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Fermentation of *Fucus vesiculosus*: Sensory evaluation and product innovation

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Fucus vesiculosus is a common macroalgae abundant in coastal regions of the North Sea, the western Baltic Sea and in both the Atlantic and Pacific oceans. While traditional consumption of seaweeds is prominent in Asian cultures, trends of consumption of seaweed-based products are growing in Western cultures. However, consumption remains relatively low despite the recognized health benefits.

Fermentation is a natural bioprocess that can improve the shelf-life, sensory and nutritional quality of fresh macroalgae. The study initially trialled two different conditions of the macroalgae: chopped into approximately 1-inch pieces and blended with an immersion blender. Both conditions were trialled using lactic acid bacteria (LAB) *Lactiplantibacillus plantarum* DSM 20174. Following this, different glucose concentrations (10 and 20%) were trialled to optimise the fermentation. Variations were also made to the algae concentrations used within the fermentation mass. Finally, the fermented and fresh macroalgae were used to develop two different product innovations, a pesto and a sauerkraut, respectively. Fermentation progress was monitored by chemical analysis. The sugars and acids were then analyzed through gas chromatogram coupled with flame ionization detector on samples across different fermentation durations. The sensory evaluation was carried out by a trained panel (n=6) and was split into two parts: a likeness of the two product innovations compared to their controls and descriptive analysis on 4 samples which were subjected to different treatments, such as raw, heat-treated, 2-day fermentation, and 12-day fermentation.

The results suggest that lactic acid concentrations were higher in 10% glucose compared to the use of 20% indicating a more optimum sugar concentration for LAB fermentation. Despite a significant reduction in pH, no lactic acid was detected from the 2-day fermentation. Spontaneous fermentations with naturally occurring LAB (from the cabbage), contained significantly higher concentrations of lactic acid compared to controlled fermentations inoculated with the LAB strain. The result from the sensory evaluation suggests that fermentation reduced the overall aroma intensity in the 12-day fermentation compared to the raw untreated sample. Additionally, both 2 and 12-day fermentations significantly reduced grassy and seaweed aromas compared to raw and heat-treated.

Keywords: aroma, fermentation, lactic acid bacteria, *Lactiplantibacillus plantarum*, macroalgae, sensory evaluation

Content

Abbreviations

ANOVA	analysis of variance
CA	cabbage and algae
CC	cabbage control
CFU	colony forming units
CK	commercial kelp
EPA	eicosapentaenoic acids
F1B	fermentation 1 blended
F1C	fermentation 1 chopped
FAO	Food and Agriculture Organisation of the United Nations
GC-FID	gas chromatography coupled with flame ionisation detector
GT10	glucose trial 10%
GT20	glucose trial 20%
HSD	honestly significant difference
HS-SPME-GC-MS	headspace solid-phase micro-extraction gas-chromatography mass spectrometry
HT	heat treated
LAB	lactic acid bacteria
MRS	De Man–Rogosa–Sharpe
PUFAs	polyunsaturated fatty acids
RCF	relative centrifugal force
RCO	random-centroid optimisation
RU	raw untreated
SF12	sensory fermentation 12-day
SF2	sensory fermentation 2-day
TPC	total phenolic content

1 Introduction

Macroalgae, more commonly known as seaweeds, are heterogeneous plants that grow abundantly on rocky solid substrates in varying water conditions and are one of the world's most underutilised renewable resources. Macroalgae are characterized and grouped according to pigmentation as brown, green and red. There are approximately 300 commercially important seaweed species which are available worldwide (Blikra et al., 2021). According to a report from the Food and Agriculture Organisation of the United Nations (FAO), it is estimated that nearly 90% of cultivated seaweed is used directly for human consumption (2022). For human consumption, brown seaweeds are the most favoured at 66.5%, followed by red seaweed at 33% and green seaweeds at 5%(Lorenzo et al., 2017). Seaweeds are the crucial primary producers in the oceanic aquatic food web (Ranga Rao & Ravishankar, 2022). Seaweed cultivation is dominated by Asian cultures as part of an oriental diet with China producing approximately 20 million tons in 2020 (FAO, 2022), while still underutilised by Western societies, it is of growing interest. As the world population is expected to increase to 9.7 billion by 2050, there is an ever-increasing demand for more sustainable food and feed production worldwide. Due to macroalga's growth conditions requiring low nutrient demand, yielding high growth rates and versatility in a growth environment (no need for freshwater supply), it poses a viable alternative to what is commonly consumed in Western cultures (Ranganathan et al., 2018).

Fucus vesiculosus also referred to as bladder wrack, is the chosen macroalgae for the investigation of fermentation and consumer acceptability as part of this thesis work. It is widely distributed within the North Atlantic coastlines. In Finland, the population of the species is stable and on the Swedish coastline, it can be found as deep as 16 m (Merck & Nordheim, 1996). The Finnish Archipelago Sea is home to 22000 islands and gives rise to approximately 15000 km of shorelines. This provides an abundance of suitable growth areas for *Fucus vesiculosus*. However, studies have shown that there is a reduction in both depth ranges and coverage within the Archipelago Sea which started in the 1970s (Kautsky et al., 2019; Merck & Nordheim, 1996; Vahteri & Vuorinen, 2016).

1.1 Nutrition and health benefits

Edible seaweeds are aquatic vegetables that are low in calories but rich in dietary fibre, essential amino acids, unsaturated fatty acids, vitamins, minerals, and phytochemicals. It is important to note as with many seasonal foods, the nutritional and chemical composition of algae will depend on species, origin, growing conditions, harvesting, and processing (Bocanegra et al., 2009). A study by Neto et al., 2018) performed various screening tests to determine and quantify the functional ingredients of four different seaweed species. The nutritional content of these seaweeds, namely *Ulva rigida, Gracilaria* sp*., Fucus vesiculosus and Saccharina latissimi*, can be seen in Table 1. In addition, a review study by Bocanegra et al. (2009) reported on a total of 12 different seaweed species and found carbohydrate, protein, lipid, and ash concentrations of seaweeds have been quantified to be 3-47%, 33-75%, 1.5-4%, and 10-35% respectively.

Table 1 Comparison of the nutritional content of four different seaweed species

	U. rigida	***Gracilaria sp.***	***F. vesiculosus***	***S.latissima***
Total content (g/100g dw)				
Carbohydrate	58	47	56	69
Protein	9	24	15	10
Lipid	0.9	0.7	3	0.5
Ash	31	29	26	20
Minerals (mg/100g dw)				
Na	2424	1595	2266	3048
K	2467	9243	4083	3869
Ca	414	200	1382	919
Mg	3759	286	836	611
Fe	110	211	8.8	185
Mn	7	16	55	0.6
Cu	3	3	3	4
Zn	3	3	3	4
Ni	1	2	2	0.3

Despite the relatively high carbohydrate content of algae, seaweeds are not considered to be a source of energy due to the low digestibility of the plant carbohydrates which are present. The polysaccharides which are present are regarded as dietary fibres instead. Some studies have investigated the total dietary fibres in different seaweed species and have reported concentrations between 30-62% (Dawczynski et al., 2007; Gómez-Ordóñez et al., 2010; MacArtain et al., 2008). The polysaccharides from seaweeds such as agar, carrageenan and alginate have traditionally been used by Western counties as stabilizing, thickening and gelling agents in the food industry (O' Connor et al., 2020).

The quantity of protein in a seaweed species will vary largely depending on season and harvesting method. For example, *Porphyra umbilicalis* (nori) has a protein content that can be up to 47% of the dry weight analysed (MacArtain et al., 2008). Some essential amino acids that have been quantified in seaweeds (*Palmaria palmata* & *Ulva* spp.) are histidine, leucine, isoleucine, and valine. In *Palmaria palmata* the levels of isoleucine and threonine are comparable to the levels found in legumes. Similarly, for *Ulva* spp. the level of histidine is comparable to the quantities found in eggs. These essential amino acids can particularly aid those people pursuing a vegetarian or vegan lifestyle. Moreover, a review by Galland-Irmouli et al. (1999) investigated the relative digestibility of water-soluble proteins *in vitro* studies from various seaweed species. They reported the highest pronase digestibility from *Ulva pertusa* (green seaweed) at 94.8% followed by *Undaria pinnatifida* (brown seaweed) at 87.2%. Interestingly, glutamic acid albeit a non-essential α-amino acid for humans, presents in flavour development and is the main contributor to the taste sensation of umami.

A study conducted by O' Connor et al. (2020), researched the effects of three different pre-treatments processes on the extraction of protein from four different seaweed samples, including *Fucus vesiculosus.* It was observed that sonication and salting out had the highest protein yield extraction at 35.1% for *Fucus vesiculosus,* followed by autoclaving at 24.3%. It was also noted that all pre-treatment methods used allowed for the detection of all essential amino acids from the samples (O' Connor et al., 2020). Typically, bioactive peptides will contain between 3 and 20 amino acid residues and their functionality is based on the amino acid composition of the algae sample (Kim & Wijesekara, 2010). Some of the bioactive peptides reported to be present in algae samples are linked with biological functions such as antihypertension, antithrombotic, antioxidant,

anticancer and antimicrobial activities while also aiding in nutrient utilisation (Elias et al., 2008; Kim & Wijesekara, 2010).

The reported lipid content of seaweeds is relatively low across seaweed species at 1.5-4% therefore its influence as an energy source is low. Polyunsaturated fatty acids (PUFAs) especially omega-3 and -6 in the form of eicosapentaenoic acids (EPA) make up approximately half of this concentration (Karleskind, 1992; MacArtain et al., 2008). The functionality of PUFAs has been widely researched and has been shown to enhance many bodily functions such as regulating blood pressure, brain development and function, and nervous systems (Karleskind, 1992).

The phytochemicals that are commonly present in seaweeds include carotenoids, phycobilin, fatty acids, sterols, tocopherol, fucoxanthin, fatty acids, and sterols to name a few. Many of these have the biological capacity and therefore can provide health benefits such as reducing the risk of diseases such as type 2 diabetes by promoting the expression of uncoupling protein (Kadam & Prabhasankar, 2010; Lordan et al., 2011; Maeda et al., 2005). Some leafy vegetables contain similar phytochemicals such as those found in seaweeds, such as cabbage. Some of these reported in studies include but are not limited to phenolic acids, flavonoids, carotenoids, alkaloids, and glycosides (Danlami, 2016). When comparing the total phenolic content (TPC) of seaweed (*Palmaria palmata*) to commonly consumed white cabbage (*Brassica oleracea capitata*), the TPC value for cabbage is 34-50 mg GAE/100g, whereas the seaweed has been reported at 456 mg GAE/100g (Martelli et al., 2020; Nawaz et al., 2018). To note also, these reported values vary greatly depending on the extraction solvent and method used. The growth and harvest of leafy terrestrial plants require a larger number of resources, fertilization, time, processing, and storage compared to the conditions required to grow seaweeds within a marine environment. This coupled with the additional phytochemicals with their correlated health benefits reported within seaweeds compared to commonly consumed plant materials, furthers the need to research the viability of this aquatic plant to hold a larger share in the food market in western cultures.

1.2 Consumer demand for seaweed

Consumer demand for seaweed products is typically dominated by Asian markets. However, interest is spreading into western cultures. This demand has been shown to be influenced by the degree of education and how adventurous the consumer may be (Nofima, 2021). The commercial seaweed market, on a global scale, is projected to grow to approximately $25 billion by 2028 according to a report by the CBI (2022). Currently, the production and processing facilities in Western cultures are too low to meet the growing demands and import rates are high. *Saccharina, laminaria* (kombu), *Pyropia yezoenis*, *Pyropia Tenera* (nori), and *Undaria* (wakame) are the most imported seaweed species from Asian countries.

A study by Palmieri & Forleo (2020), surveyed 257 consumers between April and May 2019 on the attitudes of the Italian sub-population to the potential of edible seaweeds within the western diet. Consumer scepticism towards seaweed products is expected in terms of taste, but it has been shown that various markets, such as Italian consumers, show curiosity regarding health-related characteristics (Palmieri & Forleo, 2020). The alleged health benefits, improvements to the economy and establishment of new sources of proteins were important factors influencing on acceptance of edible insects and microalgae identified in German markets (Specht et al., 2019). According to a survey conducted by Nofima, (2021), over 50% of consumers surveyed had a willingness to eat seaweed, 70% believed seaweed is healthy, and 60% believed it is safe to consume. The survey also concluded that in Norway there is a continuing growing trend for vegetarian substitutes, which are more sustainable, organic, or plant-based options, which could be an influencing positive aspect for the results of the survey. Similar views were found in a study conducted in Sweden regarding consumer options with an increasing interest towards the nutritional and sensory profiles of seaweeds (Wendin & Undeland, 2020).

