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Fermented versus unfermented macroalgae as sodium chloride replacers in bread- Impact on dough rheology, bread quality and sensory properties

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Abstract

Norway is one of the most productive places for utilization of macroalgae in the world and the world's third largest producer of wild macroalgae. Macroalgae have great potential to be used as an ingredient in foods, as they are rich in fibre and minerals, contain a balanced amino acid profile and are low in fats. It has also been proposed that macroalgae could help reduce the sodium chloride (NaCl) content in food products. Bread is one of the staple foods in the Norwegian diet and therefore one of the largest sources of NaCl. A reduction of NaCl in bread is therefore a tool to reduce the sodium intake of among the population and lower the risk for hypertension and associated diseases. As such, the present study evaluated if Norwegian brown macroalgae *Saccharina latissima* and *Alaria esculenta* (fermented and unfermented) could be used as potential NaCl replacers in bread containing whole wheat.

The effects of macroalgae and NaCl reduction on dough rheology, bread characteristics and sensory properties were investigated using various techniques. Refined (33%) and wholemeal wheat flour (66%) were used for breadmaking. A full salt control bread (0.6 g sodium/100 g flour) and three salt-reduced control breads (0.4, 0.35, 0.30 g sodium/100 g flour) were included. Incorporation of algae was in the range of 0.89-3.3% to achieve sodium contents of 0.35 or 0.3 g sodium/100 g flour, depending on the intrinsic sodium content in the algae powder. Farinograph, extensograph and SMS/Chen–Hoseney Dough Stickiness Rig analysis were applied to study the effect on dough rheology, while size exclusion high performance liquid chromatography was used to analyse the size and amounts of gluten proteins in the dough. Specific volume, H/B ratio, colour, crumb structure- and firmness were measured to determine the bread quality. Lastly, a descriptive sensory analysis was conducted by a trained sensory panel.

Addition of fermented and unfermented *S. latissima* and *A. esculenta* powder and reduction in NaCl in bread significantly increased water absorption and decreased dough development time, and dough stability in a dose-dependent manner. Further, increased levels of macroalgae and lower levels of NaCl had significant effects on extensibility, R_{max}, stickiness, the ratio of polymeric to monomeric proteins and specific volume. Textural parameters were not significantly different among the samples, whereas the colour of the bread was affected by addition of macroalgae. The results from the sensory analysis indicated that macroalgae could be used to increase the perception of salty taste, but distinct algae flavour and odour were observed by the panellists. However, incorporation of fermented *S. latissima* received lower scores for scores for certain undesirable sensory attributes than other bread with algae.

Sammendrag

Norge er en av de mest produktive stedene for utnyttelse av makroalger i verden, og verdens tredje største produsent av villhøstede av makroalger. Makroalger har et stort potensiale til å bli brukt som en viktig ingrediens i prosessering av matvarer, da det er en god kilde til fiber og mineraler, samt at de er et komplett protein og består av lite fett. Det har også blitt foreslått at makroalger kan bidra til å redusere innholdet av natrium klorid (NaCl) i matvarer. Brød er en av de største kildene til NaCl i kostholdet, som følge av de store mengdene som konsumeres. En reduksjon av NaCl i brød er derfor viktig for å redusere forekomsten av hjerte- og karsykdommer blant den norske populasjonen. På bakgrunn av dette er formålet med studien å undersøke om de norske brunalgene *Saccharina latissima* og *Alaria esculenta* (fermentert og ufermentert) kan brukes som potensielle NaCl erstattere i brød som inneholder finmalt sammalt hvetemel.

Effekten av makroalger og NaCl reduksjon på de reologiske egenskapene til deig, brød kvalitet og sensorisk kvalitet ble undersøkt ved hjelp av ulike teknikker. Raffinert (33%) og sammalt finmalt (66%) hvetemel ble brukt. Et full-salt kontroll brød (0.6 g natrium/100 g mel) og tre salt-reduserte kontroll brød (0.4, 0.35, 0.30 g natrium/100 g mel) var inkludert. Inkorporering av alger var i området 0,89-3,3 % for å oppnå natriuminnhold på 0.35 eller 0ø3 g natrium/100 g mel, avhengig av natriuminnhold i algepulveret. Farinograf, ekstensograf og SMS/Chen-Hoseney Dough Stickiness Rig-analyse ble brukt for å studere effekten på de reologiske egenskapene i deig, mens SE-HPLC ble benyttet for å analysere størrelsen og mengden av glutenproteiner i deigen. Spesifikt volum, H/B ratio, farge, krumstruktur- og fasthet ble målt for å bestemme kvaliteten på brødene. Til slutt ble en beskrivende sensorisk analyse utført av et trent panel på Nofima Ås.

