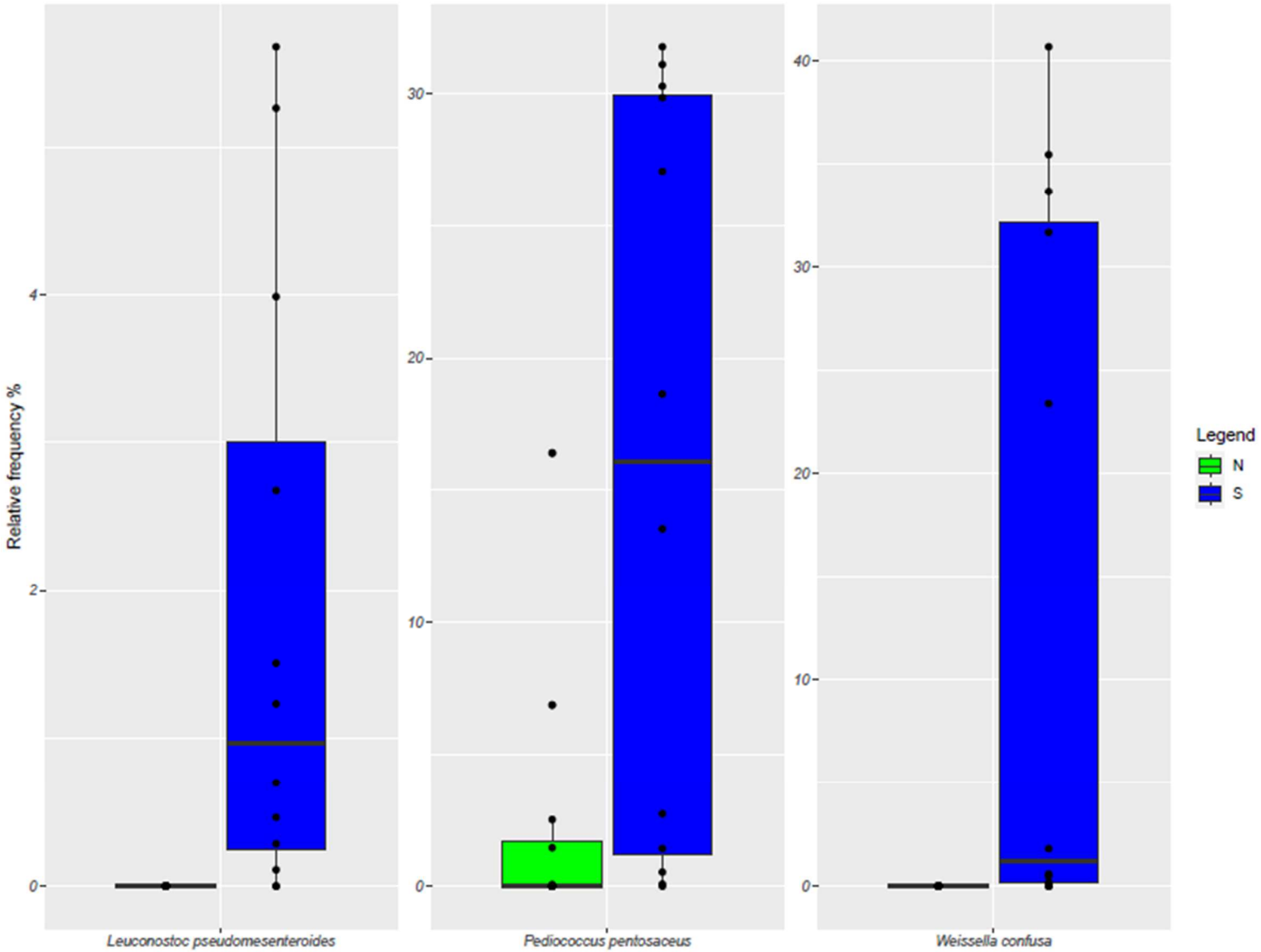
Supplementary Figure 5.2. Boxplots showing the relative abundance of bacterial Amplicon Sequencing Variants (ASVs) between the inoculated (S) and not inoculated (N) samples, fermented according to the Spanish style method.



Supplementary Figure 5.3. Chao 1, number of observed ASVs and Shannon index over time, in sample fermented according to the Greek style method.



Supplementary Figure 5.4. Boxplots showing the relative abundance of bacterial Amplicon Sequencing Variants (ASVs) between samples prepared with the recipe A (A: consisting of 10 % sea fennel sprouts and 90 % green olives) and samples prepared with the recipe B (60 % sea fennel sprouts and 40 % green olives), fermented according to the Greek style method.



Supplementary Figure 5.5. Boxplots showing the relative abundance of bacterial Amplicon Sequencing Variants (ASVs) between the inoculated (S) and not inoculated (N) samples, fermented according to the Greek style method.

Supplementary Table 5.1. Results of the pH determination of the laboratory-scale prototypes made with green olives and sea fennel sprouts.

Fermentation style	Sampling time (t, days)	Prototypes							
Spanish style		SAs		SAc		SBs		SBc	
	t_0	5.89 ± 0.01^{a}	B	5.88 ± 0.03^{a}	B	6.14 ± 0.03^{a}	A	6.21 ± 0.06^{a}	A
	t_1	5.62 ± 0.03^{b}	C	5.68 ± 0.02^{b}	BC	5.78 ± 0.08^{b}	AB	5.82 ± 0.01^{ab}	A
	t_2	5.53 ± 0.07^{b}	A	5.55 ± 0.06^{c}	A	5.50 ± 0.07^{c}	A	5.51 ± 0.02^{abc}	A
	t_3	5.25 ± 0.03^{c}	B	5.32 ± 0.02^{d}	AB	5.33 ± 0.04^{cd}	A	5.31 ± 0.02^{bcd}	AB
	t_5	5.11 ± 0.05^{cdef}	A	5.16 ± 0.02^{efg}	A	5.18 ± 0.04^{de}	A	5.19 ± 0.03^{bcd}	A
	t_6	5.11 ± 0.05^{cdef}	B	5.16 ± 0.02^{efg}	AB	5.19 ± 0.04^{de}	AB	5.21 ± 0.03^{bcd}	A
	t_7	5.17 ± 0.05^{cd}	A	5.20 ± 0.03^{ef}	A	5.27 ± 0.09^{d}	A	5.29 ± 0.04^{bcd}	A
	t_8	5.13 ± 0.03^{cde}	C	5.15 ± 0.02^{efgh}	BC	5.21 ± 0.01^{de}	B	5.30 ± 0.03^{bcd}	A
	t_9	5.09 ± 0.02^{def}	A	5.14 ± 0.01^{fgh}	A	5.12 ± 0.08^{de}	A	5.21 ± 0.06^{bcd}	A
	t_{10}	5.17 ± 0.00^{cd}	AB	5.21 ± 0.01^{ef}	AB	5.00 ± 0.15^{e}	B	5.23 ± 0.07^{bcd}	A
	t_{12}	5.12 ± 0.02^{cde}	A	5.19 ± 0.01^{ef}	A	4.47 ± 0.08^{f}	B	4.91 ± 0.26^{cde}	A
	t_{13}	5.08 ± 0.01^{def}	A	5.15 ± 0.01^{efgh}	A	4.16 ± 0.08^{g}	B	4.56 ± 0.37^{def}	B
	t_{14}	4.99 ± 0.02^{efgh}	A	5.08 ± 0.01^{hi}	A	3.91 ± 0.08^{h}	B	4.22 ± 0.32^{efg}	B
	t_{15}	5.07 ± 0.02^{def}	A	5.19 ± 0.02^{ef}	A	3.90 ± 0.08^{hi}	B	4.16 ± 0.28^{efg}	B
	t_{16}	5.11 ± 0.02^{cdef}	A	5.21 ± 0.02^{ef}	A	3.88 ± 0.08^{hij}	B	4.14 ± 0.28^{efg}	B
	t_{20}	4.99 ± 0.06^{efg}	A	5.19 ± 0.01^{ef}	A	3.75 ± 0.07^{hijkl}	B	4.02 ± 0.32^{fg}	B
	t_{21}	4.98 ± 0.07^{efgh}	A	5.18 ± 0.01^{ef}	A	3.74 ± 0.07^{hijklm}	B	4.03 ± 0.33^{fg}	B
	t_{22}	4.95 ± 0.10^{fgh}	A	5.22 ± 0.02^{e}	A	3.73 ± 0.07^{hijklm}	B	4.04 ± 0.35^{fg}	B
	t_{23}	4.88 ± 0.13^{ghi}	A	5.20 ± 0.01^{ef}	A	3.68 ± 0.07^{jklmno}	B	3.98 ± 0.35^{fg}	B
	t_{24}	4.90 ± 0.11^{gh}	A	5.22 ± 0.02^{e}	A	3.69 ± 0.06^{ijklmn}	B	4.01 ± 0.35^{fg}	B
	t_{27}	4.73 ± 0.03^{ijk}	A	5.08 ± 0.04^{ghi}	A	3.63 ± 0.07^{klmnop}	B	3.94 ± 0.35^{fg}	B
	t_{28}	4.69 ± 0.01^{jkl}	A	5.05 ± 0.05^{ij}	A	3.61 ± 0.07^{klmnop}	B	3.92 ± 0.34^{fg}	B
	t_{29}	4.64 ± 0.03^{kl}	A	5.04 ± 0.04^{ij}	A	3.56 ± 0.06^{lmnop}	B	3.90 ± 0.36^{fg}	B
	t_{30}	4.83 ± 0.03^{hij}	A	5.22 ± 0.03^{e}	A	3.78 ± 0.05^{hijk}	B	4.08 ± 0.34^{efg}	B
	t_{35}	4.58 ± 0.04^{klm}	A	4.99 ± 0.03^{jk}	A	3.63 ± 0.04^{klmnop}	B	3.97 ± 0.33^{fg}	B
	t_{36}	4.53 ± 0.05^{lmn}	B	4.95 ± 0.04^{kl}	A	3.54 ± 0.06^{mnop}	C	3.84 ± 0.31^{fg}	C
	t_{38}	4.46 ± 0.05^{mno}	B	4.91 ± 0.02^{kl}	A	3.51 ± 0.03^{nop}	D	3.92 ± 0.24^{fg}	C
	t_{42}	4.40 ± 0.04^{nop}	B	4.82 ± 0.02^{m}	A	3.52 ± 0.04^{nop}	C	3.78 ± 0.29^{fg}	C
	t_{45}	4.43 ± 0.05^{mno}	B	4.88 ± 0.03^{lm}	A	3.60 ± 0.04^{klmnop}	C	3.87 ± 0.31^{fg}	C
	t_{48}	4.25 ± 0.04^{pq}	B	4.63 ± 0.02^{no}	A	3.44 ± 0.04^{p}	C	3.71 ± 0.28^{fg}	C
	t_{50}	4.22 ± 0.04^{q}	A	4.59 ± 0.02^{op}	A	3.44 ± 0.03^{p}	B	3.67 ± 0.29^{g}	B
	t_{52}	4.32 ± 0.04^{opq}	B	4.69 ± 0.02^{n}	A	3.51 ± 0.02^{nop}	C	3.75 ± 0.27^{fg}	C
	t_{55}	4.24 ± 0.04^{pq}	B	4.61 ± 0.01^{nop}	A	3.47 ± 0.04^{op}	C	3.69 ± 0.27^{g}	C
	t_{57}	4.24 ± 0.05^{q}	B	4.62 ± 0.02^{nop}	A	3.48 ± 0.05^{nop}	C	3.71 ± 0.27^{fg}	C
	t_{63}	4.17 ± 0.05^{q}	B	4.55 ± 0.02^{p}	A	3.47 ± 0.08^{op}	C	3.75 ± 0.27^{fg}	C

Supplementary Table 5.1. (Continued).

Fermentation style	Sampling time (t, days)	Prototypes							
Greek style		GAs		GAc		GBs		GBc	
	t_0	5.75 ± 0.04^{a}	C	5.81 ± 0.06^{a}	C	6.82 ± 0.05^{a}	A	6.22 ± 0.09^{a}	B
	t_1	5.26 ± 0.03^{b}	C	5.29 ± 0.02^{b}	C	5.81 ± 0.02^{b}	A	5.68 ± 0.06^{b}	B
	t_2	5.09 ± 0.03^{c}	B	5.14 ± 0.01^{bcd}	B	5.60 ± 0.04^{b}	A	5.49 ± 0.08^{bc}	A
	t_3	5.03 ± 0.06^{cd}	B	5.03 ± 0.02^{cde}	B	5.28 ± 0.03^{c}	A	5.21 ± 0.02^{cd}	A
	t_5	4.93 ± 0.02^{cdef}	C	5.00 ± 0.01^{cdef}	B	5.07 ± 0.02^{cd}	A	5.09 ± 0.02^{cd}	A
	t_6	4.97 ± 0.01^{cde}	B	5.01 ± 0.01^{cdef}	B	5.01 ± 0.03^{de}	B	5.10 ± 0.03^{cd}	A
	t_7	5.03 ± 0.02^{cd}	B	5.08 ± 0.01^{cde}	B	5.02 ± 0.04^{de}	B	5.23 ± 0.03^{bcd}	A
	t_8	4.96 ± 0.01^{cdef}	AB	5.02 ± 0.01^{cde}	AB	4.82 ± 0.17^{e}	B	5.18 ± 0.03^{cd}	A
	t_9	4.87 ± 0.01^{defgh}	AB	5.00 ± 0.05^{cdef}	A	4.47 ± 0.32^{f}	B	5.07 ± 0.02^{cd}	A
	t_{10}	4.97 ± 0.02^{cde}	A	5.07 ± 0.05^{cde}	A	4.18 ± 0.14^{g}	B	5.12 ± 0.04^{cd}	A
	t_{12}	4.93 ± 0.03^{cdef}	B	5.05 ± 0.03^{cde}	A	3.77 ± 0.04^{h}	C	5.08 ± 0.07^{cd}	A
	t_{13}	4.90 ± 0.03^{defg}	B	5.01 ± 0.02^{cde}	AB	3.73 ± 0.03^{hi}	C	5.06 ± 0.09^{cd}	A
	t_{14}	4.83 ± 0.02^{efghi}	A	4.93 ± 0.02^{efg}	A	$3.59 \pm 0.03^{hijklmnop}$	B	4.87 ± 0.24^{de}	A
	t_{15}	4.83 ± 0.03^{efghi}	A	5.05 ± 0.01^{cde}	A	3.69 ± 0.02^{hij}	B	4.83 ± 0.51^{de}	A
	t_{16}	4.88 ± 0.07^{defgh}	A	5.05 ± 0.02^{cde}	A	3.70 ± 0.02^{hij}	B	4.48 ± 0.57^{e}	A
	t_{20}	4.87 ± 0.03^{defgh}	A	5.02 ± 0.03^{cde}	A	3.63 ± 0.03^{hijklmn}	B	3.83 ± 0.28^{f}	B
	t_{21}	4.72 ± 0.03^{hijk}	A	4.96 ± 0.02^{defg}	A	3.63 ± 0.02^{hijklmn}	B	3.76 ± 0.24^{fg}	B
	t_{22}	4.83 ± 0.02^{efghi}	A	5.05 ± 0.02^{cde}	A	3.64 ± 0.02^{hijklm}	B	3.76 ± 0.19^{fg}	B
	t_{23}	4.80 ± 0.04^{efghi}	B	5.03 ± 0.03^{cde}	A	3.62 ± 0.02^{hijklmno}	C	3.70 ± 0.16^{fgh}	C
	t_{24}	4.81 ± 0.02^{efghi}	B	5.07 ± 0.02^{cde}	A	3.66 ± 0.02^{hijk}	C	3.71 ± 0.12^{fgh}	C
	t_{27}	4.75 ± 0.03^{ghijk}	B	5.06 ± 0.03^{cde}	A	3.62 ± 0.02^{hijklmno}	C	3.65 ± 0.11^{fgh}	C
	t_{28}	4.69 ± 0.06^{ijk}	B	4.97 ± 0.03^{defg}	A	$3.57 \pm 0.02^{hijklmnopq}$	C	3.61 ± 0.11^{fgh}	C
	t_{29}	4.68 ± 0.06^{ijk}	B	4.96 ± 0.02^{defg}	A	$3.56 \pm 0.02^{hijklmnopqr}$	C	3.57 ± 0.11^{fgh}	C
	t_{30}	4.79 ± 0.01^{fghij}	B	5.15 ± 0.06^{bc}	A	3.74 ± 0.05^{hi}	C	3.82 ± 0.12^{f}	C
	t_{35}	4.61 ± 0.04^{kl}	B	4.96 ± 0.06^{efg}	A	$3.60 \pm 0.02^{hijklmnop}$	C	3.65 ± 0.08^{fgh}	C
	t_{36}	4.63 ± 0.06^{jkl}	B	4.98 ± 0.06^{cdefg}	A	$3.56 \pm 0.04^{hijklmnopqr}$	C	3.59 ± 0.08^{fgh}	C
	t_{38}	4.59 ± 0.04^{kl}	B	4.95 ± 0.03^{efg}	A	$3.50 \pm 0.04^{jklmnopqr}$	C	3.49 ± 0.06^{fgh}	C
	t_{42}	4.60 ± 0.03^{kl}	B	4.83 ± 0.14^{fgh}	A	$3.55 \pm 0.04^{ijklmnopqr}$	C	3.52 ± 0.04^{fgh}	C
	t_{45}	4.61 ± 0.05^{kl}	A	4.80 ± 0.18^{ghi}	A	3.65 ± 0.03^{hijkl}	B	3.60 ± 0.05^{fgh}	B
	t_{48}	4.35 ± 0.13^{mno}	A	4.63 ± 0.18^{ijk}	A	3.45 ± 0.05^{klmnopqr}	B	3.41 ± 0.03^{fgh}	B
	t_{50}	4.46 ± 0.19^{lmn}	A	4.65 ± 0.12^{hij}	A	$3.50 \pm 0.06^{jklmnopqr}$	B	3.43 ± 0.01^{fgh}	B
	t_{52}	4.50 ± 0.17^{lm}	A	4.65 ± 0.01^{ij}	A	$3.53 \pm 0.03^{ijklmnopqr}$	B	3.49 ± 0.02^{fgh}	B
	t_{55}	4.29 ± 0.09^{no}	B	4.56 ± 0.04^{jkl}	A	$3.49 \pm 0.06^{jklmnopqr}$	C	3.44 ± 0.02^{fgh}	C
	t_{57}	4.20 ± 0.05^{op}	B	4.54 ± 0.06^{jkl}	A	$3.51 \pm 0.08^{jklmnopqr}$	C	3.45 ± 0.02^{fgh}	C
	t_{63}	4.09 ± 0.03^{pqr}	B	4.46 ± 0.06^{klm}	A	3.42 ± 0.04^{nopqr}	C	3.40 ± 0.01^{fgh}	C
	t_{70}	4.10 ± 0.02^{pqr}	B	4.39 ± 0.03^{lmn}	A	3.41 ± 0.05^{opqr}	C	3.39 ± 0.03^{fgh}	C

Supplementary Table 5.1. (Continued).

Fermentation style	Sampling time (t, days)	Prototypes							
Greek style		GAs		GAc		GBs		GBc	
	t_{77}	4.06 ± 0.02^{pqrs}	B	4.27 ± 0.03^{nop}	A	3.37 ± 0.03^{qr}	C	3.33 ± 0.01^{gh}	C
	t_{81}	4.07 ± 0.01^{pqrs}	B	4.27 ± 0.04^{no}	A	3.36 ± 0.02^{qr}	C	3.33 ± 0.03^{gh}	C
	t_{99}	4.11 ± 0.02^{pq}	B	4.31 ± 0.04^{mno}	A	3.46 ± 0.02^{klmnopqr}	C	3.46 ± 0.02^{fgh}	C
	t_{101}	4.06 ± 0.02^{pqrs}	B	4.21 ± 0.04^{nopq}	A	3.35 ± 0.04^{qr}	C	3.33 ± 0.03^{gh}	C
	t_{115}	3.94 ± 0.05^{rstu}	B	4.17 ± 0.05^{opqr}	A	3.44 ± 0.01^{lmnopqr}	C	3.46 ± 0.02^{fgh}	C
	t_{127}	4.03 ± 0.04^{pqrst}	B	4.22 ± 0.03^{nopq}	A	$3.55 \pm 0.01^{ijklmnopqr}$	C	3.52 ± 0.02^{fgh}	C
	t_{143}	3.94 ± 0.02^{rstu}	B	4.08 ± 0.03^{qrs}	A	3.43 ± 0.02^{lmnopqr}	C	3.43 ± 0.02^{fgh}	C
	t_{156}	3.97 ± 0.01^{qrstu}	B	4.13 ± 0.03^{opqrs}	A	$3.48 \pm 0.02^{jklmnopqr}$	C	3.46 ± 0.02^{fgh}	C
	t_{190}	3.88 ± 0.03^{tu}	B	4.01 ± 0.02^{rs}	A	3.41 ± 0.04^{opqr}	C	3.35 ± 0.01^{gh}	C
	t_{219}	3.84 ± 0.03^{u}	A	3.98 ± 0.01^{s}	A	3.39 ± 0.15^{pqr}	B	3.29 ± 0.03^{h}	B
	t_{232}	3.95 ± 0.02^{qrstu}	B	4.08 ± 0.02^{qrs}	A	3.47 ± 0.04^{klmnopqr}	C	3.44 ± 0.01^{fgh}	C
	t_{247}	3.92 ± 0.04^{stu}	B	4.09 ± 0.03^{pqrs}	A	$3.53 \pm 0.04^{ijklmnopqr}$	C	3.51 ± 0.02^{fgh}	C
	t_{261}	3.83 ± 0.03^{u}	B	3.98 ± 0.02^{s}	A	3.35 ± 0.03^{r}	C	3.34 ± 0.02^{gh}	C
	t_{275}	3.85 ± 0.02^{u}	B	4.00 ± 0.03^{rs}	A	3.43 ± 0.02^{mnopqr}	C	3.42 ± 0.02^{fgh}	C
	t_{303}	3.88 ± 0.02^{tu}	B	4.00 ± 0.01^{rs}	A	3.44 ± 0.02^{lmnopqr}	C	3.40 ± 0.01^{fgh}	C
	t_{339}	3.88 ± 0.02^{tu}	B	4.01 ± 0.01^{rs}	A	3.41 ± 0.04^{opqr}	C	3.40 ± 0.02^{fgh}	C
	t_{357}	3.86 ± 0.04^{u}	B	4.02 ± 0.03^{rs}	A	3.41 ± 0.03^{opqr}	C	3.40 ± 0.02^{fgh}	C
	t_{373}	3.88 ± 0.03^{tu}	B	4.03 ± 0.03^{rs}	A	3.42 ± 0.01^{nopqr}	C	3.40 ± 0.04^{fgh}	C

The results are expressed as means of three replicates ± standard deviations. For each processing type (Spanish style or Greek style fermentation), values labelled with different small letters in the same column are significantly different ($P < 0.05$), whereas values labelled with different capital letters in the same row are significantly different ($P < 0.05$).

SAs Prototype made with 10% sea fennel sprouts and 90% green olives according to the Spanish style method
SAc Uninoculated prototype (control) made with 10% sea fennel sprouts and 90% green olives according to the Spanish style method
SBs Prototype made with 60% sea fennel sprouts and 40% green olives according to the Spanish style method
SBc Uninoculated prototype made with 60% sea fennel sprouts and 40% green olives according to the Spanish style method
GAs Prototype made with 10% sea fennel sprouts and 90% green olives according to the Greek style method
GAc Uninoculated prototype made with 10% sea fennel sprouts and 90% green olives according to the Greek style method
GBs Prototype made with 60% sea fennel sprouts and 40% green olives according to the Greek style method
GBc Uninoculated prototype made with 60% sea fennel sprouts and 40% green olives according to the Greek style method

Supplementary Table 5.2. Microbial viable counts of mesophilic lactobacilli, mesophilic lactococci, yeasts, Enterobacteriaceae and mesophilic aerobic bacteria performed on the laboratory-scale prototypes made with green olives and sea fennel sprouts.

Fermentation style	Microbial group	Sampling time (t, days)	Prototypes							
Spanish style	Mesophilic lactobacilli (Log CFU mL^{-1})		SAs		SAc		SBs		SBc	
		t_0	6.4 ± 0.2^{a}	A	1.3 ± 0.6^{cd}	C	6.5 ± 0.2^{a}	A	2.1 ± 0.2^{de}	B
		t_1	3.3 ± 0.9^{b}	AB	$< 1.0^{d}$	B	4.1 ± 1.2^{c}	A	1.8 ± 1.6^{de}	AB
		t_2	3.1 ± 0.4^{b}	A	$< 1.0^{d}$	B	4.9 ± 0.8^{bc}	A	$< 1.0^{e}$	B
		t_5	3.0 ± 0.3^{b}	B	2.2 ± 0.4^{c}	B	5.0 ± 0.6^{bc}	A	3.2 ± 0.7^{cd}	B
		t_7	3.7 ± 0.2^{b}	B	3.8 ± 0.4^{b}	B	5.0 ± 0.2^{bc}	A	4.5 ± 0.5^{c}	AB
		t_{10}	4.2 ± 0.6^{b}	B	4.5 ± 0.0^{b}	B	5.9 ± 0.1^{ab}	A	4.9 ± 0.2^{bc}	B
		t_{15}	4.0 ± 0.5^{b}	B	4.8 ± 0.8^{b}	B	7.1 ± 0.2^{a}	A	7.6 ± 0.2^{a}	A
		t_{23}	5.7 ± 0.3^{a}	B	5.2 ± 0.5^{b}	B	7.0 ± 0.2^{a}	A	7.5 ± 0.2^{a}	A
		t_{35}	6.5 ± 0.4^{a}	A	6.9 ± 0.4^{a}	A	6.7 ± 0.1^{a}	A	7.3 ± 0.3^{a}	A
		t_{50}	6.4 ± 0.6^{a}	B	7.9 ± 0.6^{a}	A	5.9 ± 0.1^{ab}	B	6.7 ± 0.2^{ab}	AB
		t_{63}	6.6 ± 0.2^{a}	B	7.2 ± 0.1^{a}	A	6.0 ± 0.0^{ab}	C	6.6 ± 0.1^{ab}	B
	Mesophilic lattococci (Log CFU mL^{-1})		SAs		SAc		SBs		SBc	
		t_0	3.6 ± 0.1^{c}	A	3.5 ± 0.3^{f}	A	3.2 ± 0.2^{g}	A	3.4 ± 0.3^{d}	A
		t_1	5.6 ± 0.7^{b}	A	5.1 ± 0.3^{e}	A	5.4 ± 0.2^{f}	A	4.4 ± 0.4^{d}	A
		t_2	6.3 ± 0.0^{ab}	AB	6.2 ± 0.0^{d}	B	6.2 ± 0.1^{bcd}	B	6.4 ± 0.1^{abc}	A
		t_5	6.7 ± 0.1^{ab}	A	6.7 ± 0.2^{c}	A	5.8 ± 0.2^{def}	B	6.3 ± 0.2^{abc}	A
		t_7	6.2 ± 0.3^{ab}	AB	6.7 ± 0.1^{c}	A	5.6 ± 0.1^{ef}	B	5.9 ± 0.7^{c}	AB
		t_{10}	6.1 ± 0.4^{ab}	A	6.9 ± 0.1^{abc}	A	6.2 ± 0.1^{bcd}	A	6.0 ± 0.5^{bc}	A
		t_{15}	6.9 ± 1.0^{ab}	A	6.8 ± 0.1^{bc}	A	7.0 ± 0.2^{a}	A	7.2 ± 0.0^{a}	A
		t_{23}	6.8 ± 0.1^{ab}	A	7.0 ± 0.1^{abc}	A	6.9 ± 0.2^{a}	A	7.1 ± 0.0^{ab}	A
		t_{35}	6.6 ± 0.6^{ab}	A	7.1 ± 0.2^{abc}	A	6.6 ± 0.2^{ab}	A	7.2 ± 0.6^{a}	A
		t_{50}	6.9 ± 0.2^{a}	A	7.4 ± 0.2^{a}	A	6.3 ± 0.1^{bc}	B	6.8 ± 0.4^{abc}	AB
		t_{63}	6.9 ± 0.0^{ab}	B	7.2 ± 0.1^{ab}	A	6.1 ± 0.1^{cde}	C	6.8 ± 0.1^{abc}	B
	Yeasts (Log CFU mL^{-1})		SAs		SAc		SBs		SBc	
		t_0	1.6 ± 0.4^{f}	A	1.7 ± 0.3^{c}	A	2.2 ± 0.2^{d}	A	2.3 ± 0.3^{c}	A
		t_1	2.3 ± 0.1^{e}	A	1.7 ± 0.6^{c}	A	2.4 ± 0.2^{d}	A	3.0 ± 0.8^{bc}	A
		t_2	3.1 ± 0.2^{d}	A	2.7 ± 0.7^{c}	A	3.8 ± 0.6^{c}	A	3.8 ± 0.9^{b}	A
		t_5	5.6 ± 0.3^{c}	A	5.5 ± 0.4^{b}	A	5.5 ± 0.5^{b}	A	5.4 ± 0.4^{a}	A
		t_7	5.8 ± 0.2^{bc}	A	6.0 ± 0.1^{ab}	A	5.8 ± 0.3^{ab}	A	6.1 ± 0.3^{a}	A
		t_{10}	5.8 ± 0.3^{bc}	A	6.1 ± 0.2^{ab}	A	6.5 ± 0.7^{ab}	A	5.9 ± 0.2^{a}	A
		t_{15}	6.3 ± 0.3^{ab}	A	6.5 ± 0.2^{ab}	A	6.3 ± 0.2^{ab}	A	6.3 ± 0.2^{a}	A
		t_{23}	6.6 ± 0.1^{a}	A	6.7 ± 0.5^{a}	A	6.6 ± 0.1^{a}	A	6.7 ± 0.4^{a}	A

Supplementary Table 5.2. (Continued).

Fermentation style	Microbial group	Sampling time (t, days)	Prototypes							
Spanish style	Yeasts (Log CFU mL^{-1})		SAs		SAc		SBs		SBc	
		t_{35}	6.4 ± 0.1^{ab}	A	6.5 ± 0.2^{ab}	A	5.9 ± 0.1^{ab}	B	6.3 ± 0.2^{a}	A
		t_{50}	6.6 ± 0.0^{a}	A	6.6 ± 0.1^{a}	A	6.4 ± 0.2^{ab}	A	6.5 ± 0.1^{a}	A
		t_{63}	6.6 ± 0.1^{a}	A	6.6 ± 0.3^{ab}	A	6.1 ± 0.1^{ab}	B	6.5 ± 0.0^{a}	AB
	Enterobacteriaceae (Log CFU mL^{-1})		SAs		SAc		SBs		SBc	
		t_0	4.0 ± 0.2^{b}	A	4.4 ± 0.3^{e}	A	3.0 ± 0.6^{c}	B	3.6 ± 0.2^{b}	AB
		t_1	6.2 ± 0.1^{a}	A	5.6 ± 0.1^{d}	AB	5.5 ± 0.1^{ab}	B	5.9 ± 0.5^{a}	AB
		t_2	6.2 ± 0.2^{a}	AB	5.9 ± 0.2^{cd}	B	6.4 ± 0.2^{a}	A	6.6 ± 0.2^{a}	A
		t_5	6.1 ± 0.3^{a}	A	6.4 ± 0.1^{abc}	A	5.4 ± 0.2^{ab}	B	6.1 ± 0.3^{a}	A
		t_7	6.0 ± 0.3^{a}	A	6.6 ± 0.1^{ab}	A	4.3 ± 0.4^{bc}	B	5.7 ± 0.8^{a}	A
		t_{10}	6.3 ± 0.1^{a}	AB	6.4 ± 0.1^{abc}	A	4.0 ± 0.2^{c}	C	5.4 ± 0.7^{a}	B
		t_{15}	5.6 ± 1.3^{ab}	AB	6.3 ± 0.1^{bc}	A	1.4 ± 1.0^{d}	C	3.6 ± 0.9^{b}	BC
		t_{23}	6.2 ± 0.2^{a}	A	6.3 ± 0.1^{bc}	A	$< 1.0^{d}$	C	1.7 ± 1.0^{c}	B
		t_{35}	4.8 ± 0.8^{ab}	B	6.9 ± 0.2^{a}	A	$< 1.0^{d}$	C	1.8 ± 0.7^{c}	C
		t_{50}	1.9 ± 0.1^{c}	B	4.8 ± 0.2^{e}	A	$< 1.0^{d}$	C	$< 1.0^{d}$	C
		t_{63}	$< 1.0^{c}$	B	2.9 ± 0.4^{f}	A	$< 1.0^{d}$	B	$< 1.0^{d}$	B
	Mesophilic aerobic bacteria (Log CFU mL^{-1})		SAs		SAc		SBs		SBc	
		t_0	6.6 ± 0.4^{bcd}	A	4.2 ± 0.2^{e}	B	6.3 ± 0.1^{abcd}	A	4.1 ± 0.2^{d}	B
		t_1	6.1 ± 0.1^{e}	A	5.8 ± 0.2^{d}	A	5.8 ± 0.2^{d}	A	5.7 ± 0.4^{c}	A
		t_2	6.3 ± 0.1^{de}	B	6.1 ± 0.1^{d}	B	6.5 ± 0.2^{abc}	B	6.9 ± 0.2^{ab}	A
		t_5	6.7 ± 0.0^{abcd}	A	6.6 ± 0.2^{c}	A	6.4 ± 0.5^{abcd}	A	6.5 ± 0.3^{abc}	A
		t_7	6.7 ± 0.0^{abcd}	A	6.7 ± 0.0^{bc}	A	6.0 ± 0.3^{cd}	B	6.6 ± 0.5^{abc}	AB
		t_{10}	6.4 ± 0.1^{cde}	AB	6.8 ± 0.1^{bc}	A	6.2 ± 0.2^{abcd}	B	6.4 ± 0.3^{bc}	AB
		t_{15}	6.9 ± 0.0^{abc}	A	7.0 ± 0.1^{abc}	A	6.6 ± 0.2^{ab}	A	6.9 ± 0.3^{ab}	A
		t_{23}	7.0 ± 0.1^{ab}	B	7.2 ± 0.0^{ab}	A	6.9 ± 0.0^{a}	B	6.9 ± 0.1^{ab}	B
		t_{35}	6.9 ± 0.1^{abc}	AB	7.3 ± 0.2^{a}	AB	6.5 ± 0.1^{abc}	B	7.4 ± 0.6^{a}	A
		t_{50}	6.9 ± 0.2^{ab}	AB	7.2 ± 0.3^{a}	A	6.4 ± 0.1^{abc}	B	6.9 ± 0.2^{ab}	AB
		t_{63}	7.1 ± 0.1^{a}	B	7.4 ± 0.0^{a}	A	6.2 ± 0.1^{bcd}	C	6.8 ± 0.1^{ab}	B
Greek style	Mesophilic lactobacilli (Log CFU mL^{-1})		GAs		GAc		GBs		GBc	
		t_0	6.7 ± 0.5^{abc}	A	1.0 ± 0.3^{j}	B	6.8 ± 0.3^{abc}	A	1.8 ± 0.1^{f}	B
		t_1	3.9 ± 0.8^{hi}	A	$< 1.0^{k}$	B	4.5 ± 1.1^{h}	A	$< 1.0^{f}$	B
		t_2	4.0 ± 0.5^{hi}	B	$< 1.0^{k}$	C	5.7 ± 0.3^{cdefg}	A	$< 1.0^{f}$	C
		t_5	4.0 ± 0.6^{hi}	AB	3.0 ± 1.1^{i}	B	5.6 ± 0.3^{cdefgh}	A	4.6 ± 1.1^{e}	AB

Supplementary Table 5.2. (Continued).