Consumers want to have the ability to make informed decisions regarding their food choices which is why it is vital to provide transparency describing any processing methods used and reasoning behind it. Fishy odours have been shown to alter consumer acceptance of seaweeds in various studies (Bruhn et al., 2019; Hung et al., 2023; Zhu et al., 2021). Fermentation of seaweed has been shown to reduce fish odours and improve sensory properties by removing undesirable aroma compounds (Seo et al., 2012).

Therefore, the process of fermentation for seaweeds has the potential to increase consumer acceptance which may be hindered by aromas or food neophobia.

1.3 Fermentation of seaweed

Fermentation is an ancient processing technique that typically utilises gram-positive lactic acid bacteria (LAB) or yeast in the production of acids from sugars and/or alcohol by oxidation/reduction mechanisms (Yadav et al., 2012). It can be used on many different food types for various purposes. The enhancements which are obtained from fermentation is microbial hydrolysis resulting in the release of intracellular compounds such as phenolic compounds and bioactive peptides (Hur et al., 2014; Reboleira et al., 2021). In addition, it also creates an acidic environment which is unfavourable for the growth of pathogenic food spoilage bacteria. This in turn generates a food product that is safer and has a longer shelf-life. A study by Salgado et al. (2021), investigated the increased nutritional value of brown seaweed waste using marine fungus *Paradendryphiella salina*. They found that the total protein content increased by more than 130%. Moreover, there was a significant reduction in the complex polysaccharides and cellulose concentrations by approximately 2.9-fold. Seaweed fermentation has a cohort of benefits and advantages as it can be used to stabilise wet biomass post-harvest, improve food safety, increase shelf-life, refine the sensory properties, and can be used as an alternative non-dairy source of probiotics (Bruhn et al., 2019). Probiotics have been known to exhibit various health effects for humans such as anti-cancerous, immunomodulatory, and cardiovascular health (Yadav et al., 2012). Additionally, a study conducted by Wang et al. (2015) found that concentrations of lactic acid as low as 0.5 % (v/v) can inhibit the growth of various pathogenic bacteria including *Salmonella* species, *E. coli*, and *Listeria monocytogenes*.

A diagram of some of the biotechnologically relevant products of seaweed fermentation can be seen in Figure 1. In commercial fermentation processes, the main structural polysaccharides are utilised in hydrolysis. Seaweeds have complex polymers such as laminarin, alginate and fucoidans for example which are present in brown seaweeds. These polymers typically cannot be utilised in fermentation with LAB. However, brown seaweeds have additional potential fermentable sugars in the form of mannitol and glucuronic acids, if mannitol fermenting cultures are used within the combination (Chades et al., 2018). Again, depending on which type of fermentation is being carried

out for seaweed processing, these sugars, along with the hydrolysed polysaccharides are converted to pyruvate through glycolysis. Furthermore, this process will continue to either ethanol and CO_2 via alcohol fermentation, or lactic acid via lactic acid fermentation. Effective pre-treatments of fresh seaweeds have been reported for the successful fermentation of macroalgae (Reboleira et al., 2021).

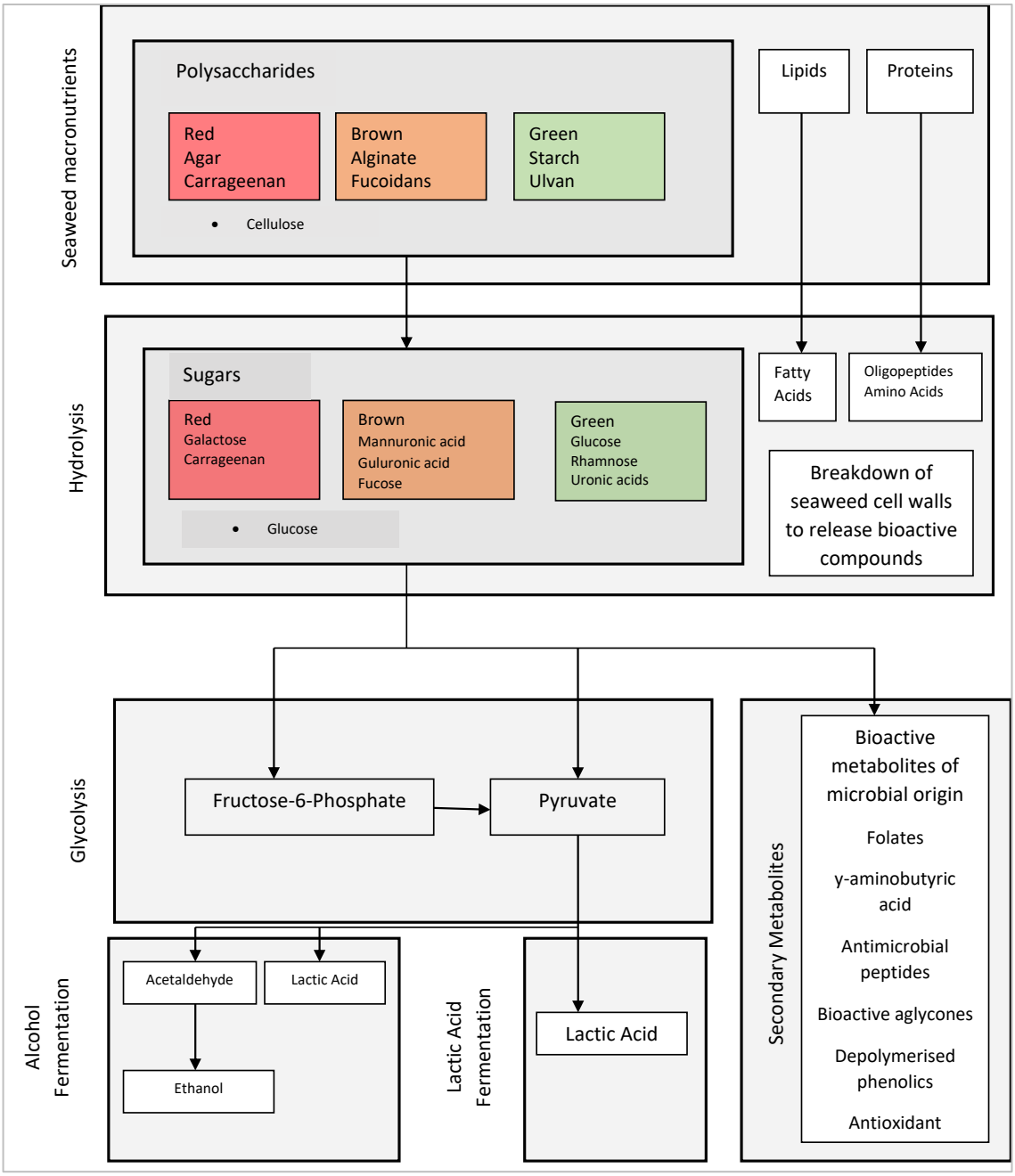

Figure 1 Seaweed fermentation added value components adapted from Reboleira et al (2021)

Zhu et al. (2021) conducted a study to investigate the effects of microbial fermentation on the fishy-odour compounds in *Laminaria japonica* (kelp). The group used *L. plantarum, P. pentosaceus* and *S. cerevisiae* for their microbial and yeast fermentations. The findings suggested that the samples which were fermented with yeast saw a large reduction in the number of aldehydes and the alcohol compounds became the most abundant. The yeast-fermented samples of seaweed saw a reduction of the overall organic volatile compounds from 44 down to 34. The use of the other two microbial fermentations did not have such a strong reduction in the fishy-odour volatile compounds in kelp (Zhu et al., 2021).

A study conducted by Hung et al. (2023) identified 51 volatile compounds in *Ulva* sp. using HS-SPME-GC-MS the concentration of each compound was measured after fermentation using five microorganisms. It was found that LAB fermentation could reduce the total amount of ketones. Ketones have been reported to produce a strong aroma, specifically short-chain aliphatic ketones including pentanone (fruity and pungent) and heptanone (cheesy and spicy) (Hung et al., 2023). Additionally, in this study and others conducted on fermented dairy products, benzaldehyde was oxidised into benzoic acid which has a sweet and pleasant odour when using lactic acid bacteria (Hung et al., 2023; Moreira et al., 2002; Sieber et al., 1995). Furthermore, a study conducted by Bruhn et al. (2019), found that heat treatment before fermentation significantly decreased the taste of salt and umami, while additionally providing a less slimy visual appearance of sugar kelp (*Saccharina latissimi*).

Microbial populations of raw fruits and vegetables can also create an environment for spontaneous fermentation. The microbial portion of fruits and vegetables typically accounts for ranges between 5.0 and 7.0 log colony forming units (CFU) g^{-1} and is usually dominated by yeasts and fungi (Sajjad et al., 2020). Lactic acid bacteria make up a considerably smaller portion of the total naturally occurring microbial count of fruit and vegetables. This spontaneous fermentation can occur when conditions are favourable in terms of low oxygen levels, water activity, temperature, and salt concentrations. The gram-negative bacteria are reduced greatly in lactic acid spontaneous fermentation. The use of a technique, known as back slopping, which involves the inoculation of a successful fermentation, is typically used to produce sauerkraut (Sajjad et al., 2020). Despite the advantages of spontaneous fermentations for stabilising and preserving fruits

and vegetables, for industrial production starter cultures are regarded as a better option. The use of starter cultures allows for a more reliable and reproducible fermented product. Additionally, there may be inadequate inhibition of spoilage organisms. Finally, it is more difficult to standardise the nutritional and rheological properties of spontaneously fermented products.

1.4 Sensory evaluation of fermented seaweed and seaweed products

It is thought that there is a higher probability of odour than taste-related problems due to a larger range of olfactory distinctions. According to a study conducted by Bushdid et al. (2014) at Rockefeller University, humans are capable of distinguishing and discriminating more than 1 trillion scents and intensities. Therefore, alterations made to processing and food formulations need additional consideration as they can cause changes in the physicochemical and sensory properties of a food product. Depending on the degree of change, which has been made to a frequently consumed food, or attempts to introduce a new product to a new market can result in rejection due to the product's aroma, taste, and texture (Cabello-Olmo et al., 2023). This can be a costly risk to some food businesses and therefore sensory evaluation is a highly dependable method to avoid potential rejection of a product on these grounds. It can also act as a tool of refinement and inform the company on quality attributes which are most important for the consumer.

Typically, panellists that are selected for sensory evaluations can be categorised as trained or consumer panellists and the selection of sensory evaluation type will depend on the purpose of the study. Trained panellists will be selected if more in-depth sensory aspects will be evaluated. For this evaluation, the panel will receive descriptive analysis training for the selected seaweed samples. In addition, the panellists will perform preference testing on product innovations. Moreover, they will be asked to identify differences in the aroma attributes which could aid or hinder the product's success. Consumer panellists are usually asked to complete basic hedonic testing to identify their preferences for one product over another. Skonberg et al. (2021) utilized a consumer panel when testing preferences of their seaweed sauerkraut style product when incorporating seaweeds at various inclusion levels. Studies conducted by both Bruhn et al. (2019) and Jönsson et al.

(2023) performed panel training for descriptive sensory analysis for appearance, odour, taste and texture. From the training of the panel, some of the words used to describe the appearance of seaweed and kelp used word such as ‘lightness’, ‘green’, ‘yellow’, ‘wet grass’, ‘slimy’, and ‘green kale’ (Bruhn et al., 2019; Jönsson et al., 2023). For aroma testing, descriptive words such as ‘sea’, ‘fishy’, ‘iron’, ‘forest floor’, ‘sweet’, ‘mushrooms’, ‘boiled green vegetables’, ‘sweet’ and ‘salty’ have been used in the same studies. Textures were also evaluated using physical touch and appearances and words such as ‘slimy’, ‘soft’, ‘rubbery’, ‘sticky’ and ‘raw’ have been used for the texture of kelp (Bruhn et al., 2019). Both studies used intensity scales for their descriptive analysis of the attributes identified from training sessions.

Some of the most notable volatile compounds, such as aldehydes and ketones, affecting consumer acceptance have been highlighted in previous research by Zhu et al. (2021). Aldehydes and ketones have been shown to produce seafood aromas and flavours in food. It has been reported that aldehydes contribute to a lower threshold for consumer satisfaction (Hung et al., 2023). Ketones have been shown to provide a fruity aroma in fermented seaweeds and are linked to increasing consumer acceptance. Zhu et al. (2021) conducted a study on the effects of microbial fermentation on the fishy-odour compounds in kelp and found that the most noteworthy odour activity values were in ketones and aldehydes. The top three most notable compounds detected in kelp were 1-octen-3-one (metallic, mushroom, dirt), (*E,Z*)-2,6-nonadienal (cucumber, green leaves), and (*E,E*)-2,4-decadienal (fatty, wax, oily). Volatile carboxylic acids may also produce aromas such as ‘liquorice’, ‘spicy’ and ‘seafood’ in some species of seaweed.