Tilsetning av fermentert og ufermentert *S. latissima* og *A. esculenta* pulver og reduksjon i NaCl i deig økte vannabsorpsjonen betydelig, samt reduserte deigens stabilitet og utviklingstid på en doseavhengig måte. Videre hadde økte nivåer av makroalger og reduserte mengder NaCl en signifikant effekt på R_{max}, ekstensibilitet og forholdet mellom polymere og monomere proteiner, samt spesifikt volum. Teksturparametere var ikke signifikant forskjellig mellom prøvene, men fargen på brødet ble påvirket ved tilsetning av makroalger. Resultatene fra den sensoriske analysen indikerte at makroalger kan brukes til å øke oppfatningen av salt smak, men distinkt algesmak og lukt ble observert av paneldeltakeren. Derimot fikk inkorporeringen av fermentert *S. latissima* lavere skår for visse uønskede sensoriske egenskaper sammenliknet med andre typene alger.

Abbreviations

ANOVA	Analysis of variance
BU	Brabender unit
Cl^-	Chloride
DA	Descriptive analysis
DDT	Dough development time
DTT	Dithiothreitol
DS	Dough stability
GS	Glutenin subunits
HMW-GS	High molecular weight- glutenin subunits
HPLC	High performance liquid chromatography
LMW-GS	Low molecular weight- glutenin subunits
NaCl	Sodium Chloride
R_{max}	Maximum resistance/ the resistance at constant deformation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis
SE-HPLC	Size exclusion high performance liquid chromatography
WA	Water absorption

Contents

1. Introduction

Over the past years there has been an increased consumption of sodium by the world's population (World Health Organization, 2012). Norwegians eat almost twice as much sodium chloride (NaCl) as recommended, where around 75% comes from processed food (Helsedirektoratet, 2011). An initiative called "Saltpartnerskapet" aims to reduce the NaCl intake in Norway by 30% from 2015 to 2025 (Helsedirektoratet, 2023). A reduction in NaCl intake is desirable because an intake that exceeds the recommendations has been shown to be a leading cause of hypertension and cardiovascular diseases (Silow et al., 2016). Further, it has also been linked to an increased risk of stroke, stomach cancer and kidney disease.

One of the staple foods of the Norwegian diet is bread, with an average daily intake of 184 g/person (Melnæs et al., 2012). Bread does not contain high concentrations of NaCl per 100 g, but since the consumption is high among the Norwegian population, bread is one of the major source of NaCl intake (Helsedirektoratet, 2019). Therefore, a reduction in NaCl content in processed products, and especially bread, is important to reduce the incidence diet-associated diseased among the population. On the other hand, NaCl has several important technological functions in bread, with a crucial impact on the quality of the finished product (Belz et al., 2012; Silow et al., 2016). By reducing NaCl in bread to values below 0.9%/100 g bread, problems can arise, such as increased dough stickiness, reduced viscoelastic properties and reduced bread volume (Adhikari et al., 2001; Belz et al., 2012; Huang & Hoseney, 1999; Silow et al., 2016). This may lead to loss of product quality, operational problems, and decreased consumer acceptability (Adhikari et al., 2001; Huang & Hoseney, 1999).

Several different approaches have been used to reduce the NaCl content in bread, including gradual reduction, NaCl replacers, taste enhancers and taste contrast to enhance NaCl perception (Gorman et al., 2023; Silow et al., 2016). Studies have investigated algae as an ingredient to reduce the NaCl content in bread products (Gorman et al., 2023). As a result of the high mineral content in macroalgae, including presence of Na, Ca, Mg, K, I, Fe and Zn, it could be used as an ingredient to reduce NaCl content in food and increase the intake of minerals (Gullón et al., 2021). Macroalgae also contain large amounts of free glutamate which evokes umami taste, and it has been shown that with increased levels of glutamate, the content of NaCl can be reduced (Nguyen et al., 2020). However, the acceptability of products with algae is limited in the western world which can be a result of undesirable sensory characteristics (Gorman et al., 2023; Lamont & McSweeney, 2021).