Fermentation style	Microbial group	Sampling time (t, days)	Prototypes			
Greek style	Mesophilic lactobacilli (Log CFU mL^{-1})		GAs	GAc	GBs	GBc
		t_7	3.6 ± 0.3^{i} B	3.7 ± 0.2^{hi} B	5.9 ± 0.3^{bcdef} A	5.2 ± 0.4^{de} A
		t_{10}	3.6 ± 0.2^{i} D	5.5 ± 0.3^{fg} C	7.3 ± 0.1^{a} A	6.0 ± 0.1^{abcde} B
		t_{15}	4.8 ± 0.2^{gh} B	4.6 ± 0.1^{gh} B	6.9 ± 0.2^{ab} A	7.0 ± 0.4^{ab} A
		t_{23}	4.9 ± 0.1^{gh} C	5.4 ± 0.4^{fg} C	6.5 ± 0.3^{abcd} B	7.4 ± 0.3^{a} A
		t_{35}	6.2 ± 0.1^{bcdef} B	6.3 ± 0.0^{bcdef} B	6.0 ± 0.3^{bcde} B	7.0 ± 0.2^{abc} A
		t_{50}	6.3 ± 0.8^{abcdef} A	6.6 ± 0.3^{abcd} A	5.7 ± 0.4^{cdefg} A	6.2 ± 0.3^{abcd} A
		t_{63}	7.3 ± 0.1^{a} A	6.9 ± 0.1^{abc} A	6.4 ± 0.4^{abcde} B	5.9 ± 0.1^{bcde} C
		t_{77}	6.9 ± 0.2^{ab} AB	7.3 ± 0.4^{a} A	6.2 ± 0.6^{abcde} BC	5.8 ± 0.1^{bcde} C
		t_{101}	6.3 ± 0.2^{abcdef} AB	7.2 ± 0.6^{ab} A	5.9 ± 0.3^{bcdef} B	5.5 ± 0.2^{bcde} B
		t_{127}	6.7 ± 0.2^{abcd} B	7.5 ± 0.0^{a} A	5.2 ± 0.2^{efgh} C	5.5 ± 0.3^{cde} C
		t_{156}	6.5 ± 0.3^{abcde} A	6.9 ± 0.2^{abc} A	5.4 ± 0.3^{defgh} B	5.8 ± 0.2^{bcde} B
		t_{190}	6.1 ± 0.2^{bcdef} B	6.7 ± 0.1^{abc} A	5.4 ± 0.2^{defgh} C	5.9 ± 0.2^{bcde} BC
		t_{219}	5.8 ± 0.2^{cdefg} AB	6.6 ± 0.1^{abcde} A	5.7 ± 0.5^{cdefg} AB	5.7 ± 0.4^{bcde} B
		t_{247}	5.7 ± 0.0^{defg} A	6.1 ± 0.1^{cdef} A	5.7 ± 0.4^{cdefgh} A	5.3 ± 0.6^{de} A
		t_{275}	5.4 ± 0.1^{efg} A	5.6 ± 0.2^{def} A	4.7 ± 0.1^{fgh} B	4.7 ± 0.2^{e} B
		t_{303}	5.3 ± 0.2^{fg} AB	5.6 ± 0.0^{efg} A	4.6 ± 0.1^{gh} B	5.6 ± 0.6^{bcde} A
		t_{339}	5.4 ± 0.1^{efg} B	5.6 ± 0.2^{efg} B	6.3 ± 0.2^{abcde} A	5.4 ± 0.1^{de} B
		t_{373}	5.6 ± 0.1^{efg} A	5.6 ± 0.0^{defg} A	5.8 ± 0.0^{bcdef} A	5.6 ± 0.2^{bcde} A
	Mesophilic lattococci (Log CFU mL^{-1})		GAs	GAc	GBs	GBc
		t_0	1.6 ± 0.9^{k} AB	$< 1.0^{h}$ B	2.6 ± 0.3^{k} A	1.9 ± 0.3^{i} AB
		t_1	3.5 ± 0.6^{ij} B	1.6 ± 0.5^{g} C	4.9 ± 0.2^{hij} A	3.6 ± 0.4^{h} B
		t_2	3.2 ± 0.7^{j} B	2.3 ± 0.7^{g} B	5.4 ± 0.1^{ghi} A	5.3 ± 0.1^{fg} A
		t_5	4.0 ± 0.7^{hij} C	4.7 ± 0.3^{f} BC	6.1 ± 0.1^{bcdef} A	5.7 ± 0.4^{def} AB
		t_7	4.5 ± 0.4^{ghi} B	5.4 ± 0.2^{ef} A	6.1 ± 0.3^{cdef} A	5.8 ± 0.2^{cdef} A
		t_{10}	5.1 ± 0.2^{fgh} C	5.6 ± 0.3^{ef} BC	7.2 ± 0.1^{a} A	5.7 ± 0.2^{def} B
		t_{15}	5.8 ± 0.2^{bcdef} B	6.0 ± 0.1^{cde} B	6.8 ± 0.2^{ab} A	6.8 ± 0.4^{ab} A
		t_{23}	6.5 ± 0.1^{abcde} B	6.7 ± 0.1^{abcd} B	6.6 ± 0.1^{abc} B	7.3 ± 0.3^{a} A
		t_{35}	6.7 ± 0.0^{abcd} A	6.6 ± 0.0^{abcd} A	6.1 ± 0.2^{cdefg} B	6.6 ± 0.3^{abc} A
		t_{50}	7.1 ± 0.4^{a} AB	7.2 ± 0.1^{a} A	6.1 ± 0.2^{bcdef} C	6.5 ± 0.1^{abcd} BC
		t_{63}	7.1 ± 0.2^{a} A	6.7 ± 0.1^{abc} AB	6.4 ± 0.3^{bcd} B	6.3 ± 0.2^{bcde} B
		t_{77}	6.9 ± 0.3^{ab} AB	7.4 ± 0.1^{a} A	6.4 ± 0.5^{bcd} BC	6.1 ± 0.2^{bcdef} C
		t_{101}	6.8 ± 0.1^{abc} AB	7.3 ± 0.4^{a} A	6.2 ± 0.2^{bcde} B	6.2 ± 0.2^{bcde} B
		t_{127}	6.8 ± 0.2^{abc} B	7.4 ± 0.1^{a} A	5.5 ± 0.1^{efgh} C	5.7 ± 0.1^{def} C
		t_{156}	6.8 ± 0.1^{abc} A	7.0 ± 0.2^{ab} A	5.9 ± 0.3^{cdefg} B	6.1 ± 0.2^{bcdef} B

Supplementary Table 5.2. (Continued).

Fermentation style	Microbial group	Sampling time (t, days)	Prototypes			
Greek style	Mesophilic lattococci (Log CFU mL^{-1})		GAs	GAc	GBs	GBc
		t_{190}	6.4 ± 0.2^{abcde} AB	6.8 ± 0.1^{abc} A	5.8 ± 0.3^{defg} B	5.9 ± 0.2^{bcdef} B
		t_{219}	6.0 ± 0.0^{abcdef} B	6.7 ± 0.2^{abcd} A	5.5 ± 0.1^{fgh} C	5.7 ± 0.2^{def} BC
		t_{247}	5.8 ± 0.1^{cdef} A	6.2 ± 0.2^{bcde} A	5.8 ± 0.3^{defg} A	5.4 ± 0.5^{efg} A
		t_{275}	5.6 ± 0.2^{defg} A	5.5 ± 0.1^{ef} A	4.7 ± 0.0^{ij} B	4.7 ± 0.1^{g} B
		t_{303}	5.4 ± 0.3^{efg} AB	5.7 ± 0.2^{e} A	4.6 ± 0.1^{j} B	5.7 ± 0.5^{def} A
		t_{339}	5.5 ± 0.1^{efg} B	5.5 ± 0.1^{ef} B	6.2 ± 0.3^{bcde} A	5.5 ± 0.1^{efg} B
		t_{373}	5.7 ± 0.1^{cdef} A	5.8 ± 0.1^{de} A	5.9 ± 0.0^{defg} A	5.7 ± 0.2^{def} A
	Yeasts (Log CFU mL^{-1})		GAs	GAc	GBs	GBc
		t_0	1.7 ± 0.2^{j} A	2.0 ± 0.5^{g} A	2.4 ± 0.3^{i} A	2.1 ± 0.1^{gh} A
		t_1	2.1 ± 0.2^{ij} B	2.3 ± 0.4^{g} B	2.5 ± 0.1^{i} AB	3.0 ± 0.0^{g} A
		t_2	2.4 ± 0.2^{i} C	2.8 ± 0.2^{fg} C	3.6 ± 0.5^{h} B	4.3 ± 0.1^{ef} A
		t_5	4.9 ± 0.3^{fg} C	5.4 ± 0.0^{cde} B	5.8 ± 0.1^{abcd} AB	5.9 ± 0.0^{abc} A
		t_7	5.6 ± 0.1^{de} B	6.1 ± 0.2^{abc} A	6.0 ± 0.1^{abcd} AB	6.0 ± 0.2^{abc} A
		t_{10}	6.0 ± 0.0^{cd} A	6.0 ± 0.2^{abc} A	6.3 ± 0.3^{a} A	6.1 ± 0.2^{a} A
		t_{15}	6.3 ± 0.1^{bc} A	6.5 ± 0.1^{ab} A	6.2 ± 0.1^{ab} A	6.5 ± 0.5^{a} A
		t_{23}	6.7 ± 0.1^{ab} A	6.8 ± 0.1^{a} A	6.6 ± 0.1^{a} AB	6.2 ± 0.3^{a} B
		t_{35}	6.7 ± 0.0^{ab} A	6.7 ± 0.1^{a} A	6.0 ± 0.0^{abcd} C	6.2 ± 0.0^{a} B
		t_{50}	6.9 ± 0.1^{a} A	6.7 ± 0.1^{a} A	6.1 ± 0.1^{abc} B	6.3 ± 0.1^{a} B
		t_{63}	6.6 ± 0.1^{ab} A	6.7 ± 0.1^{a} A	5.9 ± 0.3^{abcd} B	6.2 ± 0.1^{a} B
		t_{77}	6.6 ± 0.1^{ab} AB	6.8 ± 0.2^{a} A	6.1 ± 0.2^{abc} BC	6.0 ± 0.2^{abc} C
		t_{101}	6.4 ± 0.2^{abc} AB	6.5 ± 0.1^{ab} A	6.0 ± 0.1^{abcd} B	6.1 ± 0.2^{ab} AB
		t_{127}	5.6 ± 0.1^{de} BC	6.2 ± 0.0^{abc} A	5.3 ± 0.2^{cdef} C	5.8 ± 0.1^{abcd} B
		t_{156}	5.4 ± 0.2^{ef} A	5.8 ± 0.2^{bcd} A	5.5 ± 0.2^{bcde} A	5.6 ± 0.2^{abcd} A
		t_{190}	4.8 ± 0.1^{fg} A	5.1 ± 0.3^{de} A	5.2 ± 0.3^{def} A	5.1 ± 0.0^{cdef} A
		t_{219}	5.2 ± 0.0^{ef} A	5.0 ± 0.2^{de} AB	4.5 ± 0.4^{fg} B	5.1 ± 0.3^{bcde} AB
		t_{247}	5.1 ± 0.1^{ef} AB	5.4 ± 0.1^{cde} A	4.7 ± 0.5^{ef} B	4.8 ± 0.2^{def} AB
		t_{275}	4.4 ± 0.2^{g} A	4.5 ± 0.1^{e} A	3.8 ± 0.1^{gh} B	4.1 ± 0.2^{f} AB
		t_{303}	3.4 ± 0.2^{h} AB	3.5 ± 0.3^{f} A	1.7 ± 0.6^{i} C	2.0 ± 1.0^{h} BC
		t_{339}	2.4 ± 0.4^{i} A	2.7 ± 0.9^{g} A	1.7 ± 0.2^{i} A	1.7 ± 0.7^{h} A
		t_{373}	5.5 ± 0.1^{de} A	5.7 ± 0.1^{bcd} A	4.9 ± 0.4^{ef} B	4.4 ± 0.1^{ef} B
	Enterobacteriaceae (Log CFU mL^{-1})		GAs	GAc	GBs	GBc
		t_0	$< 1.0^{b}$ B	$< 1.0^{e}$ B	2.4 ± 0.4^{c} A	$< 1.0^{d}$ B
		t_1	1.5 ± 0.6^{a} BC	$< 1.0^{de}$ C	4.8 ± 0.3^{ab} A	2.7 ± 0.4^{c} B

Supplementary Table 5.2. (Continued).

Fermentation style	Microbial group	Sampling time (t, days)	Prototypes							
Greek style	Enterobacteriaceae (Log CFU mL^{-1})		GAs		GAc		GBs		GBc	
		t_2	< 1.0^{b}	C	< 1.0^{de}	C	5.5 ± 0.4^{a}	A	3.7 ± 0.2^{bc}	B
		t_5	1.0 ± 0.6^{ab}	B	< 1.0^{e}	B	5.6 ± 0.1^{a}	A	4.5 ± 0.5^{ab}	A
		t_7	1.5 ± 0.9^{a}	C	1.9 ± 1.1^{bcd}	BC	3.8 ± 0.8^{b}	AB	4.4 ± 0.6^{ab}	A
		t_{10}	1.7 ± 1.0^{a}	B	1.1 ± 0.8^{cde}	B	4.5 ± 0.6^{ab}	A	4.9 ± 0.1^{a}	A
		t_{15}	< 1.0^{b}	C	< 1.0^{e}	C	2.3 ± 0.8^{c}	B	5.1 ± 0.5^{a}	A
		t_{23}	< 1.0^{b}	B	$2,1 \pm 0,2^{bc}$	A	< 1.0^{d}	AB	< 1.0^{d}	AB
		t_{35}	< 1.0^{b}	B	$5,7 \pm 0,1^{a}$	A	< 1.0^{d}	B	< 1.0^{d}	B
		t_{50}	< 1.0^{b}	B	$5,3 \pm 0,4^{a}$	A	< 1.0^{d}	B	< 1.0^{d}	B
		t_{63}	< 1.0^{b}	B	$2,6 \pm 0,4^{b}$	A	< 1.0^{d}	B	< 1.0^{d}	B
		t_{77}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{101}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{127}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{156}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{190}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{219}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{247}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{275}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{303}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{339}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
		t_{373}	< 1.0^{b}	A	< 1.0^{e}	A	< 1.0^{d}	A	< 1.0^{d}	A
	Mesophilic aerobic bacteria (Log CFU mL^{-1})		GAs		GAc		GBs		GBc	
		t_0	6.8 ± 0.2^{a}	A	1.7 ± 0.3^{k}	B	6.6 ± 0.5^{ab}	A	2.2 ± 0.3^{k}	B
		t_1	3.7 ± 0.5^{g}	B	1.0 ± 0.3^{l}	C	4.8 ± 0.2^{gh}	A	3.6 ± 0.1^{j}	B
		t_2	4.1 ± 0.4^{g}	B	2.7 ± 0.2^{j}	C	5.4 ± 0.0^{fg}	A	5.1 ± 0.2^{hi}	A
		t_5	5.0 ± 0.1^{f}	B	5.2 ± 0.2^{i}	B	6.5 ± 0.3^{abcd}	A	6.2 ± 0.0^{cde}	A
		t_7	5.6 ± 0.1^{ef}	C	5.9 ± 0.1^{fgh}	B	6.2 ± 0.1^{abcde}	A	6.2 ± 0.1^{defg}	A
		t_{10}	5.8 ± 0.1^{cde}	C	6.0 ± 0.1^{efg}	BC	6.4 ± 0.1^{abcd}	A	6.2 ± 0.1^{def}	AB
		t_{15}	5.7 ± 0.2^{def}	B	6.5 ± 0.1^{cde}	A	6.5 ± 0.0^{abc}	A	6.9 ± 0.2^{abc}	A
		t_{23}	6.6 ± 0.1^{ab}	B	6.8 ± 0.1^{bc}	B	6.8 ± 0.1^{a}	B	7.2 ± 0.1^{a}	A
		t_{35}	6.8 ± 0.0^{a}	A	6.9 ± 0.0^{bc}	A	6.3 ± 0.1^{abcd}	B	7.0 ± 0.1^{ab}	A
		t_{50}	7.0 ± 0.2^{a}	A	7.1 ± 0.1^{ab}	A	6.4 ± 0.1^{abcd}	B	6.6 ± 0.2^{abcd}	B
		t_{63}	6.5 ± 0.1^{abc}	A	6.6 ± 0.0^{cd}	A	6.4 ± 0.2^{abcd}	A	6.4 ± 0.2^{bcde}	A
		t_{77}	7.0 ± 0.3^{a}	A	7.2 ± 0.2^{ab}	A	6.4 ± 0.5^{abcd}	AB	6.1 ± 0.1^{defg}	B
		t_{101}	6.8 ± 0.1^{a}	B	7.4 ± 0.4^{a}	A	6.5 ± 0.2^{abc}	B	6.5 ± 0.0^{bcde}	B

Supplementary Table 5.2. (Continued).

Fermentation style	Microbial group	Sampling time (t, days)	Prototypes							
Greek style	Mesophilic aerobic bacteria (Log CFU mL^{-1})		GAs		GAc		GBs		GBc	
		t_{127}	6.6 ± 0.4^{ab}	B	7.4 ± 0.1^{a}	A	5.5 ± 0.2^{ef}	C	5.9 ± 0.1^{defg}	C
		t_{156}	6.4 ± 0.1^{abcd}	AB	6.8 ± 0.1^{bc}	A	6.8 ± 0.3^{a}	A	6.0 ± 0.3^{defg}	B
		t_{190}	6.0 ± 0.2^{bcde}	B	6.7 ± 0.2^{bcd}	A	5.7 ± 0.2^{def}	B	6.0 ± 0.3^{defg}	B
		t_{219}	6.0 ± 0.1^{bcde}	AB	6.6 ± 0.2^{cde}	A	5.4 ± 0.4^{fgh}	B	5.4 ± 0.4^{ghi}	B
		t_{247}	5.8 ± 0.2^{cde}	AB	6.2 ± 0.1^{def}	A	5.9 ± 0.3^{cdef}	AB	5.4 ± 0.5^{ghi}	B
		t_{275}	5.6 ± 0.0^{ef}	A	5.7 ± 0.2^{ghi}	A	4.8 ± 0.1^{gh}	B	4.8 ± 0.1^{i}	B
		t_{303}	5.4 ± 0.3^{ef}	AB	5.6 ± 0.1^{ghi}	A	4.7 ± 0.2^{h}	B	5.8 ± 0.6^{efgh}	A
		t_{339}	5.5 ± 0.0^{ef}	B	5.5 ± 0.1^{hi}	B	6.3 ± 0.3^{abcde}	A	5.5 ± 0.1^{fghi}	B
		t_{373}	5.7 ± 0.0^{ef}	B	5.9 ± 0.0^{fgh}	A	5.9 ± 0.1^{bcdef}	A	5.8 ± 0.1^{efgh}	AB

The results are expressed as mean Log CFU mL^{-1} of brine of three replicates ± standard deviation. For each processing type (Spanish style or Greek style fermentation), values labelled with different small letters in the same column are significantly different ($P < 0.05$), whereas values labelled with different capital letters in the same row are significantly different ($P < 0.05$).

For legend of the prototypes see Supplementary Table 5.1.

6. Exploratory study for probiotic-enrichment of a sea fennel (*Crithmum maritimum* L.) -based preserve

6.1. Introduction

Probiotics are defined as "Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). The consumption of probiotics dates to ancient times in the form of fermented products, and the capability of these foods in improving human health was reported since very long times, being already mentioned in the Biblical scriptures and further described by Hippocrates (Ranadheera et al., 2010). Probiotics can exert a broad range of beneficial effects on the host, which have been categorized by the International Life Science Institute (ILSI) in four different areas, being (i) metabolism, (ii) inflammatory bowel diseases and irritable bowel syndrome, (iii) allergic diseases and (iv) infection. Probiotics display these beneficial effects by (i) interacting with the gut microbiota and exerting a metabolic effect thanks to enzymatic activities in the gut lumen, (ii) interacting with the gut mucus and the gut epithelium improving the mucosal barrier function and immune system, (iii) signaling to the systemic immune systems and other organ systems (Kleerebezem et al., 2019; Martinez et al., 2015; Rijkers et al., 2010; Tuohy et al., 2003).

Giving the increasing consumers demand for healthy foods capable of preventing diseases and promoting well-being beyond providing basic nutrition, the incidence of lactose intolerance and allergies against milk proteins, and the increasing trend towards vegetarian and vegan diets, the development of non-dairy probiotic foods represents an interesting opportunity for the functional food market (Bigliardi and Galati 2013; Granato et al., 2010; Lillo-Pérez et al., 2021). Probiotic-enriched foods must contain appropriately selected probiotic strains in adequate dose to confer the intended health benefits, which corresponds to a daily intake of 10^8 - 10^9 probiotic microorganisms. The viability of selected probiotic strains in a food matrix could be affected by the intrinsic characteristics of the product (pH, molecular oxygen, presence of salt, sugar, and antimicrobial compounds) and the

employed processing parameters (incubation temperature, heat treatment, packaging material and storage conditions) (Tripathi and Giri, 2014). Hence, the monitoring of viability of probiotics in food products during the intended shelf-life is of utmost importance, considering that survival and stability in these matrices are highly strain dependent (Min et al., 2019)

To date, numerous vegetables have been successfully exploited as carriers for delivering of probiotics to humans, including table olives, artichokes, and cabbage (De Bellis et al., 2021; Lavermicocca et al., 2010). Plant-based matrices are often rich in nutrients, fibers, vitamins, minerals, and dietary bioactive phytochemicals, most of which have a crucial role in the interactions with gut microorganisms (De Bellis et at., 2021). As it has been recently reviewed by the latter authors, the majority of plant-based probiotic foods have juice or smoothie texture, while only a few research studies have dealt with solid vegetable matrices (De Bellis et al., 2021).

Sea fennel (*Crithmum maritimum* L.) undoubtedly represents an ideal candidate for functionalization with probiotics, offering itself functional benefits due to the high content of vitamin C, omega 3 and omega 6, phenolic compounds, carotenoids etc (Generalić Mekinić et al. 2016; Maleš et al., 2003; Maoloni et al., 2021; Meot-Duros and Magné, 2009). The use of sea fennel in culinary preparations dates to ancient times. Forgotten for a long time, this highly aromatic herb has recently been rediscovered, being defined by various authors as “cash crop” or “emerging crop”, for its high economical potential (Atia et al., 2011; Renna, 2018) due to its application in both pharmacy and food industry. In Mediterranean countries, including Greece, France, Spain, and Italy, sea fennel is consumed as a fresh ingredient in salads or preserved in brine, olive oil or aqueous solutions of wine vinegar (Renna and Gonnella, 2012).

To the authors’ knowledge, no studies have been carried out to evaluate the potential of sea fennel- as a carrier of human probiotics, yet. Similarly, it has not yet been industrially utilized with this specific purpose. Hence, this study was aimed at evaluating the survival and stability of two

commercially available formulations of human probiotics, *Lactiplantibacillus plantarum* IMC 509 and SYNBIO®, during prolonged storage of a sea fennel-based preserve under refrigerated conditions.

6.2. Materials and methods

6.2.1. Sea fennel supply and pre-treatment

Fresh organic sea fennel sprouts (approximately 1.5 Kg) were kindly supplied by a manufacturer of sea fennel preserves (Rinci S.r.l., Castelfidardo, Ancona, Italy). They were transported to the laboratory under refrigerated conditions (+ 4 °C), washed under tap water, drained for 5 min using an industrial stainless steel vegetable strainer basket, blanched at 95 °C for 30 s by immersion in boiling water, and again drained for 5 min.

6.2.2. Probiotic bacteria strains

Lactiplantibacillus plantarum IMC 509 and SYNBIO® combination (1:1) of *Lacticaseibacillus rhamnosus* IMC 501® and *Lacticaseibacillus paracasei* IMC 502® (Silvi et al., 2003; Verdenelli et al., 2009) were kindly supplied by Synbiotec S.r.l. (Camerino, Italy).

SYNBIO® is a patented product commercialized by Synbiotec S.r.l. as a lyophilized powder, characterized by a cell concentration of 10^{11} CFU g^{-1}. The combination of the two bacterial strains of SYNBIO®, both isolated from the intestinal tract of elderly humans, was justified by Verdenelli et al. (2009) based on the results collected, where the mixture expressed higher *in vitro* adherence to intestinal cell line than the two single *Lactobacillus* strains, with beneficial effects on the bowel habits (Verdenelli et al., 2011).

Lactiplantibacillus plantarum IMC 509 is a further strain isolated by Synbiotec S.r.l. from the intestinal tract of elderly humans and well characterized for its probiotic traits (Coman and Cresci, 2014; Coman et al., 2014; Micioni Di Bonaventura et al., 2021; Verdenelli et al., 2009).

6.2.3. Preparation of sea fennel-based preserves

The amount of lactic acid to be added to the brine salt solution to reach an equilibrium pH of 3.80 was determined by preliminary assays, as follows; 35 g of blanched sea fennel sprouts were added with 105 mL of brine containing 7.0 % (w v^{-1}) NaCl and 1.0 % (w v^{-1}) fructose) and homogenized using a blender. The homogenate was added with increasing percentages of food grade lactic acid to achieve an equilibrium pH of ~3.80; pH values were assessed with a pH meter (model 300, Hanna Instruments, Padova, Italy).

Hence, 150 mL glass jars were filled with 35 g of blanched sea fennel sprouts soaked in 105 mL of brine acidified with 0.5 % (v v^{-1}) food grade lactic acid. Jars were sealed with steel caps, pasteurized at 95 °C for 5 min in boiling water, cooled in iced water, and stored at room temperature (18 ± 2 °C) for 4 weeks to allow the pH to equilibrate; pH values of the acidified brines were assessed prior (t_0) and after pasteurization (95 °C 5 min), then weekly up until the desired equilibrium pH was reached.

Once the equilibrium pH was reached, jars were separately inoculated, in triplicate, with *L. plantarum* IMC 509 and SYNBIO®, to reach a final load of ~9 Log CFU mL^{-1}(Figure 6.1). Jars were stored at 4 ± 2 °C for 44 days.

The experimental design of the study is depicted in Figure 6.2.

6.2.4. Enumeration of probiotics in the brine salt solution and on drained sea fennel sprouts

Aliquots of brine (1 mL) were aseptically collected immediately after inoculation (t_0) and 1, 7, 14, 21, 28, and 44 days of storage under refrigeration. At the end of the monitoring period, aliquots of sea fennel sprouts (10 g) were aseptically collected from the jars with stainless-steel tweezer, drained for 2 minutes using a standard #8 sieve, and homogenized with sterile 0.1% (w v^{-1}) peptone water in a Stomacher apparatus (400 Circulator, International PBI, Milan, Italy). Tenfold serial dilutions were prepared in the same diluent and aliquots (100 µL) of each dilution were subjected to enumeration of probiotic lactobacilli on De Man, Rogosa, and Sharpe (MRS) agar (VWR, Milano, Italy), incubated

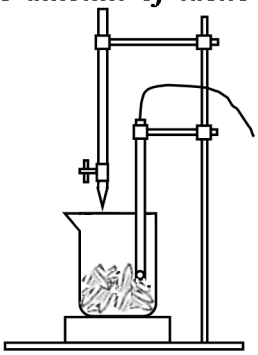
at 37 °C for 72 h. Sampling, homogenization, and preparation of serial dilutions were carried out under sterile conditions. The results of viable counting were expressed as the mean Log CFU mL^{-1} of brine or g^{-1} of sea fennel of three replicates ± standard deviation.

Figure 6.1. Probiotic-enriched sea fennel sprouts soaked in brine.

6.2.5. Statistical analysis

The results of the microbial enumeration of probiotic bacteria during the refrigerated shelf-life and at the end of the storage were subjected to one-way analysis of variance (ANOVA) with JMP Version 11.0.0 software (SAS Institute Inc., Cary, NC, USA). Differences through multiple mean comparisons were detected performing the Tukey-Kramer honest significant difference (HSD) test ($P \leq 0.05$).

Preliminary assays to determine the amount of lactic acid to be added to sea fennel based preserves to reach a final pH ~ 3.80



Preparation of sea fennel based-preserves

35 g sea fennel sprouts + 105 mL brine (7.0 % (w v^{-1}) NaCl + 1.0 % (w v^{-1}) fructose + 0.5 % (v v^{-1}) lactic acid)

Pasteurization at 95 °C for 5 min

Storage at 18 ± 2 °C for 28 days

Sampling points

t_0 | 95 °C 5 min | 7 days | 14 days | 21 days | 28 days

Probiotic bacteria inoculation

SYNBIO®
combination (1:1) of
Lacticaseibacillus rhamnosus IMC 501®
and *Lacticaseibacillus paracasei* IMC 502®

Lactiplantibacillus plantarum IMC 509

Storage at 4 ± 2 °C for 44 days

Sampling points

Inoculation | 24 hours | 7 days | 14 days | 21 days | 28 days | 44 days

pH measurement

Enumeration of probiotics in the brine salt solution

Enumeration of probiotics in the brine salt solution and on drained sea fennel sprouts

Figure 6.2. Experimental design of the study.

6.3. Results and discussion

In this exploratory study, the suitability of a sea fennel-based preserve as a carrier to deliver human probiotics was explored. Microbial safety and stability of the preserve were guaranteed by both direct acidification of the brine salt solution, where sea fennel sprouts were soaked, and by pasteurization at 95° for 5 min. According to "hurdle technology" theory, the combination of these treatments fully guarantees the death or growth inhibition of any potentially occurring food pathogen (Peng et al., 2017).

As far as the acidification of brine is concerned, it reached an equilibrium pH of 3.85 ± 0.07 after 4 weeks of incubation at room temperature since the addition of 0.5% food grade lactic acid. The markedly increase of the brine pH observed in the days after the addition of lactic acid (Figure 6.3) can be ascribed to the diffusion of this organic acid into the sea fennel tissues and hence to the previously reported buffer capacity of this herb (Corrales-Garcıa et al., 2004; Marsh, 1938).

Regarding probiotics, the commercially available formulation SYNBIO® and the strain *L. plantarum* IMC 509 were separately inoculated into the sea-fennel based preserve.

SYNBIO® has previously been exploited for the functionalization of various food products, including ripened cheese, salami, chocolate, and ice-cream, to reach a final probiotic load of approximately 10^9 CFU/daily dose of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® mixed 1:1 (Coman et al., 2012). The effects of food enrichment with SYNBIO® on bowl habits of healthy adults were also assessed with very promising results (Silvi et al., 2014). *L. plantarum* IMC 509 is currently commercialized, in association with *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®, in a vaginal formulation (Verdenelli et al., 2014). To the authors knowledge, no trials have ever been carried out with *L. plantarum* IMC 509 to develop probiotic-enriched foods.

L. plantarum IMC 509 and SYNBIO® were inoculated into the acidified brine at a final concentration of 9.2 ± 0.0 and 9.0 ± 0.1 Log CFU mL^{-1}, respectively. The enumeration of probiotics during

prolonged storage at 4 ± 2 °C revealed a slight but continuous reduction of viable counts, which attested at 8.2 ± 0.1 and 7.7 ± 0.3 Log CFU mL^{-1} for *L. plantarum* IMC 509 and SYNBIO®, respectively, after 44 days (Figure 6.4).

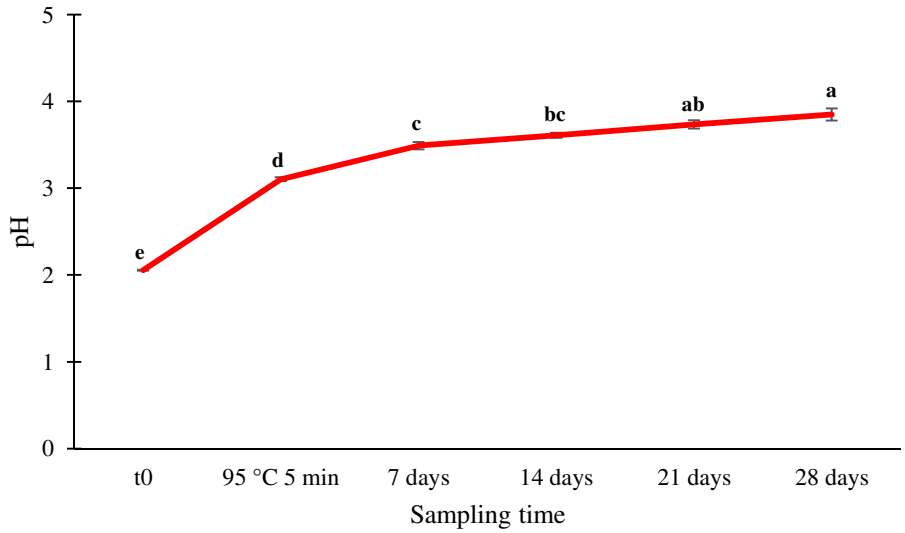


Figure 6.3. Results of pH measurements into acidified sea fennel-based preserves, Values are expressed as means ± standard deviation. For each sampling time, values labelled with different letters are significantly different ($P < 0.05$) by one-way ANOVA test.