1.5 Use of seaweed as a functional ingredient in new product development

Several studies have been conducted to understand acceptability, absorption of nutrients, nutritional enrichment, sensory characteristics, antioxidant capacity, dietary fibre utilisation and anti-inflammatory in products containing both micro and macroalgae species. There have been several product innovations using seaweeds within the food, health, packaging, and feed industries thus far (Table 2). The first study from Table 2 is by Etemadian et al. (2018), who performed extensive testing on brown seaweeds

(*Sirophysalis triodes* and *Polycladia myrica*) prior to the extraction of algae extract (total protein, crude lipids, estimations of carbohydrates, mineral content, fatty acid composition). This extract was used to season corn snacks at both 2 and 4%. After this further extensive in vitro testing was carried out. These methods included antioxidant and anti-microbiological properties, total phenol content, fatty acid composition, chemical analyses such as thiobarbituric acid, peroxide value, colour, water activity, microbial count and sensory characteristics of fortified corn snacks. After sensory evaluations, they found that the 4% incorporation of the seaweed extract produced a very mild seaweed flavour with a taste that was similar to control samples of corn snacks. Both seaweeds especially at the incorporation at 4% level were effective methods of increasing the functionality of commonly consumed snacks by children. The study by Hajal et al. (2015), also incorporated seaweed powder (*Kappaphycus alvarezii*) and *Hoodia gordonii* into a snack product in the form of a brown rice cereal bar. This study developed 10 different formulations of the cereal bar and preformed colour, texture, and sensory evaluations of the products. They found that the incorporation of seaweed powder at 2.8% scored best amongst a trained panellist of 30 people. From the sensory evaluation, the best preforming formulations were selected for proximate analysis and tested against the control sample. These results indicated a significant increase in total fibre content, ash and carbohydrate content. This study did not test for the more specific phenolic, fatty acid composition and antioxidant activity commonly associated with seaweed incorporation as per the previous study by Etemadian et al. (2018). Jenifer and Kanjana (2019) examined the impact of incorporating *Ulva lactuca,* a high-protein seaweed, into a biscuit-style product. Their research aimed to build on previous studies by not only investigating improvements in nutritional quality and consumer acceptability but also investigating the potential to increase serum protein levels in malnourished preschool children (ages 5-6). There were five different formulations at various percentages of seaweed powder incorporation between 30 and 60%. The formulation containing the 30% seaweed was supplemented to the children and they found a positive correlation between the protein serum level at this level over a two-month period. Significant differences were found an increase in weight, height, body mass index and total protein were observed. However, this study did not use a control group in their study and therefore, it may be difficult to attribute the impacts of the seaweed specifically compared to the other ingredients in the biscuit. Further research

would be needed in order to substantiate such claims. The study by Allsopp et al. (2015) however, did use a more robust method study to investigate the impact of a bread containing *Palmaria palmata* on inflammatory serum markers, lipids, thyroid function and antioxidant reducing power. The method used by this study group was a randomised parallel placebo-control human intervention study. The study concluded that there was evidence that the consumption of bread containing 5g of *Palmaria palmata* stimulated inflammation, increased the triglycerides, and altered thyroid function. They did state that these changes were not significant enough to impact health as these changes were within the normal clinical range. The final study from Table 2, developed a dehydrated soup formulation using microalgae (*Spirulina platensis*) or spinach. The soup containing the microalgae contained a higher protein content than the spinach soup 4.6 and 3.3g 100 g^{-1}, respectively. They also found through sensory evaluations using check all that apply method found that the soup with microalgae had an enhanced herb flavour, seasoning fragments and deeper dark green colour compared to the spinach soup. In addition, the microalgae dehydrated soup provided a good alternative with nutritional enrichment, with high protein, ash, fiber and antioxidant contents with good acceptability amongst panellists.

Several products which have incorporated the use of seaweed powders and microalgae within the food industry include energy bars, seasonings, pasta, bread, sweet and savoury crackers, yoghurt, cheese, soup, and fish jerky (Nova et al., 2020). Fermented seaweeds have not been seen within the European markets as widely available. Nor have fresh seaweeds been studied as widely in research areas. However, the Danish Technological Institute in collaboration with Fermentation experts and EXPERGO have developed a cereal bar containing fermented seaweed in combination with upcycling rapeseed meal as an alternative protein (Bambridge-Sutton, 2023). The research group fermented the seaweed for this product dried it and utilised microencapsulation to mask acidic aftertaste commonly associated with fermentation. This product has not yet been launched to market. This leads to a gap in the market if consumer acceptability of the products persists.

Table 2 Studies conducted containing microalgae and seaweed extracts for health and/or technological enhancement

Product	**Species**	**Benefit**	**Method of incorporation**	**Reference***
Corn snack	*Sirophysalis trinodis*	Nutritional enhancement with protein, minerals, and fatty acids	Powdered seasoning	(Etemadian et al., 2018)
Brown rice cereal bar	*Kappaphycus alvarezii*	Colour enhancement, good sensory acceptability, nutrient enrichment	Seaweed powder	(Hajal et al., 2015)
Seaweed based biscuit	*Ulva lactuca*	Dietary fibre, nutrient enhancement, protein enrichment for body mass increase for malnourished children	Powdered	(Jenifer and Kanjana, 2019)
Bread	*Palmaria palmata*	Anti-inflammatory, increased serum triglycerides, altered thyroid function	Finely chopped	(Allsopp et al., 2015)
Dehydrated soup	*Arthospira platensis*	Nutrient enrichment, antioxidant activity, colour, viscosity, and solubility enhancement	Powdered	(Los et al., 2018)

*Table adapted from (Nova et al., 2020)

Tackling the food safety issues regarding potential heavy metals, which may be present in seaweeds need to be addressed by processing to ensure consumer safety. Additionally, post-harvest deterioration of the product should be assessed to ensure its feasibility. The Commission Regulation (EC) No 1881/2006 has stated the maximum levels of toxic elements such as heavy metals in various food products, however, due to low consumption levels of macroalgae by the European population, macroalgae has not been researched and regulated. Macroalgae have the ability to store varying amounts of cadmium, inorganic arsenic, and mercury obtained from the growing environment. The EU has set limits for amounts of mercury in food and feed from algae through Regulation (EC) No 396/2005 which looks at pesticides and is set at a default level of 0.01 mg/kg. The regulation limit is relatively low when compared to other marine products for human consumption which are set in Commission Regulation (EC) No 1881/2006, which can further complicate processes of bringing new products containing seaweeds to the European markets (Commission Regulation (EC) No 1881/2006). There are some species of seaweed which do not need to be authorised by the novel food Regulation (EU) 2017/2470 which have been approved in the Novel Food Catalogue. Some of the species which have been approved include those most consumed red (*Gracilaria verrucosa, Chondrus crispus, Palmaria palmate*), brown (*Ascophyllum nodosum, Fucus vesiculosus, Himanthalia elongata),* and green (*Entermorpha* sp*., Ulva fenestrata, Monostroma nitidum*). A study by Bruhn et al. (2019) found that the fermentation of sugar kelp significantly reduced the amount of two harmful heavy metals mercury and cadmium as well as reduced the salt content of the seaweed. Therefore, seaweed fermentation may aid in producing food products which are safer for human consumption as well as aiding other aspects such as self-life, improved sensory properties and better consumer acceptance.

1.6 Aim of the study

This thesis work aimed to develop two products containing fermented *Fucus vesiculosus*. For this, various trials using lactic acid bacteria to ferment the seaweed were tested to attempt to optimise this fermentation process. The study also focused on testing the quality attributes of raw to processed seaweeds in addition to the sensory characteristics of the seaweed-containing products.

2 Materials and methods

2.1 Seaweed collection

The *Fucus vesiculosus* was harvested on two separate occasions, the first batch on the 15th of August and the second batch on the 5th of September 2023. The area in which the seaweed was collected was the surrounding area of Boskär, Turku Archipelago region. Once received at the unit on the 18th of August and 8th of September respectively, the batches were transported in sea water and stored under UV light to prevent autolysis at a refrigeration temperature of 4 ℃ as intermediate storage. The two batches were processed within two to three days of arrival date by washing under tap water to remove debris and leafy parts of the thallus were sectioned into containers and stored in the freezer at -20 °C.

Additionally, a packet of dried powdered organic kelp (*Ascophyllum nodosum),* Saltlife brand) was purchased from the Ruohonjuuri Turku store. This kelp was harvested and processed on the coast of Canada.

2.2 Preparation of lactic acid bacteria

The preparation of lactic acid bacteria was conducted according to the unit protocol for fermentation of Baltic herring (Kivinen, 2023). MRS broth (LAB094), Agar (LAB223) and MRS-glycerol broth (MQ water: glycerol in 80:20 ratio) were prepared according to manufacturer instructions. A pure culture of *Lactiplantibacillus plantarum* DSM 20174 was prepared on the MRS agar plate from the glycerol stock. The incubation total period of the plate was 67 hours at 30 ℃ compared to 72 hours as there was sufficient growth on the plates. One colony was transferred to 1 mL MRS broth in Eppendorf tubes and further incubated for 24 hours again at 30 ℃. The Eppendorf tubes were then centrifuged (5801R) at 2500 rcf at 8 ℃ for 20 minutes. In the laminar flow cabinet, the supernatant was poured off, 5 mL of MRS-glycerol broth was added to the falcon tube, 1 mL was added into the Eppendorf tube and flushed to mix the bacteria pallet and then added to the falcon tube. This 6 mL of MRS-glycerol with bacteria pallet was dispensed back

across 6 Eppendorf tubes, labelled (MM LB 1-6) and stored in the freezer at -80 ℃ until further use.

2.3 Growing and washing of bacteria

The starter cultures were inoculated in 50 mL of MRS broth using two methods, one using a 1µl and by pipetting 100 µl into falcon tubes. The two methods were used to determine any difference between growth methods. The inoculated MRS broth was incubated at 30 ℃ for 24 hours. The falcon tubes were then centrifuged at 2500 rcf at 8 ℃ for 20 minutes. The supernatant was poured off and 50 mL of saline was added. The centrifuge conditions were repeated with the saline and then once again the supernatant was poured off and repeated once as per the method by Kivinen (2023). Once the bacteria were washed, it was resuspended in 10 mL of saline (dilution depends on intended use).

2.4 Seaweed pasteurisation

To reduce the potential of spontaneous fermentation and to control the procedures, the macroalgae was subject to different pasteurisation temperatures and durations. There are two preparations of seaweed being used for fermentation, which include one batch of blended seaweed and one chopped from the blade section. In the first, second, and third trials the unit protocol of Kivinen (2023), pasteurisation for herring was adapted for seaweeds, which was followed at 80 ℃ in a water bath for 10 minutes, mixing the sample bottles at 5 minutes. Samples were then cooled to below 40 ℃ before inoculation. For the fourth trial and first sauerkraut trial, seaweed was pasteurised at 95 ℃ in a water bath for 10 minutes. For the sensory evaluation samples, 2-day and 12-day fermentations and the second sauerkraut trial, the seaweed was pasteurised at 95 ℃ in a water bath for 15 minutes. The commercial store-bought powdered kelp product was not pasteurised before fermentation as it had already been processed and heat treated.

2.5 Fermentation optimisation trials

Various fermentation techniques and methods from the literature were employed throughout the thesis project in attempts to optimise a protocol for fermenting fresh *Fucus vesiculosus* (Bao et al., 2018; Bruhn et al., 2019; Hung et al., 2023; Kivinen, 2023). During the various trials (Table 3b), seaweed conditions (chopped/blended), glucose concentrations, heat treatment, inoculation rates of LAB, and durations of fermentation were altered.

The first and second trials used and adapted was the unit protocol for fermentation of Baltic herring from Kivinen (2023) with the conditions of the seaweed, chopped and blended. The fermentation mass of which can be seen in Table 4. These samples will be referred to as F1C and F1B, C indicating chopped sample and B indicating blended sample. Adjustments were made for the quantity of algae used, and no additional NaCl was added to the fermentation. Once the seaweed was pasteurised at 80 ℃ for 10 minutes and allowed to cool to below 40 ℃, 10% of *L. plantarum* DSM 20174 at $2.1x10^8$ CFU was added to the fermentation mass. A 2% glucose solution was added at 67% of the fermentation using laboratory reagent grade D-glucose anhydrous from Fisher Scientific (Code: G/0450/60, Lot: 2213383). The bottles were closed and shaken prior to being added to the incubator. The pH was measured at the start of fermentation and each day thereafter. Fermentation processes for macroalgae were carried out at 37 °C for 5 days with monitoring and sample collection as per method by Bao et al. (2018). Every 24 hours duplicate samples were collected for sugar, acid and CFU analysis.