1.1 Aim of study

The aim of this study was to investigate the use of the Norwegian brown macroalgae *Saccharina latissima* and *Alaria esculenta* as potential sodium chloride replacers in bread containing whole wheat. Different levels of NaCl and the brown macroalgae were used to study the effect on rheological properties of dough, chemical properties of gluten, the quality and sensory properties of bread consisting of 1/3 refined wheat flour and 2/3 whole wheat flour. The sodium levels in the breads were equal (1% NaCl/100 g flour). Fermented and unfermented samples of *S. latissima* and *A. esculenta* were included in this thesis to investigate if processing of macroalgae via fermentation has an impact on the properties of the bread.

2. Theory

2.1 Macroalgae

Macroalgae are mainly consumed and produced in Asian countries like Japan and China (Chapman et al., 2015; Hurtado et al., 2022; Kumar et al., 2008), but recently the interest has increased in Western countries because of increased awareness of sustainable marine food resources and with the increased focus on nutrition and health (Roohinejad et al., 2017). Marine macroalgae are eukaryotic, multicellular, macroscopic and autotrophic organisms (Leandro et al., 2020). Based on the pigmentation, the organization of photosynthetic membranes and other morphological features, macroalgae are divided into three large groups: green algae (*Chlorophyta*), brown algae (*Phaeophyceae*) and red algae (*Rhodophyta*). Among these, brown algae are currently consumed the most, accounting for 66.5% of the global consumption, whereas red and green algae are responsible for 33% and 5%, respectively (Afonso et al., 2019).

The composition of macroalgae varies according to type of species, season, water temperature, geography and surrounding nutrient levels (Mišurcová, 2011). Additionally, factors such as sea level, atmospheric CO_2 and UV-distribution can also contribute to variations (Sunny, 2017). Due to these variations, generalization of the composition is difficult. However, certain elements characterize the different main groups of macroalgae (Holdt & Kraan, 2011). Common for all three (green, brown and red) is the high content of ash (macro-minerals and trace elements) and polysaccharides. The type and structure of the polysaccharides are species-specific and differ between the three groups. Green macroalgae mainly contain sulphated polysaccharides (sulphated galactans and xylans) while red species mainly contain carrageenans or agars. The main component in brown algae, on the other hand, are structural cell wall polysaccharides such as alginates and fucoidans (Kumar et al., 2008). Alginate consists of L-guluronic acid and D-mannuronic acid residues and provides both flexibility and strength to the plants. The variation in the chemical structure of alginate in different brown algae species results in different physical properties. Brown algae also contain storage polysaccharides, notably laminarin (β-1, 3 glucan). Lastly, cellulose and hemicellulose are present in macroalgae, which are neutral polysaccharides. Non-starch polysaccharides in algae are dietary fibres, as they cannot be digested by humans.

Macroalgae in general have a high content of minerals and vitamins due to the marine habitat and the high exposure to sunlight (MacArtain et al., 2007). Most of the brown species have

high contents of calcium, magnesium, potassium, iron and sodium, and high concentration of iodine (Afonso et al., 2019; Kumar et al., 2008). The iodine concentration has been reported to range from 1.6 mg/100 g to 816.5 mg/100 g (Teas et al., 2004), as it is actively taken up from seawater (Smyth, 2021). In comparison, cod, an animal source known for its high iodine content, contains 279 µg/100 g (Mattilsynet, 2022). Dairy products are also known as a source of iodine in the diet but have lower contents (milk: 19 µg/100 g, yoghurt: 16 µg/100 g). The iodine content in macroalgae depends on factors such as area of the plant, stage of growth, species, season and geographical location (Smyth, 2021). For humans, iodine is essential to produce thyroid hormones (Zimmermann, 2009) and important for brain development (Andersson et al., 2007). Mild to moderate iodine deficiency is a global issue, which also seem to be increasing in Norway due to a decrease in consumption of dairy products and white fish and increase in consumption of plant-based foods (Brantsæter et al., 2018). With this perspective, macroalgae utilization offers the opportunity to enrich food using a plant-based iodine source. However, 1 g of unprocessed brown algae can exceed EFSA`s upper tolerable daily iodine limit of 600 µg by several times (EFSA, 2014). Regular consumption of macroalgae with a high iodine content such as *S. latissima (*Biancarosa *et al., 2018)* can lead to an excessive intake and adverse effects on the thyroid function (EFSA, 2014). Therefore, the maximum amounts of algae added to food products should take their iodine contents into account, to avoid excessive intake among consumers (Nielsen et al., 2020).