This finding might be feasibly ascribed to the exposure of the inoculated microorganisms to stress conditions, such as the high concentration of sea fennel phenolic compounds and essential oils with an acknowledged antimicrobial activity (Gann, 2013; Nabet et al., 2017; Zaika et al., 1983) but mostly the low pH of the brine due to the addition of 0.5% of lactic acid to guarantee microbial safety and stability of the sea fennel-based preserve. In fact, though lactic acid is the major product of sugar fermentation and virtually acts as an antimicrobial agent against competing microorganisms, its accumulation, and hence the prolonged exposure to acidic conditions, usually results in death of lactic acid bacteria, and especially, of probiotics (Papadimitriou et al., 2016).

Notwithstanding this, at the end of the monitoring period, the enumeration of probiotics adhering on drained sea fennel sprouts revealed bacterial loads comparable to those found in the brine salt solution ($P > 0.05$) and attesting at 8.1 ± 0.0 Log CFU g^{-1} for *L. plantarum* IMC 509 and 7.0 ± 0.2 Log CFU

g^{-1} for SYNBIO®, respectively (Figure 6.5). Both these loads were comparable to quantities of probiotics in processed foods established as efficient for benefiting human health (10^6-10^7 CFU g^{-1}), (Talwalkar et al., 2004), and equivalent to 10^8-10^9 CFU of probiotics provided by a daily consumption of 100 g or 100 ml of probiotic-enriched foods, (Jayamanne and Adams, 2006).

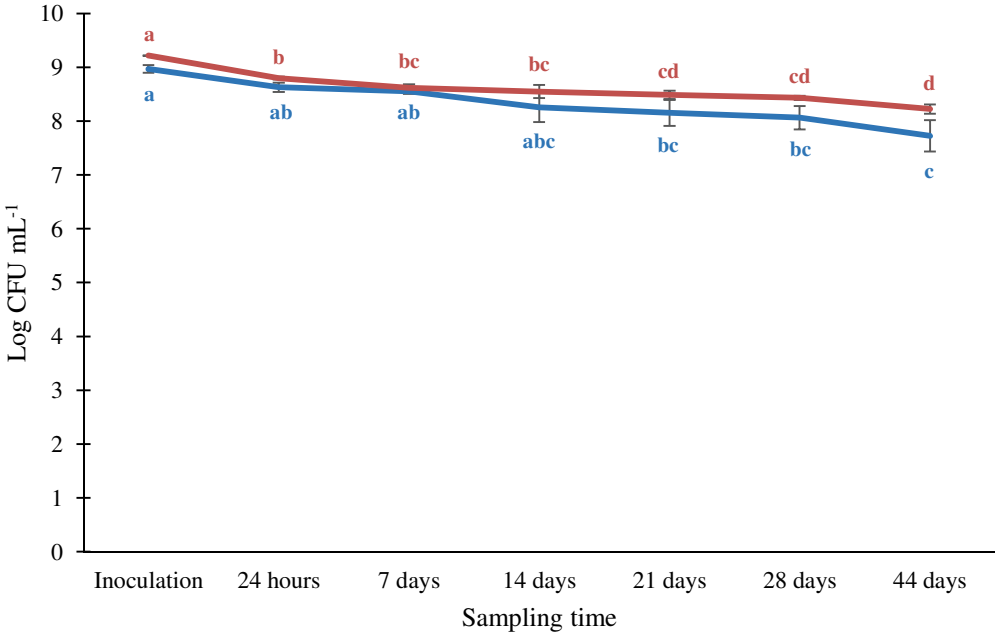


Figure 6.4. Results of the enumeration of the two assayed probiotic formulations (*Lactiplantibacillus plantarum* IMC 509 and SYNBIO® combination (1:1) of *Lacticaseibacillus rhamnosus* IMC 501® and *Lacticaseibacillus paracasei* IMC 502®), in an acidified sea fennel based preserve, during a refrigerated storage of 44 days. Results are expressed as mean value of CFU mL^{-1} ± standard deviation. Values labelled with different letters in the same growth curve are significantly different ($P < 0.05$) by one-way ANOVA test.

This finding clearly suggests the occurrence, in the specific substrate assayed, of sea fennel-derived nutrients able to support the survival of the inoculated probiotic strains, in agreement to what has previously been suggested for other probiotic-enriched foods (Coman et al., 2012). Even the addition of 1% fructose to the brine salt solution might have had a beneficial impact since this carbohydrate has previously been reported to enhance the survival of probiotic lactobacilli (Savijoki et al., 2019). In addition to this, the specific storage conditions applied, such as the use of glass jars hermetically sealed with stainless steel caps and refrigeration at 4 ± 2 °C, might also have contributed to the survival of the assayed probiotic strains at the right dose. In fact, glass has been reported as an ideal packaging material for probiotic-enriched foods, being characterized by an extremely low oxygen

permeability, which promotes the survival of probiotic lactic acid bacteria isolated from the human intestinal tract with a high sensitivity to high oxygen levels (Tripathi and Giri, 2014).

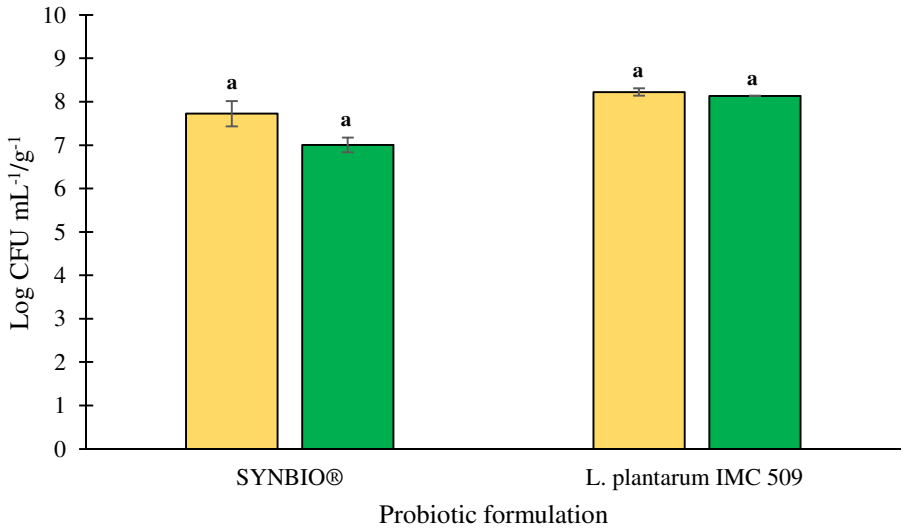


Figure 6.5. Loads of SYNBIO® and *Lactiplantibacillus plantarum* IMC 509 in ☐ brine and on ☐ drained sea fennel leaves, assessed after 44 days storage under refrigeration. For each probiotic formulation, values labelled with different letters are significantly different ($P < 0.05$) by one-way ANOVA test.

Even storage at 4 - 5 °C has previously been recommended to guarantee a prolonged viability of probiotic bacteria, which is inversely correlated to temperature (Tripathi and Giri, 2014).

6.4. Conclusions

The results overall collected in the present study demonstrated that artificially acidified sea fennel sprouts in brine can be an excellent vehicle to deliver probiotics to humans, given the high viability of both the probiotics assayed during storage under refrigeration. In more detail, for both *L. plantarum* IMC 509 and SYNBIO® viable counts higher than the minim loads suggested for health beneficial effects were found either in the brine or on drained sea fennel after 44 days of storage at 4 °C. On the one hand these findings suggest the anchorage of the probiotic cells on the vegetable tissues of sea fennel, and on the other hand the good adaptation of all the assayed probiotic strains to the sea fennel-based substrate. Hence, the consumption of 100 gram/die of drained sea fennel sprouts soaked in acidified brine carrying more than 1 billion of *L. plantarum* IMC 509 or a mixture of *L. rhamnosus*

IMC 501® and *L. paracasei* IMC 502® will guarantee the intake of 9 Log CFU g^{-1} of probiotics, ensuring beneficial effects on human health.

Further *in vivo* feeding trials are needed to assess the capability of the probiotic-enriched sea fennel-based preserve of delivering viable bacteria into the human gastrointestinal tract.

7. Microbiological safety and stability of innovative green sauces made with sea fennel (*Chritmum maritimum* L.)

The complete content of this section have been submitted to the Journal "Food Research International".

7.1. Introduction

Due to modern lifestyle and eating habits, today's consumers are increasingly interested in ready-to-eat meals, characterized by either familiar or unexpected innovative flavors. Hence, production of numerous foods, including sauces, has shifted from home-made to commercial practice.

Sauces are defined in the Codex Alimentarius as "*substances added to food to enhance aroma and taste*". They have always represented an important part of the worldwide culture and, considering the increase of their application in everyday life, they are gaining importance from both a nutritive and an economic point of view (García-Casal et al., 2016; Sikora et al., 2008). Hundreds of sauces are commercially produced worldwide, including very popular preparations and condiments, such as HP sauce, ketchup, mustard, Tabasco, and mayonnaise.

Sauces can be classified according to different criteria, including geographical origin (e.g.: Italian, Indian, Japanese sauces etc.), serving temperature (e.g.: warm or cold sauces), flavor (e.g.: mild or hot sauces), acidity (e.g.: low-acid or acid sauces), sweetness (e.g.: sweet or salty sauces), color (e.g.: brown sauces, pink sauces, green sauces, etc.).

Besides to palatability and nutritional value, microbial safety and stability of commercial sauces are key issues for both consumers and food producers. Absence of pathogenic microorganisms and microbiological stability during prolonged shelf-life can be achieved by (i) addition of organic acids to lower pH; (ii) addition of sugars or salt for water activity reduction; (iii) addition of chemical (e.g.: sorbic and benzoic acid) or natural (e.g.: essential oils) preservatives; (iv) application of thermal (e.g.: pasteurization, sterilization) or non-thermal (e.g.: high-pressure) preservation techniques.

Microbial safety and stability are commonly established by challenge tests and shelf-life studies, respectively.

Microbial challenge tests are usually conducted to validate processes that are intended to deliver some degree of lethality against a target organism or multiple target organisms. For the designing of these tests, multiple factors must be considered, including the selection of appropriate pathogens or surrogates, the inoculation level and procedure, the duration of the study, the incubation conditions, and the analysis of the collected samples (NACMCF, 2010).

Microbial shelf-life tests are useful in determining how rapidly microbiological changes occur in a refrigerated or ambient-stored food during its distribution and storage. Since humidity and temperature are known to accelerate microbial changes, accelerated shelf-life studies are carried out by storing the product at elevated stress conditions (e.g.: high temperatures and/or humidity), thus allowing the collection of useful data for a proper shelf-life estimation. For the designing of appropriate accelerated shelf-life tests, the likelihood of the product to support the growth of spoilage microorganisms must be carefully evaluated, by focusing on the history of the product, e.g.: how it is formulated, produced, packaged, stored, and consumed.

Starting from previous experiences gained by the research team while developing innovative fermented sea fennel-based preserves (Maoloni et al., 2021), the present study was aimed at assessing the microbiological risk and stability of pilot-scale prototypes of newly developed shelf-stable green sauces made with sea fennel (*Crithmum maritimum* L.) and other horticultural products. Sea fennel, also known as rock samphire or St. peter's herb, is a facultative halophyte that grows spontaneously in the proximity of the sea, on maritime cliffs and sandy beaches. It is widely distributed in Mediterranean countries (Italy, Croatia, Turkey, Greece, and North Africa), but it is also cultivated in many European regions (Atia et al., 2011; Generalić Mekinić et al., 2016; Meot-Duros and Magné, 2009). This highly aromatic herb is characterized by the presence of several bioactive compounds,

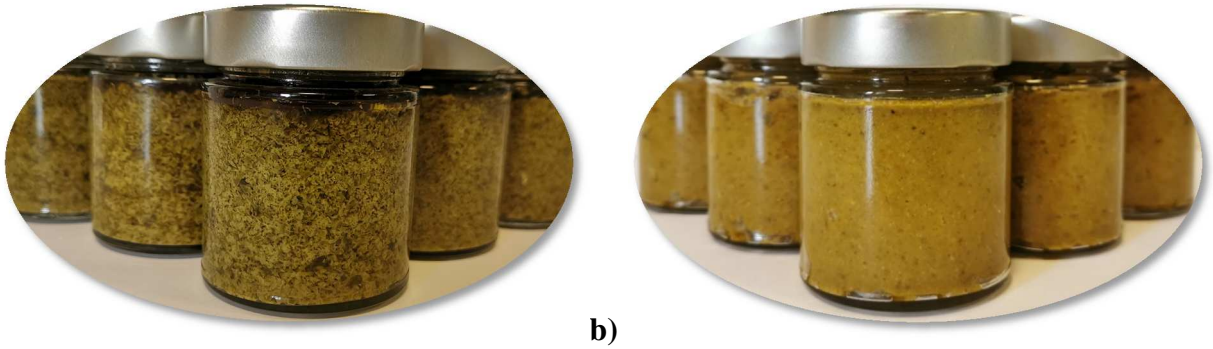
like C vitamin, polyphenols, carotenoids, essential ω-3 and ω-6 fatty acids and essential oils, which are particularly suitable to prevent the development of chronic degenerative diseases. Indeed, in the past, it was used in folk medicine as antiscorbutic, appetizer, tonic, carminative, and diuretic. Sea fennel has also uses in cosmetic, thanks to its essential oils, and in culinary, where it is consumed cooked, fresh in salads, as unfermented preserves in brine or olive oil, and even as sauces used for seasoning of fish or meats (Atia et al., 2011; Franke, 1982; Generalić Mekinić et al., 2016; Guil-Guerrero and Rodríguez-García, 1999; Jallali et al., 2014; Ksouri et al., 2012; Meot-Duros and Magné, 2009; Nabet et al., 2017; Siracusa et al., 2011).

In the present study, industrial-scale prototypes of two innovative shelf-stable green sauces produced with sea fennel as the main ingredient have been subjected to both accelerated shelf-life and microbial challenge tests, using selected strains of different test microorganisms, being *Staphylococcus aureus* and *Bacillus cereus*, with the aim to assess: (i) the microbiological stability of the two sauces subjected to thermal treatments conventionally exploited at industrial level for pasteurization ($F_{85}^{7} = 2\ min$ or $F_{95}^{7} = 5\ min$); (ii) the microbial risk associated to the potential growth of heat resistant pathogens in the new developed sauces once subjected to mild heat treatments, more respectful of the nutritional and sensory traits of the product ($F_{75}^{7} = 1 - 2\ min$).

7.2. Materials and methods

7.2.1. Setting of the study

Accelerated shelf-life tests (ASLT) and microbial challenge tests (MCT) were performed between July and October 2020 onto pilot-scale prototypes of new shelf-stable green sauces manufactured with sea fennel young sprouts as a main ingredient (Figure 7.1) at a local industrial producer of vegetable preserves and sauces (Rinci S.r.l., Castelfidardo, Ancona, Italy), according to the recipes shown in Table 7.1. The experimental design of the study is depicted in Figure 7.2.



a) **b)**

Figure 7.1. Industrial-scale prototypes of the two innovative shelf-stable green sauces manufactured with sea fennel (*Chritmum maritimum* L.) according to recipe 1 (panel **a**) and recipe 2 (panel **b**) (see Table 7.1 for the list of ingredients and Table 7.2 for measured physico-chemical parameters).

Two recipes were optimized by the food technologist working at Rinci according to the following general criteria: i) selection of ingredients characterized by high nutritional quality and/or palatability, the latter being influenced by smell, taste and physical characteristics, such as texture, hardness, color, etc.; ii) compatibility of the selected ingredients in blends; iii) achievement of fixed pH and a_w values in the final products, namely: (i) pH < 3.9 and a_w > 0.94 - 0.96, for recipe 1; (ii) pH > 4.6 - 5.0 and $a_w \leq 0.93$ for recipe 2.

Table 7.1. Ingredients used for the manufacture of the two innovative shelf-stable sea fennel-based sauces. For each sauce, equilibrium pH and a_w expected after pasteurization are reported.

Ingredient	**Recipe 1** (%)	**Recipe 2** (%)
Sea fennel	43.5	32.0
Green olives	14.0	9.7
Olive oil	40.0	28.6
Elephant garlic	0.5	-
Garlic in powder	-	0.4
Dried tomatoes	-	4.3
Cashews	-	23.0
Salt (NaCl)	2.0	2.0
pH	< 3.9	> 4.6 - 5.0
a_w	> 0.94 - 0.96	≤ 0.93

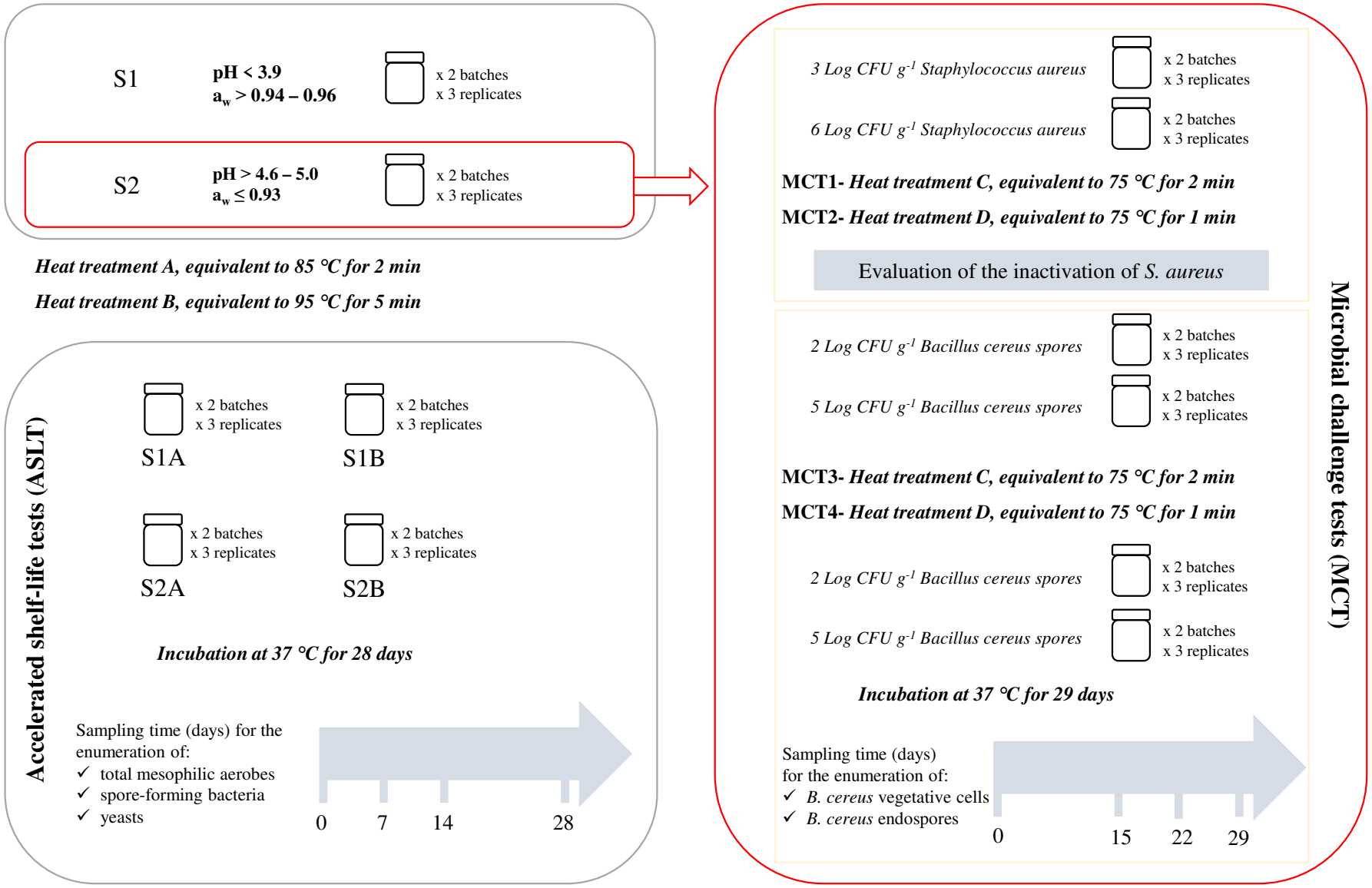


Figure 7.2. Experimental design of the study.

7.2.2. Microorganisms

Four test microorganisms were used in the microbial challenge tests, namely *Staphylococcus aureus* ATCC 2921 plus DSM 20231, and *Bacillus cereus* ATCC 11778 plus DSM 31. The ATCC and DSMZ strains were purchased from the American Type Culture Collection (https://www.atcc.org) and the Deutsche Sammlung von Mikroorganismen und Zell Kulturen (https://www.dsmz.de), respectively; they were reconstituted following the manufacturers' instructions. Prior to their sub-culturing, all the strains were maintained at -80 °C in a mixture of glycerol and Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke Hampshire, United Kingdom), at a 2:3 (v v^{-1}) ratio.

7.2.3. Preparation of the bacterial inocula

The two *S. aureus* strains were sub-cultured in BHI broth, incubated at 37 °C for 24 h; each broth culture was centrifuged at 4'000 rpm for 10 min, and the resulting pellet was resuspended in 10 mL of sterile physiological solution (NaCl 0.85%, w v^{-1}). The last two steps (centrifugation followed by resuspension in sterile diluent) were repeated twice, to remove any residues of growth medium, debris from dead cells, and bacterial metabolites. Prior to use as an inoculum, each suspension was subjected to cell density determination by viable counting on BHI agar incubated at 37 °C for 24 - 48 h. Aliquots of the two microbial suspensions were individually inoculated, in equal amount, in the test sauce to reach the desired final concentrations.

The two *B. cereus* strains were sub-cultured in Nutrient Broth (NB), containing 5g L^{-1} of peptone (Oxoid), 3 g L^{-1} of meat extract (Oxoid) and 0.01 g L^{-1} of manganese sulphate monohydrate ($MnSO_4$ x H_2O, Chimica Centro, Recanati, MC, Italy), with long-term incubation at 30 °C to encourage sporulation. Sporulation rate and spore density were checked every 10 days by viable counting onto BHI agar. To this end, two 5-mL aliquots of each broth culture were sampled under sterile conditions and subjected to either thermal treatment at 80 °C for 10 min, followed by cooling in iced water (for enumeration of spores) or no heat treatment (for enumeration of vegetative cells). Once a sporulation

rate of 100 % was reached (after 30 days), each broth culture was centrifuged at 4'000 rpm for 10 min, and the pellet resuspended in 10 mL of sterile physiological solution. Aliquots of the two *B. cereus* spore suspensions were individually inoculated, in equal amounts, in the test sauce to reach the desired final concentrations.

7.2.4. Pilot-scale production of the sea fennel-based green sauces

For each recipe, two production batches were manufactured at pilot-scale by Rinci S.r.l.; for each batch, ~ 30 Kg of sauce was prepared with the ingredients listed in Table 7.1, as briefly described as follows. Organic young sea fennel sprouts were manually harvested from a local sea fennel crop producer (Azienda Agricola Paccasassi Del Conero Società Semplice Agricola Di Galeazzi Luca, Velieri Francesco e Babbini Alessandro, Castelfidardo, Ancona, Italy), washed in a sodium hypochlorite (100 mg Kg^{-1}) solution, rinsed with tap water to reach a residual sodium hypochlorite concentration < 0.02 mg Kg^{-1}, blanched at 90 °C for 2 min, rapidly cooled, and drained by centrifugation. Organic horticultural products listed in Table 7.1 were purchased from local retailers and treated by washing and rinsing, as previously described. Both sea fennel sprouts and horticultural products were finely minced using a vacuum cutter, up until a homogeneous paste was obtained. Immediately after production, pH and a_w were determined as detailed in Section 7.2.5; pH was adjusted to the desired value using lactic acid 80 % food grade. For each recipe and production batch, three technical replicates consisting of 156 mL glass jars filled with 140 g of sauce and sealed with disposable steel caps were prepared.

The jars were stabilized by using different pasteurization treatments as detailed in Sections 7.2.6 and 7.2.7. After pasteurization, pH and a_w were measured to verify that the sauces were at equilibrium for these two parameters. To this end, aliquots of the sauces were aseptically collected and subjected to the determinations detailed in Section 7.2.5. Equilibrium pH and a_w of the two sauces before and after pasteurization are shown in Table 7.2.

7.2.5. pH and a_w determination

The pH was measured by using a pH meter equipped with an HI2031 solid electrode (Hanna Instruments, Padova, Italy). The water activity (a_w) was evaluated by using an Aqualab 4TE apparatus (Meter Group, Pullman, USA), in accordance with the ISO 21807:2004 standard method. For both pH and a_w, the results were expressed as mean of two production batches and three technical replicates ± standard deviation.

Table 7.2. Equilibrium pH and a_w of the two innovative shelf-stable sea fennel-based sauces (S) produced according to recipe 1 (S1) or 2 (S2).

Sauce	Stabilization treatment	pH	a_w
S1	unheated control	3.52 ± 0.01[b]	0.952 ± 0.002[a]
S1	heat treatment A:reference $F_{85}^{7} = 2\ min$; measured $F_{85}^{7} = 2.2\ min$	3.54 ± 0.00[ab]	0.947 ± 0.003[a]
S1	heat treatment B: reference $F_{95}^{7} = 5\ min$; measured $F_{95}^{7} = 5.6\ min$	3.55 ± 0.02[a]	0.946 ± 0.002[a]
S2	unheated control	4.61 ± 0.01[b]	0.921 ± 0.002[a]
S2	heat treatment A: reference $F_{85}^{7} = 2\ min$; measured $F_{85}^{7} = 2.7\ min$	4.69 ± 0.01[a]	0.916 ± 0.002[a]
S2	heat treatment B. reference $F_{95}^{7} = 5\ min$; measured $F_{95}^{7} = 5.6\ min$	4.69 ± 0.01[a]	0.915 ± 0.003[a]
S2	heat treatment C: reference $F_{75}^{7} = 2\ min$; measured $F_{75}^{7} = 2.6\ min$	4.64 ± 0.01[b]	0.917 ± 0.001[a]
S2	heat treatment D: reference $F_{75}^{7} = 1\ min$; measured $F_{75}^{7} = 1.7\ min$	4.64 ± 0.01[b]	0.917 ± 0.002[a]

The results are expressed as mean values of two production batches and three technical replicates ± standard deviation. For each recipe (S1 or S2) values labelled with different letters in the same column are significantly different ($P < 0.05$).

7.2.6. Accelerated shelf-life tests (ASLT)

The effectiveness of two heat treatments commonly exploited at industrial level for pasteurization of vegetable-based products was evaluated through accelerated shelf-life tests. For each recipe and production batch, 6 technical replicates were subjected to the following reference (theoretical) treatments at the slowest heating point: (i) heat treatment A, $F_{85}^{7} = 2\ min$ (3 replicates); (ii) heat treatment B, $F_{95}^{7} = 5\ min$ (3 replicates); no heat treatment (control, 3 replicates).

For each heat treatment assayed, sealed jars were immersed in boiling water so that the latter covered the jars 3-4 cm higher. After heat treatments, the jars were rapidly cooled in cold water until they reached a temperature of 25 ± 1 °C.

Preliminary heat penetration tests were performed by using a food temperature meter equipped with an immersion thermocouple probe (model Testo 108, Testo, Milan, Italy) inserted directly inside the jars through a 5 mm hole made on the lid. The probes were placed at the slowest heating point of the sauces. Time and temperature data were continuously acquired by means of data loggers connected with a computer either during the heating or cooling processes. Two separate heat penetration runs were carried out, with 2 replicates in each run. Data collected through these preliminary tests were used for estimating the equivalent F-values of the real processes by elaborating the time-temperature profiles registered at the coldest point according to the log-linear Bigelow model. A Z-value of 7 °C was used, based on the experimental data collected by Bertolatti et al. (2001) for different groups of *S. aureus*. Aliquots (10 g) of the heat treated and unheated (control) sauces were aseptically collected prior to incubation (t_0) and after 7, 14, and 28 days of incubation at 37 °C and subjected to the following microbiological analyses. Briefly, each aliquot was added with 90 mL of sterile 0.1% (w v^{-1}) peptone water and homogenized with a Stomacher apparatus (400 Circulator, International PBI, Milan, Italy) for 2 min at 230 rpm. Each homogenate was ten-fold serially diluted in the same diluent and subjected to the enumeration of: (i) total mesophilic aerobes on BHI agar (Oxoid) incubated at 30 °C for 72 h; (ii) yeasts on Rose Bengal Chloramphenicol agar (VWR International, Milan, Italy) incubated at 25 °C for 5 days; (iii) spore-forming bacteria on BHI agar, as detailed in Section 7.2.3. The results of the viable counts were expressed as mean Log CFU g^{-1} of sauce of two production batches and three technical replicates ± standard deviation.

7.2.7. Microbial challenge tests (MCT)

For the sole sauce manufactured according to recipe 2, the following microbial challenge tests (MCT) were performed as detailed below:

- MCT1. High (6 Log CFU g^{-1}) and low (3 Log CFU g^{-1}) level inoculation of *S. aureus* followed by reference heat treatment C, $F_{75}^{7} = 2\ min$.

- MCT2. High (6 Log CFU g^{-1}) and low (3 Log CFU g^{-1}) level inoculation of *S. aureus* followed by reference heat treatment D, $F_{75}^{7} = 1\ min$.
- MCT3. High (5 Log CFU g^{-1}) and low (2 Log CFU g^{-1}) level inoculation of *B. cereus* followed by reference heat treatment C, $F_{75}^{7} = 2\ min$ and incubation at 37 °C for 29 days.
- MCT4. High (5 Log CFU g^{-1}) and low (2 Log CFU g^{-1}) level inoculation of *B. cereus* followed by reference heat treatment D, $F_{75}^{7} = 1\ min$ and incubation at 37 °C for 29 days.

For each MCT, and each production batch, 3 technical replicates were separately spiked with the mixture of the test strains listed in Section 7.2.2. The bacterial suspensions made of 24 h-stationary phase cells (*S. aureus*) or endospores (*B. cereus*) were inoculated drop-wise into each jar on several different locations and carefully mixed with a sterile spatula, to ensure the homogeneous distribution of the inoculum across the sauce.

Preliminary heat penetration tests were performed, as previously described in section 7.2.6.

The first two MTC were carried out to evaluate the inactivation of *S. aureus* by the two mild heat treatments, whereas the remaining two MTC were aimed at evaluating the ability of the heat-treated sauce to inhibit the growth of *B. cereus* during its storage.

In more detail, in the challenge tests aimed at verifying the survival of *S. aureus*, aliquots (10 g) of the sauce, aseptically collected prior and immediately after heating, were subjected to viable counting of the test microorganism onto Baird Parker agar base (VWR) supplemented with Egg Yolk Emulsion with Potassium Tellurite (VWR), incubated at 37 °C for 48 h.

In the challenge tests aimed at verifying the survival and growth of *B. cereus*, aliquots (10 g) of the sauce, aseptically collected prior and after heat treatment, as well as after 15, 22 and 29 days of incubation at 37°C were subjected to viable counting of both vegetative cells and spores onto Brilliance *Bacillus cereus* agar base (Oxoid) supplemented with Brilliance *Bacillus cereus* Selective Supplement (Oxoid), incubated at 37 °C for 24 h.

The results of the viable counts, assayed in the challenge tests, were expressed as mean Log CFU g^{-1} of sauce of two production batches and three technical replicates ± standard deviation.

7.2.8. Statistical analysis

Data overall collected were subjected to one-way analysis of variance (ANOVA), using the Tukey-Kramer's Honest Significant Difference (HSD) at a level of significance set at 0.05 ($P < 0.05$). Statistical analyses were carried out with the software JMP® Version 11.0 (SAS Institute Inc., Cary, NC).

7.3. Results

7.3.1. Accelerated shelf-life tests

The Log reductions of the microbial accelerated shelf-life tests carried out onto the two new sea fennel-based sauces are shown in Table 7.3, whereas in Figure 7.3 the growth curves are shown, respectively. Supplementary Table 7.1 reports the Log CFU g^{-1}. A few trends emerged from the comparative evaluation of data; overall, loads of total mesophilic aerobes and spore-forming bacteria were significantly lower in the experimental sauces than the corresponding controls, irrespective of the recipe and sampling time; for yeasts, loads < 1 Log CFU g^{-1} were always seen, except for the controls analyzed immediately after sauce production (t_0). For the sauce manufactured according to recipe 1, heat treatment B ($F_{95}^{7} = 5\ min$) produced a significantly higher bacterial reduction in respect to heat treatment A ($F_{85}^{7} = 2\ min$); by contrast, in the sauce produced according to recipe 2, no significant differences were seen between the two industrial-like heat treatments applied (A and B), both leading to a significant decline of the bacterial groups under study. Finally, for both total mesophiles and spore formers, the initial contamination level seemed to affect the load recorded after 28 days of incubation at 37 °C, with the heat-treated sauce produced according to recipe 1 showing a complete dying off of all the microbial groups enumerated after 28 days of incubation.

7.3.2. Microbial challenge tests for assessing growth potential of S. aureus and B. cereus

The Log reductions for the low- and high-level challenge tests carried out on the sauce made according to recipe 2 using *S. aureus* and *B. cereus* as test microorganisms, are summarized in Table 7.4. Growth curves are displayed in Figure 7.4, whereas the Log CFU g^{-1} are reported in Supplementary Table 7.2. Both the heat treatments assayed were able to kill *S. aureus* cells, irrespective of the initial inoculation level. Concerning *B. cereus,* during the monitoring period, two different pictures emerged from the enumeration of endospores and vegetative cells, with differences depending on the initial inoculation level. In more detail, the loads of the vegetative cells remain almost stable, during storage, irrespective of the heat treatment, whereas for endospores, a significative reduction was seen during the monitoring period.

Table 7.3. Results of the accelerated shelf-life tests (ASLT) performed on the pilot-scale prototypes of the innovative shelf-stable sea fennel-based sauces (S) produced according to recipe 1 (S1) or 2 (S2). Total mesophilic aerobes, spore-forming bacteria, and yeasts were enumerated at t_0, and after 7 (t_7), 14 (t_{14}) and 28 (t_{28}) days of incubation at 37 °C.