Table 3a Sample abbreviations main conditions which undergone further testing

Sample Abbreviation	Description
F1C	First Fermentation Chopped seaweed in Trials 1
F1B	First Fermentation Blended seaweed in Trials 1
GT10	Glucose Trial 10% in Trial 3
GT20	Glucose Trial 20% in Trial 3
SF12	Sensory Fermentation 12 days, Algae addition on Day 0 in Trial 5
SF2	Sensory Fermentation 2 days, Algae addition on Day 6 in Trial 6
CK	Commercial Kelp Powder in Trial 7
CA	Cabbage and Algae in the second sauerkraut trial
CC	Cabbage Control in the second sauerkraut trial

Table 3b Summary of the various trials and methods used for fermentation of seaweed

Trial	Seaweed Condition	Glucose Solution	Heat Treatment	LAB Inoculation	Incubation	Sampling	Additional Steps
Trials 1 & 2 (F1C) *	Chopped	2%	80°C for 10 min	10% DSM 20174	37°C for 5 days	Every 24 hours	-
(F1B) *	Blended	2%	80°C for 10 min	10% DSM 20174	37°C for 5 days	Every 24 hours	-
Trial 3 (GT10) *	Chopped	10%	80°C for 10 min	9%	37°C for 7 days, then 30°C until day 16	Every 24 hours	Isolate algae with more glucose and LAB on day 16
(GT20) *		20%	80°C for 10 min	8.7%	37°C for 7 days, then 30°C until day 16	Every 24 hours	Isolate algae with more glucose and LAB on day 16
Trial 4	Chopped	10%	95°C for 10 min	1%	30°C for 11 days	Every 24 hours	LAB grown before seaweed addition, Seaweed addition on Day 2
Trial 5 (SF12) *	Chopped	10%	95°C for 15 min	1%	30°C for 12 days	Every 24 hours	Algae addition on Day 0
Trial 6 (SF2) *	Chopped	10%	95°C for 15 min	1%	30°C for 12 days	Every 24 hours	Similar to Trial 4, Algae addition on Day 6
Trial 7 (CK) *	Commercial Kelp Powder	10%	No heat treatment	0.1% DSM 20174 & 0.1% DSM 13273	30°C for 12 days	Every 24 hours	-
		Salt/Salt solution					
Sauerkraut (first strategy)	Chopped	2.5% sol/using algae NaCl	95°C for 10 min	Natural fermentation	Room temperature (17-19°C)	Day 8-10	Using red cabbage
Sauerkraut (second strategy) (CA & CC) *	Chopped	Dry NaCl: 2.5%	95°C for 15 min	Natural fermentation	Room temperature	Day 8	Using red cabbage, Control: No algae

*Abbreviations of the trial names can be seen in Table 3a above

Table 4 Fermentation mass of main methods of fermentation trials in which further chemical analysis was conducted

	Fermentation 1		**Glucose variation**		**Commercial kelp**		**Sensory fermentation**	
Ingredient	*F1C*	*F1B*	*GT10*	*GT20*	*DSM 20174*	*DSM 13273*	*SF2*	*SF12*
Algae (w/v)	23%	49%	28%	28%	5%	5%	4.9%	4.85%
Sugar solution (v/v)	67%	41%	62.3%	63%	94.6%	94.6%	94%	94.2%
Starter culture (v/v)	10%	10%	9%	8.7%	0.1%	0.1%	0.97%	0.97%

Abbreviations of the trial names can be seen in Table 3a above

The third trial was conducted on only chopped seaweed samples and was testing two glucose concentrations, 10% and 20% referred to as GT10 and GT20 in the results section. Other parts of the method remained the same such as the pasteurisation temperature and sampling every 24 hours. The incubation temperature started at 37 ℃, however, on day 7 of this fermentation the temperature of the incubator was dropped to 30 ℃ due to instrument demand. The fermentation continued at this temperature until day 16. From this fermentation, two pieces of algae were removed and isolated into a falcon tube, where an additional 5 mL of the 10% and 20% glucose solution, with another 100 µL of LAB and finally 2 mL of the previous fermentation broth. This was done to see if the pH would continue to drop with the algae and LAB receiving fresh growing conditions.

The fourth trial used a fermentation broth, meaning the algae was not added on day 0 as per the previous trials. This was conducted to allow the LAB to grow and proliferate within the fermentation mass before the addition of seaweed. The 10% glucose solution was inoculated with 500 µL of the LAB strain and incubated at 30 ℃. Then on day 2 of the fermentation, a reduced quantity of algae at 5% was heat treated at an increased temperature of 95 ℃ for 10 minutes. Once the algae had cooled to below 40 ℃, it was added to the fermentation broth. Sampling was again conducted every 24 hours. Fermentation continued at this temperature for 11 days.

The fifth and sixth trials were conducted for the sensory evaluations and utilized the previous conditions of the fourth trial in terms of the fermentation mass. However, the fifth trial (referred to as SF12 in results) incorporated the algae on day 0 and the sixth trial (referred to as SF2 in results) used the fermentation broth technique, and the algae was only added on day 6 after pH and brix readings and sampling. The algae for both these trials were pasteurised at an increased duration of 95 ℃ for 15 minutes compared to 10 minutes used for all previous trials. The algae for both trials were added at 5 % of the fermentation mass, the 10% glucose solution was approximately 94% of the fermentation mass and the starter LAB culture was added at 1% of the overall fermentation mass. These trials were done in a batch of 10, and the pH and brix readings and sampling were taken in duplicates every 24 hours. The fifth trial using the technique of algae addition on day 0 was incubated at 30 ℃ for 12 days, and on day 12 of this fermentation, the sensory evaluation was conducted on the seaweed. The sixth trial which used the fermentation

broth method of algae addition on day 6 is referred to as the 2-day fermentation for the sensory evaluation as the seaweed from this trial was used for sensory evaluation on day 8 of the overall fermentation, due to the seaweed only being within the fermentation for a total of 48 hours. The readings and sampling of extra samples not used in the sensory evaluations were monitored through pH and Brix for 10 days.

As a final trial for the inoculated strain of *L. plantarum* a commercial store-bought powdered kelp (referred to as CK in results) was used to test the viability of LAB in its ability to ferment the dry substrate. Two different strains of *L. plantarum* were used for this trial, the DSM 20174 which had been used in all previous trials and DSM 13273. Each strain was tested in duplicate using the same conditions and fermentation mass. There was a difference in the growth rates of the LAB strains, the DSM 20174 had a recorded growth rate of $2.3x10^6$ log CFU whereas the DSM 13273 had a growth rate of $3.4x10^7$ log CFU when inoculated. Additionally, no pasteurisation or any heat treatment was used for the powdered kelp species. The monitoring of pH and brix as well as sampling was the same as the previous trials of every 24 hours, and the incubation temperature was 30 ℃ for a total of 12 days.

For the product innovation of the sauerkraut product, the fermentation trial used a natural spontaneous fermentation with the naturally occurring LAB from the red cabbage instead of inoculation of the LAB *L. plantarum* strain. The fermentation also excluded the use of any additional glucose solutions as the cabbage contains sufficient fermentable sugars for the naturally occurring LAB. Two different strategies were used when conducting this fermented product. The first of these was testing the viability of using the NaCl content from the algae and a control sample of 2.5% brine solution. For this trial as previously stated, the algae were pasteurised at 95℃ for 10 minutes, and the algae were added to the fermentation mass at 3.4%, the red cabbage made up 28% of the fermentation mass and the remaining mass was made up of the NaCl solution and in the control sample, it was boiled and cooled water. This fermentation was carried out at room temperature in the laboratory which varied between 17-19 ℃. To ensure the cabbage and algae were fully submerged and maintained anaerobic conditions, food-grade plastic bags were filled with water and placed inside the fermentation containers. Bubble formation was additionally monitored in these fermentation samples and on day 2 the seal of the containers was

slightly opened to allow the release of the CO^2 build up. The pH and brix were recorded on day 8 to day 10 where fermentation was ended, and samples were frozen at -20 ℃.
The second trial of the sauerkraut products used a different method of incorporating the NaCl. Instead of using a solution, 2.5% NaCl was added dry to both samples and used to draw out liquid from the cabbage. The control in this trial was sauerkraut without algae. The algae for the product innovation were pasteurised at a higher temperature for a longer duration of 95 ℃ for 15 minutes. As per the previous trial on the sauerkraut, the fermentation was carried out at room temperature. The fermentation was monitored, and sampling was conducted in the same manner as in previous trials. The products of this trial were used for the sensory evaluation and GC-FID analysis, referred to as CC and CA, control and product-containing algae. The fermentation ended on day 8 when the pH reached the desired value of approximately 3.8 by transferring to the freezer at -20 ℃.

2.6 Product innovations

As previously stated, two product innovations were developed utilizing *Fucus vesiculosus,* namely sauerkraut and pesto. The ingredients and quantities incorporated can be seen in Table 5. For the sauerkraut product, as explained in 2.5 Fermentation optimization trials, used the technique of spontaneous natural fermentation. The red cabbage was used to disguise the pigmentation leaking from the algae. Also, juniper berries and caraway seeds were used in the base recipe at 0.95g and 0.2g. The control and algae sauerkraut from the second trial were used for the sensory evaluations.

For the pesto product, previously fermented algae from the chopped 10% glucose trial were used as a functional ingredient. The total amount of fermented algae added to the batch was 9g. The ingredients were blended using an immersion blender and then split into two separate batches, control, and algae pesto. The algae were added into the blender and blitzed one final time to fully incorporate the product. Then the two batches were stored in the refrigerator overnight at 4 ℃. Before the sensory evaluations, the pesto was removed from the refrigerator and left to sit at room temperature for two hours.

Table 5 Fermentation mass of spontaneous fermentation and functional ingredient product innovations

	Sauerkraut			**Pesto**	
Ingredient	*Control*	*Algae*		*Control*	*Algae*
Cabbage (w/v)	96.08%	91%	Basil (w/v)	12.59%	12.06%
Algae (w/v)	/	4.50%	Algae (w/v)	/	4.17%
Salt (w/v)	2.60%	2.50%	Pinenuts (w/v)	7.74%	7.42%
Juniper berry (w/v)	1.07%	1.06%	Garlic (w/v)	2.17%	2.08%
Carraway seed (w/v)	0.23%	0.22%	Parmesan (w/v)	22.76%	21.80%
			Olive oil (v/v)	54.72%	52.43%

The conditions which were tested in the sensory evaluations as well as the product innovations can be seen in Figure 2 below. The sensory evaluation setup can be seen in the bottom left of the figure.

Raw and processed	Product innovations
	
	

Figure 2 Images of the 4 different seaweed treatments (raw, heat treated, 2-day, 12-day fermented), full evaluation setup, and both product innovations with their control sample.

2.7 Sugar, acids, pH, Brix, and colony forming units monitoring

The pH of the samples from every 24 hours of each of the fermentation trials was measured using the LLG-labware digital pH meter 5 and recorded. The Brix readings were taken as the fermentations were being conducted using the Hanna instrument HI 96801. The digital instrument is an optical tool that employs the measurement of refractive index to determine the percentage Brix of sugar in aqueous solutions.

The protocol for sugar and acid analysis was adapted from Kalpio (2014) using GC methods. The sample preparations were adjusted from the protocol to reduce the quantities of each sample to account for 1 mL compared to the 5 mL in the protocol. The ratios were kept the same and can be seen in Table 5 below. References internal standards, which were used in the quantitative analysis are xylitol for sugars and tartaric acids for acids. Calculations from these standards were used against the concentrations of measured compounds in the sample to quantify the exact concentrations from each day of fermentation. The frozen samples were thawed and once the dilutions (B-E) were made then they were vortexed (approx. 30 seconds). The samples were then filtered through RTFE syringe filters and 300 µL of the filtrates were transferred into autosampler bottles. Then all samples and standards were dried in heat blocks at 50 ℃ under nitrogen gas. Once the samples were dried then they were stored open cap in a desiccator overnight. The volatile components are determined by TMS derivatives and require derivatisation by TMS using hexamethyldisilane and trimethylchlorosilane (Tri-sil) in pyridine. This was added to all samples and standards using a needle and syringe the following day in the fume hood before the GC-analysis. The samples were then vortexed (Vortex-Genie, Springfield, MA) for 5 minutes heated at 60 ℃ for 30 minutes and allowed to cool.
The GC-FID instrument used was Shimadzu GC-2010Plus with Autoinjector AOC-20i with flame ionization detector and LabSolutions software for quantification. The analytical conditions are helium as a carrier gas with an injection temperature of 210 ℃ and an injection volume of 1µl. The total analysis time for sugars and acids is just under 29 minutes (Kalpio, 2014).