Further, brown macroalgae generally have a low Na/K ratio, a factor regarded as beneficial for cardiovascular health (Afonso et al., 2019). A unique property of algae is that they contain vitamin B_{12}, which is not present in any land plant material (Kumar et al., 2008). Brown algae do however contain less vitamin B_{12} compared to red and green algae (Luhila et al., 2022). Results by Luhila et al. (2022) showed that the brown algae *Fucus vesiculosus* had the lowest B_{12} content (4.14±0.36 µg/100 g DW) compared to the red and green algae investigated, whereas in meat like raw Norwegian beef the B_{12} content is 1.2µg/100 g (Mattilsynet, 2022).

The protein content in brown algae is commonly low and varies from 5-15% (Kumar et al., 2008). The amino acid composition of macroalgae is close to proteins from leguminous plants and ovalbumin (Kumar et al., 2008), and consists of all essential amino acids, but they are not nutritionally complete proteins (Mišurcová, 2011). The lipid content in brown algae is regarded as low (<2%) compared to the other nutrients (MacArtain et al., 2007), but contains several ω-6 fatty acids (Miyashita et al., 2013).

2.1.1 Macroalgae industry in Norway

Several brown algae species can be found along the coastline of Norway, which is considered to be one of the world's most productive places for utilization of macroalgae (Biancarosa et al., 2018). Norway's wild macroalgae harvest was 1 734 477 tonnes in the period from 2009 to 2018, and it is the third largest producer of wild macroalgae after Chile and China (Hurtado et al., 2022). Furthermore, Norway was the largest producer of cultivated macroalgae in Europe but is only responsible for 0.46% of the total production (wild and cultivated) of macroalgae in the world. The most relevant edible algae in Norway are *S. latissima* (sugar kelp) and *A. esculenta* (winged kelp), both of which are brown algae cultivated on a commercial scale (Biancarosa et al., 2018). For *A. esculenta*, a protein content of 9.11 g/100 g has been reported while the lipid content was 1.5 g/100 g (Mæhre et al., 2014). Polyunsaturated fatty acids were the dominant fatty acids in both *S. latissima* and *A. esculenta* with contents of 203 mg/100 g DW and 231 mg/100 g DW, respectively (Biancarosa et al., 2018). The concentrations of iodine were found to be considerably higher in *S. latissima* (460 mg/100 g DW) compared to *A. esculenta*, where they ranged between 22 and 38 mg/100 g DW (Biancarosa et al., 2018; Mæhre et al., 2014). Differences in Fe concentration (16 mg/100 g DW in *S. latissima* and 7.2 mg/100 g DW in *A. esculenta)*, and Na (2.4 g/100 g for *S. latissima* and 1.6 g/100 g for *A. esculenta*) but no notable differences in Ca and Mg were found between the two species.

However, there are challenges associated with using macroalgae as an ingredient. As a result of macroalgae being a raw material with high water content, further processing of the raw material is required to extend the shelf life (Blikra et al., 2021). Processing technologies such as drying, fermentation or freezing (Hurtado et al., 2022), can stabilize the macroalgae biomass and increase the shelf life (Blikra et al., 2021). Further, a treatment referred to as low temperature blanching is used to reduce the iodine content in brown macroalgae, more specifically *S. latissima* (Nielsen et al., 2020). Such post-harvest processing operations are crucial for establishing supply chains for macroalgae.

Macroalgae produced along the Norwegian coastline are mainly utilized for production of thickeners (alginate, carrageenan) (Mæhre et al., 2014). However, in the last years research projects have had an increasing focus on the use of algae for production of industrial biofuels, compounds of medical and pharmaceutical value (Biancarosa et al., 2018), or inclusion of macroalgae in a wide range of food products like ice cream, meat, cheese and bread (Mouritsen et al., 2012). In addition, food companies have also started to explore applications

of macroalgae in their products. Orkla Ocean AS is a Norwegian-based supplier of macroalgae products and aims to increase the commercial use of macroalgae with the greatest focus on food applications. Additionally, there are several small companies that harvest or cultivate macroalgae for incorporation into food products. Tekslo Seaweed (Sjøsaker) and Lofoten Seaweed are two examples on such companies, and their products include snacks, pasta and spices (Lofoten Seaweed, 2023; Tekslo Seaweed, 2023).