Sampling time (Δ)	**Sample**					
	S1	**S1A**	**S1B**	**S2**	**S2A**	**S2B**
	Total mesophilic aerobes (Log CFU g^{-1} reductions)					
$\Delta(t_0\text{-}t_7)$	1.0 ± 0.1^{b} A	0.7 ± 0.2^{a} AB	0.3 ± 0.3^{a} B	0.2 ± 0.0^{ab} A	0.2 ± 0.1^{a} A	0.1 ± 0.1^{b} A
$\Delta(t_0\text{-}t_{14})$	1.2 ± 0.1^{b} A	1.0 ± 0.7^{a} A	0.3 ± 0.3^{a} A	0.1 ± 0.0^{b} A	0.3 ± 0.3^{a} A	0.4 ± 0.1^{ab} A
$\Delta(t_0\text{-}t_{28})$	1.7 ± 0.2^{a} A	1.7 ± 0.2^{a} A	0.9 ± 0.8^{a} A	0.2 ± 0.0^{a} A	0.5 ± 0.2^{a} A	0.6 ± 0.2^{a} A
	Spore-forming bacteria (Log CFU g^{-1} reductions)					
$\Delta(t_0\text{-}t_7)$	0.6 ± 0.2^{b} A	0.3 ± 0.2^{b} AB	0.0 ± 0.0^{a} B	0.1 ± 0.1^{a} B	0.8 ± 0.3^{a} A	0.6 ± 0.3^{a} AB
$\Delta(t_0\text{-}t_{14})$	1.2 ± 0.2^{a} A	0.6 ± 0.4^{ab} B	0.0 ± 0.0^{a} B	0.1 ± 0.2^{a} A	0.4 ± 0.1^{a} A	0.4 ± 0.2^{a} A
$\Delta(t_0\text{-}t_{28})$	1.7 ± 0.3^{a} A	1.3 ± 0.2^{a} A	0.0 ± 0.0^{a} B	0.0 ± 0.1^{a} B	0.8 ± 0.1^{a} A	0.5 ± 0.2^{a} A
	Yeasts (Log CFU g^{-1} reductions)					
$\Delta(t_0\text{-}t_7)$	3.1 ± 0.0^{a} A	0.0 ± 0.0^{a} B	0.0 ± 0.0^{a} B	3.7 ± 0.1^{a} A	0.0 ± 0.0^{a} B	0.0 ± 0.0^{a} B
$\Delta(t_0\text{-}t_{14})$	3.1 ± 0.0^{a} A	0.0 ± 0.0^{a} B	0.0 ± 0.0^{a} B	3.7 ± 0.1^{a} A	0.0 ± 0.0^{a} B	0.0 ± 0.0^{a} B
$\Delta(t_0\text{-}t_{28})$	3.1 ± 0.0^{a} A	0.0 ± 0.0^{a} B	0.0 ± 0.0^{a} B	3.7 ± 0.1^{a} A	0.0 ± 0.0^{a} B	0.0 ± 0.0^{a} B

The results are expressed as mean Δ of the Log CFU g^{-1} of sauce of two production batches and three technical replicates ± standard deviation. For each recipe values labelled with different capital letters in the same row are significantly different ($P < 0.05$), whereas values labelled with different small letters in the same column are significantly different ($P < 0.05$).

S1: sauce manufactured according to recipe 1 (unheated control)
S1A: sauce manufactured according to recipe 1 subjected to heat treatment A, reference $F_{85}^{7} = 2\ min$; measured $F_{85}^{7} = 2.2\ min$
S1B: sauce manufactured according to recipe 1 subjected to heat treatment B, reference $F_{95}^{7} = 5\ min$; measured $F_{95}^{7} = 5.6\ min$
S2: sauce manufactured according to recipe 2 (unheated control)
S2A: sauce manufactured according to recipe 2 subjected to heat treatment A, reference $F_{85}^{7} = 2\ min$; measured $F_{85}^{7} = 2.7\ min$
S2B: sauce manufactured according to recipe 1 subjected to heat treatment B, reference $F_{95}^{7} = 5\ min$; measured $F_{95}^{7} = 5.6\ min$

Table 7.4. Results of the microbial challenge tests (MCT) performed on the pilot-scale prototypes of the new sauce manufactured according to recipe 2. Two MCT were performed with the inoculation of *Staphylococcus aureus*. Two further MCT were performed with the inoculation of *Bacillus cereus* spores. *S. aureus* was enumerated immediately after the inoculation (t_0) and after pasteurization ($t_{75\ °C\ 1\ min}$ or $t_{75\ °C\ 2\ min}$), whereas vegetative cells and endospores of *Bacillus cereus* were enumerated immediately after the inoculation (t_0), after pasteurization ($t_{75\ °C\ 1\ min}$ and $t_{75\ °C\ 2\ min}$) and after 15 (t_{15}), 22 (t_{22}) and 29 (t_{29}) days of incubation at 37 °C.

Pasteurization	Mean Log reductions (Δ)	Low inoculation level			High inoculation level		
		Staphylococcus aureus	*Bacillus cereus*		*Staphylococcus aureus*	*Bacillus cereus*	
reference $F_{75}^{7} = 1\ min$; measured $F_{75}^{7} = 1.7\ min$			vegetative cells	endospores		vegetative cells	endospores
	Δ (t_0-$t_{75\ °C\ 1\ min}$)	3.4 ± 0.0	0.2 ± 0.2^{a} A	0.0 ± 0.0^{c} A	6.6 ± 0.0	0.1 ± 0.2^{a} A	0.0 ± 0.0^{c} A
	Δ (t_0-t_{15})	-	0.4 ± 0.6^{a} A	1.3 ± 0.1^{b} A	-	0.1 ± 0.3^{a} A	0.7 ± 0.1^{b} A
	Δ (t_0-t_{22})	-	0.5 ± 0.1^{a} A	1.1 ± 0.4^{b} A	-	0.1 ± 0.6^{a} A	0.9 ± 0.2^{b} A
	Δ (t_0-t_{29})	-	0.1 ± 0.2^{a} B	2.3 ± 0.1^{a} A	-	0.2 ± 0.4^{a} B	2.0 ± 0.2^{a} A
reference $F_{75}^{7} = 2\ min$; measured $F_{75}^{7} = 2.6\ min$			vegetative cells	endospores		vegetative cells	endospores
	Δ (t_0-$t_{75\ °C\ 2\ min}$)	3.2 ± 0.1	0.2 ± 0.3^{a} A	0.2 ± 0.2^{c} A	6.3 ± 0.1	0.0 ± 0.0^{a} A	-0.1 ± 0.1^{c} A
	Δ (t_0-t_{15})	-	0.2 ± 0.1^{a} B	1.4 ± 0.1^{b} A	-	0.0 ± 0.0^{a} B	1.4 ± 0.3^{b} A
	Δ (t_0-t_{22})	-	0.3 ± 0.1^{a} B	0.8 ± 0.1^{bc} A	-	0.4 ± 0.3^{a} B	1.3 ± 0.0^{b} A
	Δ (t_0-t_{29})	-	0.2 ± 0.2^{a} B	2.4 ± 0.1^{a} A	-	0.1 ± 0.1^{a} B	2.0 ± 0.0^{a} A

The results are expressed as mean Log reductions (Δ) of two production batches and three technical replicates ± standard deviation. For each inoculum level, values labelled with different capital letters in the same row are significantly different ($P < 0.05$), whereas, for each one of the two assayed thermal treatments, values labelled with different small letters in the same column are significantly different ($P < 0.05$).

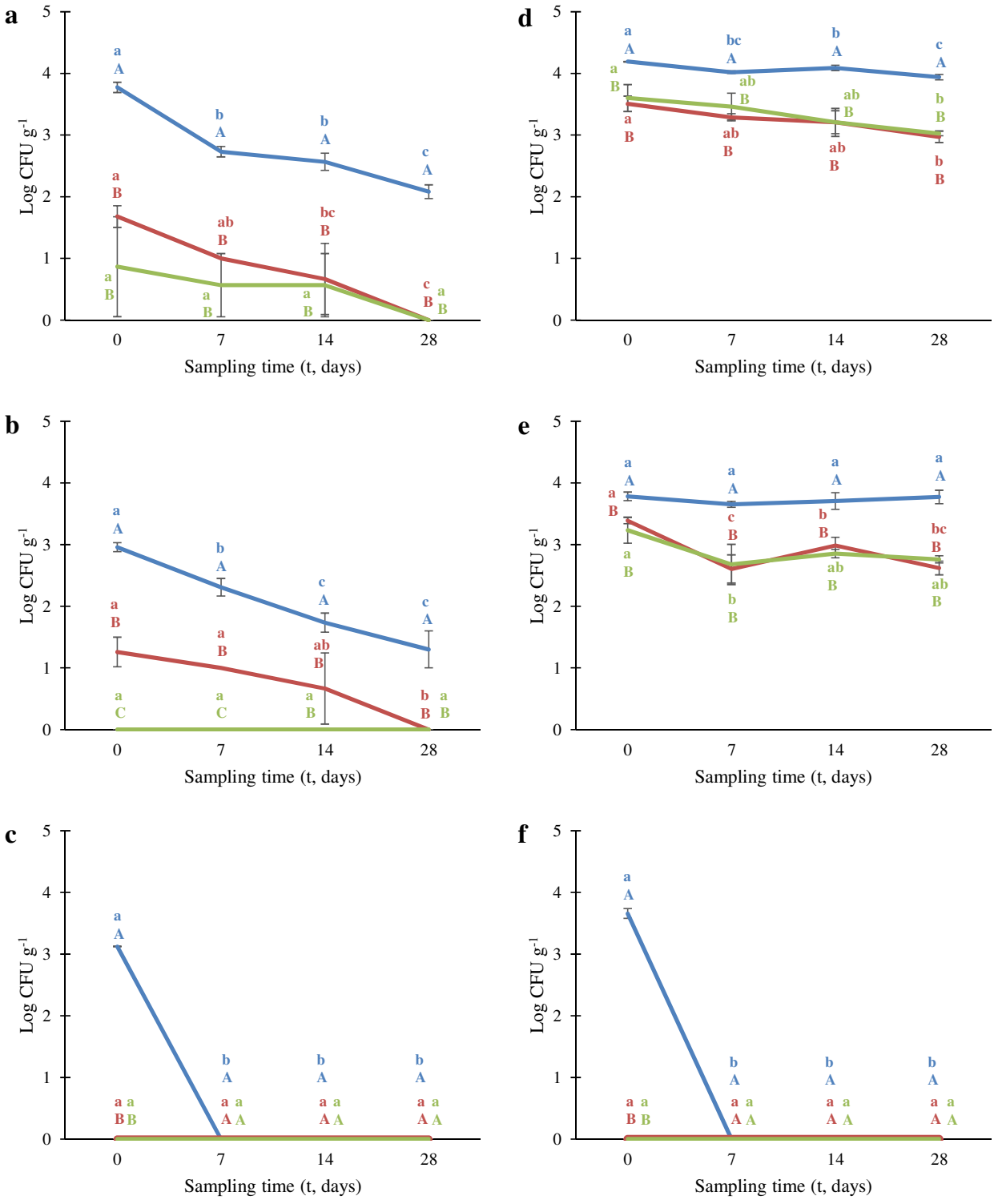


Figure 7.3. Results of the accelerated shelf-life tests (ASLT) performed on the pilot-scale prototypes of the new sauces manufactured according to recipe 1 (panels **a**, **b,** and **c**) and 2 (panels **d**, **e** and **f**). Total mesophilic aerobes (panel **a** and **d**), spore-forming bacteria (panels **b** and **e**), and yeasts (panels **c** and **f**) were enumerated on the prototypes subjected to heat treatment A, $F_{85}^{7} = 2\ min$ (S1A and S2A), or B, $F_{95}^{7} = 5\ min$ (S1B and S2B), and on untreated controls (S1 and S2), immediately after heating (t_0) and after 7 (t_7), 14 (t_{14}), and 28 (t_{28}) days of incubation at 37 °C. The results are expressed as mean Log CFU g^{-1} of sauce of two production batches and three technical replicates ± standard deviation. For each panel, values labelled with different small letters in the same growth curve are significantly different ($P < 0.05$), whereas values labelled with different capital letters between different growth curves are significantly different ($P < 0.05$).
For legend of prototypes see Table 7.3.

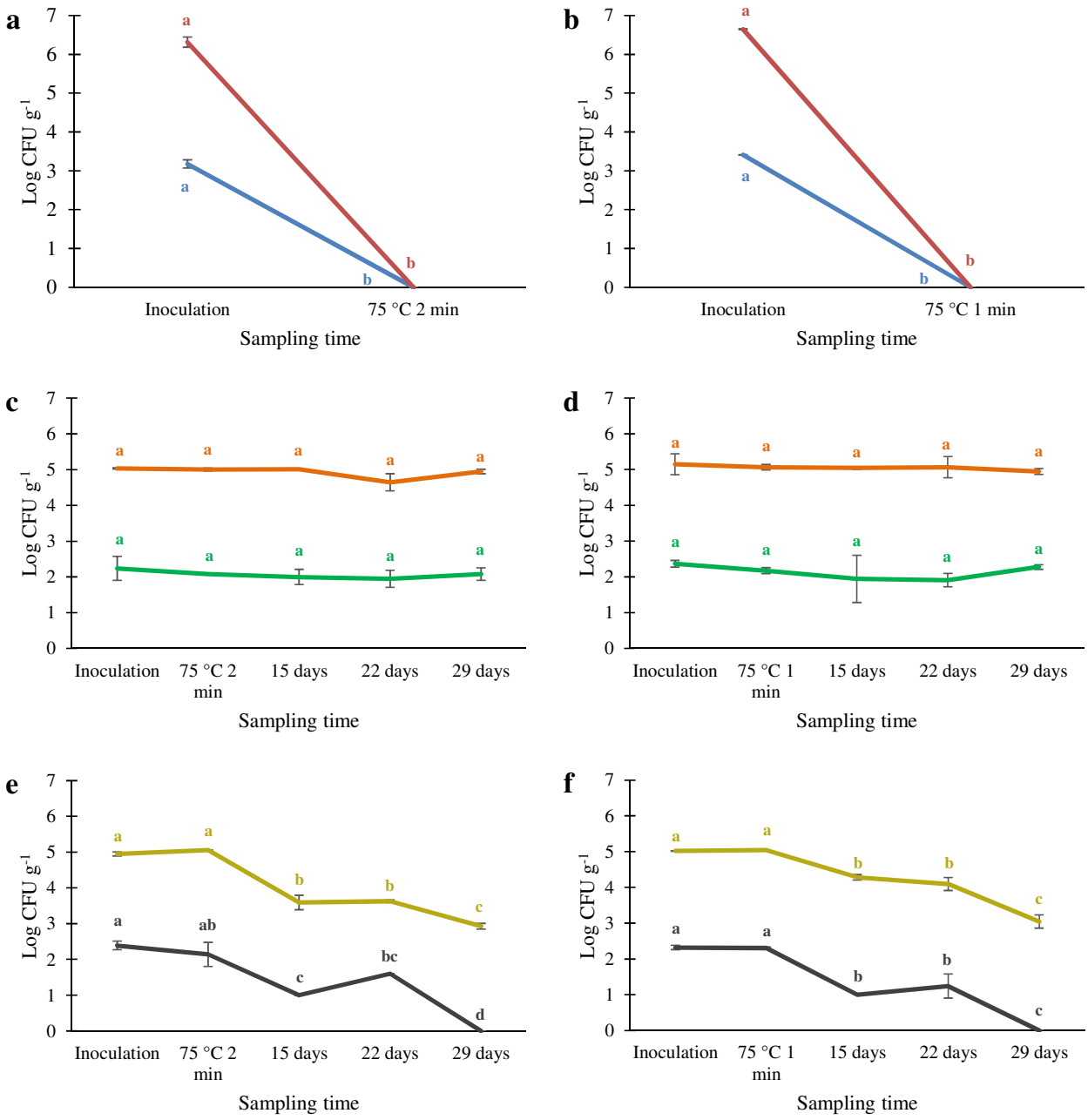


Figure 7.4. Results of the four microbial challenge tests (MCT) performed on the pilot-scale prototypes of the new sauce manufactured according to recipe 2. MCT 1 and MCT 2 were performed with the inoculation of *Staphylococcus aureus* at a high ▬ or low ▬ level, followed by heat treatment C, $F_{75}^{7} = 2\ min$ (panel **a**) or heat treatment D, $F_{75}^{7} = 1\ min$ (panel **b**), respectively. MCT 3 and MCT 4 were performed with the inoculation of *Bacillus cereus* endospores followed by heat treatment C, $F_{75}^{7} = 2\ min$ (panels **c** and **e**) or heat treatment D, $F_{75}^{7} = 1\ min$ (panels **d** and **f**), respectively. Vegetative cells (panels **c** and **d**) at a high ▬ and low ▬ inoculation level, and endospores (panels **e** and **f**) at a ▬ high and ▬ low inoculation level, were enumerated at the time of the inoculation, after heat treatment ($t_{75°C\ 1\ min}$, $t_{75°C\ 2\ min}$) and after 15 (t_{15}), 22 (t_{22}) and 29 (t_{29}) days of incubation at 37 °C. The results are expressed as mean Log CFU g^{-1} of sauce of two production batches and three technical replicates ± standard deviation. For each panel, values labelled with different letters in the same growth curve are significantly different ($P < 0.05$).

7.4. Discussion

Manufacture of new sea fennel-based sauces

In the present study, two green sauces not available on either the Italian or international markets were developed, by using young sea fennel sprouts as a main ingredient. Green sauces are a family of cold, uncooked sauces usually composed of one or more horticulture products and/or herbs or greens, a salt-containing water phase, and vegetable oils or fat-free oil substitutes (Smittle, 2000). Examples of popular commercially available green sauces are *Chimichourri*, an Argentinian uncooked sauce traditionally made with finely chopped parsley, garlic, and red peppers, added with vinegar and olive oil, and *Pesto,* easily one of the most appreciated and worldwide consumed sauces. *Pesto* can potentially be made with any herb or greens, but according to the traditional Italian recipe, it is prepared with basil, olive oil, cheese (usually *Parmesan*, but sometimes even *Pecorino*) and nuts (often pine nuts, but sometimes walnuts or other nuts, as well).

Besides to sea fennel, both the recipes herein formulated included vegetable high acid ingredients ($pH \leq 4.6$) such as tomatoes and green olives, as well as low-acid ingredients, such as cashews; virgin olive oil was also added together with lactic acid and NaCl to reach the desired equilibrium pH and a_w. In commercial green sauces, organic acids are typically added to lower pH and hence prevent the growth of pathogenic and spoilage microorganisms. Acetic acid is the most utilized; however, due to its pungent odor, it is often replaced with the odorless citric acid or lactic acid (herein used for acidification), or a mixture of these organic acids.

Stabilization of the new sauce prototypes by pasteurization

Pasteurization is included among thermal treatments commonly exploited at industrial level to inactivate microorganisms and endogenous enzymes in foods, including vegetables and vegetable-based products (Aamir et al., 2013). It is recognized as a relatively mild heat treatment, involving

heating of food product at temperatures below 100 °C, and commonly in the range 65 - 85 °C (Aamir et al., 2013).

However, the application of intense time-temperature combinations for the inactivation of the most heat resistant food-borne pathogens and/or spoilage microorganisms (according to the main goal of the pasteurization process for acid and low-acid foods) can lead to the deterioration of physical, chemical, and organoleptic properties of the treated foods, thus explaining the growing interest of industrial producers towards the use of mild preservation techniques, able to guarantee safety and stability of foods without compromising their nutritional and sensory traits. This is especially true for vegetables (Peng et al., 2017).

The Gram positive, rod-shaped, spore-forming, anaerobic bacterium *Clostridium botulinum* is recognized as the most heat-resistant pathogen in low-acid foods, since its spores (and especially those produced by the proteolytic strains belonging to group 1) might survive the thermal processing applied to these products; hence, thermal processes must target this organism to get safe preservation at room temperature if no other effective hurdles are present. However, the occurrence of additional hurdles (e.g.: pH or a_w control, addition of sodium chloride, combination of the above controlling factors, etc.) allows food manufacturers to apply milder thermal processes to stabilize their products (Merialdi et al., 2016).

For *C. botulinum* group 1 (producing toxins A, B, F and including proteolytic, mesophilic strains), minimum pH for growth is 4.6, whereas minimum a_w is 0.94 with 10% NaCl; for *C. botulinum* group 2 (producing toxins B, E and F, and including non-proteolytic, psychrotrophic strains), minimum pH for growth is 5.0, whereas minimum a_w is 0.97 with 5% NaCl. In both groups, toxin can be produced at growth-permissive a_w values, at pH < 5.2 (ICMSF, 1996a).

Regarding the two innovative sea fennel-based sauces herein manufactured, they were both characterized by pH and a_w values which do not allow the growth of this pathogen.

In the present study, four pasteurization treatments were assayed for the stabilization of the two sauces, namely: (i) two industrial-like heat treatments (equivalent to 85 °C for 2 min or 95 °C for 5 min, Z = 7 °C) characterized by temperature-time couples comparable to those commonly exploited for pasteurization of commercially available vegetable-based juices and preserves (86 °C - 96 °C for ~2 min, Peng et al., 2017); (ii) two mild heat treatments (equivalent to 75 °C for 1 min or 75 °C for 2 min, Z = 7 °C) previously exploited for laboratory-scale pasteurization of fluid milk and orange juice (Kabir et al., 2021).

Accelerated growth tests to assess the commercial stability of the sauce prototypes subjected to standard pasteurization treatments

It is known that thermo-tolerant spoilage microorganisms and endospores survive pasteurization and can germinate or grow, causing the deterioration of pasteurized products (Aamir et al., 2013).

In accelerated shelf-life tests, accelerated conditions (e.g.: elevated temperature or humidity) are applied to the food products to predict their shelf-life over extended periods of time (up to 1 year). The main advantage of these tests is the obtaining of results in a significantly shorter time than standard shelf-life tests, which require the waiting for results after the actual shelf-life of the product (NACMCF, 2010).

As data overall collected from the accelerated shelf-life tests carried out in the present study were evaluated, both the untreated sauces showed comparable contamination levels of total mesophilic aerobes, spore formers, and yeasts. In the sauce manufactured according to recipe 1 the two stabilization treatments assayed led to a more drastic microbial reduction in respect with the sauce made according to recipe 2. This finding might be feasibly explained by the differences occurring between the two sauces in terms of: (i) pH and a_w, with the sauce prepared according to recipe 1 being characterized by a markedly lower pH (3.5) in respect with the other sauce (4.7); (ii) the ingredients used, with the sauce manufactured according to recipe 2 including dried vegetables (powdered garlic

and dried tomato) and fruit (cashews) known for their generally high load of thermo-tolerant spore-forming bacteria usually originating from contact with soil, dust, or wastewater during harvest and post-harvest periods (Alp and Bulantekin, 2021; Heyndrickx, 2011).

According to hurdle technology, aimed at producing safe, stable, nutritious, tasty, and economic foods, the combined use of different preservation factors or techniques ('hurdles'), including the lowering of pH and a_w, leads to the achievement of mild but reliable preservation effects (Leistner and Gorris, 1995).

For yeasts, in both the sauces the two heat treatments led to a complete die-off; yeasts and molds are considered as potential spoilage microorganisms in commercial vegetable-based products, since they are known to thrive in high acid conditions. For the control of these microorganisms, a mild pasteurization (~2 min at 70 °C) is recommended (Put and De Jong, 1982), thus leading to six log reductions in ascospores of the target microorganism *Saccharomyces cerevisiae* (D60 22.5 minutes, Z 5.5 °C). For the sole sauce manufactured according to recipe 1, characterized by a lower pH (3.5) than sauce 2, a complete die-off of total mesophilic bacteria and spore formers was also seen along the monitoring period, depending on the heat treatment: as early as time zero (treatment B) or after 14 days of storage at 37 °C (treatment A). Again, the latter evidence might be feasibly ascribed to both the specific substrate including sea fennel as a main ingredient with ascertained antimicrobial activities (Meot-Duros et al., 2008) and to non-permissive growth conditions (pH < 3.9).

Microbial challenge tests to assess S. aureus survival or B. cereus growth in sea-fennel based sauce subjected to mild pasteurization treatments (75 °C for 1 min or 75 °C for 2 min).

Four microbial challenge tests were carried out onto the sauce produced according to recipe 2, using *S. aureus* and *B. cereus* as test microorganisms. According to the National Advisory Committee on Microbiological Criteria for Foods (2010), *S. aureus* is among the human pathogens of concern for multiplication in foods with: a_w > 0.90 - 0.92 and pH > 4.6 - 5.0 or a_w > 0.94 - 0.96 and pH = 4.2 -

4.6, whereas *B. cereus* is included among pathogens potentially growing in foods with a_w > 0.92 - 0.96 and pH > 4.6 - 5.0; however, a certain variability emerges from the available literature regarding these parameters, with *minimum* a_w = 0.83 and pH = 4.0 for growth of *S. aureus* and minimum a_w = 0.92 and pH = 4.3 for growth of *B. cereus* when other conditions are near optimum (Fermanian et al., 1997; Finlay et al., 1999; ICMSF, 1996b; Kramer and Gilbert, 1989; Osimani et al., 2018b; Sutherland and Limond, 1993).

Regarding the dose that cause illness, it attests at 10^4 - 10^9 cells per gram of food for *B. cereus* (Tewari and Abdullah, 2015) and at 10^5 - 10^8 cells per gram of food for *S. aureus* (Montville and Matthews, 2008; Seo and Bohach, 2007).

S. aureus is a facultative anaerobe, which can grow under both aerobic and anaerobic conditions. However, under anaerobic conditions, its multiplication occurs at a much slower rate (Stewart, 2003). *B. cereus* is a facultative anaerobic microorganism, as well, with the ability to survive at various levels of oxygenation (Duport et al., 2016). For the latter microbe, it has previously been reported that vegetative cells grown under aerobic conditions are less resistant to heat and acid than the vegetative cells grown anaerobically or microaerobically (Mols et al., 2009).

Regarding the production of toxins, limits are pH = 4.5 - 9.6 and a_w = 0.87 - 0.99 for *S. aureus* and pH = 5.5 - 10.0 for *B. cereus* (Fermanian et al., 1997; Finlay et al., 1999; ICMSF, 1996b; Kramer and Gilbert, 1989; Sutherland and Limond, 1993).

A strain-dependant variability has previously been documented in the physical growth parameters (e.g. pH and a_w) of both pathogens (Stewart, 2003; Wijnands, 2008).

In the challenge tests herein carried out, a cocktail of two strains for each test microorganism was spiked into the sea fennel-based sauce prepared according to recipe 2. This choice was again supported by what previously reported in a technical guidance on challenge tests and durability studies for assessing shelf-life of ready-to-eat foods (EURL *Lm*, 2021), about the exploitation of at

least 2 strains to account for strain-dependant variations in survival or growth of the test microorganism. In such a technical document, it is recommended that one strain with known growth characteristics is used (e.g.: a deposited type or reference strain) together with one or more freely chosen strains, with known or even unknown growth traits (e.g.: wild isolates). Given these premises, for each test microorganism, at least one reference strain purchased from International Culture Collections (ATCC or DSMZ) was used in the microbial challenge tests herein carried out.

Two inoculum levels were selected for each test microorganism, consisting of a high- and a low-level inoculum; this parameter has previously been reported to affect the observations resulting from microbial challenge studies (NACMCF, 2010), with high inoculum level mimicking a population nearing stationary phase and a low inoculum level being representative of the introduction of low-level contaminants.

In the present study, the first two microbial challenge tests (MCT1 and MCT2) were aimed at evaluating the ability of two mild heat treatments equivalent to 75 °C for 2 min or 75 °C for 1 min to inactivate *S. aureus* cells (Z = 7 °C) in the sea fennel-based sauce with pH = 4.7 and a_w < 0.92.

S. aureus is Gram-positive, facultative anaerobic, non-motile bacterium; it is a commensal and opportunistic pathogen responsible for staphylococcal food poisoning, a gastrointestinal illness caused by eating foods contaminated with enterotoxins produced by this bacterium, as well as hospital and community infections (Hennekinne et al., 2012). In food products, *S. aureus* is known to survive under dry and stressful conditions, including high salt concentrations (up to 15% NaCl) and low a_w values (0.83 - 0.86) (Medveďová and Valík, 2012). It does not produce spores and it can grow in a wide range of temperatures (from 7 to 48.5 °C) (Kadariya et al., 2014). Humans are the main vehicle for transmission of *S. aureus* to foods, during improper food processing and handling. Indeed, up to 50% of people have been estimated to carry this microorganism on their hands and nasal mucosa lining the nasal cavity (Arbuthnott et al., 1990; Kadariya et al., 2014). *S. aureus* is not thermoduric;

however pre-formed enterotoxins are highly stable and highly heat-resistant, being not inactivated by boiling, cooking, pasteurization, etc. (Necidova et al., 2016). As a rule, in unheated foods, *S. aureus* does not represent a concern, being a poorly competitive microorganism when compared to the resident microbiota (Kennedy et al., 2005) whereas in foods subjected to mild heat treatments designed to kill heat sensitive bacterial pathogens (e.g.: *Salmonella* or *Escherichia coli* O157:H7), *S. aureus* may survive and prevail over the weakened competing microflora (Kennedy et al., 2005). To date, various foods have been reported to serve as an optimum growth medium for *S. aureus*, including meat and meat-based products, milk and dairy products, bakery, ready-to-eat foods, and even vegetables (Kadariya et al., 2014).

In a past research study aimed at investigating the thermal inactivation of *S. aureus*, this latter was recognized as an ideal target microorganism in designing mild thermal food treatments (e.g.: cooking or pasteurization), being more thermo-tolerant than *Listeria monocytogenes* (Kennedy et al., 2005). According to these authors, pasteurization at 75 °C for 1 min rather than at 70 °C for 2 min is recommended for the thermal inactivation of heat-resistant *S. aureus* strains.

The results overall collected in the challenge test herein carried out clearly suggested that both the mild heat treatments assayed were effective to kill *S. aureus* cells, irrespective of the initial inoculation level; these findings fully confirmed what previously suggested by Kennedy et al. (2005).

Two further microbial challenge tests were carried out with *B. cereus* (MCT3 and MCT4), to evaluate the ability of sea-fennel based sauce subjected to mild in-container pasteurization treatments to inhibit the growth of this toxigenic species, across a 30 day-period.

Bacillus cereus is a Gram-positive, spore-forming, facultative anaerobic, rod-shaped bacterium responsible for food poisoning, an acute intoxication due to the production of two types of toxins: the emetic toxin (cerulide), that is produced during the bacterial growth phase in the food and cause vomiting, and the diarrheal toxins, namely haemolysin BL (HBL), non haemolytic enterotoxin (NHE)

and cytotoxin K (CYTK), that are produced during the bacterial growth in the small intestine and cause diarrhea (Ehling-Schulz et al., 2006). Symptoms of gastroenteritis caused by *B. cereus* are generally mild and short-lived (up to 24 hours).

Notwithstanding the numerous available studies aimed at understanding the correlation between diarrheal syndrome, very few data are known regarding the dose response relationship, and 10^3 to 10^8 number of cells per gram food were estimated to be necessary to induce diarrheal symptoms (Granum and Lund, 1997).

This pathogen is widespread in natural environments (e.g.: soil) as well as in several foods, including vegetables (Jenson and Moir, 2003; Osimani et al., 2018b). It produces endospores, that can easily survive harsh environments as well as various food processing (e.g.: cooking, pasteurization, freezing, drying, etc.). Some strains require heat activation for spores to germinate and outgrow (Osimani et al., 2018b).

In general, the presence of *B. cereus* spores in food products, including vegetables (Osimani et al., 2018b) can be due to many potential factors, such as the initial contamination of the raw materials or subsequent temperature abuse during transportation and distribution (Burgess et al., 2010). Moderate heat treatments ($T < 75$ °C) commonly applied at industrial level to inactivate vegetative cells of pathogens, are known to favor the germination and growth of *Bacillus* spores (Andersson et al., 1995). In more detail, at temperatures comprised between 25 and 37 °C, optimum pH for germination is neutral, whereas at lower temperatures (ranging from 6 to 14 °C) it shifts to acid side (pH 5.0) (Sakata et al., 1975). More recently it has been reported that germination of *B. subtilis* spores can be induced by acid treatment (pH 3 for 30 min) (Leser et al., 2008), thus suggesting that at pH values ~ 4.6, as those characterizing the sauce, herein assayed, they may germinate.

The microbial challenge tests herein performed with *B. cereus* clearly showed a slight but progressive reduction of the spore counts of this test microorganism during storage, alongside stable loads of

vegetative cells. This finding seems to suggest that germination of *B. cereus* spores was not followed by an active multiplication of the resulting vegetative cells, thus in turn suggesting an inhibitory effect of the specific substrate towards the test microorganism. The germination of *B. cereus* spores after the two heat treatments herein carried out agrees well with what reported in the scientific literature about the higher germination rate of the endospores of this food pathogen after preliminary activation by heating at temperatures in the range 65 - 95 °C for various times (Deeth, 2017). Moreover, a complication with germination of *B. cereus* spores is known, given the occurrence of both slow-germinating and fast-germinating spores, with the latter requiring less intense heat-activation treatment than slow-germinating spores (Deeth, 2017).

According to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), less than 3 Log CFU g^{-1} growth above the initial inoculum level across all replicates is a pass criterion in challenge studies aimed at evaluating the capacity of a food product to inhibit the growth of *B. cereus* (CFP, 2022).

Regarding the sauce herein assayed, the latter finding might be explained by the incorporated hurdles, namely the low a_w (≤ 0.92) and acid pH (≤ 4.7) as well as the occurrence of natural antimicrobials (e.g.: sea fennel essential oils). For the first hurdle, the effect of low a_w values on germination of *B. cereus* spores has previously been demonstrated (Rao et al., 2018); analogously, the ability of organic acids to enhance the demineralization of bacterial spores and, hence, to suppress their germination has also been elucidated (Inukai et al., 1984; Nishihara et al., 1981). Even for sea fennel essential oils, an antimicrobial activity towards food poisoning and spoilage bacteria has already been demonstrated by various authors (Houta et al., 2015; Ruberto et al., 2000)**.** Hence, as even clarified by Aamir et al. (2013), though pasteurization represents a hurdle for microbial control, it is not sufficient by itself to guarantee the safety and quality of pasteurized vegetables, thus justifying the exploitation of additional hurdles, such as reduction of water activity, change in pH and incorporation of food additives or preservatives, to control undesired microbes.