Table 6 Sample preparations for GC-analysis adapted from protocol from Kalpio (2014)

Sample	Content			
A	100 MQ-H_2O			
B	90 MQ- H_2O	5 xylitol std	5 tartaric acid std	
C	95 MQ- H_2O	5 seaweed*		
D	85 MQ- H_2O	5 xylitol std	5 tartaric acid std	5 seaweed*
E*	80 MQ- H_2O	5 xylitol std	5 tartaric acid std	10 seaweed*
F	50 of sugar and 50 acid std sol into autosampler bottles			

The numbers are shown in percentages of total volume. E* increased concentrations of seaweeds in the samples, seaweed* refers to the liquid phase of fermentation mass

Table 7 Sugar and acid standard solutions concentrations used for internal standards in GC-analysis

Sugar Standards	Concentrations g/L	Manufacturer
D-Fructose	4.904	Sigma Aldrich chemistry, No. F-0127
D-Glucose	6.916	Merck, 1.08337.0250
Sucrose	7.348	Merck, S1600000
Xylitol	5.007	Sigma Aldrich chemistry, Lot SLBS9890
Acid Standards	**Concentrations g/L**	
Malic acid	5.044	Sigma Aldrich chemistry, Lot SLBW9277
Citric acid	7.265	Merck, A1202000
Quinic acid	5.388	Sigma Aldrich chemistry, Lot BCBW90003
Ascorbic acid	4.932	VWR PROLABO, No. 0500920
Tartaric acid	5.003	Sigma Aldrich chemistry, Lot 41447-100MG
Lactic acid	5.041	Sigma Aldrich chemistry, Lot L1750-10G

The correction factors were calculated for each of the standards to quantify the concentrations of the sugars and acids within the samples across the fermentations:

$$Formula\ for\ correction\ factor\ K = \frac{Area_{std}}{Conc_{std}} = K * \frac{Area_{analyte}}{Conc_{analyte}}$$

$$Lactic\ acid\ K = \frac{381862}{2.5015} = K\frac{137314}{2.5005}$$

$$Lactic\ acid\ K = \frac{152653.208}{54914.6171} = 2.77$$

From this, the equation was manipulated to calculate the concentrations of each compound by the following:

$$Formula\ for\ concentration\ of\ analyte = \frac{Area_{analyte} * Conc_{std}}{Area_{std} * K}$$

$$Day\ 5\ chopped\ lactic\ acid\ conc\ = \frac{10004 * 5.003}{125905 * 2.779} = 0.1430\ g/L$$

Colony forming units were the method used to monitor the amount of lactic acid bacteria before, during and after fermentation. The method was used before fermentation to determine the bacterial number required for fermentation. The MRS agar plates were used and 100 µl of diluted samples were spread on the plates and incubated for 72 hours at 30 ℃ (Kivinen, 2023). Samples which were collected during the fermentation from the liquid portion of the jars used this method also but were performed the week following the fermentation. Samples were stored at -80 ℃ until analysis was carried out. Duplicates of CFU from each day were performed to obtain the average amount of colonies from each phase of fermentation. Colonies were counted within the 20-300 range.

2.8 Sensory evaluation

Sensory evaluation training and analyses were planned and carried out with reference to Heymann, (2010). The panel was recruited from students at the food science unit at the University of Turku. There were 6 members in the sensory panel who took part in the training and final evaluation. Compusense software was used both for training and evaluation.

Two 90-minute training sessions were held in which the panel developed terminology and phrase banks to describe the seaweed under appearance, aroma, and texture attributes. Additionally, the total list of descriptive words was reduced from 41 to 14 (Table 8). Additionally, scale training and attributes were developed so that each member understood various intensity and attribute names (Kemp et al., 2018). During the training sessions, it was decided that a 10-point scale was more suitable for the final evaluation. Descriptive testing was carried out on these selected attributes for appearance, aroma, and texture on the following samples, raw, heat treated, 12-day fermented, and 2-day fermented. Furthermore, the two product innovations and control samples were evaluated under the aroma attributes and likeness/acceptability of the products. These samples were placed into brown jars and the panel were instructed to evaluate solely on the aroma attributes.

Table 8 Selected terminology for descriptive analysis and their definitions from training sessions

Attribute	Definition
Appearance	
Green	The degree of green in the sample
Brown	The degree of brown in the sample
Matte/Glossy	Degree of dryness or wetness on the sample
Redness	Red colour detectable in the sample
Aroma/Smell	
Aroma intensity	Total aroma intensity of the sample
Grassy	Freshly cut grass
Metallic	Degree of metallic aroma
Seaweed	Fresh seaweed/seashore/salt
Wet Muddy	Staleness/earthy/forest floor of sample
Texture	
Smooth/Grainy	Surface level smoothness or graininess
Elastic	Elastic resistance/stretchy
Soft/Hard	Physical softness or hardness of leaves
Tear ability	Ease of tearing sample
Rubbery	The surface of the sample feels like rubber

2.9 Statistics

Results of pH, Brix, CFU, sugars and acids are given as means from duplicate samples. The sugars and acids also used ± standard deviations of the means where both samples contained the sugar or acid. Statistical analysis for sensory evaluation results used 2-way ANOVA and post hoc of Tukey's HSD using a significance value of $p < 0.1$(*), 0.05(**), and 0.01(***) were conducted using Compusense software.

3 Results

3.1 Fermentation process

Generally, the inoculated samples with LAB from the methods tested did not reach the desired pH value of 4.5 within the fermentation duration, apart from the sensory samples, SF2 and SF12 which can be seen in Figure 3a and Figure 4. The SF2, which used the fermentation broth method, reached the desired pH range by day 4. Whereas SF12, the seaweed was added on day 0, the pH did not reach the desired pH value until day 11, and the sensory evaluation was conducted on day 12. In all samples, the LAB did experience some sublethal injury (SI)[1] during the inoculation (Table 9). However, the SF2 sample had recovered to initial inoculation levels by day 9 of the fermentation. Moreover, the GC-FID results for sugars and acids (Table 10) did not detect any lactic acid within SF2 with the instrument parameters. Despite the drop in pH within the sample, there were no notable acids detected with the instrument parameters.

The Brix (Figure 3b) readings in the sensory fermentation samples (SF2, SF12) as well as the commercial kelp (CK) did not reduce significantly in the duration of the fermentation. This may further indicate that the LAB within the sample either did not survive post-inoculation or the LAB which did survive was not able to obtain the sufficient environmental conditions required to proliferate and utilize the sugars within the fermentation mass. There was a drop in the brix readings from the sauerkraut samples as well as within the glucose trial (GT10, GT20). The drop in the brix readings for the sauerkraut products can be noted at day 7 in the fermentation. This is where we expect the fermentation to be optimal and the LAB to proliferate exponentially and peak at the stationary phase of growth. This is where most of the available sugars will be utilized by the naturally present bacteria from the cabbage leaves.

[1] Sublethal injury (SI) can defined as “a consequence of exposure to a chemical, physical process or change in conditions from growth media that damages but does not kill a microorganism” (Hurst, 1977). Cellular injury may result in the compromise of the permeability barrier within the cell wall and/or membrane, affecting functional cell components like ribosomes and structural DNA, referred to as metabolic damage, or a combination of both. Cells that are sub lethally injured exhibit sensitivity to selective components that uninjured cells resist, rendering them incapable of growth on selective media typically employed for detecting foodborne pathogens in the food industry.

To get dilution factors of the samples within a countable range for CFU, some of the samples were tested multiple times which meant defrosting the sample material several times (Table 9). This may have affected the true CFU within the original fermentation liquid. Therefore, the results of the CFU are only here used to indicate whether the LAB survived within the fermentation and roughly indicate the reduction in the overall range.

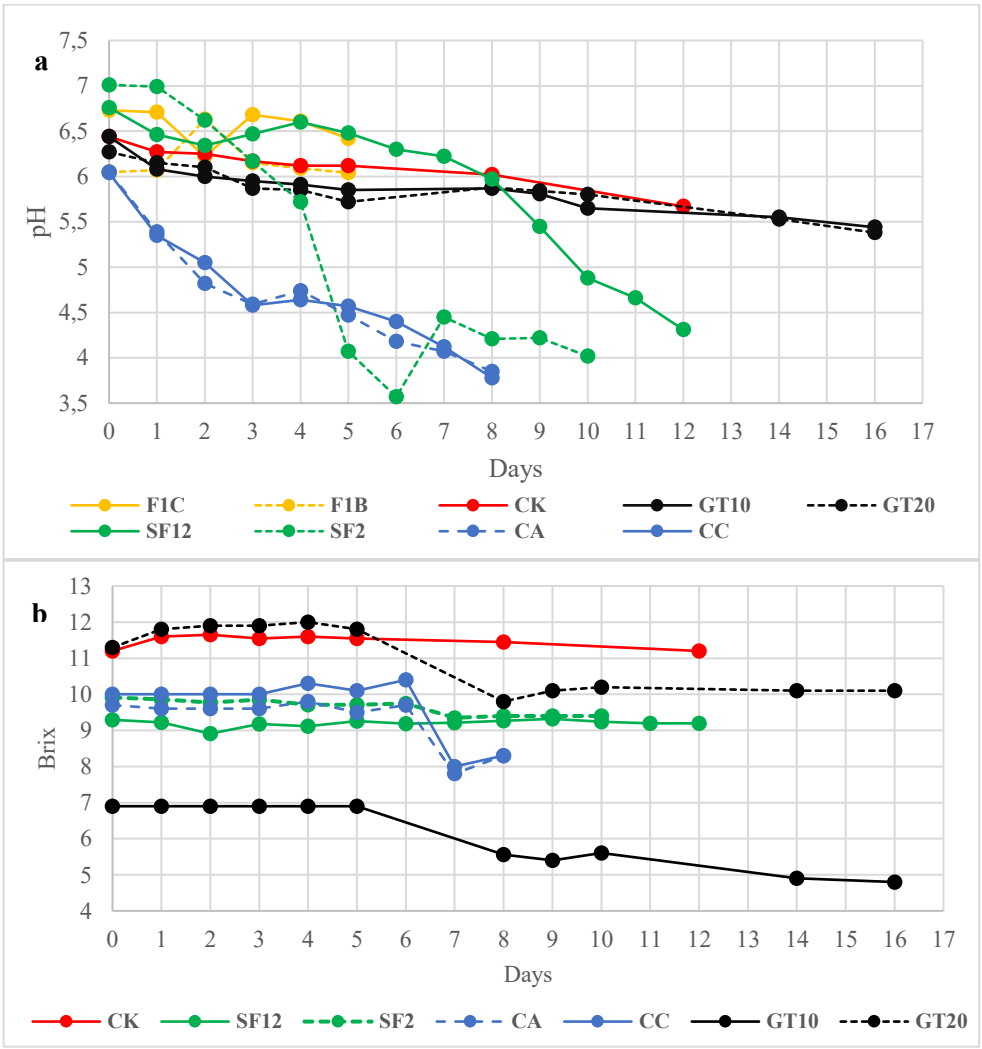

Figure 3 The fermentation process. Abbreviations can be seen in Table 3a. Data as averages (n=3) apart from SF2 & SF12 which were averages (n=10) sample readings **a** The pH range during the process across the different trials **b** The Brix readings across the various trials, excluding the first fermentation, readings taken every 24 hrs.

The spontaneous fermentation method used for the product innovation of the sauerkraut style product exhibited a much steadier and rapid decrease in pH compared to the inoculated samples. When combining this with the drop in Brix (Figure 4), would indicate that there was successful fermentation in these samples. This was further confirmed by sugar and acid analysis. However, within 3 days the pH had dropped to the initial expected pH of 4.5 which is commonly associated with the first gaseous phase (drop in pH and production of $CO_{2)}$ by *Leuconostoc mesenteroides*. After which, the second non-gaseous phase, dropped the pH further to the desired pH of 3.8 by the action of other LAB such as *Lactobacillus brevis*, *Pediococcus pentosaceus* and *Lactobacillus plantarum* species. Furthermore, the action of these bacteria can be correlated with the drop in Brix by days 7 and 8 (Figure 4) and sugars quantified by GC-FID (Table 10) where sucrose and fructose are utilized first by the LAB.