2.1.2 Effect of macroalgae and macroalgae-derived ingredients on product properties

Hydrocolloids, such as alginate and carrageenan, are the most valuable ingredients extracted from algae used in food products (Mæhre et al., 2014). Khalil et al. (2017) defined hydrocolloids as *long chain of hydrophilic polymers (mostly polysaccharides) that are characterized by their capability to form viscous dispersion and/or gels when dispersed in water*. These molecules exert several functional properties in food products, such as binding water, acting as stabilizers, thickeners, gelling agents and fillers (Jafar & Gisoo, 2012). The primary reason for using hydrocolloids in food products is their ability to modify the rheology of food systems. Unrefined macroalgae exhibit functional properties that include swelling, oil and water retention (Elleuch et al., 2011), which are associated with their proteins and polysaccharides (Quitral et al., 2022).

Macroalgae can be included in fresh, dried, defrosted, fermented, or cooked form, and currently the dried form is the most commonly used (Hurtado et al., 2022). Studies have evaluated its incorporation into products like spreadable processed cheese (Tohamy et al., 2018), yoghurt (Robertson et al., 2016), pasta (El-Baz et al., 2017; Kadam & Prabhasankar, 2010), meat products (Cofrades et al., 2017; Gullón et al., 2021; Inguglia et al., 2017) and bread (Arufe et al., 2018; Gorman et al., 2023; Lamont & McSweeney, 2021; Mamat et al., 2014). These studies have generally reported increased mineral contents (Tohamy et al., 2018) but reduced acceptability by consumers (Robertson et al., 2016). However, there are examples of products that were not negatively affected by algae addition (Cofrades et al., 2017; Gullón et al., 2021). Incorporation of algae at optimized levels resulted in decreased cooking loss, as well as hardness and small or no differences in appearance in meat products (Gullón et al., 2021). Algae were not reported to affect the product stability in frankfurters, patties and restructured steaks, where the technology constraints associated with NaCl reduction can be palliated by the presence of macroalgae, which has been attributed to their dietary fibers (Cofrades et al., 2017). Macroalgae have been shown to affect the sensory quality of meat

products both positively and negatively, where the effect is dependent on the type and concentration of macroalgae.

To increase the consumption of algae it should ideally be included into food products that are widely consumed. One of the staple foods of the Norwegian diet is bread, with an average daily intake of 184 g/person (Melnæs et al., 2012). This would make bread a convenient vehicle, even if only small amounts of algae were added. However, the addition of algae can affect textural as well as sensory properties. To better understand such effects on dough and bread properties, a brief discussion of the chemistry of bread making and the role of NaCl is given below in chapter 2.2.

2.2 Bread

Flour, water, NaCl and yeast are ingredients essential to the breadmaking process (Parenti et al., 2020). Nonessential ingredients such as dairy products, enzymes or fat can be added to dough to enhance the palatability, machinability and shelf life (Sun et al., 2023). Once flour particles become hydrated, a protein network develops which gives dough its viscoelastic properties and bread its structure (Delcour & Hoseney, 2010). The flour used in breadmaking plays a critical role for the product characteristics, including colour, texture, the consistency of the bread crumb and loaf volume (Parenti et al., 2020). The protein content in the flour, more precisely the concentration and profile of the proteins, is especially important to produce a good-quality loaf of bread (Delcour & Hoseney, 2010). Water or other liquid ingredients also serve as both solvents and plasticizers. Most bread doughs contain anywhere from 60% to 75% water (Mondal & Datta, 2008).

Baker's yeast, *Saccharomyces cerevisiae*, is responsible for the gas production in the dough (Delcour & Hoseney, 2010). It converts the fermentable carbohydrates present in the flour (i.e., glucose, and to a lesser degree maltose, fructose, raffinose and fructans) into carbon dioxide and ethanol, which the viscoelastic dough is responsible for retaining. A result of this is a lifted, light and leavened bread. Yeast and its metabolites also play an important role in the rheological properties of dough and the bread quality (texture, volume and taste) (Struyf et al., 2017). The main yeast metabolites (except for $C0_2$) glycerol, ethanol and succinic acid has been showed to soften the dough, altering the configuration of the gluten network which decreases viscosity and extensibility, and reduce the stiffness and extensional viscosity, respectively (Meerts et al., 2018).