7.5. Conclusions

The results overall collected through the accelerated growth tests carried out on the two newly developed green sauces subjected to industrial-like heat treatments equivalent to 85 °C for 2 min or alternatively 95 °C for 5 min (estimated on the basis of Z = 7 °C) demonstrated their shelf-stability during 1 month storage in conditions of thermal abuse; moreover, the microbial challenge tests carried out on the sauce with pH = 4.7 and aw < 0.92 with *S. aureus* revealed that, both the milder heat treatments assayed ($F_{75}^{7} = 1 - 2\ min$) were able to kill the vegetative cells of this food pathogen; in addition, an inhibition of the growth of *B. cereus* was seen during storage at 37 °C of the same sauce challenged with this food pathogen.

7.6. Supplementary data

Supplementary Table 7.1. Results of the accelerated shelf-life tests (ASLT) performed on the pilot-scale prototypes of the innovative shelf-stable sea fennel-based sauces (S) produced according to recipe 1 (S1) or 2 (S2). Total mesophilic aerobes, spore-forming bacteria, and yeasts were enumerated at t_0, and after 7 (t_7), 14 (t_{14}) and 28 (t_{28}) days of incubation at 37 °C.

Sampling time (days)	Sample											
	S1		**S1 A**		**S1 B**		**S2**		**S2 A**		**S2B**	
	Total mesophilic aerobes (Log CFU g^{-1})											
t_0	3.8 ± 0.1^{a}	A	1.7 ± 0.2^{a}	B	$< 1.0^{a}$	B	4.2 ± 0.0^{a}	A	3.5 ± 0.1^{a}	B	3.6 ± 0.2^{a}	B
t_7	2.7 ± 0.1^{b}	A	1.0 ± 0.0^{ab}	B	$< 1.0^{a}$	B	4.0 ± 0.0^{bc}	A	3.3 ± 0.1^{ab}	B	3.5 ± 0.2^{ab}	B
t_{14}	2.6 ± 0.1^{b}	A	$< 1.0^{bc}$	B	$< 1.0^{a}$	B	4.1 ± 0.0^{b}	A	3.2 ± 0.2^{ab}	B	3.2 ± 0.2^{ab}	B
t_{28}	2.1 ± 0.1^{c}	A	$< 1.0^{c}$	B	$< 1.0^{a}$	B	3.9 ± 0.0^{c}	A	3.0 ± 0.1^{b}	B	3.0 ± 0.0^{b}	B
	Spore-forming bacteria (Log CFU g^{-1})											
t_0	3.0 ± 0.1^{a}	A	1.3 ± 0.2^{a}	B	$< 1.0^{a}$	C	3.8 ± 0.1^{a}	A	3.4 ± 0.0^{a}	B	3.2 ± 0.2^{a}	B
t_7	2.3 ± 0.1^{b}	A	1.0 ± 0.0^{a}	B	$< 1.0^{a}$	C	3.7 ± 0.0^{a}	A	2.6 ± 0.2^{c}	B	2.7 ± 0.3^{b}	B
t_{14}	1.7 ± 0.2^{c}	A	$< 1.0^{ab}$	B	$< 1.0^{a}$	B	3.7 ± 0.1^{a}	A	3.0 ± 0.1^{b}	B	2.9 ± 0.1^{ab}	B
t_{28}	1.3 ± 0.3^{c}	A	$< 1.0^{b}$	B	$< 1.0^{a}$	B	3.8 ± 0.1^{a}	A	2.6 ± 0.1^{bc}	B	2.8 ± 0.1^{ab}	B
	Yeasts (Log CFU g^{-1})											
t_0	3.1 ± 0.0^{a}	A	$< 1.0^{a}$	B	$< 1.0^{a}$	B	3.7 ± 0.1^{a}	A	$< 1.0^{a}$	B	$< 1.0^{a}$	B
t_7	$< 1.0^{b}$	A	$< 1.0^{a}$	A	$< 1.0^{a}$	A	$< 1.0^{b}$	A	$< 1.0^{a}$	A	$< 1.0^{a}$	A
t_{14}	$< 1.0^{b}$	A	$< 1.0^{a}$	A	$< 1.0^{a}$	A	$< 1.0^{b}$	A	$< 1.0^{a}$	A	$< 1.0^{a}$	A
t_{28}	$< 1.0^{b}$	A	$< 1.0^{a}$	A	$< 1.0^{a}$	A	$< 1.0^{b}$	A	$< 1.0^{a}$	A	$< 1.0^{a}$	A

The results are expressed as mean Log CFU g^{-1} of sauce of two production batches and three technical replicates ± standard deviation. For each recipe values labelled with different capital letters in the same row are significantly different ($P < 0.05$), whereas values labelled with different small letters in the same column are significantly different ($P < 0.05$).

S1: sauce manufactured according to recipe 1 (unheated control)
S1A: sauce manufactured according to recipe 1 subjected to heat treatment A, reference $F_{85}^{7} = 2\ min$; measured $F_{85}^{7} = 2.2\ min$
S1B: sauce manufactured according to recipe 1 subjected to heat treatment B, reference $F_{95}^{7} = 5\ min$; measured $F_{95}^{7} = 5.6\ min$
S2: sauce manufactured according to recipe 2 (unheated control)
S2A: sauce manufactured according to recipe 2 subjected to heat treatment A, reference $F_{85}^{7} = 2\ min$; measured $F_{85}^{7} = 2.7\ min$
S2B: sauce manufactured according to recipe 1 subjected to heat treatment B, reference $F_{95}^{7} = 5\ min$; measured $F_{95}^{7} = 5.6\ min$

Supplementary Table 7.2. Results of the microbial challenge tests (MCT) performed on the pilot-scale prototypes of the new sauce manufactured according to recipe 2. Two MCT were performed with the inoculation of *Staphylococcus aureus*. Two further MCT were performed with the inoculation of *Bacillus cereus* spores. *S. aureus* was enumerated immediately after the inoculation (t_0) and after pasteurization ($t_{75\ °C\ 1\ min}$ or $t_{75\ °C\ 2\ min}$), whereas vegetative cells and endospores of *Bacillus cereus* were enumerated immediately after the inoculation (t_0), after pasteurization ($t_{75\ °C\ 1\ min}$ and $t_{75\ °C\ 2\ min}$) and after 15 (t_{15}), 22 (t_{22}) and 29 (t_{29}) days of incubation at 37 °C.

		Low inoculation level			**High inoculation level**		
Pasteurization	**Sampling time**	***Staphylococcus aureus***	***Bacillus cereus***		***Staphylococcus aureus***	***Bacillus cereus***	
reference $F_{75}^{7} = 1\ min$; measured $F_{75}^{7} = 1.7\ min$			vegetative cells	endospores		vegetative cells	endospores
	t_0	3.4 ± 0.0^{a}	2.4 ± 0.1^{a} A	2.3 ± 0.1^{a} A	6.6 ± 0.0^{a}	5.1 ± 0.3^{a} A	5.0 ± 0.0^{a} A
	$t_{75\ °C\ 1\ min}$	$< 1.0^{b}$	2.2 ± 0.1^{a} A	2.3 ± 0.0^{a} A	$< 1.0^{b}$	5.1 ± 0.1^{a} A	5.0 ± 0.0^{a} A
	$t_{15\ days}$	-	1.9 ± 0.7^{a} A	1.0 ± 0.0^{b} A	-	5.0 ± 0.0^{a} A	4.3 ± 0.1^{b} B
	$t_{22\ days}$	-	1.9 ± 0.2^{a} A	1.2 ± 0.3^{b} A	-	5.1 ± 0.3^{a} A	4.1 ± 0.2^{b} A
	$t_{29\ days}$	-	2.3 ± 0.1^{a} A	$< 1.0^{c}$ B	-	4.9 ± 0.1^{a} A	3.0 ± 0.2^{c} B
reference $F_{75}^{7} = 2\ min$; measured $F_{75}^{7} = 2.6\ min$			vegetative cells	endospores		vegetative cells	endospores
	t_0	3.2 ± 0.1^{a}	2.2 ± 0.3^{a} A	2.4 ± 0.1^{a} A	6.3 ± 0.1^{a}	5.0 ± 0.0^{a} A	4.9 ± 0.1^{a} A
	$t_{75\ °C\ 2\ min}$	$< 1.0^{b}$	2.1 ± 0.0^{a} A	$2,1 \pm 0,3^{ab}$ A	$< 1.0^{b}$	5.0 ± 0.0^{a} A	5.1 ± 0.0^{a} A
	$t_{15\ days}$	-	2.0 ± 0.2^{a} A	1.0 ± 0.0^{c} B	-	5.0 ± 0.0^{a} A	3.6 ± 0.2^{b} B
	$t_{22\ days}$	-	1.9 ± 0.2^{a} A	1.6 ± 0.0^{bc} A	-	4.6 ± 0.2^{a} A	3.6 ± 0.0^{b} B
	$t_{29\ days}$	-	2.1 ± 0.2^{a} A	$< 1.0^{d}$ B	-	4.9 ± 0.1^{a} A	2.9 ± 0.1^{c} B

The results are expressed as mean Log CFU g^{-1} of two production batches and three technical replicates ± standard deviation. For each inoculum level, values labelled with different capital letters in the same row are significantly different ($P < 0.05$), whereas, for each one of the two assayed thermal treatments, values labelled with different small letters in the same column are significantly different ($P < 0.05$).

8. Development of sea fennel (*Crithmum maritimum* L.) dried spices: microbial, color and sensory traits

8.1. Introduction

Spices are defined according to the International Standards Organization (ISO) as "vegetable products or mixtures thereof, free from extraneous matter, used for flavoring, seasoning and imparting aroma in foods" (Wu et al., 2012). Fresh spices are characterized by a high moisture content, thus representing a highly perishable product, requiring the implementation of preservation strategies to prevent biological degradation. Dehydration is one of the oldest techniques used in food preservation, which aims to extend shelf-life of products reducing the moisture content to reach low water activity values able to inhibit microbial growth (Jin et al., 2018; Tapia et al., 2020).

Different drying methods have been used in herbs and spices dehydration, including convective air drying, microwave drying and freeze-drying. In convective air drying, the product is exposed to a flow of hot air, which evaporates and removes moisture from the surface of the product. The process involves two transport mechanisms occurring simultaneously and in opposite directions: i) a flow of heat from the hot air to the surface of the product, and from the surface to the inner part of the product; and ii) a flow of matter from the inner part of the product, like water or steam, to the surface, and from the surface to the air, as steam. This method is widely used in herbs drying due to the low cost of application (Chua et al., 2019). In microwave drying, heat is generated within the product due to the absorption of microwave energy by water molecules. Subsequently, evaporation of water occurs on the inside of the product and is followed by diffusion of water from the inside to the surface of the product as steam. The capability of this method in the production of high-quality dried herbs in a short processing time is pointed out in several studies. Nevertheless, there is a need for further research aiming at the evaluation of specific microwave drying processes targeted at different plant species, characterized by different microwave energy absorption capacities, in order to have a wider

industrial use of this technology (Chua et al., 2019; Heindl and Müller, 2007; Kathirvel at al., 2006; Wray and Ramaswamy, 2015). Another preservation technique is freeze-drying where moisture is removed by sublimation of water from solid to vapor state. This method is widely used for the storage of fruits and vegetables as it preserves bioactive compounds, due to the lack of liquid water and oxygen during the process and the use of low temperature. This method is widely used for production of high-value products, despite the high cost required (Bhatta et al., 2020).

In addition to its use in the culinary field as flavoring agent able to impart aroma to food, some spices like basil, thyme, coriander, cumin, oregano, and parsley have been used for medicinal purpose in the traditional Mediterranean diet, due to the high concentration of bioactive compounds, consisting mainly of polyphenols but also of essential oils (Bower et al., 2016; Embuscado, 2015; Sachan et al., 2018; Viuda-Martos et al., 2010). *Crithmum maritimum* L., commonly known as sea fennel, is a highly aromatic herb belonging to the Apiaceae family like parsley, coriander and cumin (Aćimović, 2019), characterized by the presence of several bioactive compounds including polyphenols and essential oils. Sea fennel, apart from the food use, has been largely considered for its nutritional and healthy properties, therefore its use in the production of dried spices represents an opportunity in the exploitation of its economic potential (Atia et al., 2011; Renna, 2018). To date, sea fennel has already been exploited for production of dried spices by different authors (Giungato et al., 2019; Renna and Gonnella, 2012; Renna et al., 2017).

Given these premises, this study aims to evaluate four different methods of preserving dried sea fennel spices, namely room temperature drying, oven drying, microwave drying and freezing, evaluating for each method the drying performance (weight loss and water activity), the microbiological quality, color and sensory properties.

8.2. Materials and methods

8.2.1. Sea fennel supply and moisture content determination

Two harvest batches of organic sea fennel sprouts (approximately 4.5 Kg each) were kindly provided by a local producer of sea fennel-based preserves (Rinci S.r.l., Castelfidardo, Ancona, Italy). Sea fennel was transported to the laboratory under refrigerated conditions (4 ± 2 °C), then washed under tap water and dried with paper towels, and finally stored in plastic bags at 4 °C until use. Prior to the drying experiments, sea fennel was chopped to obtain portions about 1.5 - 2 cm in length to facilitate water evaporation. In order to assess the initial moisture content, four aliquots (50 g) of chopped sea fennel for each batch were dried in an oven at 105 °C for 24 h.

8.2.2. Drying methods

Sea fennel was dried using four different methods: room temperature drying (RTD), oven drying (OD), microwave drying (MD) and freeze-driying (FD). Each experiment was carried out in triplicate, dividing approximately 1 Kg of sea fennel, chopped as described in section 8.2.1, into three portions of approximately 330 g.

In the drying experiments performed at room temperature (RTD) and in the oven (OD), sea fennel was uniformly distributed on aluminum trays, which had been previously perforated at the bottom to prevent water stagnation. The former were performed in an environment with a controlled temperature of 18-20 °C, while the latter were performed in an Heraeus function line B12/UB12 incubator (Thermo Fisher Scientific, Waltham, MA, USA) setting the temperature at 45 °C. For both the methods, each tray was weighed at regular intervals, and the drying process was stopped when a constant weight was reached. Microwave drying (MD) was performed using an Amstrad WP 820 microwave oven (Amstrad, Brentwood, Essex, United Kingdom) with a maximum power of 800 W. Sea fennel was placed in a circular way avoiding the centre of the microwave plate and processed at a high power level for 2 min, at a medium power level for 3 min, and finally at a low power level

until a constant weight was reached. Sea fennel was weighed at regular intervals of 1 min. Freeze-drying was carried out using a VirTis Wizard 2.0 freeze-dryer (SP scientific, Warminster, PA, USA). The drying time of 24 hours was determined by preliminary tests. Dried sea fennel samples were coded as RTDSF, ODSF, MDSF, FDSF when dried at room temperature, oven, microwave and freeze-dried, respectively.

8.2.3. Weight loss calculation and water activity measurement

For all the drying experiments, each of the three replicates was weighted individually before and after the drying process to calculate the weight loss. Results were expressed as mean % weight loss, referred to sea fennel initial weight, of the three replicates ± standard deviation.

Fresh and dried sea fennel samples were subjected to water activity (a_w) measurement through an Aqualab 4TE apparatus (Meter Group, Pullman, USA), in accordance with the standard ISO 21807:2004 method. Fresh sea fennel was coded as FRESHSF.

8.2.4. Microbiological evaluation

Ten g of fresh or dried sea fennel were added with 90 mL of 0.1 % (w v^{-1}) sterile peptone water, then homogenised using a Stomacher 400 Circulator apparatus (International PBI, Milan, Italy) for 2 min at 230 rpm. The homogenate was ten-fold serially diluted in 0.1 % (w v-1) sterile peptone water and subjected to the enumeration of: (i) mesophilic aerobic bacteria and (ii) spore-forming bacteria on Plate Count Agar (PCA) (VWR, International, Radnor, PA, USA) incubated at 30 °C for 72 h; for the enumeration of the spore-forming bacteria, the homogenate was subjected to heat treatment at 80 °C for 10 min followed by cooling in ice water, to inactivate the vegetative cells prior to the analysis. (iii) Enterobacteriaceae on violet red bile glucose agar (VRBGA) (VWR) incubated at 37 °C for 24 h; (iv) yeasts and moulds on Rose Bengal Chloramphenicol agar (VWR) incubated at 25 °C for 5 days. The results of the microbial counts were expressed as mean Log CFU g^{-1} of three replicates ± standard deviation.

8.2.5. Color assessment

The colorimetric profile of fresh and dried sea fennel was defined through a Chroma Meter CR-200 (Minolta, Japan) determining lightness (L), redness-greenness (a*: + red; − green) and yellowness-blueness (b*: + yellow; − blue) coordinates according to the CIELab color space system with a D65 light source. Moreover, the chroma (C) was calculated using the formula $[(a^{*2} + b^{*2})]^{1/2}$. To perform the color assessment, whole fresh leaves were bound together to create a homogeneous surface, while for dried samples were ground to obtain a powder. The instrument was calibrated using standard white coordinates, and the colorimetric readings were performed in triplicate. Results were expressed as mean values ± standard deviation.

8.2.6. Sensory analysis

A sensory analysis was performed on dried sea fennel by 10 non-smoker panelists, half male and female, aged between 25 and 40 years, as described by Maoloni et al., 2021 with slight modifications. Briefly, preliminary training sessions were performed to choose attributes to define dried sea fennel sensory properties. Samples were coded with three-digit random numbers, then aliquots (1 g) were presented to the panel at room temperature. Each panelist assigned a score between 1 and 9, where 1 expressed the lowest and 9 the highest intensity, respectively, to i) twelve odor and flavor descriptors (herbal, woody, spicy, kerosene-like, fruity, camphor, earthy, tobacco, celery, fresh, carrot and sea breeze), ii) four taste descriptors (sour, bitter, salty and sweet), iii) two color descriptors (color appreciation and green ratio). The global acceptance was also evaluated using a 9-point hedonic scale, where 1 expressed the lowest (extremely disliked) and 9 the highest (extremely liked) degree of liking (Peryam and Pilgrim, 1957). The results were expressed as mean value ± standard deviation.

8.2.7. Statistical analysis

One-way analysis of variance (ANOVA) was performed to detect differences between the different drying methods. Each harvest batch was individually subjected to the Tukey-Kramer honest

significant difference (HSD) test with criterion of significance set at $P \leq 0.05$ using JMP Version 11.0.0 software (SAS Institute Inc., Cary, NC, USA).

8.3. Results

8.3.1. Drying parameters

The initial sea fennel moisture content resulted to be 85.89 ± 0.20 and 83.21 ± 0.35 % for batch 1 and 2, respectively, whereas the relative water activity values were 1.00 ± 0.01 (batch 1) and 0.99 ± 0.01 (batch 2).

The drying parameters, describing the time for each drying experiment, the weight loss and the final water activity value reached, are reported in Table 8.1.

Table 8.1. Drying parameters, weight loss and water activity of dried sea fennel for each treatment.

Samples	Drying method	Time		Weight loss (%)		Water activity (a_w)	
		Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2
RTDSF	**Drying at 18-20 °C**	16 days	16 days	84.68 ± 0.26^{b}	82.69 ± 0.34^{a}	0.58 ± 0.09^{a}	0.49 ± 0.02^{a}
ODSF	**Oven drying at 45 °C**	4 days	4 days	85.73 ± 0.22^{a}	82.94 ± 0.09^{a}	0.21 ± 0.06^{b}	0.28 ± 0.02^{b}
MDSF	**Microwaving drying**	15 minutes	8 minutes	85.67 ± 0.26^{a}	82.63 ± 0.66^{a}	0.20 ± 0.03^{b}	0.40 ± 0.06^{a}
FDSF	**Freeze-drying**	24 hours	24 hours	85.34 ± 0.39^{ab}	83.15 ± 0.50^{a}	0.26 ± 0.05^{b}	0.16 ± 0.04^{c}

Weight loss and water activity are expressed as mean value ± standard deviation. Values labelled with different letters in the same column are significantly different ($P < 0.05$).

Microwave drying was the faster drying method, followed by freeze-drying, oven drying and room temperature drying. All the assayed methods resulted able in determining a weight loss (%) comparable to the initial moisture content of the two harvest batches. Regarding water activity, higher values were generally reported for the drying experiments performed at room temperature; in batch 2, similar values were collected both for the microwave drying and the room temperature drying.

8.3.2. Microbial enumeration

The results of the viable counts are reported in Table 8.2. Mesophilic aerobic bacteria, spore-forming bacteria and Enterobacteriaceae were generally present in similar or higher concentration in the dried

Table 8.2. Microbial viable counts of mesophilic aerobic bacteria, spore-forming bacteria, Enterobacteriaceae, yeasts and molds on fresh and dried sea fennel samples.

Harvest batch	Samples	Mesophilic aerobic bacteria	Spore-forming bacteria	Enterobacteriaceae	Yeasts	Molds
1	FRESHSF	6.0 ± 0.1^{b}	2.3 ± 0.2^{c}	4.6 ± 0.1^{ab}	4.9 ± 0.2^{a}	3.8 ± 0.1^{b}
	RTDSF	6.3 ± 0.3^{ab}	2.8 ± 0.3^{bc}	4.4 ± 0.1^{b}	4.6 ± 0.3^{ab}	4.0 ± 0.2^{ab}
	ODSF	7.0 ± 0.5^{a}	4.7 ± 0.2^{a}	5.7 ± 1.0^{a}	2.2 ± 0.2^{c}	4.3 ± 0.2^{a}
	MDSF	2.9 ± 0.2^{c}	2.8 ± 0.2^{bc}	$< 1.0^{c}$	$< 1.0^{d}$	$< 1.0^{c}$
	FDSF	6.2 ± 0.1^{b}	3.2 ± 0.0^{b}	5.6 ± 0.0^{a}	4.1 ± 0.2^{b}	4.0 ± 0.2^{ab}
2	FRESHSF	4.7 ± 0.2^{b}	2.7 ± 0.2^{bc}	3.3 ± 0.4^{bc}	3.6 ± 0.4^{b}	3.5 ± 0.1^{a}
	RTDSF	4.7 ± 0.2^{b}	3.4 ± 0.2^{b}	2.9 ± 0.1^{c}	3.2 ± 0.4^{b}	4.0 ± 0.4^{a}
	ODSF	4.7 ± 0.6^{b}	4.3 ± 0.6^{a}	3.5 ± 0.1^{b}	2.2 ± 0.2^{c}	3.9 ± 0.3^{a}
	MDSF	2.4 ± 0.1^{c}	2.5 ± 0.1^{c}	$< 1.0^{d}$	$< 1.0^{d}$	$< 1.0^{b}$
	FDSF	5.5 ± 0.1^{a}	3.3 ± 0.1^{bc}	4.2 ± 0.1^{a}	4.4 ± 0.0^{a}	3.9 ± 0.1^{a}

The results are expressed as mean Log CFU g^{-1} of three replicates ± standard deviation. For each harvest batch, values labelled with different letters in the same column are significantly different ($P < 0.05$).

FRESHSF: fresh sea fennel
RTDSF: room temperature dried sea fennel
ODSF: oven dried sea fennel
MDSF: microwave dried sea fennel
FDSF: freeze-dried sea fennel

samples than in the fresh ones. The only exception was represented by microwave dried samples, which were characterized by significant lower concentrations of mesophilic aerobic bacteria and Enterobacteriaceae than fresh samples. More in detail, microwave drying determined a strong reduction in Enterobacteriaceae, yeasts and molds with concentrations values under the detection limit (< 1.0 Log CFU g^{-1}). Yeasts and molds exhibited different behaviors related to both the drying method employed and the sea fennel harvest batch. Generally, a significant lower yeast concentration was observed in oven dried samples than in the fresh plant.

8.3.3. Color evaluation

The results related to the color assessment are reported in Table 8.3.

Table 8.3. Colorimetric profile of fresh and dried sea fennel.

Harvest batch	Samples	L	a*	b*	C
1	**FRESHSF**	38.05 ± 1.45^{d}	-8.31 ± 1.21^{b}	10.08 ± 1.46^{d}	13.07 ± 1.88^{d}
	RTDSF	55.34 ± 0.57^{b}	-12.11 ± 0.24^{c}	31.84 ± 0.35^{b}	34.07 ± 0.41^{b}
	ODSF	49.30 ± 2.21^{c}	-6.88 ± 0.50^{a}	26.11 ± 0.68^{c}	27.00 ± 0.77^{c}
	MDSF	51.13 ± 1.47^{c}	-13.40 ± 0.70^{d}	33.92 ± 0.76^{a}	36.47 ± 0.95^{a}
	FDSF	60.24 ± 0.59^{a}	-17.08 ± 0.26^{e}	32.57 ± 0.45^{b}	36.77 ± 0.52^{a}
2	**FRESHSF**	39.19 ± 1.29^{c}	-7.27 ± 0.57^{a}	8.44 ± 0.84^{d}	11.14 ± 1.00^{d}
	RTDSF	57.17 ± 0.94^{a}	-14.50 ± 0.19^{d}	34.46 ± 0.50^{a}	37.39 ± 0.52^{a}
	ODSF	50.38 ± 1.75^{b}	-9.52 ± 0.33^{b}	28.44 ± 0.79^{c}	29.99 ± 0.84^{c}
	MDSF	50.19 ± 0.83^{b}	-12.68 ± 1.71^{c}	33.49 ± 0.49^{b}	35.84 ± 0.70^{b}
	FDSF	57.99 ± 0.94^{a}	-17.95 ± 0.43^{e}	33.41 ± 0.67^{b}	37.92 ± 0.78^{a}

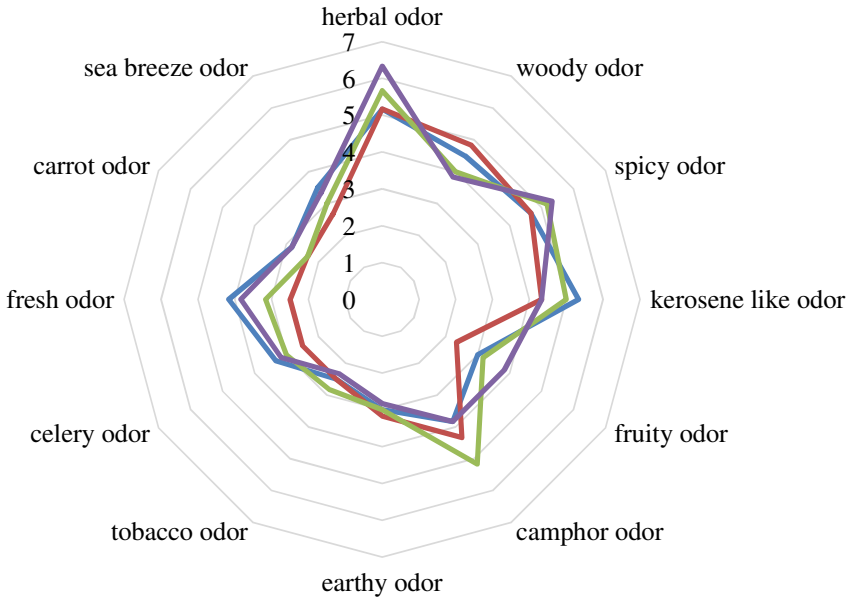
CIE Lab color parameters: L, lightness; a*, redness-greenness; b*, yellowness-blueness; C, chroma. Results are expressed as mean value of three replicates ± standard deviation. For each harvest batch, values labelled with different letters in the same column are significantly different ($P < 0.05$).

For legend of the samples see Table 8.2.

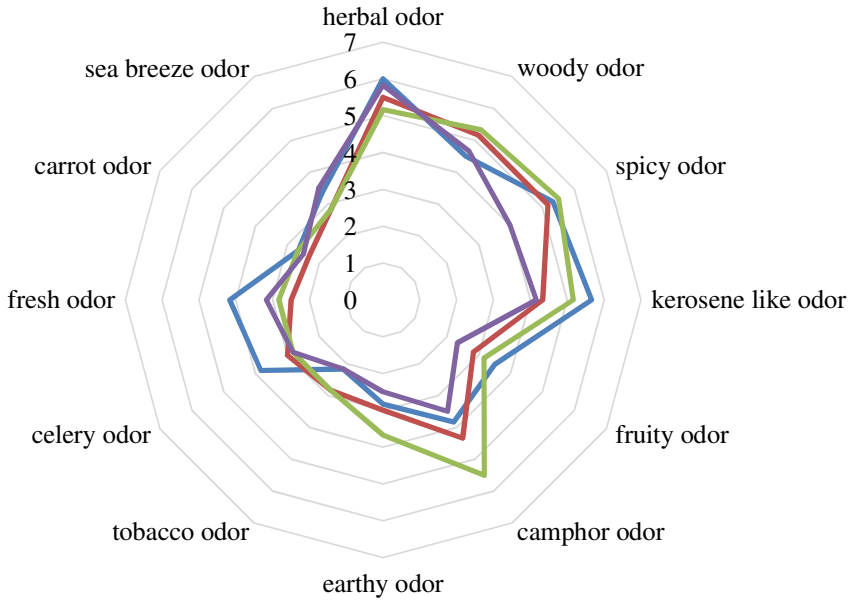
The drying process resulted in an increase in lightness (L) in all the dried samples compared to the fresh ones. The highest values were recorded in freeze-drying for batch 1 and in both freeze-drying and room temperature drying for batch 2. The red (+)/green (-) color attribute (a*) exhibited different behavior related to the drying method and to the harvest batches. Whereas, the yellow (+)/blue (-) color attribute (b*) and chroma resulted higher in all the dried samples than in the fresh ones.

8.3.4. Sensory analysis

The results of the sensory analysis are depicted in Figure 8.1.

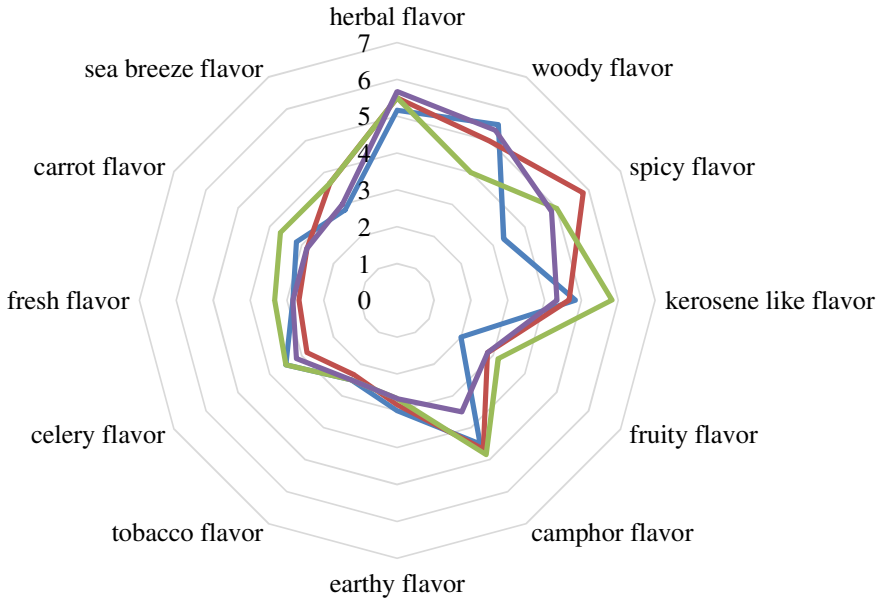


a)

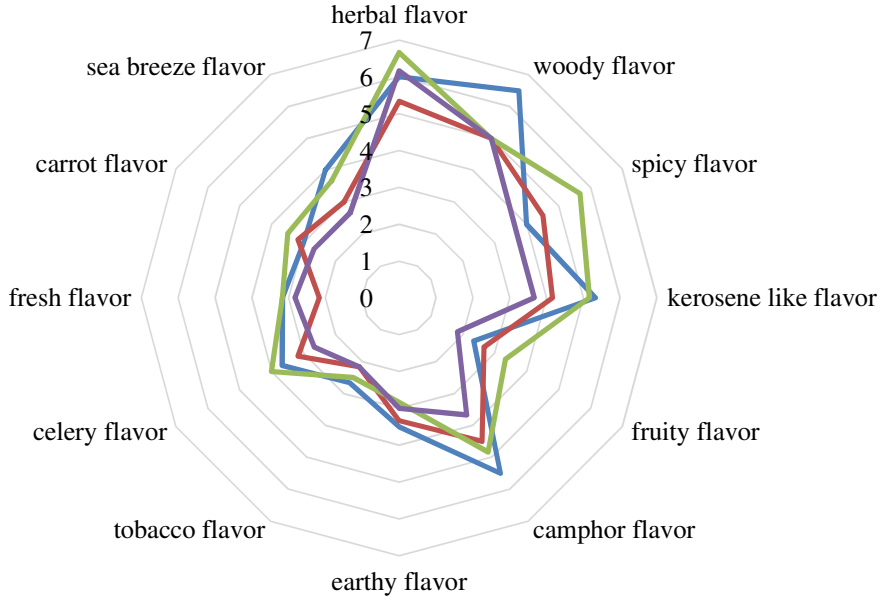


b)

Figure 8.1. Results of sensory analysis performed on dried sea fennel for the harvest batch 1 (panels **a**, **c** and **e**) and the harvest batch 2 (panels **b**, **d**, and **f**); RTDSF, ODSF, MDSF, FDSF. Each sample was evaluated, by a trained panel, consisting of 10 non-smoker tasters aged between 25 and 40, for the presence and intensity of i) twelve odor (panels **a** and **b**) and flavor descriptors (panels **c** and **d**), being herbal, woody, spicy, kerosene-like, fruity, camphor, earthy, tobacco, celery, fresh, carrot and sea breeze, ii) four taste descriptors (panels **e** and **f**), being sour, bitter, salty and sweet, iii) two color descriptors (panels **e** and **f**), being color appreciation and green ratio.