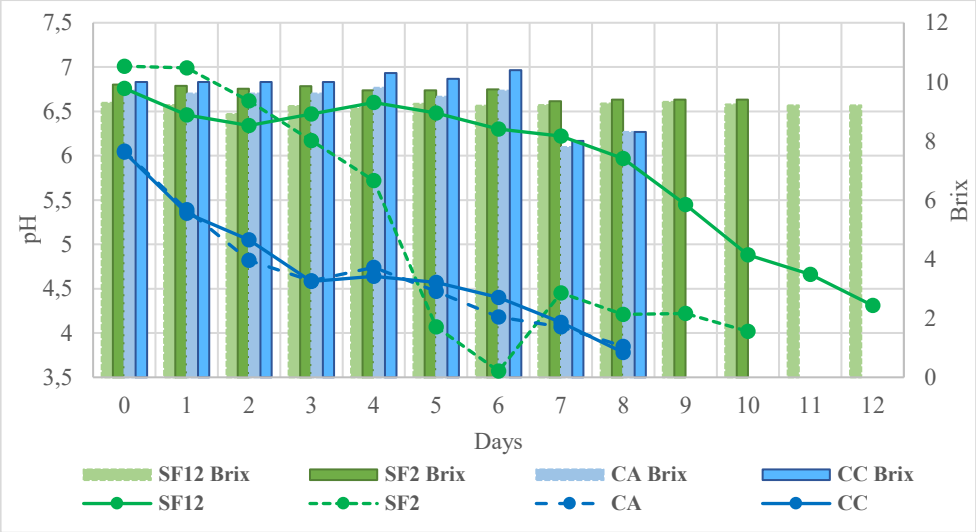

Figure 4 Comparison of the pH and Brix readings for the sensory samples and the spontaneous fermentation of sauerkraut products. Abbreviations can be seen in Table 3a. Data as averages (n=3) apart from SF2 & SF12 which were averages (n=10) sample readings

Table 9 Colony forming units of lactic acid bacteria on MRS agar

F1C			F1B			GT10			GT20			SF12			SF2		
Day	*CFU*	*Log*	*Day*	*CFU*	*Log*	*Day*	*CFU*	*Log*	*Day*	*CFU*	*Log*	*Day*	*CFU*	*Log*	*Day*	*CFU*	*Log*
0	n.d		0	n.d		0	13	raw	0	8	raw	0	8	raw	0	10	raw
1	n.d		1	n.d		2	33	raw	2	3	raw	2	5	raw	4	52	10^4
2	n.d		2	n.d		5	1.67	10^5	5	2	raw	5	94	10^4	7	147	10^4
3	34	raw	3	n.d		8	5.89	10^3	8	1.86	10^2	12	195	10^4	9	50	10^6
4	74.5	raw	4	n.d													
5	161	raw	5	n.d													

CK			CA			CC		
Day	*CFU*	*Log*	*Day*	*CFU*	*Log*	*Day*	*CFU*	*Log*
0	n.d		0	28	raw	0	22	raw
2	n.d		4	125	10^4	4	169	raw
5	7.8	10^6	6	33	10^4	6	25	10^3
12	5.1	10^6	8	1	10^6	8	65	10^4

*n.d = not detected, various dilution factors tested or not within countable range (30-300 CFU). Abbreviations can be seen in Table 3a. Means calculated across duplicate MRS plate counts and displayed as as log CFU/100µL

3.2 Sugars and acids

An example chromatogram of the TSM derivatives of the liquid phase of the fermented day 8 sauerkraut seaweed can be seen in Figure 4. Glucose and fructose were the dominating sugars detected within the samples. As seen in Figure 4, two peaks were detected for glucose and three for fructose, the peak areas were added and the equation from materials and methods was used to quantify the total of each sugar. The dominating acid was lactic acid and can be seen between the two Tri-Sil peaks with a retention time of 3.4 mins.

The dominating sugar detected amongst the samples was D-glucose which can be seen in Table 10. The levels varied significantly between samples and within the days of fermentations, seeing spikes of increases from start to finish of each trial. This was particularly problematic amongst the fermentations 1 between the chopped and blended samples. These variations in the quantities of sugars detected may be due to the sampling method of the liquid phase of the fermentation bottle. Sucrose was also detected in small quantities and only the chopped sample on days 2 and 5. Further testing would need to be carried out to determine if this was a result of fermentation. In the glucose trial (GT10 and GT20), sample GT10 saw a reduction in the overall D-glucose from the start to the end of fermentation indicating some utilisation of the available sugars. The GT20 in contrast increased over the same time. When coupled with the levels of lactic acid concentrations, GT10 produced over double the quantity compared to GT20, indicating that the glucose levels added for the 10% trial were more suitable for the LAB to proliferate. Although the sensory fermentation samples (SF2 and SF12) saw a reduction in the overall sugar content between the start and end of fermentation, lactic acid was only detected in very small quantities (30 ± 3.78 mg/L) on day 12 from SF12. Quinic acid was the only other detected acid within the samples with the set instrument parameters of the GC-FID.

In comparison, the natural fermentation in the sauerkraut style products detected some of all sugars tested, glucose, fructose, and sucrose. The latter two were used first by any bacteria present within the fermentation and levels of glucose had risen by the end of fermentation. In addition, lactic, citric, quinic, and malic acids were detected in both samples, with lactic acid being the dominating acid in both samples.

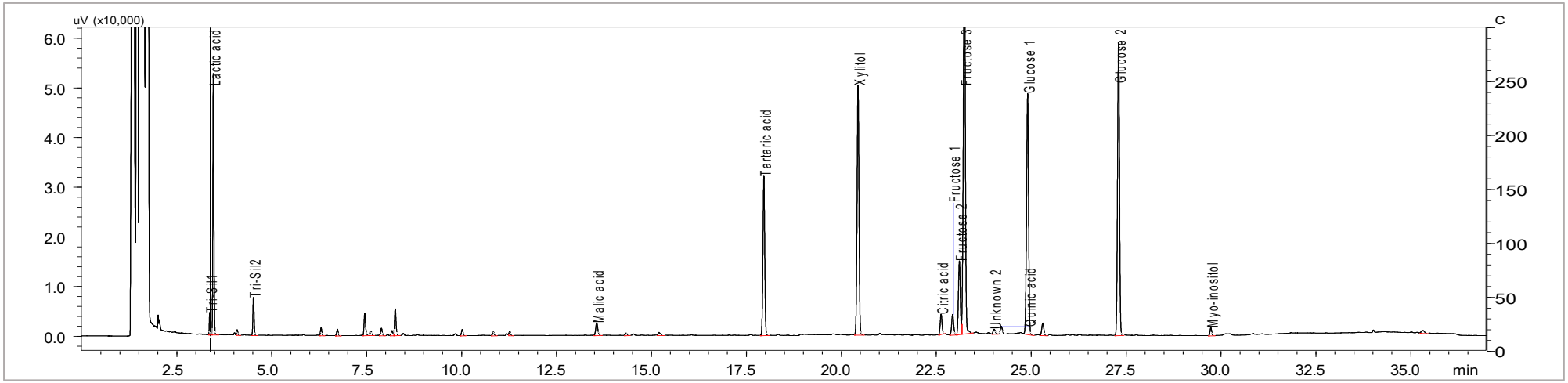

Figure 4 Chromatogram of sample the sauerkraut with algae (CA) day 8 as an example from the GC-FID results with identified compounds, tartaric acid and xylitol are internal standards.

Table 10 Contents of sugars and acids in the liquid phase of the different seaweed fermentations

		D-glucose	**Sucrose**	**D-fructose**	**Total sugars**	**Lactic acid**	**Citric acid**	**Ascorbic acid**	**Quinic acid**	**Malic acid**	**Total acids**
Condition	*DAY*	*mean**	*mean*	*mean*		*mean*	*mean*	*mean*	*mean*	*mean*	
F1C	0	2.27 ± 0.19	n.d	n.d	2.27	n.d	300 ± 0.04*	0.06 ± n.a	n.d	n.d	0.36
	2	4.44 ± 1.51	0.48 ± n.a	n.d	4.92	20.01 ± 6.50*	1.02 ± 0.01	0.05 ± 0.02	n.d	n.d	1.09
	4	2.86 ± 0.10	n.d	n.d	2.87	40 ± 14.8*	0.41 ± 0.05	n.d	n.d	n.d	0.45
	5	12.54 ± 12.52	0.04 ± n.a	n.d	12.58	130 ± 17.6*	3.23 ± 3.8	0.11 ± 0.05	n.d	n.d	3.47
F1B	0	6.8 ± 0.67	n.d	n.d	6.8	n.d	1.09 ± 0.07	0.07 ± n.a	n.d	n.d	1.16
	2	3.07 ± 0.08	n.d	n.d	3.07	30 ± 10.90*	0.38 ± 0.03	0.06 ± 0.02	n.d	n.d	0.47
	4	5.14 ± 0.43	n.d	n.d	5.14	40 ± 1.49*	1.13 ± 0.12	0.09 ± n.a	n.d	n.d	1.26
	5	4.81 ± 0.3	n.d	n.d	4.81	40 ± 0.64*	1.13 ± 0.17	0.09 ± n.a	n.d	n.d	1.26
GT10	0	41.04 ± 2.76	n.d	4.38 ± 0.30*	41.04	0.02 ± n.a	0.26 ± 0.12	n.d	n.d	n.d	0.28
	2	39.83 ± 0.87	n.d	4.08 ± 0.42*	39.83	40 ± 0.2*	0.37 ± 0.01	n.d	n.d	n.d	0.41
	5	41.5 ± 0.54	n.d	7.39 ± 0.61*	41.51	60 ± 23.60*	0.21 ± 0.09	n.d	n.d	n.d	0.27
	8	31.98 ± 12.08	n.d	6.87 ± 0.93*	31.99	130 ± 21.4*	0.41 ± 0.1	n.d	n.d	n.d	0.54

Table 10 cont.

		D-glucose	Sucrose	D-fructose	Total sugars	Lactic acid	Citric acid	Ascorbic acid	Quinic acid	Malic acid	Total acids
Condition	*DAY*	*mean**	*mean*	*mean*		*mean*	*mean*	*mean*	*mean*	*mean*	
GT20	0	68.36 ± 15.72	n.d	3.21 ± 2.93*	68.36	0.02 ± n.a	0.29 ± 0.02	n.d	n.d	n.d	0.31
	2	56.46 ± 17.44	n.d	5.46 ± 0.71*	56.47	60 ± 38.5*	0.42 ± 0.1	n.d	n.d	n.d	0.48
	5	96.97 ± 78.52	n.d	14.6 ± 4.85*	96.98	80 ± 70.1*	0.43 ± 0.47	n.d	n.d	n.d	0.51
	8	100.24 ± 30.15	n.d	18.7 ± 0.01*	100.26	80.01 ± 67.8*	0.31 ± 0.4	n.d	n.d	n.d	0.39
SF12	0	74.68 ± 7.23	n.d	17.3 ± 2.61*	74.7	n.d	n.d	n.d	n.d	n.d	0
	2	73.06 ± 3.36	n.d	10.4 ± 3.51*	73.07	n.d	n.d	n.d	n.d	n.d	0
	5	68.18 ± 21.23	n.d	11.0 ± 2.77*	68.19	n.d	n.d	n.d	n.d	n.d	0
	12	67.61 ± 8.55	n.d	9.04 ± 4.42*	67.62	30 ± 3.78*	n.d	n.d	0.55 ± 0.06	n.d	0.58
SF2	0	75.9 ± 3.39	n.d	16.3 ± 5.81*	75.92	n.d	n.d	n.d	n.d	n.d	0
	4	70.13 ± 2.7	n.d	7.65 ± 0.36*	70.14	n.d	n.d	n.d	0.24 ± 0.03	n.d	0.24
	7	63.08 ± 14	0.02 ± n.a	16.3 ± 10*	63.12	n.d	n.d	n.d	340 ± 2.10*	n.d	0.34
	9	66.97 ± 2.31	n.d	9.95 ± 0.19*	66.98	n.d	n.d	n.d	0.36 ± 0.05	n.d	0.36

Table 10 cont.

		D-glucose	Sucrose	D-fructose	Total sugars	Lactic acid	Citric acid	Ascorbic acid	Quinic acid	Malic acid	Total acids
Condition	*DAY*	*mean (*)*	*mean*	*mean*		*mean*	*mean*	*mean*	*mean*	*mean*	
CK	0	70.48 ± 3.91	0.09 ± n.a	4.41 ± 0.28*	70.57	n.d	0.2 ± 0.03	n.d	n.d	n.d	0.2
	2	65.66 ± 4.5	0.02 ± n.a	5.62 ± n.a*	65.69	n.d	0.23 ± 0.2	n.d	n.d	n.d	0.23
	5	69.78 ± 1.64	n.d	7.65 ± 1.34*	69.79	n.d	0.18 ± 0.08	n.d	n.d	n.d	0.18
	12	64.83 ± 0.28	n.d	n.d	64.83	70 ± 14.7*	0.12 ± 0.03	n.d	n.d	n.d	0.19
CC	0	6.62 ± 2.76	0.46 ± 0.19	272.0 ± 100*	7.35	n.d	0.56 ± 0.01	n.d	n.d	0.11 ± 0.03	0.67
	4	22.94 ± 5.85	0.38 ± 0.19	1.58 ± 0.58	24.9	540 ± 525.0*	1.07 ± 1.02	n.d	0.45 ± 0.43	0.44 ± 0.44	2.5
	6	23.99 ± 0.46	0.11 ± 0.02	1.85 ± 0.01	25.95	950 ± 139*	0.7 ± 0.6	n.d	0.57 ± 0.07	0.52 ± 0.05	2.74
	8	16.66 ± 4.35	0.06 ± 0.03	1.25 ± 0.35	17.97	3.04 ± 1.71	1.24 ± 0.75	n.d	0.57 ± 0.32	0.53 ± 0.35	5.38
CA	0	3.17 ± 1.34	0.23 ± 0.09	119.0 ± 50*	3.52	n.d	0.17 ± 0.14	n.d	0.03 ± n.a	0.05 ± 0.03	0.25
	4	15.13 ± 2.34	n.d	1.07 ± 0.16	16.2	440 ± 81.5*	0.77 ± 0.17	n.d	0.32 ± 0.05	0.27 ± 0.05	1.8
	6	12.37 ± 0.16	0.08 ± n.a	992 ± 1.54*	13.44	1260 ± 50.03	0.48 ± 0.52	n.d	0.36 ± 0.03	0.32 ± 0.03	2.42
	8	9.47 ± 1.14	n.d	790 ± 80.0*	10.26	1690 ± 17.82*	0.34 ± 0.25	n.d	270 ± 2.79*	0.21 ± 0.02	2.51

(*) Values of means (± standard deviation) of duplicate samples (n=2) in g/L unless individually marked with an asterisk* then that value is in mg/L. Abbreviations can be seen in Table 3a. n.d = not detected in samples with instrument parameters, n.a = not available within the standard deviations column due to only being detected in one of the duplicate samples.