2.2.1 Composition of refined flour and whole wheat flour

Triticum aestivum L., also known as hexaploid bread wheat, is the wheat species most widely used for breadmaking around the world (Parenti et al., 2020). The wheat kernel can roughly be divided into three parts, i.e., the endosperm, the germ and multiple histological outer layers that make up the bran (Hemdane et al., 2016). Refined wheat flour mainly consists of ground endosperm, where almost all the bran and germ have been removed. In contrast, whole wheat flour contains the entire wheat kernel, although in roller-milled whole wheat flour it has been ground, separated and then recombined (Atwell & Finnie, 2016). The composition and nutritional value of whole wheat flour is affected by processing (Gómez et al., 2020). Further, compositional differences of flour can also be due to differences between genotypes and environmental conditions (Atwell & Finnie, 2016). In Table 1, the average composition of unenriched refined wheat and whole wheat flours on the Norwegian market is presented (Mattilsynet, 2022).

Table 1. Composition of Norwegian unenriched refined wheat flour and whole wheat flour (Mattilsynet, 2022).

Constituent	**Refined wheat flour**	**Whole wheat flour**
Carbohydrates (g/100 g)	68.1	57.9
(Dietary fiber (g/100 g))	(5.0)	(13.0)
Protein (g/100 g)	12.1	13.4
Lipids (g/100 g)	1.4	2.1
Calcium (mg/100 g)	21.0	36.0
Iron (mg/100 g)	1.2	3.3
Magnesium (mg/100 g)	35.0	110.0
Phosphorus (mg/100 g)	123.0	303.0
Potassium (mg/100 g)	188.0	420.0

Whole wheat flour contains more protein, lipids, dietary fibre (mainly insoluble), minerals and vitamins compared to refined wheat flour (Gómez et al., 2020; Mattilsynet, 2022). That is a result of the dietary fibre and minerals being concentrated in the bran and germ fractions (Hemdane et al., 2016).

2.2.2 Cereal proteins and the gluten network

The protein content in commercial wheat varies from 8% to 16% (Delcour & Hoseney, 2010). A broad classification of proteins in cereals was developed by T.B. Osborne, based on the extraction in a series of solvents (Osborne, 1924). The four Osborne fractions are referred to as albumins, globulins, prolamins and glutelins. Albumins are soluble in water, globulins are

soluble in dilute saline, prolamins are soluble in 60-70% aqueous alcohol and lastly glutelins are insoluble in all the solvents mentioned but can be extracted via alkali. Also, sodium dodecyl sulphate (SDS) and reducing agents such as dithiothreitol (DTT) are used to extract glutenins (Wilson et al., 1981). Figure 1 presents the profile of wheat protein fractions obtained by the Osborne fractionation (Osborne, 1924; Sharma et al., 2020). The main wheat proteins, both in terms of concentration as well as functionality are the storage proteins in the endosperm, also called gluten or gluten forming proteins (Shewry, 2019).