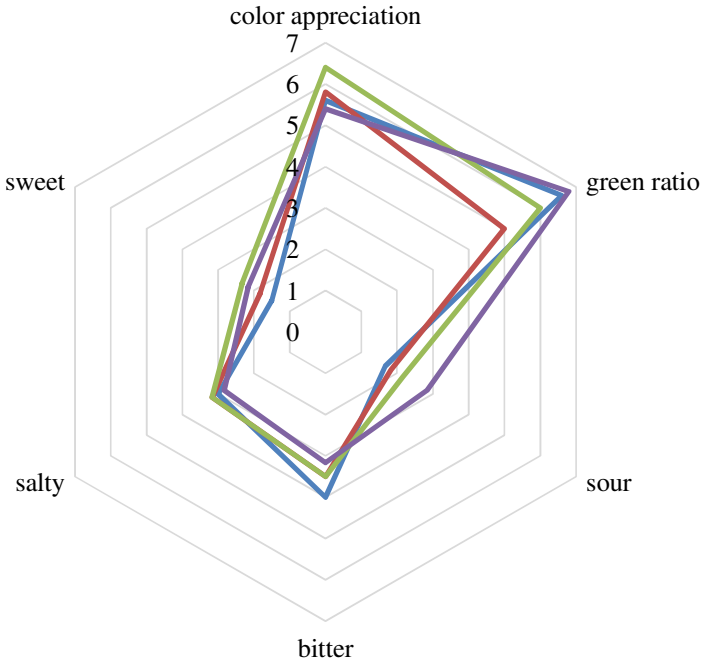
c)



d)

Figure 8.1. (Continued).

e)



f)

Figure 8.1. (Continued). Each descriptor was evaluated by assigning 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. **RTDSF**: room temperature dried sea fennel, **ODSF**: oven dried sea fennel, **MDSF**: microwave dried sea fennel, **FDSF**: freeze-dried sea fennel.

As the odor descriptors were taken into consideration, room dried samples (RTDSF) were characterized by kerosene-like and herbal notes, oven dried (ODSF)and freeze-dried (FDSF) samples by herbal, woody and spicy notes, whereas microwave dried (MDSF) samples by herbal, camphor

and spicy notes. As regarding the flavor descriptors, the main perceived aromatic notes in oven dried (ODSF) and freeze-dried samples were the same already described for the odor descriptors, while in the room temperature (RTDSF) and microwave dried (MDSF) samples woody and kerosene-like notes emerged, respectively. All the assayed samples were characterized by a bitter and salty taste. In freeze-dried samples, sweet and sour notes were also perceived in batch 1 and 2, respectively. As the color attributes were considered, the higher color appreciation was gained by microwave dried samples, while the higher green ratio scores were registered for freeze-dried samples. Microwave dried samples generally received a greater overall appreciation with values of 6.7 ± 1.2 and 6.5 ± 0.8 for the global liking in batch 1 and 2, respectively.

8.4. Discussion

Numerous culinary herbs and spices have been used for centuries as food flavoring, food preservative agents and in medical practices. Also, sea fennel is used as an ingredient of many dishes for its interesting sensory attributes and it is acknowledged for its nutritional and healthy properties, having high content in polyphenols, mainly chlorogenic acid, and essential oils, mainly terpene hydrocarbons and oxygenated terpenes (Generalić Mekinić et al., 2018; Jallali et al., 2014; Maoloni et al., 2021; Meot-Duros and Magné, 2009; Montesano et al., 2018). Traditionally, this plant serves as a condiment in salads, owing to its spicy and salty taste, or it may be pickled. Leaves can be consumed fresh in salads, or cooked in soups and sauces. Flower top and stalk infusion have been used as herbal teas (Rico et al., 2020). With the present research we evaluated the possibility of preserving this fresh plant as spice through four different treatments (room temperature drying, oven drying, microwave drying and freeze-drying).

All the treatments considered resulted able in removing almost completely the moisture content from the fresh plant, determining water activity values below 0.6 and thus ensuring a protection of the dried product from microbial spoilage during shelf-life. Water activity (a_w) is one of the most important

factors affecting microbial growth and toxin production by microorganisms, and its reduction is the main purpose of dehydration in order to achieve food preservation. More in detail, each microorganism is characterized by a minimum a_w for growth and toxin production and a_w values below 0.6 are reported in literature as inhibiting for microbial proliferation (Alp and Bulantekin, 2021; Tapia et al., 2020).

Regarding microbial counts, in dried samples were generally detected higher or similar concentrations for all the assayed microbial groups than in the fresh plant. Exceptions to this behavior were represented by oven drying at 45 °C, determining a significant reduction in yeasts population, and by microwave drying, determining a significant reduction for all the assayed microbial groups, apart from spore-forming bacteria. The behavior generally observed in room temperature, oven, and freeze-drying methods, could be explained considering the reported incapability in determining a microbial reduction of the applied temperatures in the first two methods, namely 18-20 and 45 °C, and of the traditional freeze-drying process (Cătunescu et al., 2019; Chitrakar et al., 2019; Duan et al., 2007). Furthermore, the increase in the microbial concentration of some microbial groups could be explained considering the weight loss registered in all the dried samples, and thus could just be apparent. Similarly, the reduction in the yeast population determined by oven drying could be caused by the applied temperature of 45 °C (Van Uden, 1985). Finally, the reduction in the microbial load of mesophilic aerobic bacteria, yeasts and molds in microwave drying could be explained considering the recognized sterilizing capability of microwave, which, in turn, could be attributed to a thermal effect or to a combination of thermal and non-thermal effects (Trivedi et al., 2011). Despite the documented capability of microwaves to kill bacterial spores (Celandroni et al., 2004; Park et al., 2006), some authors reported a higher resistance to microwave radiation for the bacterial spore than vegetative bacteria (Kim et al., 2009; Najdovski et al., 1991). These last findings could explain the microbial counts of spore-forming bacteria reported in this study for microwave dried sea fennel; the application of different power levels and/or a prolonged process could be assayed for a better

evaluation of spore inactivation by microwaves. Spices are low water activity foods (a_w < 0.7) in which microbial growth is inhibited, but microorganisms survived the drying process can remain viable during storage, thus representing a potential hazard if the spice is added to ready-to-eat foods or in the final phase of cooking (Bourdoux et al., 2016; Chitrakar et al., 2019). In the last years, foodborne outbreaks related to the consumption of dried herbs and spices have been reported by the European Food Safety Authority (EFSA, 2013), mainly caused by *Bacillus cereus*, *Salmonella senftenberg* and *Clostridium perfringens*. As stated in this study, the microbial contamination of the fresh plant represents one of the main factors affecting microbiological quality of spices, thus proper cleaning operations, including the use of sanitizing solutions, could allow the obtaining of high microbiological quality dried spices (Pahariya at al., 2022).

Regarding color, fresh sea fennel showed slightly lower values for lightness (L), chroma (C) and (b*) color attribute and slightly higher values for (a*) color attribute, if compared with the results reported by Renna et al. (2017). As the CIE L*a*b* color parameters of dried samples were considered, the results overall collected in this study for oven drying, microwave drying and freeze-drying agreed with data previously reported by Renna et al. (2017). The sensory evaluation revealed the presence of odor and flavor notes typical of the fresh plant, i.e. herbal and kerosene-like notes (Maoloni et al., 2021). The application of different dehydration methods could lead to changes in the composition of the volatile compounds fraction with consequent modification of sensory parameters, mainly through non-specific evaporation of compounds and/or specific degradation of some compounds (Lechtenberg et al., 2007). Herbal and spicy aromatic notes, and a bitter and salty taste were already described in dried sea fennel by Renna and Gonnella, (2012). In contrast with our results, the authors pointed out strong differences between oven dried and freeze-dried samples, possibly determined by the higher temperature (65 °C) applied in the oven drying (Turek and Stintzing, 2013). Finally, the high color and overall appreciation recorded for microwave dried sea fennel agreed with data collected by Renna et al. (2017).

8.5. Conclusions

All the drying treatments resulted able in removing the moisture content to achieve a water activity value < 0.6 for all the samples, thus making them microbiologically stable during the storage.

The importance of proper cleaning operation aiming to reduce the microbial contamination of the fresh plant is pointed out, especially if drying technologies incapable in microbial load reduction, like room temperature drying, oven drying at low temperature or freeze-drying, are employed. Further study, using different microwave power combination and/or different timing could be interesting to deepen the knowledge about the ability of microwaves in killing microbial spores in sea fennel. Finally, a greater appreciation for microwave dried spices was expressed by the panel.

Follow up studies will be required, aiming to assess the effect of each drying method on sea fennel bioactive compounds, in order to have a clearer overview of the potential of each drying method in the production of high value spices with possible positive effect on human health.

9. Conclusive remarks

The present Ph.D. thesis project started with the intuition that sea fennel (*Crithmum maritimum* L.) may have a high economical potential, related both to the presence of several bioactive compounds, like vitamin C, polyphenols, essential oils and essential ω-3 and ω-6 fatty acids, and to the strong link with the culinary tradition in the Marche region, for the production of high nutritional value preserves, sauces and spices.

The phytochemical study on the fresh plant, cultivated in the Conero Natural Park (Marche, Central Italy) area, allowed the detection of chlorogenic acid as the main phenolic compound together with the identification of compounds new for sea fennel (saponins and hydroxylated fatty acids), with potential pharmacological activity.

The formulation of a multiple strain starter culture, through the characterization of lactic acid bacteria for their pro-technological and sensory criteria, resulted as a valuable strategy to control the fermentation process of sea fennel, either alone or mixed with green olives (*Olea europaea* L. cv. Ascolana tenera), to produce microbiologically stable and safe preserves. During a spontaneous fermentation process, it is generally observed a progressive increase in lactic acid bacteria combined with a decrease in Enterobacteriaceae concentration, as highlighted in this thesis studying bacterial dynamics occurring in kimchi manufacture. The inoculation of the multiple strain starter favored a faster acidification and reduction in the Enterobacteriaceae concentration. The results overall collected about sea fennel fermentation confirmed the great potential of this plant for the production of foods with complex sensory traits and potential beneficial health effects.

Furthermore, the high viability of both the probiotic formulations *Lactiplantibacillus plantarum* IMC 509 and SYNBIO® during a prolonged refrigerated storage, in artificially acidified sea

fennel sprouts in brine, suggested this substrate as an excellent vehicle to deliver probiotics to humans.

Accelerated shelf-life tests carried out on the new sea fennel-based sauces subjected to conventional in-container pasteurizations ($F_{85}^{7} = 2\ min$ or $F_{95}^{7} = 5\ min$), proved the shelf-stability of the sauces during 1 month storage in thermal abuse (37 °C). Whereas, microbial challenge tests showed that both the mild in-container pasteurizations ($F_{75}^{7} = 1 - 2\ min$) assayed were able to kill *Staphylococcus aureus* vegetative cells; additionally, an inhibition of the growth of *Bacillus cereus* was reported during prolonged storage at 37 °C.

Finally, four different drying methods (room temperature, oven, microwave and freeze-drying) resulted able in removing the moisture content allowing the production of dried spices with water activity values below 0.6, thus stable from a microbiological point of view during the storage. Further studies targeted to assess the effect of each drying method on sea fennel bioactive compounds, may be useful for choosing the best method to produce of high value dried spices with possible positive effect on human health.

The overall collected results confirmed the intuition regarding the great potential of sea fennel for the production of high nutritional value preserves, sauces and spices linked to the territory, with potential impact on the manufacturing sector.

10. References

Aamir, M., Ovissipour, M., Sablani, S.S., Rasco, B., 2013. Predicting the quality of pasteurized vegetables using kinetic models: a review. Int. J. Food Sci. Volume 2013, Article ID 271271.

Aćimović, M.G., 2019. Nutraceutical potential of Apiaceae. In: Mérillon, J.-M., Ramawat, K.G. (Eds.), Bioactive Molecules in Food, Reference Series in Phytochemistry. Springer Nature, Switzerland AG, pp. 1311-1341.

Adamberg, K., Kask, S., Laht, T.M., Paalme, T., 2003. The effect of temperature and pH on the growth of lactic acid bacteria: a pH-auxostat study. Int. J. Food Microbiol. 85, 171-183.

Adouani, I., Du, M., Hang, T.J., 2013. Identification and determination of related substances in diosmin bulk drug and pharmaceutical formulations by HPLC and HPLC-MS. Chromatographia 76, 499-508.

Agudelo, A., Carvajal, M., Martinez-Ballesta, M.D.C., 2021. Halophytes of the Mediterranean Basin - Underutilized species with the potential to be nutritious crops in the scenario of the climate change. Foods 10, 119.

Ahmed, A.A.O., Kita, A., Nemś, A., Miedzianka, J., Foligni, R., Abdalla, A.M.A., Mozzon, M., 2020. Tree-to-tree variability in fruits and kernels of a *Balanites aegyptiaca* (L.) Del. population grown in Sudan. Trees 34, 111-119.

Aliotta, G., Pollio, A., 1994. Useful plants in renal therapy according to Pliny the Elder. Am. J. Nephrol. 14, 399-411.

Alonso-Salces, R.M., Korta, E., Barranco, A., Berrueta, L.A., Gallo, B., Vicente, F., 2001. Pressurized liquid extraction for the determination of polyphenols in apple. J. Chromatogr. A 933, 37-43.

Alp, D., Bulantekin, Ö., 2021. The microbiological quality of various foods dried by applying different drying methods: a review. Eur. Food Res. Technol. 247, 1333-1343.

Alves-Silva, J.M., Guerra, I., Gonçalves, M.J., Cavaleiro, C., Cruz, M.T., Figueirinha, A., Salgueiro, L., 2020. Chemical composition of *Crithmum maritimum* L. essential oil and hydrodistillation residual water by GC-MS and HPLC-DAD-MS/MS, and their biological activities. Ind. Crops Prod. 149, 112329.

Amoruso, F., Signore, A., Gómez, P.A., Martínez-Ballesta, M.D.C., Giménez, A., Franco, J.A., Fernández, J.A., Egea-Gilabert, C., 2022. Effect of saline-nutrient solution on yield, quality, and shelf-life of sea fennel (*Crithmum maritimum* L.) plants. Horticulturae 8, 127.

Anagnostopoulos, D.A., Goulas, V., Xenofontos, E., Vouras, C., Nikoloudakis, N., Tsaltas, D., 2020. Benefits of the use of lactic acid bacteria starter in green cracked Cypriot table olives fermentation. Foods 9, 17.

Andersson, A., Ronner, U., Granum, P.E., 1995. What problems does the food industry have with the spore-forming pathogens *Bacillus cereus* and *Clostridium perfringens*? Int. J. Food Microbiol. 28, 145-155.

Aquilanti, L., Zannini, E., Zocchetti, A., Osimani, A., Clementi, F., 2007. Polyphasic characterization of indigenous lactobacilli and lactococci from PDO Canestrato Pugliese cheese. LWT - Food Sci. Technol. 40, 1146-1155.

Arbuthnott, J.P., Coleman, D.C., Azavedo, J.S.D., 1990. Staphylococcal toxins in human disease. J. Appl. Bacteriol. 69, 101S-107S.

Arroyo-López, F.N., Querol, A., Bautista-Gallego, J., Garrido-Fernández, A., 2008. Role of yeasts in table olive production. Int. J. Food Microbiol. 128, 189-196.

Arroyo-López, F.N., Romero-Gil, V., Bautista-Gallego, J., Rodríguez-Gómez, F., Jiménez-Díaz, R., García-García, P., Querol, A., Garrido-Fernández, A., 2012. Yeasts in table olive processing: desirable or spoilage microorganisms? Int. J. Food Microbiol. 160, 42-49.

Arya, S.S., Devi, S., Ram, K., Kumar, S., Kumar, N., Mann, A., Kumar, A., Chand, G., 2019. Halophytes: the plants of therapeutic medicine. In: Hasanuzzaman M., Nahar K., Öztürk M. (Eds.), Ecophysiology, Abiotic Stress Responses and Utilization of Halophytes. Springer, Singapore, pp. 271-287.

Ashaolu, T.J., Reale, A., 2020. A holistic review on Euro-Asian lactic acid bacteria fermented cereals and vegetables. Microorganisms 8, 1176.

Atia, A., Barhoumi, Z., Mokded, R., Abdelly, C., Smaoui, A., 2011. Environmental eco-physiology and economical potential of the halophyte *Crithmum maritimum* L. (Apiaceae). J. Med. Plant Res. 5, 3564-3571.

Balatsouras, G., Tsibri, A., Dalles, T., Doutsias, G., 1983. Effects of fermentation and its control on the sensory characteristics of Conservolea variety green olives. Appl. Environ. Microbiol. 46, 68-74.

Barrett, D.M., 2003. Pickling. In: Caballero, B., Finglas, P., Toldra, F., (Eds). Encyclopedia of Food Sciences and Nutrition (2nd ed.). Academic Press, Cambridge, Massachusetts, pp 4563-4566.

Bautista-Gallego, J., Arroyo-López, F.N., Rantsiou, K., Jiménez-Díaz, R., Garrido-Fernández, A., Cocolin, L., 2013. Screening of lactic acid bacteria isolated from fermented table olives with probiotic potential. Food Res. Int. 50, 135-142.

Behera, S.S., El Sheikha, A.F., Hammami, R., Kumar, A., 2020. Traditionally fermented pickles: how the microbial diversity associated with their nutritional and health benefits?. J. Funct. Foods 70, 103971.

Belleggia, L., Milanović, V., Ferrocino, I., Cocolin, L., Haouet, M.N., Scuota, S., Maoloni, A., Garofalo, C., Cardinali, F., Aquilanti, L., Mozzon, M., Foligni, R., Pasquini, M., Trombetta, M.F., Clementi, F., Osimani, A., 2020. Is there any still undisclosed biodiversity in Ciauscolo salami? A new glance into the microbiota of an artisan production as revealed by high-throughput sequencing. Meat Sci. 165, 108128.

Bemelmans, W.J.E., Muskiet, F.A.J., Feskens, E.J.M., De Vries, J.H.M., Broer, J., May, J.F., Meyboom-de Jong, B., 2000. Associations of alpha-linolenic acid and linoleic acid with risk factors for coronary heart disease. Eur. J. Clin. Nutr. 54, 865-871.

Ben Amor, N., Ben Hamed, K., Debez, A., Grignon, C., Abdelly, C., 2005. Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. Plant Sci. 168, 889-899.

Ben Hamed, K., Debez, A., Chibani, F., Abdelly, C., 2004. Salt response of *Crithmum maritimum*, an oleagineous halophyte. Trop. Ecol. 45, 151-160.

Bertolatti, D., Munyard, S.J., Grubb, W.B., Binns, C.W., 2001. Thermal inactivation of antimicrobial-resistant Gram-positive cocci in chicken meat: D and Z value determinations. Int. J. Environ. Health Res. 11, 257-266.

Bevilacqua, A., Altieri, C., Corbo, M.R., Sinigaglia, M., Ouoba, L.I.I., 2010a. Characterization of lactic acid bacteria isolated from Italian Bella di Cerignola table olives: selection of potential multifunctional starter cultures. J. Food Sci. 75, M536-M544.

Bevilacqua, A., Cannarsi, M., Gallo, M., Sinigaglia, M., Corbo, M.R., 2010b. Characterization and implications of *Enterobacter cloacae* strains, isolated from Italian table olives “Bella di Cerignola”. J. Food Sci. 75, M53-M60.

Bevilacqua, A., Gallo, M., Corbo, M.R., Sinigaglia, M., 2013. Modelling the survival of *Enterobacter cloacae* in a model olive cover brine solution. Int. J. Food Sci. Technol. 48, 1366-1370.

Bhatta, S., Stevanovic Janezic, T., Ratti, C., 2020. Freeze-drying of plant-based foods. Foods 9, 87.

Bigliardi, B., Galati, F., 2013. Innovation trends in the food industry: the case of functional foods. Trends Food Sci. Technol. 31, 118-129.

Blana, V.A., Grounta, A., Tassou, C.C., Nychas, G.J.E., Panagou, E.Z., 2014. Inoculated fermentation of green olives with potential probiotic *Lactobacillus pentosus* and *Lactobacillus plantarum* starter cultures isolated from industrially fermented olives. Food Microbiol. 38, 208-218.

Boskou, D., Camposeo, S., Clodoveo, M.L., 2015. Table olives as sources of bioactive compounds. In: Boskou, D. (Ed.), Olive and Olive Oil Bioactive Constituents. AOCS press, Urbana, IL, pp. 217-259.

Botta, C., Cocolin, L., 2012. Microbial dynamics and biodiversity in table olive fermentation: culture-dependent and-independent approaches. Front. Microbiol. 3, 245.

Bourdoux, S., Li, D., Rajkovic, A., Devlieghere, F., Uyttendaele, M., 2016. Performance of drying technologies to ensure microbial safety of dried fruits and vegetables. Compr. Rev. Food Sci. Food Saf. 15, 1056-1066.

Boutellaa, S., Zellagui, A., Öztürk, M., Bensouici, C., Ölmez, Ö.T., Menakh, M., Duru, M.E., 2019. HPLC-DAD profiling and antioxidant activity of the n- butanol extract from aerial parts of Algerian *Crithmum maritimum* L.. Acta Sci. Nat. 6, 8-16.

Bower, A., Marquez, S., de Mejia, E.G., 2016. The health benefits of selected culinary herbs and spices found in the traditional Mediterranean diet. Crit. Rev. Food Sci. Nutr. 56, 2728-2746.

Brand-Williams, W., Cuvelier, M. E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25-30.

Breidt, F., McFeeters, R.F., Perez-Diaz, I., Lee, C.H., 2013. Fermented vegetables. In Doyle, M.P., Buchanan, R.L. (Eds). Food microbiology: Fundamentals and Frontiers. ASM Press, Washington, DC, pp. 841-855.

Burczyk, J., Wierzchowska-Renke, K., Głowniak, K., Głowniak, P., Marek, D., 2002. Geographic and environmental influences on the variation of essential oil and coumarins in *Crithmum maritimum* L. J. Herbs Spices Med. Plants. 9, 305-311.

Burgess, S.A., Lindsay, D., Flint, S.H., 2010. Thermophilic bacilli and their importance in dairy processing. Int. J. Food Microbiol. 144, 215-225.

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11, 2639-2643.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581-583.

Calvo, J., Calvente, V., de Orellano, M.E., Benuzzi, D., Sanz de Tosetti, M.I., 2007. Biological control of postharvest spoilage caused by *Penicillium expansum* and *Botrytis cinerea* in apple by using the bacterium *Rahnella aquatilis*. Int. J. Food Microbiol. 113, 251-257.

Campus, M., Değirmencioğlu, N., Comunian, R., 2018. Technologies and trends to improve table olive quality and safety. Front. Microbiol. 9, 617.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of highthroughput community sequencing data. Nat. Methods. 7, 335-336.

Carballo, J., 2012. The role of fermentation reactions in the generation of flavor and aroma of foods. In: Mehta, B.M., Kamal-Eldin, A., Iwanski, R.Z. (Eds.), Fermentation: Effects on Food Properties. CRC press Taylor & Francis Group, Boca Raton, FL, pp. 51-83.

Carocho, M., Ferreira, I.C.F.R., 2013. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol., 51, 15-25.

Carrió, E., Vallès, J., 2012. Ethnobotany of medicinal plants used in eastern Mallorca (Balearic Islands, Mediterranean Sea). J. Ethnopharmacol. 141, 1021-1040.

Cătunescu, G.M., Muntean, M., Marian, O., David, A.P., Rotar, A.M., 2019. Comparative effect of gamma irradiation, drying and freezing on sensory, and hygienic quality of parsley leaves. LWT - Food Sci. Technol. 115, 108448.

Celandroni, F., Longo, I., Tosoratti, N., Giannessi, F., Ghelardi, E., Salvetti, S., Baggiani, A., Senesi, S. (2004). Effect of microwave radiation on *Bacillus subtilis* spores. J. Appl. Microbiol. 97, 1220-1227.

CFP (Conference for Food Protection), 2022. CFP Guidance document on using the NACMCF parameters for challenge study protocols for retail food operators and regulators. Conference for Food Protection 2023 Biennial Meeting Houston, Texas, April 24 – 28, Conference-developed guides and documents. Available online: http://www.foodprotect.org/media/guide/using-the-nacmcf-parameters-for-challenge-study-protocols-for-retail-food-operators-and-regulators.pdf.

Chang, H.C., 2018. Healthy and safe Korean traditional fermented foods: Kimchi and chongkukjang. J. Ethn. Foods. 5, 161-166.

Chang, H.-W., Kim, K.-H., Nam, Y.-D., Roh, S.W., Kim, M.-S., Jeon, C.O., Oh, H.-M., Bae, J.-W., 2008. Analysis of yeast and archaeal population dynamics in kimchi using denaturing gradient gel electrophoresis. Int. J. Food Microbiol. 126, 159-166.

Chang, J.-H., Shim, Y.Y., Cha, S.-K., Chee, K.M., 2010. Probiotic characteristics of lactic acid bacteria isolated from kimchi. J. Appl. Microbiol. 109, 220-230.

Cheigh, H., 1999. Production, characteristics and health functions of kimchi. Acta Hortic. 483, 405-420.

Cheng, H., 2010. Volatile flavor compounds in yogurt: a review. Crit. Rev. Food Sci. Nutr. 50, 938-950.

Chitrakar, B., Zhang, M., Adhikari, B., 2019. Dehydrated foods: are they microbiologically safe?. Crit. Rev. Food Sci. Nutr. 59, 2734-2745.

Choi, B.-R., Kwon, E.-Y., Kim, H.-J., Choi, M.-S., 2018. Role of synbiotics containing D-allulose in the alteration of body fat and hepatic lipids in diet-induced obese mice. Nutrients 10, 1797.

Choi, M.H., Park, Y.H., 1999. Growth of *Pichia guilliermondii* A9, an osmotolerant yeast, in waste brine generated from kimchi production. Bioresour. Technol. 70, 231-236.

Chranioti, C., Kotzekidou, P., Gerasopoulos, D., 2018. Effect of starter cultures on fermentation of naturally and alkali-treated cv. Conservolea green olives. LWT - Food Sci. Technol. 89, 403-408.

Chua, L.Y., Chong, C.H., Chua, B.L., Figiel, A., 2019. Influence of drying methods on the antibacterial, antioxidant and essential oil volatile composition of herbs: a review. Food Bioprocess Technol. 12, 450-476.

Chung, H.-K., Shin, D., Chung, K.R., Choi, S.Y., Woo, N., 2017. Recovering the royal cuisine in Chosun Dynasty and its esthetics. J. Ethn. Foods 4, 242-253.

CMTD, 2022. Meeting of the Committee for the Common Organisation of the Agricultural Markets - Arable crops and olive oil, 2022-04-01.

Cocolin, L., Alessandria, V., Botta, C., Gorra, R., De Filippis, F., Ercolini, D., Rantsiou, K., 2013. NaOH-debittering induces changes in bacterial ecology during table olives fermentation. PLoS One 8, e69074.

Coman, M.M., Cecchini, C., Verdenelli, M.C., Silvi, S., Orpianesi, C., Cresci, A., 2012. Functional foods as carriers for SYNBIO®, a probiotic bacteria combination. Int. J. Food Microbiol. 157, 346-352.

Coman, M.M., Cresci, A., 2014. Probiotics and prebiotics for human health: innovations and new trends. Edizioni Accademiche Italiane trademark of OmniScriptum, Chisinau, MD.

Coman, M.M., Verdenelli, M.C., Cecchini, C., Silvi, S., Orpianesi, C., Boyko, N., Cresci, A., 2014. In vitro evaluation of antimicrobial activity of *Lactobacillus rhamnosus* IMC 501®, *Lactobacillus paracasei* IMC 502® and SYNBIO® against pathogens. J. Appl. Microbiol. 117, 518-527.

Corrales-Garcıa, J., Peña-Valdivia, C.B., Razo-Martínez, Y., Sánchez-Hernández, M., 2004. Acidity changes and pH-buffering capacity of nopalitos (*Opuntia* spp.). Postharvest Biol. Technol. 32, 169-174.

Cornara, L., La Rocca, A., Marsili, S., Mariotti, M. G., 2009. Traditional uses of plants in the Eastern Riviera (Liguria, Italy). J. Ethnopharmacol. 125, 16-30.

Corrêa, R.C., Di Gioia, F., Ferreira, I.C., Petropoulos, S.A., 2020. Wild greens used in the Mediterranean diet. In: Preedy, V.R., Watson, R.R. (Eds.), The Mediterranean Diet. Academic Press, Cambridge, Massachusetts, pp. 209-228.

Corsetti, A., Perpetuini, G., Schirone, M., Tofalo, R., Suzzi, G., 2012. Application of starter cultures to table olive fermentation: an overview on the experimental studies. Front. Microbiol. 3, 248.

Cunsolo, F., Ruberto, G., Amico, V., Piattelli, M., 1993. Bioactive metabolites from Sicilian marine fennel, *Crithmum maritimum*. J. Nat. Prod. 56, 1598-1600.

De Andrade Neves, N., Stringheta, P.C., Gómez-Alonso, S., Hermosín-Gutiérrez, I., 2018. Flavonols and ellagic acid derivatives in peels of different species of jabuticaba (Plinia spp.) identified by HPLC-DAD-ESI/MSn. Food Chem. 252, 61-71.

De Bellis, P., Sisto, A., Lavermicocca, P., 2021. Probiotic bacteria and plant-based matrices: an association with improved health-promoting features. J. Funct. Foods 87, 104821.

De Bellis, P., Valerio, F., Sisto, A., Lonigro, S.L., Lavermicocca, P., 2010. Probiotic table olives: microbial populations adhering on olive surface in fermentation sets inoculated with the probiotic strain *Lactobacillus paracasei* IMPC2.1 in an industrial plant. Int. J. Food Microbiol. 140, 6-13.

Deeth, H., 2017. Optimum thermal processing for extended shelf-life (ESL) milk. Foods 6, 102.

De la Borbolla y Alcalá, J.M.R., Diez, M.F., Cancho, F.G., 1964. Empleo de cultivos puros de lactobacilos en la preparacion de aceitunas verdes. Grasas y Aceites (Seville), 15, 6-11.

Della, A., Paraskeva-Hadjichambi, D., Hadjichambis, A.C., 2006. An ethnobotanical survey of wild edible plants of Paphos and Larnaca countryside of Cyprus. J. Ethnobiol. Ethnomed. 2, 1-9.

De Lorgeril, M., Salen, P. 2004. Alpha-linolenic acid and coronary heart disease. Nutr. Metab. Cardiovasc. Dis. 14, 162-169.

Di Cagno, R., Surico, R.F., Siragusa, S., De Angelis, M., Paradiso, A., Minervini, F., De Gara, L., Gobbetti, M., 2008. Selection and use of autochthonous mixed starter for lactic acid fermentation of carrots, French beans or marrows. Int. J. Food Microbiol. 127, 220-228.

Duan, X., Zhang, M., Mujumdar, A.S., 2007. Studies on the microwave freeze drying technique and sterilization characteristics of cabbage. Dry. Technol. 25, 1725-1731.

Duport, C., Jobin, M., Schmitt, P., 2016. Adaptation in *Bacillus cereus*: from stress to disease. Front. Microbiol. 7, 1550.

EFSA (European Food Safety Authority), 2013. Panel on Biological Hazards (BIOHAZ). 2013. Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). EFSA J. 11(1):3025. Available online: http://www.efsa. europa.eu/en/efsajournal/pub/3025.

Ehling-Schulz, M., Guinebretiere, M.H., Monthán, A., Berge, O., Fricker, M., Svensson, B., 2006. Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. FEMS Microbiol. Lett. 260, 232-240.

Elomari, M., Coroler, L., Hoste, B., Gillis, M., Izard, D., Leclerc, H., 1996. DNA relatedness among *Pseudomonas* strains isolated from natural mineral waters and proposal of *Pseudomonas veronii* sp. nov. Int. J. Syst. Evol. Microbiol. 46, 1138-1144.

Embuscado, M.E., 2015. Spices and herbs: natural sources of antioxidants–a mini review. J. Funct. Foods 18, 811-819.

Engels, W.J.M., Dekker, R., De Jong, C., Neeter, R., Visser, S., 1997. A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese. Int. Dairy J. 7, 255-263.

EURL *Lm* (European Union Reference Laboratory for *Listeria monocytogenes*), 2021. Technical guidance document on challenge tests and durability studies for assessing shelf-life of ready-to-eat foods related to *Listeria monocytogenes*. Version 4 of 1 July 2021. Available online: https://ec.europa.eu/food/system/files/2021-07/biosafety_fh_mc_tech-guide-doc_listeria-in-rte-foods_en_0.pdf.

FAO/WHO (2001) Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a joint FAO/WHO expert consultation, Cordoba

Farag, N.F., Farag, M.A., Abdelrahman, E.H., Azzam, S.M., El-Kashoury, E.S.A., 2015. Metabolites profiling of *Chrysanthemum pacificum* Nakai parts using UPLC-PDA-MS coupled to chemometrics. Nat. Prod. Res. 29, 1342-1349.

Fermanian, C., Lapeyre, C., Fremy, J.M., Claisse, M., 1997. Diarrhoeal toxin production at low temperature by selected strains of *Bacillus cereus*. J. Dairy Res. 64, 551-559.

Fernández-Díez, M.J, de Castro, R., Garrido Fernández, A., González Cancho, F., González Pellisso, F., Nosti Vega, M., Heredia Moreno, A., Mosquera, I.M., Rejano Navarro, L., Durán Quintana, M.C., 1985. Green table olives. In: Derivados I.d.l.G.y.s. (Ed.), Biotechnology of Table Olives. CSIC, Madrid, pp. 53-123.

Fessard, A., Remize, F., 2017. Why are *Weissella* spp. not used as commercial starter cultures for food fermentation?. Fermentation 3, 38.

Finlay, W.J.J., Logan, N.A., Sutherland, A.D., 1999. Semiautomated metabolic staining assay for *Bacillus cereus* emetic toxin. Appl. Environ. Microbiol. 65, 1811-1812.

Franke, W., 1982. Vitamin C in sea fennel (*Crithmum maritimum*), an edible wild plant. Econ. Bot. 36, 163-165.

Galanakis, C.M., 2021. Functionality of food components and emerging technologies. Foods 10, 128.

Gann, L.D., 2013. Antimicrobial activity of essential oils and their components against lactic acid bacteria. Master's Thesis, University of Tennessee.

García-Casal, M.N., Peña-Rosas, J.P., Malavé, H.G., 2016. Sauces, spices, and condiments: definitions, potential benefits, consumption patterns, and global markets. Ann. N. Y. Acad. Sci. 1379, 3-16.