3.3 Sensory evaluation

The narrowed down list of 14 key attributes used for descriptive analysis had 4 regarding visual appearance (green, brown, matte/glossy, red), 5 regarding aroma (overall intensity, grassy, seaweed, muddy, metallic), and 5 regarding texture (smooth surface, soft/hard, rubbery, elastic, tearability). These attributes were used on the raw untreated (RU), heat treated (HT) at 95 ℃ for 15 minutes, sensory fermentation 2-day (SF2) and sensory fermentation 12-day (SF12) and can be seen in Figure 5a.

Comparing the visual appearance of HT to RU, SF2, and SF12 the heat treatment caused an enhancement of the brown appearance and reduction of green pigmentation. Fermentation also enhanced the glossy appearance of the seaweed. The red appearance attribute was scored on a present or not present option rather than a 10-point intensity scale. The presence of redness in the appearance is more likely related to the freezing and thawing of samples rather than the processing of samples.

When comparing aromas of RU and HT to SF2 and SF12, the fermentation significantly decreased the grassy and seaweed aromas at an alpha value of 0.01. The correlation between the selected attributes and samples can be seen in the biplot PCA in Figure 5b. Additionally, the longer fermentation of 12 days showed the potential of fermentation to greatly reduce the overall aroma intensity compared to the other methods used.

The texture attributes showed no significant results across the various processes using the statistical methods of 2-way ANOVA and Tukey's post hoc. This may be more indicative of the part of the thallus which was being evaluated rather than the process the seaweeds were subjected to.

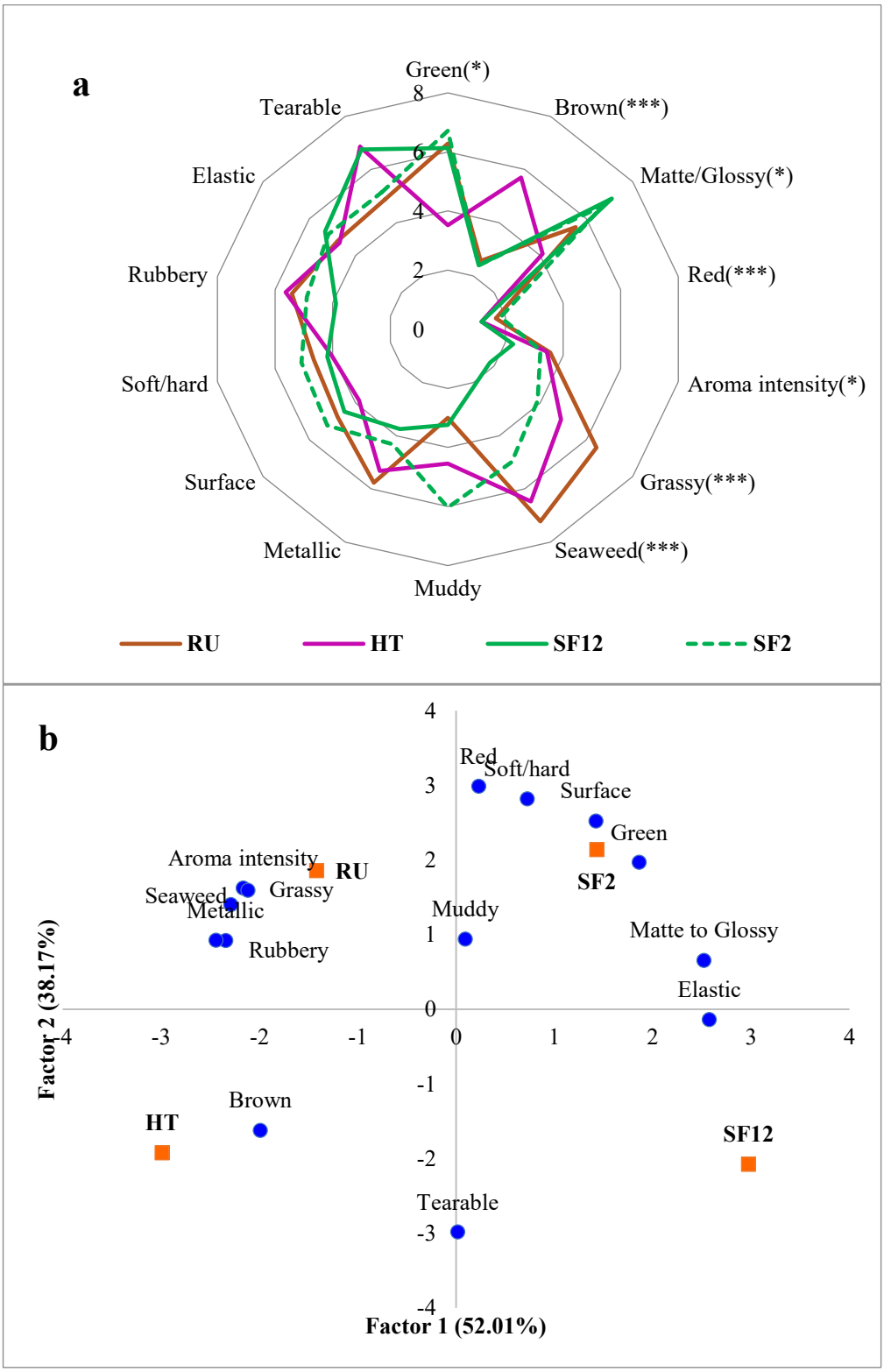

Figure 5 a Development in visual appearance, aroma, and texture attributes during the processing steps from (RU) raw untreated over (HT) heat treatment to (SF2, SF12) fermented *Fucus vesiculosus*. Significance at different alpha levels = 0.1 (*), 0.05 (**), 0.01 (***). **b** Biplot PCA depicts the correlation matrix between the different processes and the higher rating in the attributes.

The second portion of the sensory evaluation was carried out on the product innovations, the sauerkraut and pesto as well as their respective controls (not containing algae). This section only focused on detecting the aroma attributes (Figure 6a) which were determined by the panel during the training sessions and in turn ranking the products 1-4 on their preferences (Figure 6b). The sauerkraut-style products exhibited more intense aroma profiles which were identified during the training. Muddy and seaweed attributes were significantly more intense ($p < 0.01$) within the sauerkraut with and without algae. Metallic aromas were also detected as significantly higher ($p < 0.1$) in the sauerkraut products. Within the comments section of the evaluation when the panellist was asked for justification of their preferences, 3/6 panellists mentioned that sauerkraut is a more unfamiliar product to them and that the basil within the pesto product disguised the other aromas. Further likeness/preference and taste testing would need to be carried out by a larger consumer panel to determine which may be more suited for consumer acceptance. However, from this evaluation, the results indicated that the pesto products were preferred over that of the sauerkraut products within this panel.

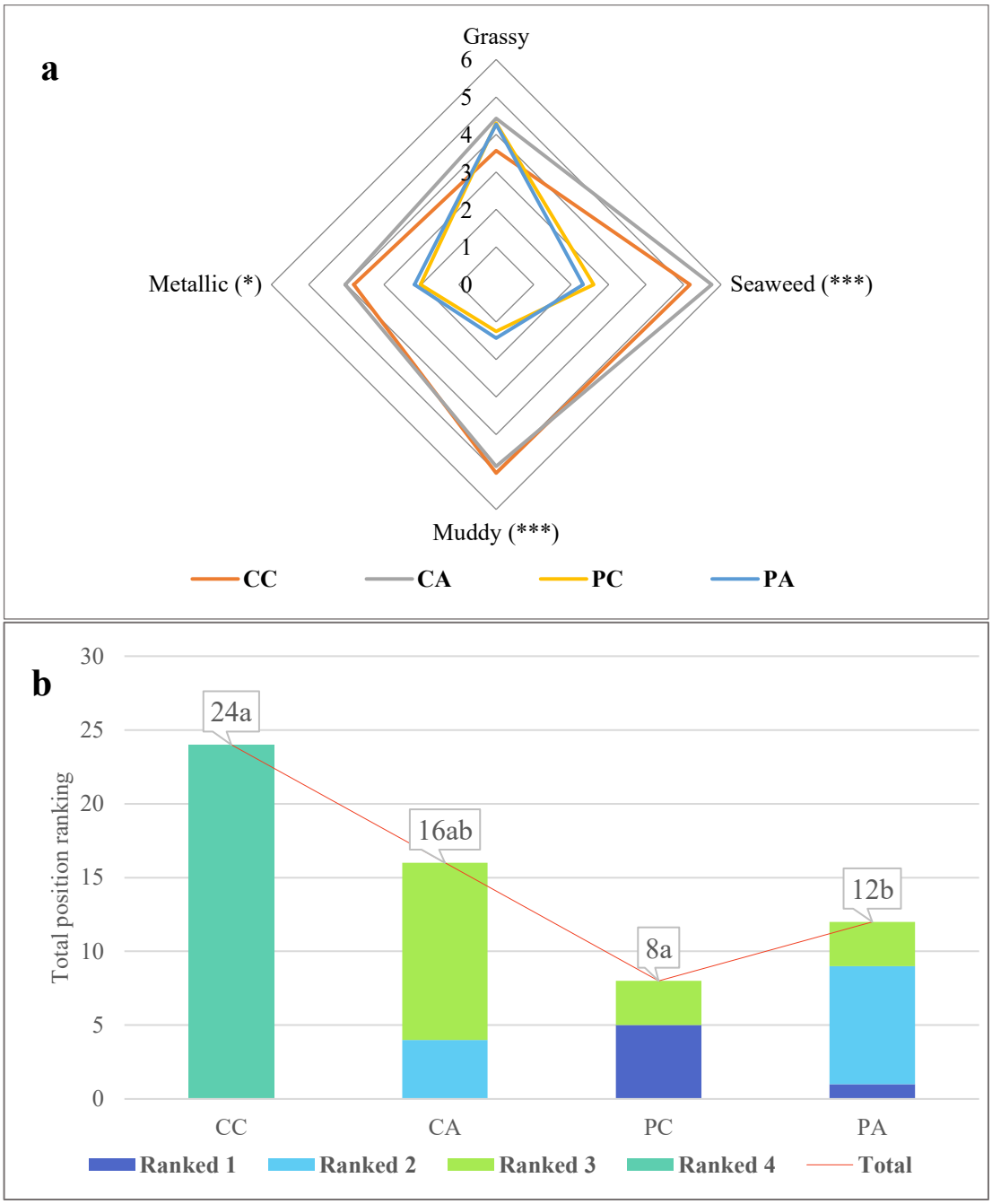

Figure 6 a Aroma attribute tested for the product innovations, (CC) cabbage control, (CA) cabbage algae, (PC) pesto control, and (PA) pesto algae. Significance at different alpha levels = 0.1 (*), 0.05 (**), 0.01 (***). **b** Preference ranking amongst the panel (n=6) of each of the product innovations and their respective controls, position ranking 1-4 from most preferred (1) to least preferred (4). Significant differences between products are indicated by a and b at a significant level of $p < 0.05$.

4 Discussion

4.1 Fermentation process

The use of a single LAB strain, *L. plantarum* DSM 20174, was not an efficient or effective method when attempting to ferment *Fucus vesiculosus.* On average, the lactic acid (Table 10) produced from the inoculated fermentation with this strain of LAB was relatively low compared to that produced in other studies (Bruhn et al., 2019; Lin et al., 2020; Skonberg et al., 2021; Sudhakar & Dharani, 2022). For example, a study by Lin et al. (2020) was able to obtain lactic acid the highest lactic acid concentration on *Gracilaria* sp. of 19.32 g/L when utilising hydrolysis as a pre-treatment. Similar concentrations of lactic acid were obtained from the study by Skonberg et al. (2021) when trialling seaweed within a sauerkraut product. However, these concentrations were only obtained by day 7 of the fermentation. When detected within the samples of this study, the range of lactic acid was between 0.02 – 0.13 g/L with a large reduction in the LAB from early in the process according to CFU counts, indicating potential sublethal injury to the strain. In the study by Bruhn et al. (2019), *L. plantarum* was also used for fermentation on *Saccharina latissimi.* However, it was standardised manufactured LAB which incorporated the required amounts of dextrose as a carrier for the bacteria. Although the study did not perform sugars and acids analysis to indicate the amount of lactic acid produced, they did report a stabilisation of pH at 4.5 within 40 hours and an increase in LAB by a factor of 100 in terms of CFU. It can be assumed that this dried and powdered form of LAB would be more stable with less susceptibility to sublethal injury when exposed to different growth environments or conditions. In a study conducted by Bao et al. (2018), a method of mixed fermentation was used for four *L. plantarum* strains and *B. subtilis* on *Spirulina platensis* in powdered form. Additionally, they used the random-centroid optimisation (RCO) method in trying to gain a better understanding of which factors had the most influence on the success of LAB within the fermentation with targets of maximal biomasses of probiotics. Similarly, this study looked at inoculation size, glucose concentrations, fermentation time and temperature. They found that fermentation temperature and time had the largest influence on the viability of bacteria within the fermentation of powdered microalgae. With their combination of bacteria for fermentation, they were able to obtain a rapid pH drop to approximately 4.5 within 12

hours. This is vastly different from the results of this study, where a pH of 4.5 was only reached by two samples, SF2 on day 5 and SF12 on day 11 (Figure 3a). Moreover, we observed differences in the influence of the conditions in which the macroalgae was in (chopped vs blended) as well as glucose concentrations. In the F1B sample, the LAB (Table 9) did not recover within the five days of fermentation, while there was some recovery within the F1C sample, the initial inoculation levels were not reached. However, F1C produced 3 times more lactic acid than that of the blended sample (Table 10). This may be due to the additional surface area which the LAB was exposed to or potential compounds which did not favour the proliferation of this strain of LAB. There was a notable variation in the glucose concentration in both samples when quantified through GC-FID. This may be a result of sampling error (improper shaking prior to sampling) or may be due to the D-glucose which was added was not in solution for this trial and therefore did not dissolve sufficiently upon addition. Furthermore, the glucose trial had similar results in terms of lactic acid production and recovery of LAB, indicating that the glucose concentration of 10% was better suited to the LAB strain when compared to the 20%. This may due to the higher osmotic pressure of cells at a higher glucose concentration level which can inhibit the metabolic activities and thus reducing the fermentation efficiency. Additionally, the bacteria may struggle to maintain their cellular function which in turn will lower the lactic acid production.

The SF2 was the sample which incorporated the fermentation broth with the seaweed only being added on day 6 and showed the most promising initial results in terms of pH drop and drop in Brix (Figure 4). However, the only acid which was detected using the GC-FID at the set parameters was quinic and that was at low quantities of 0.24 – 0.36 g/L from day 4 to day 9 (Table 10). Therefore, this sample was not successful in fermentation and was more likely a result of some contamination within the fermentation mass. The incorporation of the GC-FID method to confirm or deny was essential in other to determine the success of the fermentation. There may have been some other acids or compounds with smaller molecular weights which could not be detected at the high temperatures of 210 °C within the GC-FID.

The dominant acid which was produced during the first two trials was citric acid in samples F1C, F1B, GT10, and GT20 and can be seen in Table 10. The F1C sample contained the highest concentration at 3.23 g/L of the total acids detected at 3.47 g/L.

Citric acid fermentations are most associated with the filamentous fungus *Aspergillus niger* (Kirimura & Yoshioka, 2019). However, it is possible to be produced by LAB, the quantities of citric acid produced are strain dependant and there are low reporting rates for *L. plantarum* (Punia Bangar et al., 2022). Due to the high amounts of citric acid detected and much lower quantities of lactic acid in the samples, it may be possible that there was contamination within these samples. This may be a result of the heat treatment not being sufficient to inactivate the natural microorganisms present in the seaweed.

In contrast to the inoculated samples and trials, the natural spontaneous fermentation conducted by the LAB found on the red cabbage leaves was 25 times more successful. This considers initial pH drop, utilisation of available sugar content, and lactic acid production as a more successful fermentation. As mentioned, there are commonly two different phases with sauerkraut fermentation, namely the initial gaseous phase, in which CO_2 is produced, followed by the non-gaseous phase which continues to drop the pH further creating an acidic environment in which other pathogenic bacteria cannot proliferate. From the samples which were analysed, the control sample produced nearly double the amount of lactic acid, 3.04 g/L, compared to the sample with algae which produced only 1.69 g/L of lactic acid. This may indicate that the algae could potentially hinder the amount of lactic acid which can be produced within the product. In theory, this may just be due to having a less fermentable substrate for LAB to work on. In addition, because this process was a spontaneous fermentation, the levels of naturally occurring LAB present were not standardised or monitored prior to the start of fermentation. However, these samples were only analysed in duplicates and the standard deviation for the control on the final day of fermentation was relativity high at ± 1.71. With a standard deviation that high it would be recommended to run more replicates to gain a better understanding of the true potential of the algae to hinder the fermentation within the sauerkraut. Furthermore, sauerkraut is generally fermented for three to four weeks so it may have stabilised if the fermentation was carried out for a longer period (Zabat et al., 2018). Furthermore, a study by Skonberg et al. (2021) investigated the inclusion rates of sugar kelp and winged kelp to a sauerkraut style product on fermentation kinetics. They found that the kelp species and inclusion level significantly impacted most of the variables tested for such as pH, LAB counts, and lactic acid levels. Winged kelp performed best with the set testing parameters. Moreover, they found similar results to this study in terms

of higher levels of seaweed did impact the performance of the fermentation as well as the concentration of fermentable sugars within the brine. However, they did investigate the total phenolics as well as the antioxidant activity and found them to be higher with the increased inclusion of seaweed.

4.2 Potential inhibitors and challenges to seaweed fermentation

Phlorotannin, mannitol and laminarin, all of which are abundant in brown seaweeds, possess potential antimicrobial effects that could inhibit LAB proliferation by suppressing microbial degradation. Their presence in *Fucus vesiculosus* might have contributed to the reduced lactic acid production and LAB viability. Understanding the concentrations and interactions of these compounds with LAB could provide insights into optimising fermentation conditions for better outcomes. A study by Chades et al. (2018) invested the potential for *Clostridia* to ferment mannitol extracts from brown seaweeds for bioethanol production and found that 11 of the 41 strains were effective in utilising mannitol with ethanol being the dominant end-product. This may be a potential avenue for increasing the use of seaweeds in industries before entering the food industry and contributing to a circular economy. Furthering the importance of the selection of combination bacteria for full utilisation of brown seaweeds when fermenting.

The high buffering capacity of fresh seaweed might have resisted pH changes, posing an additional challenge for LAB fermentation. This is mainly due to the complexity of the polysaccharides associated with fresh seaweeds. This buffering capacity has the potential to delay the acidification process, thereby impacting the overall efficiency of fermentation (Fabris et al., 2020). Addressing the buffering capacity through pre-treatment of seaweed or adjusting fermentation parameters could improve LAB performance.

4.3 Sensory evaluation attributes

The sensory evaluation of seaweed products showed notable differences in visual appearance, and aroma across various treatments, underscoring the influence of processing methods on the sensory attributes of seaweed. The heat treatment significantly enhanced the brown appearance and reduced the green pigmentation of the seaweed compared to the raw untreated, 2-day sensory fermentation, and 12-day sensory fermentation samples. The red appearance attribute was noted as more related to freezing and thawing processes rather than the fermentation or heat treatment, suggesting that sample handling also plays a crucial role in visual characteristics. Fermentation, on the other hand, was found to enhance the glossy appearance of the seaweed, potentially due to the excretion of polysaccharides by microorganisms, which create a sheen on the surface.

The aroma profile of the seaweed was most significantly affected by fermentation. Both SF2 and SF12 showed a marked decrease in grassy and seaweed aromas compared to RU and HT samples. This reduction in specific aromas at a significant level ($\alpha = 0.01$) suggests that fermentation may alter or degrade certain volatile compounds responsible for these smells. The longer fermentation duration (SF12) further decreased the overall aroma intensity, indicating that extended fermentation could be a viable method for mellowing strong seaweed odours, potentially making the product more appealing to consumers who are sensitive to intense aromas. Hung et al. (2023) investigated the effects of microbial fermentation on aroma compounds by using GC-olfactometry. They found that not only did fermentation reduce unpleasant odours, but it also enhanced the pleasant aromas associated with the seaweed.

Interestingly, texture attributes did not show significant differences across the various treatments. This lack of significant results could be due to variability in the thallus parts being evaluated, suggesting that future studies should control for the specific parts of the seaweed used in sensory evaluation to obtain more consistent texture data. It may also indicate that the processing methods used do not drastically alter the texture, or that the sensory panel was not sensitive enough to detect minor textural differences. However, this was a contradicting finding to that by Bruhn et al. (2019), who found that fermentation of sugar kelp reduced the slimy appearance.

In evaluating the sauerkraut and pesto products, the sauerkraut exhibited more intense aroma profiles, with significant muddy and seaweed attributes ($p < 0.01$) and higher metallic aromas ($p < 0.1$). These findings may imply that sauerkraut, whether with or without algae, possesses stronger and potentially less familiar aroma profiles to the panellists. The comments from the panellists suggested a general unfamiliarity with sauerkraut, and the basil in the pesto masked some of the algae's aromas, making the pesto more acceptable. However, Skonberg et al. (2021) used a larger consumer panel (n = 100) when evaluating their sauerkraut style products with various inclusion rates of two different seaweeds (sugar kelp and winged kelp). The panel were presented with a 9-point hedonic scale (1 = dislike intensely, 9 = like intensely) and asked to rate the various products on colour, aroma, flavour, texture, and overall liking. The results indicated that a 25% sugar kelp inclusion rate in sauerkraut was most preferred followed closely by 50% winged kelp amongst the panel. The panel used for the consumer evaluation was more familiar with both seaweed and sauerkraut products which may have had a positive correlation with the products used. Therefore, the products selected for introduction to the market should consider as always, the preferences amongst the general population of introduction. The preference for the pesto product amongst the panel in this study highlights the potential of using herbs and other ingredients to mask or complement the strong aromas of fermented seaweed. Although the panellists were not asked to evaluate the product innovations, red cabbage was used to mask any leaching of brown/red colours of the seaweeds to the more commonly used white cabbage.

4.4 Methodological considerations

Upon completion of this thesis, several key methodological considerations have been drawn that should be considered for future research when fermenting fresh seaweed, *Fucus vesiculosus* LAB. Firstly, the combination of different strains of LAB should be further explored to enhance the process and overcome the challenges mentioned in the above section. Secondly, the use of the RCO method seemed a useful method to trial multiple conditions and understand the degree of impact of each condition. This method was used in previous studies such as that performed by Bao et al. (2018) and it seemed to be an effective method in streamlining the trial process. The third consideration would be

the management of the inhibitory compounds found in fresh seaweeds, those compounds were also named in the section above as phlorotannin, mannitol and laminarin. If time constraints were not an issue within this work, testing on *Fucus vesiculosus* to determine the exact degree and impact of these compounds would be performed prior to starting the fermentation process. These compounds can be reduced by different pre-treatment methods or through the use of combinations of various bacterial strains which are less susceptible to sublethal injury due to exposure to the compounds.

To further enhance the results of the descriptive analysis, the use of reference compounds and samples may prove an effective method. For this study, they were not used due to having a limited time frame but also a lack of the relevant compounds for the seaweed products. Lastly, stringent food safety measures, particularly concerning heavy metals and sterilization techniques, must be prioritized to ensure safety when performing sensory evaluations. Therefore, taste testing was not carried out as part of this thesis work. However, if time constraints again were not an issue, testing would be carried out to determine the suitability of the product for taste testing. This would provide future insight into the acceptability and marketability of the final product. These considerations provide a foundation for advancing the field and developing high-quality, safe, and appealing fermented seaweed products.

5 Conclusions

The unique attributes of fermented *Fucus vesiculosus* products present significant market potential. Innovations in fermentation processes that address the challenges identified, such as optimising LAB strains, managing inhibitory compounds, and enhancing sensory attributes, could lead to the development of novel, health-promoting food products. The growing interest in functional foods and sustainable ingredients supports the commercial viability of such innovations. The findings underscore the importance of understanding how different processing methods, especially fermentation, impact the sensory attributes of seaweed products. The decrease in grassy and seaweed aromas through fermentation presents an opportunity to develop milder seaweed products that may have broader consumer appeal. Moreover, the results suggest that incorporating familiar flavours, such as basil in pesto, can enhance consumer acceptance of seaweed-based products. The sensory attributes of fermented seaweed products are influenced by the production of volatile compounds. These compounds contribute to the flavour, aroma, and overall acceptability of the final product.

6 References/Literature

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