García-Villalba, R., Espín, J.C., Tomás-Barberán, F.A., Rocha-Guzmán, N.E., 2017. Comprehensive characterization by LC-DAD-MS/MS of the phenolic composition of seven *Quercus* leaf teas. J. Food Compos. Anal. 63, 38-46.

Gardner, N.J., Savard, T., Obermeier, P., Caldwell, G., Champagne, C.P., 2001. Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. Int. J. Food Microbiol. 64, 261-275.

Garofalo, C., Bancalari, E., Milanović, V., Cardinali, F., Osimani, A., Savo Sardaro, M.L., Bottari, B., Bernini, V., Aquilanti, L., Clementi, F., Neviani, E., Gatti, M., 2017. Study of the bacterial diversity of foods: PCR-DGGE versus LH-PCR. Int. J. Food Microbiol. 242, 24-36.

Garrido-Fernández, A., Fernandez-Diez, M.J., Adams, M.R., 1997. Table olives: production and processing. Chapman & Hall, London.

Generalić Mekinić, I., Blažević, I., Mudnić, I., Burčul, F., Grga, M., Skroza, D., Jerčić, I., Ljubenkov, I., Boban, M., Miloš, M., Katalinić, V., 2016. Sea fennel (*Crithmum maritimum* L.): phytochemical profile, antioxidative, cholinesterase inhibitory and vasodilatory activity. J. Food Sci. Technol. 53, 3104-3112.

Generalić Mekinić, I., Šimat, V., Ljubenkov, I., Burčul, F., Grga, M., Mihajlovski, M., Lončar, R., Katalinić, V., Skroza, D., 2018. Influence of the vegetation period on sea fennel, *Crithmum maritimum* L. (Apiaceae), phenolic composition, antioxidant and anticholinesterase activities. Ind. Crops Prod. 124, 947-953.

Giungato, P., Renna, M., Rana, R., Licen, S., Barbieri, P., 2019. Characterization of dried and freeze-dried sea fennel (*Crithmum maritimum* L.) samples with headspace gas-chromatography/mass spectrometry and evaluation of an electronic nose discrimination potential. Food Res Int. 115, 65-72.

Gouvea, D.R., Buqui, G.A., Lopes, J.L.C., Diniz, A., Lopes, N.P., 2017. An UPLC-MS/MS method for determination of vicenin-2 and lychnopholic acid in rat plasma and its application to a pharmacokinetic study. J. Braz. Chem. Soc. 28, 427-434.

Granato, D., Branco, G.F., Nazzaro, F., Cruz, A.G., Faria, J.A., 2010. Functional foods and nondairy probiotic food development: trends, concepts, and products. Compr. Rev. Food Sci. Food Saf. 9, 292-302.

Granato, D., do Prado-Silva, L., Alvarenga, V.O., Zielinski, A.A.F., Bataglion, G.A., de Morais, D.R., Eberlin, M.N., Sant'Ana, A. de S., 2016. Characterization of binary and ternary mixtures of green, white and black tea extracts by electrospray ionization mass spectrometry and modeling of their in vitro antibacterial activity. LWT - Food Sci. Technol. 65, 414-420.

Granum, P. E., Lund, T., 1997. *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett. 157, 223-228.

Gras, A., Garnatje, T., Marín, J., Parada, M., Sala, E., Talavera, M., Vallès, J., 2021. The power of wild plants in feeding humanity: a meta-analytic ethnobotanical approach in the catalan linguistic area. Foods 10, 61.

Gu, G., Ottesen, A., Bolten, S., Wang, L., Luo, Y., Rideout, S., Lyu, S., Nou, X., 2019. Impact of routine sanitation on the microbiomes in a fresh produce processing facility. Int. J. Food Microbiol. 294, 31-41.

Guarrera, P.M., 2006. Usi e tradizioni della flora italiana. Medicina popolare e etnobotanica, Aracne editrice, Rome.

Güçlü-Üstündağ, Ö., Mazza, G., 2007. Saponins: Properties, applications and processing. Crit. Rev. Food Sci. Nutr. 47, 231-258.

Guil-Guerrero, J.L., Gimenez-Martinez, J.J., Torija-Isasa, M.E., 1998. Mineral nutrient composition of edible wild plants. J. Food Compos. Anal. 11, 322-328.

Guil-Guerrero, J.L., Rodríguez-García, I., 1999. Lipids classes, fatty acids and carotenes of the leaves of six edible wild plants. Eur. Food Res. Technol. 209, 313-316.

Guil-Guerrero, J.L., Rodríguez-García, I., Torija-Isasa, M.E., 1997. Nutritional and toxic factors in selected wild edible plants. Plant Foods Hum. Nutr. 51, 99-107.

Guil-Guerrero, J., Torija-Isasa, M.E., 2002. Edible wild plants. In: Majundar, D.K., Govil, J.N., Singh, V.K. (Eds.), Recent progress in medicinal plants, vol. VIII. SciTech Publising LLC, Texas, pp. 431-466.

Guil-Guerrero, J.L., Torija-Isasa, M.E., Gimenez-Martinez, J.J., 1996a. Nutritional composition of rock samphire (*Crithmum maritimum* L.). Alimentaria (Espana). 272, 65-72.

Guil-Guerrero, J.L., Torija-Isasa, M.E., Gimenez-Martinez, J.J., Rodríguez-García, I., 1996b. Identification of fatty acids in edible wild plants by gas chromatography. J. Chromatogr. A. 719, 229-235.

Han, X., Shen, T., Lou, H., 2007. Dietary polyphenols and their biological significance. Int. J. Mol. Sci. 8, 950-988.

Haouet, M.N., Altissimi, M.S., Mercuri, M.L., Baldassarri, C., Osimani, A., Clementi, F., Ortenzi, R., 2017. Evaluation of the safety of Milano-type dry fermented sausages produced by a fast drying technology. Ital. J. Food Sci. 29, 1-9.

Heindl, A.G., Müller, J., 2007. Microwave drying of medicinal and aromatic plants. Stewart Postharvest Rev. 4,5.

Hennekinne, J.A., De Buyser, M.L., Dragacci, S., 2012. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiol. Rev. 36, 815-836.

Heperkan, D., 2013. Microbiota of table olive fermentations and criteria of selection for their use as starters. Front. Microbiol. 4, 143.

Heyndrickx, M., 2011. The importance of endospore-forming bacteria originating from soil for contamination of industrial food processing. Appl. Environ. Soil Sci. Volume 2011, Article ID 561975.

Hong, Y., Yang, H.-S., Chang, H.-C., Kim, H.-Y., 2013. Comparison of bacterial community changes in fermenting *kimchi* at two different temperatures using a denaturing gradient gel electrophoresis analysis. J. Microbiol. Biotechnol. 23, 76-84.

Houta, O., Akrout, A., Najja, H., Neffati, M., Amri, H., 2015. Chemical composition, antioxidant and antimicrobial activities of essential oil from *Crithmum maritimum* cultivated in Tunisia. J. Essent. Oil-Bear. Plants 18, 1459-1466.

Hurtado, A., Reguant, C., Bordons, A., Rozès, N., 2012. Lactic acid bacteria from fermented table olives. Food Microbiol. 31, 1-8.

Hurtado, A., Reguant, C., Esteve-Zarzoso, B., Bordons, A., Rozès, N., 2008. Microbial population dynamics during the processing of Arbequina table olives. Food Res. Int. 41, 738-744.

Hynes, W.L., Ferretti, J.J., Gilmore, M.S., Segarra, R.A., 1992. PCR amplification of streptococcal DNA using crude cell lysates. FEMS Microbiol. Lett. 94, 139-142.

ICMSF (International Commission on Microbiological Specifications for Foods), 1996a. *Clostridium botulinum*. In: Roberts, T.A., Baird-Parker, A.C., Tompkin, R.B. (Eds.), Micro-organisms in foods 5 Microbiological specifications of food pathogens. Blackie Academic and Professional, London, pp. 66-111.

ICMSF (International Commission on Microbiological Specifications for Foods), 1996b. *Bacillus cereus*. In: Roberts, T.A., Baird-Parker, A.C., Tompkin, R.B. (Eds.), Micro-organisms in foods 5 Microbiological specifications of food pathogens. Blackie Academic and Professional, London, pp. 20-35.

Inukai, S., Kikuchi, J., Watanabe, T., 1984. Antimicrobial action of salts of organic acids on genus *Bacillus*, 2: release of divalent cations from *Bacillus* spores treated with sodium citrate. Shokuhin Eiseigaku Zasshi 25, 132-136.

IOOC (International Olive Oil Council), 2022a. Economic affairs and promotion unit. European table olive production. Available online: https://www.internationaloliveoil.org/wp-content/uploads/2021/12/OT-CE-901-07-12-2021-P.pdf

IOOC (International Olive Oil Council), 2022b. Economic affairs and promotion unit. World table olive production. Available online: https://www.internationaloliveoil.org/wp-content/uploads/2021/12/OT-W901-07-12-2021-P.pdf

IOOC (International Olive Oil Council), 2004. Trade Standard Applying to Table Olives. IOOC. Available online: https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-OT-NC1-2004-Eng.pdf

Jaehae, L., Kyeongsoon, H., Chaelin, P., Ilgwon, K., Jeongwon, K., Dukno, Y., Montanari, M., Naomichi, I., Cwiertka, K.J., Younghee, S., et al., 2015. Humanistic Understanding of Kimchi and Kimjang Culture (1st ed.). World Institute of Kimchi, Gwangju, Korea.

Jaiswal, R., Müller, H., Müller, A., Karar, M.G.E., Kuhnert, N., 2014. Identification and characterization of chlorogenic acids, chlorogenic acid glycosides and flavonoids from *Lonicera henryi* L. (Caprifoliaceae) leaves by LC-MS^n. Phytochemistry 108, 252-263.

Jallali, I., Zaouali, Y., Missaoui, I., Smeoui, A., Abdelly, C., Ksouri, R., 2014. Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and *Inula crithmoïdes* L. Food Chem. 145, 1031-1038.

Jang, D.-J., Chung, K.R., Yang, H.J., Kim, K.-S., Kwon, D.Y., 2015. Discussion on the origin of *kimchi*, representative of Korean unique fermented vegetables. J. Ethn. Foods. 2, 126-136.

Jayamanne, V.S., Adams, M.R., 2006. Determination of survival, identity and stress resistance of probiotic bifidobacteria in bio-yoghurts. Lett. Appl. Microbiol. 42, 189-194.

Jenson, I., Moir, C.J., 2003. *Bacillus cereus* and other *Bacillus* species. In: Hocking, A.D. (Ed.), Foodborne microorganisms of public health significance (6^{th} ed.). Australian Institute of Food Science and Technology (NSW Branch), Sydney, pp. 445-478.

Jeong, S.H., Jung, J.Y., Lee, S.H., Jin, H.M., Jeon, C.O., 2013. Microbial succession and metabolite changes during fermentation of dongchimi, traditional Korean watery kimchi. Int. J. Food Microbiol. 164, 46-53.

Jesionek, W., Majer-Dziedzic, B., Choma, I.M., 2015. Separation, identification, and investigation of antioxidant ability of plant extract components using TLC, LC-MS, and TLC-DPPH•. J. Liq. Chromatogr. Relat. Technol. 38, 1147-1153.

Jiang, S., Cai, L., Lv, L., Li, L., 2021. *Pediococcus pentosaceus*, a future additive or probiotic candidate. Microb. Cell Fact. 20, 45.

Jin, W., Mujumdar, A.S., Zhang, M., Shi, W., 2018. Novel drying techniques for spices and herbs: a review. Food Eng. Rev. 10, 34-45.

Jo, S.Y., Choi, E.A., Lee, J.J., Chang, H.C., 2015. Characterization of starter kimchi fermented with *Leuconostoc kimchi* GJ2 and its cholesterol-lowering effects in rats fed a high-fat and high-cholesterol diet. J. Sci. Food Agric. 95, 2750-2756.

Jung, J.Y., Jeong, J.-W., Lee, S.-Y., Jin, H.M., Choi, H.W., Ryu, B.-G., Han, S.-S., Kang, H.K., Chung, E.J., Choi, K.-M., 2019. Complete genome sequence of *Leuconostoc kimchii* strain NKJ218, isolated from homemade kimchi. Microbiol. Resour. Announc. 8, e00367-19.

Jung, M.Y., Kim, T.-W., Lee, C., Kim, J.Y., Song, H.S., Kim, Y.B., Ahn, S.W., Kim, J.S., Roh, S.W., Lee, S.H., 2018. Role of jeotgal, a Korean traditional fermented fish sauce, in microbial dynamics and metabolite profiles during kimchi fermentation. Food Chem. 265, 135-143.

Jung, J.Y., Lee, S.H., Jeon, C.O., 2014. Kimchi microflora: history, current status, and perspectives for industrial kimchi production. Appl. Microbiol. Biotechnol. 98, 2385-2393.

Jung, J.Y., Lee, S.H., Kim, J.M., Park, M.S., Bae, J.-W., Hahn, Y., Madsen, E.L., Jeon, C.O., 2011. Metagenomic analysis of kimchi, a traditional Korean fermented food. App. Environ. Microbiol. 77, 2264-2274.

Kabir, M.N., Aras, S., George, J., Wadood, S., Chowdhury, S., Fouladkhah, A.C., 2021. High-pressure and thermal-assisted pasteurization of habituated, wild-type, and pressure-stressed *Listeria monocytogenes*, *Listeria innocua*, and *Staphylococcus aureus*. LWT - Food Sci. Technol. 137, 110445.

Kadariya, J., Smith, T.C., Thapaliya, D., 2014. *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. Biomed Res. Int. Volume 2014, Article ID 827965.

Kado C.I., 2006. *Erwinia* and related genera. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, NY, pp. 443-450.

Kahala, M., Blasco, L., Joutsjoki, V., 2012. Molecular characterization of spoilage bacteria as a means to observe the microbiological quality of carrot. J. Food Prot. 75, 523-532.

Kailis, S.G., Kiritsakis, A., 2017. Table olives: processing, nutritional, and health implications. In: Shahidi, F., Kiritsakis, A., (Eds.), Olives and Olive Oil as Functional Foods: Bioactivity, Chemistry and Processing. John Wiley & Sons Ltd, Hoboken, NJ, pp. 295-324.

Kathirvel, K., Naik, K.R., Gariepy, Y., Orsat, V., Raghavan, G.S.V., 2006. Microwave drying-a promising alternative for the herb processing industry. 2006 ASAE Annual Meeting. American Society of Agricultural and Biological Engineers.

Kennedy, J., Blair, I.S., McDowell, D.A., Bolton, D.J., 2005. An investigation of the thermal inactivation of *Staphylococcus aureus* and the potential for increased thermotolerance as a result of chilled storage. J. Appl. Microbiol. 99, 1229-1235.

Kiai, H., Hafidi, A., 2014. Chemical composition changes in four green olive cultivars during spontaneous fermentation. LWT - Food Sci. Technol. 57, 663-670.

Kim, E., Cho, Y., Lee, Y., Han, S.-K., Kim, C.-G., Choo, D.-W., Kim, Y.-R., Kim, H.-Y. A., 2017. Proteomic approach for rapid identification of *Weissella* species isolated from Korean fermented foods on MALDI-TOF MS supplemented with an in-house database. Int. J. Food Microbiol. 243, 9-15.

Kim, M.-J., Lee, H.-W., Kim, J.Y., Kang, S.E., Roh, S.W., Hong, S.W., Yoo, S.R., Kim, T.-W., 2020. Impact of fermentation conditions on the diversity of white colony-forming yeast and analysis of metabolite changes by white colony-forming yeast in kimchi. Food Res. Int. 136, 109315.

Kim, S.Y., Shin, S.J., Song, C.H., Jo, E.K., Kim, H.J., Park, J.K., 2009. Destruction of *Bacillus licheniformis* spores by microwave irradiation. J. Appl. Microbiol. 106, 877-885.

Kleerebezem, M., Binda, S., Bron, P.A., Gross, G., Hill, C., van Hylckama Vlieg, J.E., Lebeer, S., Satokari, R., Ouwehand, A.C., 2019. Understanding mode of action can drive the translational pipeline towards more reliable health benefits for probiotics. Curr. Opin. Biotechnol. 56, 55-60.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1.

Kothari, D., Lee, W. Do, Jung, E.S., Niu, K.M., Lee, C.H., Kim, S.K., 2020. Controlled fermentation using autochthonous *Lactobacillus plantarum* improves antimicrobial potential of Chinese chives against poultry pathogens. Antibiotics 9, 1-17.

Koyro, H.W., Khan, M.A., Lieth, H., 2011. Halophytic crops: a resource for the future to reduce the water crisis?. Emir. J. Food Agric. 23, 01-16.

Kramer, J.M., Gilbert, R.J., 1989. *Bacillus cereus* and other *Bacillus* species. In: Doyle, M.P. (Ed.), Foodborne Bacterial Pathogens. Marcel Dekker, New York, pp. 21-70.

Ksouri, R., Ksouri, W.M., Jallali, I., Debez, A., Magné, C., Hiroko, I., Abdelly, C., 2012. Medicinal halophytes: potent source of health promoting biomolecules with medical, nutraceutical and food applications. Crit. Rev. Biotechnol. 32, 289-326.

Kulisic-Bilusic, T., Blažević, I., Dejanović, B., Miloš, M., Pifat, G., 2010. Evaluation of the antioxidant activity of essential oils from caper (*Capparis spinosa*) and sea fennel (*Crithmum maritimum*) by different methods. J. Food Biochem. 34, 286-302.

Kumar, K., Srivastav, S., Sharanagat, V.S., 2021. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrason. Sonochem. 70, 105325.

Kumar, S., Chandra, P., Bajpai, V., Singh, A., Srivastava, M., Mishra, D.K., Kumar, B., 2015. Rapid qualitative and quantitative analysis of bioactive compounds from *Phyllanthus amarus* using LC/MS/MS techniques. Ind. Crops Prod. 69, 143-152.

Lavermicocca, P., Rossi, M., Russo, F., Srirajaskanthan, R., 2010. Table olives: a carrier for delivering probiotic bacteria to humans. In: Preedy, V.R., Watson, R.R., Olives and Olive Oil in Health and Disease Prevention. Academic Press, Cambridge, Massachusetts, pp. 735-743.

Leal-Sánchez, M.V., Ruiz-Barba, J.L., Sánchez, A.H., Rejano, L., Jiménez-Dıaz, R., Garrido, A., 2003. Fermentation profile and optimization of green olive fermentation using *Lactobacillus plantarum* LPCO10 as a starter culture. Food Microbiol. 20, 421-430.

Leavitt, S.D., Fankhauser, J.D., Leavitt, D.H., Porter, L.D., Johnson, L.A., St. Clair, L.L., 2011. Complex patterns of speciation in cosmopolitan "rock posy" lichens–Discovering and delimiting cryptic fungal species in the lichen-forming Rhizoplaca melanophthalma species-complex (Lecanoraceae, Ascomycota). Mol. Phylogenetics Evol. 59, 587-602.

Lechtenberg, M., Zumdick, S., Gerhards, C., Schmidt, T.J., Hensel, A., 2007. Evaluation of analytical markers characterising different drying methods of parsley leaves (*Petroselinum crispum* L.). Pharmazie-Int. J. Pharm. Sci. 62, 949-954.

Lee, H.-W., Yoon, S.-R., Kim, S.-J., Lee, H.M., Lee, J.Y., Lee, J.-H., Kim, S.H., Ha, J.-H., 2017a. Identification of microbial communities, with a focus on foodborne pathogens, during kimchi manufacturing process using culture-independent and -dependent analyses. LWT - Food Sci. Technol. 81, 153-159.

Lee, K.-H., Bong, Y.-J., Lee, H.A., Kim, H.-Y., Park, K.-Y., 2016. Probiotic effects of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* isolated from kimchi. J. Korean Soc. Food Sci. Nutr. 45, 12-19.

Lee, M., Song, J.H., Jung, M.Y., Lee, S.H., Chang, J.Y., 2017b. Large-scale targeted metagenomics analysis of bacterial ecological changes in 88 kimchi samples during fermentation. Food Microbiol. 66, 173-183.

Lee, M., Song, J.H., Park, J.M., Chang, J.Y., 2019. Bacterial diversity in Korean temple kimchi fermentation. Food Res. Int. 126, 108592.

Lee, M.-E., Jang, J.-Y., Lee, J.-H., Park, H.-W., Choi, H.-J., Kim, T.-W., 2015a. Starter cultures for kimchi fermentation. J. Microbiol. Biotechnol. 25, 559-568.

Lee, N.-K., Han, K.J., Son, S.-H., Eom, S.J., Lee, S.-K., Paik, H.-D., 2015b. Multifunctional effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi. LWT - Food Sci. Technol. 64, 1036-1041.

Lee, S.H., Jung, J.Y., Lee, S.H., Jeon, C.O., 2011. Complete genome sequence of *Leuconostoc kimchii* strain C2, isolated from Kimchi. J. Bacteriol. 193, 5548.

Leistner, L., Gorris, L.G., 1995. Food preservation by hurdle technology. Trends Food Sci. Technol. 6, 41-46.

Leser, T.D., Knarreborg, A., Worm, J., 2008. Germination and outgrowth of *Bacillus subtilis* and *Bacillus licheniformis* spores in the gastrointestinal tract of pigs. J. Appl. Microbiol. 104, 1025-1033.

Li, J., Zhang, S., Zhang, M., Sun, B., 2019. Novel approach for extraction of grape skin antioxidants by accelerated solvent extraction: Box-Behnken design optimization. J. Food Sci. Technol. 56, 4879-4890.

Lillo-Pérez, S., Guerra-Valle, M., Orellana-Palma, P., Petzold, G., 2021. Probiotics in fruit and vegetable matrices: opportunities for nondairy consumers. LWT - Food Sci. Technol. 151, 112106.

Linares-Morales, J.R., Gutiérrez-Méndez, N., Rivera-Chavira, B.E., Pérez-Vega, S.B., Nevárez-Moorillón, G.V., 2018. Biocontrol processes in fruits and fresh produce, the use of lactic acid bacteria as a sustainable option. Front. Sustain. Food Syst., 2, 50.

Liu, J., Henkel, T., 2002. Traditional Chinese Medicine (TCM): Are polyphenols and saponins the key ingredients triggering biological activities? Curr. Med. Chem. 9, 1483-1485.

Liu, X., Narbad, A., 2018. Lactic acid bacteria-based food fermentations. In Chen, W., Narbard A. (Eds.). Lactic Acid Bacteria in Foodborne Hazards Reduction. Springer, Singapore, pp. 141-181.

Liu, L.H., Wang, N.Y., Wu, A.Y.J., Lin, C.C., Lee, C.M., Liu, C.P., 2018. *Citrobacter freundii* bacteremia: risk factors of mortality and prevalence of resistance genes. J. Microbiol. Immunol. Infect. 51, 565-572.

Lu, H., Tian, Z., Cui, Y., Liu, Z., Ma, X., 2020. Chlorogenic acid: a comprehensive review of the dietary sources, processing effects, bioavailability, beneficial properties, mechanisms of action, and future directions. Compr. Rev. Food Sci. Food Saf. 19, 3130-3158.

Lucchetti, L., Zitti, S., Taffetani, F., 2019. Ethnobotanical uses in the Ancona district (Marche region, Central Italy). J. Ethnobiol. Ethnomed. 15, 1-33.

Lucena-Padrós, H., Jiménez, E., Maldonado-Barragán, A., Rodríguez, J.M., Ruiz-Barba, J.L., 2015. PCR-DGGE assessment of the bacterial diversity in Spanish-style green table-olive fermentations. Int. J. Food Microbiol. 205, 47-53.

Magnusson, J., Jonsson, H., Schnürer, J., Roos, S., 2002. *Weissella soli* sp. nov., a lactic acid bacterium isolated from soil. Int. J. Syst. Evol. Microbiol. 52, 831-834.

Majcherczyk, J., Surówka, K., 2019. Effects of onion or caraway on the formation of biogenic amines during sauerkraut fermentation and refrigerated storage. Food Chem. 298, 125083.

Maleš, Z., Zuntar, I., Nigović, B., Plazibat, M., Vundać, V. B., 2003. Quantitative analysis of the polyphenols of the aerial parts of rock samphire - *Crithmum maritimum* L. Acta Pharm. 53, 139-144.

Maoloni, A., Milanović, V., Osimani, A., Cardinali, F., Garofalo, C., Belleggia, L., Foligni, R., Mannozzi, C., Mozzon, M., Cirlini, M., Spaggiari, M., Reale, A., Boscaino, F., Di Renzo, T., Haouet, M.N., Staccini, B., Di Bella, S., Aquilanti, L., 2021. Exploitation of sea fennel (*Crithmum maritimum* L.) for manufacturing of novel high-value fermented preserves. Food Bioprod. Process. 127, 174-197.

Marsh, G. (1938). Buffering action of nonacid vegetables. Hilgardia 11, 315-341.

Marsilio, V., Seghetti, L., Iannucci, E., Russi, F., Lanza, B., Felicioni, M., 2005. Use of a lactic acid bacteria starter culture during green olive (*Olea europaea* L cv Ascolana tenera) processing. J. Sci. Food Agric. 85, 1084-1090.

Martinez, R.C.R., Bedani, R., Saad, S.M.I., 2015. Scientific evidence for health effects attributed to the consumption of probiotics and prebiotics: an update for current perspectives and future challenges. Br. J. Nutr. 114, 1993-2015.

Martins-Noguerol, R., Matías, L., Pérez-Ramos, I.M., Moreira, X., Muñoz-Vallés, S., Mancilla-Leytón, J.M., Francisco, M., García-González, A., DeAndrés-Gil, C., Martínez-Force, E., Millán-Linares, M.D.L., Pedroche, J., Figueroa, M.E., Moreno-Pérez, A.J., Cambrollé, J., 2022a. Differences in nutrient composition of sea fennel (*Crithmum maritimum*) grown in different habitats and optimally controlled growing conditions. J. Food Compos. Anal. 106, 104266.

Martins-Noguerol, R., Pérez-Ramos, I.M., Matías, L., Moreira, X., Francisco, M., García-González, A., Troncoso-Ponce, A.M., Thomasset, B., Martínez-Force, E., Moreno-Pérez, A.J., Cambrollé, J., 2022b. *Crithmum maritimum* seeds, a potential source for high-quality oil and phenolic compounds in soils with no agronomical relevance. J. Food Compos. Anal. 104413.

Masoodi, M., Mir, A.A., Petasis, N.A., Serhan, C.N., Nicolaou, A., 2008. Simultaneous lipidomic analysis of three families of bioactive lipid mediators leukotrienes, resolvins, protectins and related hydroxy-fatty acids by liquid chromatography/electrospray ionisation tandem mass spectrometry. Rapid Commun. Mass Spectrom. 22, 75-83.

Medveďová, A., Valík, Ľ., 2012. *Staphylococcus aureus*: characterisation and quantitative growth description in milk and artisanal raw milk cheese production. In: Eissa, A.A. (Ed.), Structure and Function of Food Engineering. IntechOpen, Rijeka, Croatia, pp. 71-102.

Menon, R.R., Kumari, S., Kumar, P., Verma, A., Krishnamurthi, S., Rameshkumar, N., 2019. *Sphingomonas pokkalii* sp. nov., a novel plant associated rhizobacterium isolated from a saline tolerant pokkali rice and its draft genome analysis. Syst. App. Microbiol. 42, 334-342.

Meot-Duros, L., Cérantola, S., Talarmin, H., Le Meur, C., Le Floch, G., Magné, C., 2010. New antibacterial and cytotoxic activities of falcarindiol isolated in *Crithmum maritimum* L. leaf extract. Food Chem. Toxicol. 48, 553-557.

Meot-Duros, L., Le Floch, G., Magné, C., 2008. Radical scavenging, antioxidant and antimicrobial activities of halophytic species. J. Ethnopharmacol. 116, 258-262.

Meot-Duros, L., Magné, C., 2009. Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. Plant Physiol Biochem. 47, 37-41.

Merialdi, G., Ramini, M., Parolari, G., Barbuti, S., Frustoli, M.A., Taddei, R., Pongolini, S., Ardigò, P., Cozzolino, P., 2016. Study on potential *Clostridium botulinum* growth and toxin production in Parma ham. Ital. J. Food Saf. 5, 69-74.

Mheen, T.I., Kwon, T.W., 1984. Effect of temperature and salt concentration on kimchi fermentation. Korean J. Food Sci. Technol. 16, 443-449.

Micioni Di Bonaventura, M.V., Coman, M.M., Tomassoni, D., Micioni Di Bonaventura, E., Botticelli, L., Gabrielli, M.G., Rossolini, G.M., Di Pilato, V., Cecchini, C., Amedei, A., Silvi, S., Verdenelli, M.C., Cifani, C., 2021. Supplementation with *Lactiplantibacillus plantarum* IMC 510 modifies microbiota composition and prevents body weight gain induced by cafeteria diet in rats. Int. J. Mol. Sci. 22, 11171.

Milanović, V., Osimani, A., Garofalo, C., Belleggia, L., Maoloni, A., Cardinali, F., Mozzon, M., Foligni, R., Aquilanti, L., Clementi, F., 2020. Selection of cereal-sourced lactic acid bacteria as candidate starters for the baking industry. Plos One, 15, e0236190.

Min, M., Bunt, C.R., Mason, S.L., Hussain, M.A., 2019. Non-dairy probiotic food products: an emerging group of functional foods. Crit. Rev. Food Sci. Nutr. 59, 2626-2641.

Misihairabgwi, J., Cheikhyoussef, A., 2017. Traditional fermented foods and beverages of Namibia. J. Ethn. Foods. 4, 145-153.

Mols, M., Pier, I., Zwietering, M.H., Abee, T., 2009. The impact of oxygen availability on stress survival and radical formation of *Bacillus cereus*. Int. J. Food Microbiol. 135, 303-311.

Montaño, A., Bobillo, M., Marshall, V.M., 1993. Effect of sodium chloride on metabolism of two strains of *Lactobacillus plantarum* isolated from fermenting green olives. Lett. Appl. Microbiol. 16, 315-318.

Montaño, A., Sánchez, A.H., López-López, A., de Castro, A., Rejano, L., 2010. Chemical composition of fermented green olives: acidity, salt, moisture, fat, protein, ash, fiber, sugar, and polyphenol. In Preedy, V.R., Watson, R.R. (Eds.). Olives and Olive Oil in Health and Disease Prevention. Academic Press, Cambridge, Massachusetts, pp. 291-297.

Montesano, F.F., Gattullo, C.E., Parente, A., Terzano, R., Renna, M., 2018. Cultivation of potted sea fennel, an emerging Mediterranean halophyte, using a renewable seaweed-based material as a peat substitute. Agriculture 8, 96.

Montville, T.J., Matthews, K.R., 2008. Food microbiology: an introduction (2nd ed.). ASM Press, Washington D.C.

Mota-Gutierrez, J., Ferrocino, I., Rantsiou, K., Cocolin, L., 2019. Metataxonomic comparison between internal transcribed spacer and 26S ribosomal large subunit (LSU) rDNA gene. Int. J. Food Microbiol. 290, 132-140.

Mozzon, M., Foligni, R., Mannozzi, C., 2020a. Brewing quality of hop varieties cultivated in central Italy based on multivolatile fingerprinting and bitter acid content. Foods 9, 541.

Mozzon, M., Foligni, R., Mannozzi, C., Zamporlini, F., Raffaelli, N., Aquilanti, L., 2020b. Clotting properties of *Onopordum tauricum* (Willd.) aqueous extract in milk of different species. Foods 9, 692.

Mozzon, M., Pacetti, D., Frega, N.G., Lucci, P., 2015. Crude palm oil from interspecific hybrid *Elaeis oleifera*× *E. guineensis*: alcoholic constituents of unsaponifiable matter. J. Am. Oil Chem.' Soc. 92, 717-724.

Muck, R.E., 2010. Silage microbiology and its control through additives. Rev. Bras. Zootec. 39, 183-191.

Nabet, N., Boudries, H., Chougui, N., Loupassaki, S., Souagui, S., Burló, F., Hernández, F., Carbonell-Barrachina, A.A., Madani, K., Larbat, R., 2017. Biological activities and secondary compound composition from *Crithmum maritimum* aerial parts. Int. J. Food Prop. 20, 1843-1855.

NACMCF (National Advisory Committee on Microbiological Criteria for Foods), 2010. Parameters for determining inoculated pack/challenge study protocols. J. Food Protect. 73, 140-202.

Najdovski, L., Dragaš, A.Z., Kotnik, V., 1991. The killing activity of microwaves on some non-sporogenic and sporogenic medically important bacterial strains. J. Hosp. Infect. 19, 239-247.

Najjaa, H., Abdelkarim, B.A., Doria, E., Boubakri, A., Trabelsi, N., Falleh, H., Tlili, H., Neffati, M., 2020. Phenolic composition of some Tunisian medicinal plants associated with anti-proliferative effect on human breast cancer MCF-7 cells. EuroBiotech J. 4, 104-112.

Naveed, M., Hejazi, V., Abbas, M., Kamboh, A.A., Khan, G.J., Shumzaid, M., Ahmad, F., Babazadeh, D., FangFang, X., Modarresi-Ghazani, F., WenHua, L., XiaoHui, Z., 2018. Chlorogenic acid (CGA): a pharmacological review and call for further research. Biomed. Pharmacother. 97, 67-74.

Necidova, L., Bogdanovicova, K., Harustiakova, D., Bartova, K., 2016. Pasteurization as a means of inactivating staphylococcal enterotoxins A, B, and C in milk. J. Dairy Sci. 99, 8638-8643.

Nishihara, T., Kanoh, Y., Uchida, Y., Kondo, M., 1981. Studies on the bacterial spore coat.(10) Role of surface metals in germination of *Bacillus megaterium* spores. Nippon Saikingaku Zasshi 36, 767-774.

Oh, H.-M., Cho, Y.-J., Kim, B.K., Roe, J.-H., Kang, S.-O., Nahm, B.H., Jeong, G., Han, H.-U., Chun, J., 2010. Complete genome sequence analysis of *Leuconostoc kimchii* IMSNU 11154. J. Bacteriol. 192, 3844-3845.

Ohloff, G., Flament, I., Pickenhagen, W., 1985. Flavor chemistry. Food Rev. Int. 1, 99-148.

Osimani, A., Garofalo, C., Aquilanti, L., Milanović, V., Clementi, F., 2015. Unpasteurised commercial boza as a source of microbial diversity. Int. J. Food Microbiol. 194, 62-70.

Osimani, A., Milanović, V., Cardinali, F., Garofalo, C., Clementi, F., Pasquini, M., Riolo, P., Ruschioni, S., Isidoro, N., Loreto, N., Franciosi, E., Tuohy, K., Petruzzelli, A., Foglini, M.,

Gabucci, C., Tonucci, F., Aquilanti, L., 2018a. The bacterial biota of laboratory-reared edible mealworms (*Tenebrio molitor* L.): from feed to frass. Int. J. Food Microbiol. 272, 49-60.

Osimani, A., Aquilanti, L., Clementi, F., 2018b. *Bacillus cereus* foodborne outbreaks in mass catering. Int. J. Hosp. Manag. 72, 145-153.

Osimani, A., Zannini, E., Aquilanti, L., Mannazzu, I., Comitini, F., Clementi, F., 2009. Lactic acid bacteria and yeasts from wheat sourdoughs of the Marche region. Ital. J. Food Sci. 21, 269-286.

Othman, N.B., Roblain, D., Chammen, N., Thonart, P., Hamdi, M., 2009. Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. Food Chem. 116, 662-669.

Oyama-Okubo, N., Tsuji, T., 2013. Analysis of floral scent compounds and classification by scent quality in tulip cultivars. J. Jpn. Soc. Hortic. Sci. 82, 344-353.

Özcan, M., 2000. The use of yogurt as starter in rock samphire (*Crithmum maritimum* L.) fermentation. Eur. Food Res. Technol. 210, 424-426.

Özcan, M.M., Pedro, L.G., Figueiredo, A.C., Barroso, J.G., 2006. Constituents of the essential oil of sea fennel (*Crithmum maritimum* L.) growing wild in Turkey. J. Med. Food 9, 128-130.

Özcan, M.M., Uslu, N., Figueredo, G., Juhaimi, F.A., Ghafoor, K., Babiker, E.E., Alsawmahi, O.N., Özcan, M.M., Ahmed, I.A.M., 2019. The effect of fermentation process on bioactive properties, essential oil composition and phenolic constituents of raw fresh and fermented sea fennel (*Crithmum maritimum* L.) leaves. Indian J. Tradit. Knowl. (IJTK), 18, 800-804.

Pacifico, S., Galasso, S., Piccolella, S., Kretschmer, N., Pan, S.P., Nocera, P., Lettieri, A., Bauer, R., Monaco, P., 2018. Winter wild fennel leaves as a source of anti-inflammatory and antioxidant polyphenols. Arab. J. Chem. 11, 513-524.

Pahariya, P., Fisher, D.J., Choudhary, R., 2022. Comparative analyses of sanitizing solutions on microbial reduction and quality of leafy greens. LWT - Food Sci. Technol. 154, 112696.

Panagou, E.Z., Tassou, C.C., 2006. Changes in volatile compounds and related biochemical profile during controlled fermentation of cv. Conservolea green olives. Food Microbiol. 23, 738-746.

Pannelli, G., Alfei, B., Santinelli, A., 2001. "Varietà di olivo nelle Marche", ASSAM. Available at: http://www.assam.marche.it/component/jdownloads/send/97-biodiversita-repertorio-vegetali/1645-scheda-ascolana-tenera

Papadimitriou, K., Alegría, Á., Bron, P.A., De Angelis, M., Gobbetti, M., Kleerebezem, M., Lemos, J.A., Linares, D.M., Ross, P., Stanton, C., Turroni, F., van Sinderen, D., Varmanen, P., Ventura, M., Zúñiga, M., Tsakalidou, E., Kok, J., 2016. Stress physiology of lactic acid bacteria. Microbiol. Mol. Biol. Rev. 80, 837-890.

Park, D.K., Bitton, G., Melker, R., 2006. Microbial inactivation by microwave radiation in the home environment. J. Environ. Health 69, 17.

Park, E.-J., Chun, J., Cha, C.-J., Park, W.-S., Jeon, C.O., Bae, J.-W., 2012. Bacterial community analysis during fermentation of ten representative kinds of kimchi with barcoded pyrosequencing. Food Microbiol. 30, 197-204.

Park, S., Ji, Y., Park, H., Lee, K., Park, H., Beck, B.R., Shin, H., Holzapfel, W.H., 2016. Evaluation of functional properties of lactobacilli isolated from Korean white kimchi. Food Control. 69, 5-12.

Pateira, L., Nogueira, T., Antunes, A., Venâncio, F., Tavares, R., Capelo, J., 1999. Two chemotypes of *Crithmum maritimum* L. from Portugal. Flavour Fragr. J. 14, 333-343.

Patra, J.K., Das, G., Paramithiotis, S., Shin, H.S., 2016. Kimchi and other widely consumed traditional fermented foods of Korea: A review. Front. Microbiol. 7, 1493.

Pavela, R., Maggi, F., Lupidi, G., Cianfaglione, K., Dauvergne, X., Bruno, M., Benelli, G., 2017. Efficacy of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils against *Culex quinquefasciatus* Say and *Spodoptera littoralis* (Boisd.). Ind. Crops Prod. 109, 603-610.

Pelagatti, O., & Brighigna, A. (1981). The revival and use of dried lactic acid bacteria for table olive preparation by the Sivigliano system. Industrie Alimentari (Italy). Volume: 20, Issue 3, pp. 182-184.

Peng, J., Tang, J., Barrett, D.M., Sablani, S.S., Anderson, N., Powers, J.R., 2017. Thermal pasteurization of ready-to-eat foods and vegetables: critical factors for process design and effects on quality. Crit. Rev. Food Sci. Nutr. 57, 2970-2995.

Penland, M., Deutsch, S. M., Falentin, H., Pawtowski, A., Poirier, E., Visenti, G., Le Meur, C., Maillard, M.B., Thierry, A., Mounier, J., Coton, M., 2020. Deciphering microbial community dynamics and biochemical changes during Nyons black olive natural fermentations. Front. Microbiol. 11, 586614.

Pereira, C.G., Barreira, L., da Rosa Neng, N., Nogueira, J.M.F., Marques, C., Santos, T.F., Varela, J., Custódio, L., 2017. Searching for new sources of innovative products for the food industry within halophyte aromatic plants: In vitro antioxidant activity and phenolic and mineral contents of infusions and decoctions of *Crithmum maritimum* L. Food Chem. Toxicol. 107, 581-589.

Peres, C.M., Alves, M., Hernandez-Mendoza, A., Moreira, L., Silva, S., Bronze, M.R., Vilas-Boas, L., Peres, C., Malcata, F.X., 2014. Novel isolates of lactobacilli from fermented Portuguese olive as potential probiotics. LWT - Food Sci. Technol. 59, 234-246.

Perpetuini, G., Prete, R., Garcia-Gonzalez, N., Khairul Alam, M., Corsetti, A., 2020. Table olives more than a fermented food. Foods 9, 178.

Peryam, D.R., Pilgrim, F.J., 1957. Hedonic scale method of measuring food preferences. Food Technol. 11, 9-14.

Pferschy-Wenzig, E.M., Bauer, R., 2015. The relevance of pharmacognosy in pharmacological research on herbal medicinal products. Epilepsy Behav. 52, 344-362.

Picchi, G., Pieroni, A., 2005. Atlante dei prodotti tipici: Le erbe. AGRA editrice. RAI-Eri, Rome.

Pietrzak, W., Nowak, R., Olech, M., 2014. Effect of extraction method on phenolic content and antioxidant activity of mistletoe extracts from *Viscum album* subsp. abietis. Chem. Pap. 68, 976-982.

Pino, A., De Angelis, M., Todaro, A., Van Hoorde, K., Randazzo, C.L., Caggia, C., 2018. Fermentation of Nocellara Etnea table olives by functional starter cultures at different low salt concentrations. Front. Microbial. 9, 1125.

Polatoğlu, K., Karakoç, Ö.C., Yücel, Y.Y., Gücel, S., Demirci, B., Başer, K.H.C., Demirci, F., 2016. Insecticidal activity of edible *Crithmum maritimum* L. essential oil against Coleopteran and Lepidopteran insects. Ind. Crops Prod. 89, 383-389.

Poltronieri, P., D'Urso, O.F., Blaiotta, G., Morea, M., 2008. DNA arrays and membrane hybridization methods for screening of six *Lactobacillus* species common in food products. Food Anal. Methods 1(3), 171-180.

Proteggente, A.R., Pannala, A.S., Paganga, G., van Buren, L., Wagner, E., Wiseman, S., van de Put, F., Dacombe, C., Rice-Evans, C.A., 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radic. Res. 36, 217-233.

Pulido, R., Bravo, L., Saura-Calixto, F., 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 48, 3396-3402.

Put, H.M.C., De Jong, J., 1982. Heat resistance studies of yeasts; vegetative cells versus ascospores: erythromycin inhibition of sporulation in *Kluyveromyces* and *Saccharomyces* species. J. Appl. Bacteriol. 53, 73-79.

Quattrini, M., Korcari, D., Ricci, G., Fortina, M.G., 2020. A polyphasic approach to characterize *Weissella cibaria* and *Weissella confusa* strains. J. Appl. Microbiol. 128, 500-512.

Ragaert, P., Devlieghere, F., Debevere, J., 2017. Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. Postharvest Biol. Technol. 44, 185–194.

Ramos, C.L., Schwan, R.F., 2017. Technological and nutritional aspects of indigenous Latin America fermented foods. Curr. Opin. Food Sci. 13, 97–102.

Ranadheera, R.D.C.S., Baines, S.K., Adams, M.C., 2010. Importance of food in probiotic efficacy. Food Res. Int. 43, 1-7.

Randazzo, C.L., Ribbera, A., Pitino, I., Romeo, F.V., Caggia, C., 2012. Diversity of bacterial population of table olives assessed by PCR-DGGE analysis. Food Microbiol. 32, 87-96.

Rao, L., Feeherry, F.E., Ghosh, S., Liao, X., Lin, X., Zhang, P., Li, Y., Doona, C.J., Setlow, P., 2018. Effects of lowering water activity by various humectants on germination of spores of *Bacillus* species with different germinants. Food Microbiol. 72, 112-127.

Ray, R.C., Joshi, V.K., 2015. Fermented foods: past, present and future. In: Ray, R.C., Montet, D. (Eds.), Microorganisms and Fermentation of Traditional Foods. CRC Press, Boca Raton, pp. 1-36.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231-1237.

Reale, A., Di Renzo, T., Zotta, T., Preziuso, M., Boscaino, F., Ianniello, R., Storti, L.V., Tremonte, P., Coppola, R., 2016. Effect of respirative cultures of *Lactobacillus casei* on model sourdough fermentation. LWT - Food Sci. Technol. 73, 622-629.

Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1924&from=en.

Rejano, L., Montaño, A., Casado, F.J., Sánchez, A.H., de Castro, A., 2010. Table olives: varieties and variations. In: Preedy, V.R., Watson, R.R. (Eds.), Olives and Olive Oil in Health and Disease Prevention. Academic Press, New York, pp. 5-15.

Renna, M., 2018. Reviewing the prospects of sea fennel (*Crithmum maritimum* L.) as emerging vegetable crop. Plants 7, 92.

Renna, M., Gonnella, M., 2012. The use of the sea fennel as a new spice-colorant in culinary preparations. Int. J. Gastron. Food Sci. 1, 111-115.

Renna, M., Gonnella, M., Caretto, S., Mita, G., Serio, F., 2017. Sea fennel (*Crithmum maritimum* L.): from underutilized crop to new dried product for food use. Genet. Resour. Crop Evol. 64, 205-216.

Repajić, M., Cegledi, E., Kruk, V., Pedisić, S., Çinar, F., Kovačević, D.B., Žutić, I., Dragović-Uzelac, V., 2020. Accelerated solvent extraction as a green tool for the recovery of polyphenols and pigments fromwild nettle leaves. Processes 8, 803.

Resurreccion, A.V.A. (1998). Consumer sensory testing for product development. A Chapman & Hall food science book. Aspen Publishers, Inc, Gaithersburg, Maryland.

Rico, D., Albertos, I., Martinez-Alvarez, O., Lopez-Caballero, M.E., Martin-Diana, A.B., 2020. Use of sea fennel as a natural ingredient of edible films for extending the shelf life of fresh fish burgers. Molecules 25, 5260.

Riediger, N.D., Othman, R., Fitz, E., Pierce, G.N., Suh, M., Moghadasian, M.H., 2008. Low n-6: n-3 fatty acid ratio, with fish-or flaxseed oil, in a high fat diet improves plasma lipids and beneficially alters tissue fatty acid composition in mice. Eur. J. Nutr. 47, 153-160.

Rijkers, G.T., Bengmark, S., Enck, P., Haller, D., Herz, U., Kalliomaki, M., Kudo, S., Lenoir-Wijnkoop, I., Mercenier, A., Myllyluoma, E., Rabot, S., Rafter, J., Szajewska, H., Watzl, B., Wells, J., Wolvers, D., Antoine, J.M., 2010. Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. J. Nutr. 140, 671S-676S.

Rizzello, C.G., Coda, R., Wang, Y., Verni, M., Kajala, I., Katina, K., Laitila, A., 2019. Characterization of indigenous *Pediococcus pentosaceus*, *Leuconostoc kimchii*, *Weissella cibaria* and *Weissella confusa* for faba bean bioprocessing. Int. J. Food Microbiol. 302, 24-34.

Rodrigues, M.J., Soszynski, A., Martins, A., Rauter, A.P., Neng, N.R., Nogueira, J.M.F., Varela, J., Barreira, L., Custódio, L., 2015. Unravelling the antioxidant potential and the phenolic composition of different anatomical organs of the marine halophyte *Limonium algarvense*. Ind. Crops Prod. 77, 315-322.

Rodríguez, H., Curiel, J.A., Landete, J.M., de las Rivas, B., de Felipe, F.L., Gómez-Cordovés, C., Mancheño J.M., Muñoz, R., 2009. Food phenolics and lactic acid bacteria. Int. J. Food Microbiol. 132, 79-90.

Romojaro, A., Botella, M.Á., Obón, C., Pretel, M.T., 2013. Nutritional and antioxidant properties of wild edible plants and their use as potential ingredients in the modern diet. Int. J. Food Sci. Nutr. 64, 944-952.

Roncolini, A., Milanović, V., Aquilanti, L., Cardinali, F., Garofalo, C., Sabbatini, R., Clementi, F., Belleggia, L., Pasquini, M., Mozzon, M., Foligni, R., Trombetta, M.F., Haouet, M.N., Altissimi, M. S., Di Bella, S., Piersanti, A., Griffoni, F., Reale, A., Niro, S., Osimani, A., 2020. Lesser mealworm (*Alphitobius diaperinus*) powder as a novel baking ingredient for manufacturing high-protein, mineral-dense snacks. Food Res. Int. 131, 109031.

Ruberto, G., Baratta, M.T., Deans, S.G., Damien Dorman, H.J., 2000. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. Planta Med. 66, 687-693.

Ruiz-Barba, J.L., Jiménez-Díaz, R., 1994. Vitamin and amino acid requirements of *Lactobacillus plantarum* strains isolated from green olive fermentations. J. Appl. Bacteriol. 76, 350-355.

Sabatini, N., Marsilio, V., 2008. Volatile compounds in table olives (*Olea Europaea* L., Nocellara del Belice cultivar). Food Chem. 107, 1522-1528.

Sachan, A., Kumar, S., Kumari, K., Singh, D., 2018. Medicinal uses of spices used in our traditional culture: Worldwide. J. Med. Plants Stud. 6, 116-122.

Sakata, T., Suami, S., Kakimoto, D., 1975. Factors affecting the germination of *Bacillus subtilis* spores. Mem. Fac. Fish., Kagoshima Univ. 24, 139-147.

Sánchez, A.H., De Castro, A., Rejano, L., Montaño, A., 2000. Comparative study on chemical changes in olive juice and brine during green olive fermentation. J. Agric. Food Chem. 48, 5975-5980.

Sánchez, A., García, P., Rejano, L., Brenes, M., Garrido, A., 1995. The effects of acidification and temperature during washing of Spanish-style green olives on the fermentation process. J. Sci. Food Agric. 68, 197-202.

Sánchez-Faure, A., Calvo, M.M., Pérez-Jiménez, J., Martín-Diana, A.B., Rico, D., Montero, M.P., Gómez-Guillén, M. del C., López-Caballero, M.E., Martínez-Alvarez, O., 2020. Exploring the potential of common iceplant, seaside arrowgrass and sea fennel as edible halophytic plants. Food Res. Int. 137, 109613.

Sapei, L., Hwa, L., 2014. Study on the kinetics of vitamin C degradation in fresh strawberry juices. Procedia Chem. 9, 62-68.

Savijoki, K., Nyman, T.A., Kainulainen, V., Miettinen, I., Siljamäki, P., Fallarero, A., Sandholm, J., Satokari, R., Varmanen, P., 2019. Growth mode and carbon source impact the surfaceome dynamics of *Lactobacillus rhamnosus* GG. Front. Microbiol. 10, 1272.

Savo, V., Giulia, C., Maria, G.P., David, R., 2011. Folk phytotherapy of the Amalfi coast (Campania, Southern Italy). J. Ethnopharmacol. 135, 376-392.

Schleifer, K.H., 2019. Leuconostocaceae fam. nov. *Bergey's Man.* Syst. Archaea Bact.

Selman, J.D., 1994. Vitamin retention during blanching of vegetables. Food Chem. 49, 137-147.

Senatore, F., Napolitano, F., Ozcan, M., 2000. Composition and antibacterial activity of the essential oil from *Crithmum maritimum* L. (Apiaceae) growing wild in Turkey. Flavour Fragr. J. 15, 186-189.

Seo, K.S., Bohach, G.A., 2007. *Staphylococcus aureus*. In: Doyle, M.P., Beuchat, L.R. (Eds.), Food Microbiology: Fundamentals and Frontiers (3rd ed.). ASM Press, Washington D.C., pp. 493-518.

Serag, A., Baky, M.H., Döll, S., Farag, M.A., 2020. UHPLC-MS metabolome based classification of umbelliferous fruit taxa: a prospect for phyto-equivalency of its different accessions and in response to roasting. RSC Adv. 10, 76-85.

Sikora, M., Badrie, N., Deisingh, A.K., Kowalski, S., 2008. Sauces and dressings: a review of properties and applications. Crit. Rev. Food Sci. Nutr. 48, 50-77.

Silva, D.B., Turatti, I.C.C., Gouveia, D.R., Ernst, M., Teixeira, S.P., Lopes, N.P., 2014. Mass Spectrometry of flavonoid vicenin-2, based sunlight barriers in *Lychnophora* species. Sci. Rep. 4, 1-8.

Silvi, S., Verdenelli, M.C., Cecchini, C., Coman, M.M., Bernabei, M.S., Rosati, J., De Leone, R., Orpianesi, C., Cresci, A., 2014. Probiotic-enriched foods and dietary supplement containing SYNBIO positively affects bowel habits in healthy adults: an assessment using standard statistical analysis and Support Vector Machines. Int. J. Food Sci. Nutr. 65, 994-1002.

Silvi, S., Verdenelli, M.C., Orpianesi, C., Cresci, A., 2003. EU project Crownalife: functional foods, gut microflora and healthy ageing: isolation and identification of *Lactobacillus* and *Bifidobacterium*

strains from faecal samples of elderly subjects for a possible probiotic use in functional foods. J. Food Eng. 56, 195-200.

Simopoulos, A.P., 1999. Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr. 70, 560s-569s.

Siracusa, L., Kulisic-Bilusic, T., Politeo, O., Krause, I., Dejanovic, B., Ruberto, G., 2011. Phenolic composition and antioxidant activity of aqueous infusions from *Capparis spinosa* L. and *Crithmum maritimum* L. before and after submission to a two-step in vitro digestion model. J. Agric. Food Chem. 59, 12453-12459.

Smid, E.J., Kleerebezem, M., 2014. Production of aroma compounds in lactic fermentations. Annu. Rev. Food Sci. Technol. 5, 313-326.

Smittle, R.B., 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. J. Food Prot. 63, 1144-1153.

Snyder, A., Breidt Jr, F., Andress, E.L., Ingham, B.H., 2020. Manufacture of traditionally fermented vegetable products: best practice for small businesses and retail food establishments. Food Prot. Trends 40, 251-263.

Sokal, R.R., Sneath, P.H.A. (1963). Principles of numerical taxonomy. WH Freeman, San Francisco.

Song, H.S., Whon, T.W., Kim, J., Lee, S.H., Kim, J.Y., Kim, Y.B., Choi, H.-J., Rhee, J.-K., Roh, S.W., 2020. Microbial niches in raw ingredients determine microbial community assembly during kimchi fermentation. Food Chem. 318, 126481.

Souid, A., Della Croce, C.M., Frassinetti, S., Gabriele, M., Pozzo, L., Ciardi, M., Abdelly, C., Ben Hamed, K., Magné, C., Longo, V., 2021. Nutraceutical potential of leaf hydro-ethanolic extract of the edible halophyte *Crithmum maritimum* L. Molecules. 26, 5380.

Souid, A., Della Croce, C.M., Pozzo, L., Ciardi, M., Giorgetti, L., Gervasi, P.G., Abdelly, C., Magné, C., Ben Hamed, K., Longo, V., 2020. Antioxidant properties and hepatoprotective effect of the edible halophyte *Crithmum maritimum* L. against carbon tetrachloride-induced liver injury in rats. Eur. Food Res. Technol. 246, 1393-1403.

Sparg, S.G., Light, M.E., Van Staden, J., 2004. Biological activities and distribution of plant saponins. J. Ethnopharmacol. 94, 219-243.

Stewart, C.M., 2003. *Staphylococcus aureus* and staphylococcal enterotoxins. In: Hocking A.D. (Ed.), Foodborne Microorganisms of Public Health Significance (6th ed.). Australian Institute of Food Science and Technology (NSW Branch), Sydney, pp. 359-379.

Sun, H., Ge, X., Lv, Y., Wang, A., 2012. Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in food and feed. J. Chromatogr. A 1237, 1-23.

Surowsky, B., Froehling, A., Gottschalk, N., Schlüter, O., Knorr, D., 2014. Impact of cold plasma on *Citrobacter freundii* in apple juice: inactivation kinetics and mechanisms. Int. J. Food Microbiol. 174, 63-71.

Sutherland, A.D., Limond, A.M., 1993. Influence of pH and sugars on the growth and production of diarrhoeagenic toxin by *Bacillus cereus*. J. Dairy Res. 60, 575-580.

Taccari, M., Aquilanti, L., Polverigiani, S., Osimani, A., Garofalo, C., Milanović, V., Clementi, F., 2016. Microbial diversity of type I sourdoughs prepared and back-slopped with wholemeal and refined soft (*Triticum aestivum*) wheat flours. J. Food Sci. 81, M1996-M2005.

Talwalkar, A., Miller, C.W., Kailasapathy, K., Nguyen, M.H., 2004. Effect of packaging materials and dissolved oxygen on the survival of probiotic bacteria in yoghurt. Int. J. Food Sci. Technol. 39, 605-611.

Tamang, B., Tamang, J.P., 2010. *In situ* fermentation dynamics during production of *gundruk* and *khalpi*, ethnic fermented vegetables products of the Himalayas. Indian J. Microbiol. 50, 93-98.

Tapia, M.S., Alzamora, S.M., Chirife, J., 2020. Effects of water activity (a_w) on microbial stability as a hurdle in food preservation. In: Barbosa-Cánovas, G.V., Fontana Jr., A.J., Schmidt, S.J., Labuza, T.P. (Eds.), Water Activity in Foods: Fundamentals and Applications (2nd ed.), John Wiley & Sons, Inc., Hoboken, NJ, pp. 323-355.

Tardío, J., Sánchez-Mata, M.D.C., Morales, R., Molina, M., García-Herrera, P., Morales, P., Díez-Marqués, C., Fernández-Ruiz, V., Cámara, M., Pardo-de-Santayana, M., Matallana-González, M.C., Ruiz-Rodríguez, B.M., Sánchez-Mata, D., Torija-Isasa, M.E., Guil-Guerrero, J.L., Boussalah, N., 2016. Ethnobotanical and food composition monographs of selected Mediterranean wild edible plants. In: De Cortes Sánchez-Mata, M., Tardío, J. (Eds.), Mediterranean Wild Edible Plants. Springer, New York, pp. 273-470.

Tavoletti, S., Foligni, R., Mozzon, M., Pasquini, M., 2018. Comparison between fatty acid profiles of old and modern varieties of *T. turgidum* and *T. aestivum*: a case study in central Italy. J. Cereal Sci. 82, 198-205.

Tewari, A., Abdullah, S., 2015. *Bacillus cereus* food poisoning: international and Indian perspective. J. Food Sci. Technol. 52, 2500-2511.

Tomasik, P. J., Tomasik, P., 2003. Probiotics and prebiotics. Cereal Chem. 80, 113-117.

Torres, S., Verón, H., Contreras, L., Isla, M.I., 2020. An overview of plant-autochthonous microorganisms and fermented vegetable foods. Food Sci. Human Wellness. 9, 112-123.

Torres-Rodríguez, I., Rodríguez-Alegría, M.E., Miranda-Molina, A., Giles-Gómez, M., Conca Morales, R., López-Munguía, A., Bolívar, F., Escalante, A., 2014. Screening and characterization of

extracellular polysaccharides produced by *Leuconostoc kimchii* isolated from traditional fermented *pulque* beverage. Springerplus 3, 583.

Touwaide, A., Pollio, A., Aliotta, G., Piomelli, D., De Santo, N.G., 1997. Medicinal plants for the treatment of urogenital tract pathologies according to Dioscorides' De Materia Medica. Am. J. Nephrol. 17, 241-247.

Tripathi, M.K., Giri, S.K., 2014. Probiotic functional foods: survival of probiotics during processing and storage. J. Funct. Foods 9, 225-241.

Trivedi, N., Patadia, M., Kothari, V., 2011. Biological applications of microwaves. Int. J. Life Sci. Technol. 4, 37.

Tuohy, K.M., Probert, H.M., Smejkal, C.W., Gibson, G.R., 2003. Using probiotics and prebiotics to improve gut health. Drug Discov. Today, 8, 692-700.

Turek, C., Stintzing, F.C., 2013. Stability of essential oils: a review. Compr. Rev. Food Sci. Food Saf. 12, 40-53.

Van Uden, N., 1985. Temperature profiles of yeasts. Adv. Microb. Physiol. 25, 195-251.

Ventura, Y., Wuddineh, W.A., Myrzabayeva, M., Alikulov, Z., Khozin-Goldberg, I., Shpigel, M., Samocha, T.M., Sagi, M., 2011. Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. Sci. Hortic. 128, 189-196.

Verdenelli, M.C., Coman, M.M., Cecchini, C., Silvi, S., Orpianesi, C., Cresci, A., 2014. Evaluation of antipathogenic activity and adherence properties of human *Lactobacillus strains* for vaginal formulations. J. Appl. Microbiol. 116, 1297-1307.

Verdenelli, M.C., Ghelfi, F., Silvi, S., Orpianesi, C., Cecchini, C., Cresci, A., 2009. Probiotic properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faeces. Eur. J. Nutr. 48, 355-363.

Verdenelli, M.C., Silvi, S., Cecchini, C., Orpianesi, C., Cresci, A., 2011. Influence of a combination of two potential probiotic strains, *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® on bowel habits of healthy adults. Lett. Appl. Microbiol. 52, 596-602.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J.A., 2010. Spices as functional foods. Crit. Rev. Food Sci. Nutr. 51, 13-28.

Vrhovsek, U., Masuero, D., Gasperotti, M., Franceschi, P., Caputi, L., Viola, R., Mattivi, F., 2012. A versatile targeted metabolomics method for the rapid quantification of multiple classes of phenolics in fruits and beverages. J. Agric. Food Chem. 60, 8831-8840.

Wagner, H., Bladt, S., 1996. Plant drug analysis: a thin layer chromatography atlas, 2nd ed. Springer Science & Business Media, Berlin, Germany.

Wang, L., Weller, C.L., 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol. 17, 300-312.

Wijnands, L.M., 2008. *Bacillus cereus* associated food borne disease: quantitative aspects of exposure assessment and hazard characterization. PhD thesis, Wageningen University and Research, The Netherlands.

Wouters, D., Bernaert, N., Conjaerts, W., Van Droogenbroeck, B., De Loose, M., De Vuyst, L., 2013a. Species diversity, community dynamics, and metabolite kinetics of spontaneous leek fermentations. Food Microbiol. 33, 185-196.

Wouters, D., Grosu-Tudor, S., Zamfir, M., De Vuyst, L., 2013b. Bacterial community dynamics, lactic acid bacteria species divesity and metabolite kinetics of traditional Romanian vegetable fermentations. J. Sci. Food Agric. 93, 749-760.

Wray, D., Ramaswamy, H.S., 2015. Novel concepts in microwave drying of foods. Dry. Technol. 33, 769-783.

Wu, M., Guo, P., Tsui, S.W., Chen, H., Zhao, Z., 2012. An ethnobotanical survey of medicinal spices used in Chinese hotpot. Food Res. Int. 48, 226-232.

Xiao, H.W., Pan, Z., Deng, L.Z., El-Mashad, H.M., Yang, X.H., Mujumdar, A.S., Gao, Z.-J., Zhang, Q., 2017. Recent developments and trends in thermal blanching - A comprehensive review. Inf. Process. Agric. 4, 101-127.

Xiong, T., Guan, Q., Song, S., Hao, M., Xie, M., 2012. Dynamic changes of lactic acid bacteria flora during Chinese sauerkraut fermentation. Food Control 26, 178-181.

Xiong, T., Li, X., Guan, Q., Peng, F., Xie, M., 2014. Starter culture fermentation of Chinese sauerkraut: growth, acidification and metabolic analyses. Food Control 41, 122-127.

Yamani, M.I., 1993. Fermentation of brined tunrnip roots using *Lactobacillus plantarum* and *Leuconostoc mesenteroides* starter cultures. World J. Microbiol. Biotechnol. 9, 176-179.

Yensen N.P., 2008. Halophyte uses for the twenty-first century. In Khan, M.A., Weber, D.J. (Eds.). Ecophysiology of High Salinity Tolerant Plants. Springer, Dordrecht, pp. 367-396.

Yu, H.-S., Jang, H.J., Lee, N.-K., Paik, H.-D., 2019. Evaluation of the probiotic characteristics and prophylactic potential of *Weissella cibaria* strains isolated from kimchi. LWT - Food Sci. Technol. 112, 108229.

Zafeiropoulou, V., Tomou, E.M., Douros, A., Skaltsa, H., 2021. The effect of successive harvesting on the volatile constituents of two essential oils of cultivated populations of sea fennel (*Crithmum maritimum* L.) in Greece. J. Essent. Oil-Bear. Plants 24, 1-11.

Zafeiropoulou, V., Tomou, E.-M., Karioti, A., Skaltsa, H., Ioannidou, O., 2020. Sea fennel: phytochemical analysis of Greek wild and cultivated *Crithmum maritimum* L. populations, based on HPLC-PDA-MS and NMR methods. J. Pharmacogn. Phytochem. 9, 998-1004.

Zago, M., Lanza, B., Rossetti, L., Muzzalupo, I., Carminati, D., Giraffa, G., 2013. Selection of *Lactobacillus plantarum* strains to use as starters in fermented table olives: oleuropeinase activity and phage sensitivity. Food Microbiol. 34, 81-87.

Zaika, L.L., Kissinger, J.C., Wasserman, A.E., 1983. Inhibition of lactic acid bacteria by herbs. J. Food Sci. 48, 1455-1459.

Zenobi, S., Fiorentini, M., Zitti, S., Aquilanti, L., Foligni, R., Mannozzi, C., Mozzon, M., Orsini, R., 2021. *Crithmum maritimum* L.: first results on phenological development and biomass production in Mediterranean areas. Agronomy, 11, 773.

Acknowledgements/Ringraziamenti

This Ph.D. thesis was funded by the Marche Region under the project POR Marche FSE 2014/2020 Progetto “Dottorato Innovativo” - Borse di studio per Dottorato di ricerca per l’innovazione del sistema regionale”- Edizione Anno Accademico 2018/2019-“Sviluppo di nuove conserve, salse e spezie biologiche funzionali a base di finocchio marino (*Crithmum maritimum* L.) e altri prodotti orticoli”.

At the end of this Ph.D. program I would like to thank several people that were fundamental in my reaching of this important goal.

First of all, thanks to my supervisor Prof. Lucia Aquilanti, for giving me the opportunity to undertake this Ph.D. course, for teaching and supporting me and for being my mentor. I had spent three great years under her supervision.

Thanks to Prof. Andrea Osimani, Prof. Cristiana Garofalo, Dr. Vesna Milanović, Dr. Federica Cardinali, for their valuable support and suggestions during my entire Ph.D. course.

I would like to thank Prof. Rudolf Bauer, for giving me the opportunity to collaborate with his research group at the Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-Universität, Graz, and for teaching me with patience, willingness and competence. I will always keep my stay in Graz in good memory.

Thanks to Dr. Luca Galeazzi, for contributing in my entire Ph.D. and for teaching me with patience and competence.

I would like to thank my colleagues Luca, Cristiana and Giorgia, without you these three years would not have been the same.

Thanks to the colleagues I met at Karl-Franzens-Universität, Teresa, Evangelia and Pia, I had spent three great months with you.

A special thanks to my roommate in Graz, Simrat, I will always keep in good memory our chats and our dinners.

Un ringraziamento speciale va alla mia famiglia, ai miei genitori Flavia e Giovanni e ai miei fratelli Emanuele e Melania. Siete il mio punto di riferimento e la mia forza più grande. Senza di voi non sarei quella che sono. Grazie per avermi aiutato a realizzare i miei obiettivi. Vi voglio bene.

Un ringraziamento speciale va a Riccardo, il mio compagno, la mia famiglia. Grazie per essermi stato vicino in questi anni, sostenendomi nelle difficoltà e spronandomi a dare il massimo. Sei una persona speciale, ti amo.

Un ringraziamento alle “pavone” Stefania, Silvia e Liudmila. Ormai non riusciamo a vederci molto spesso ma siete nel mio cuore. Vi voglio bene.