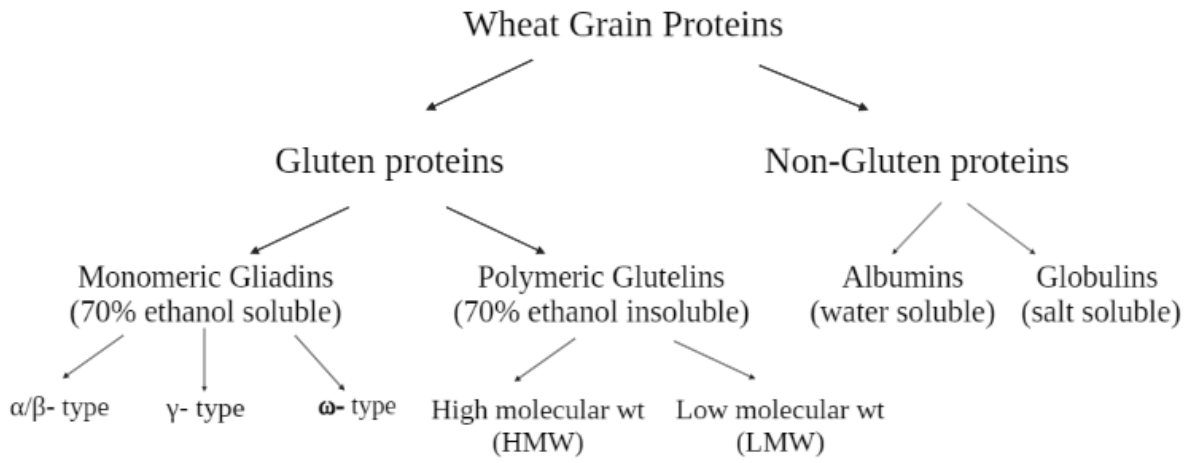

Figure 1. Nomenclature and classification of wheat storage proteins (Osborne, 1924; Sharma et al., 2020).

Gluten can be defined as "*the proteinaceous rubbery mass that remains when a dough made out of wheat flour and water is washed to remove starch and water soluble material*" (Wieser, 2007). Gluten consists of hundreds of proteins, which together form a network. The gluten proteins are presents as monomeric gliadins or as oligo- and polymeric glutelins (Figure 1), which are linked by interchain disulphide bonds. In general, the amino acid composition of gluten proteins is characterized by high contents of glutamine (38%) and proline (20%), and a low content of amino acids with charged side groups (Delcour & Hoseney, 2010). Gluten is considered one of the most complex protein networks in nature (Wieser, 2007). This is mainly due to the numerous components, their different size variability caused by genotype, growing condition and the different technological processes.

Gliadins are a heterogeneous mixture of proteins with molecular masses from 30 to 75 kDa and assumed to be monomeric proteins in their native state (Ooms & Delcour, 2019). In literature, they are classified into the four groups α-gliadins, β-gliadins, γ-gliadins and ω-

gliadins, however α- and β-gliadins can be considered as one group called α/β- type (Wieser, 2007). What separates them from each other are their differences in mobility in acid polyacrylamide gel electrophoresis (acid-PAGE), as well as their amino acid profile, especially glutamine, proline, cysteine, phenylalanine and tyrosine (Wieser, 2007). Glutenins are polymeric and consists of glutenin subunits (GS) linked through disulphide bonds. They can be separated into two groups by using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), called the high molecular weight (HMW) and low molecular weight (LMW) (Shewry, 2019). HMW-GS have a molecular weight from 65 000 to 90 000, whereas the molecular weight of LMW-GS varies from 30 000 to 60 000 (Delcour & Hoseney, 2010).

The differences in structure between the gliadins and glutenins provide them with different functionalities (Ooms & Delcour, 2019). This is especially important during formation of dough. Belton (1999) divided the formation of dough into two stages. The first stage is known as the energy input stage and hydration stage, where the dough goes through deformation during mixing (Belton, 1999). In the second stage, depolymerization and (re)polymerisation of the dough occur. The most important interactions for development of the three-dimensional gluten network are the intermolecular disulphide (SS) bonds between glutenins and intermolecular hydrogen bonds involving the glutamine residues, but electrostatic and hydrophobic interactions may contribute to stability as well (Ooms & Delcour, 2019).

Glutenins are mainly responsible for the formation of the polymeric protein network, which provides cohesiveness and elasticity to the dough (Ooms & Delcour, 2019). Gliadins on the other hand act as plasticizers and contribute to dough viscosity, as well as extensibility. Based on this, gluten has been described as a "two-component glue" (Wieser, 2007) and both protein types are important for appropriate viscoelastic properties of dough and the end product's quality. Various factors influence the formation of gluten networks (Ooms & Delcour, 2019). Non-starch polysaccharides, especially insoluble fibers, have been demonstrated across studied to disrupt the continuity of the gluten network (Ooms & Delcour, 2019; Sun et al., 2023; Zhou et al., 2021). Further, dietary fiber competes with the gluten proteins for water, which leads to delayed and impaired gluten hydration (Sun et al., 2023).

2.2.3 Bread baking

In general, the bread baking process can be divided into three basic operations (Delcour & Hoseney, 2010). These include mixing or dough formation, fermentation and baking. Mixing is responsible for blending the ingredients (Bloksma, 1990; Delcour & Hoseney, 2010) and development of a viscoelastic gluten network in which HMW-GS are linked to LMW-GS through SS-bonds, and gliadins through non-covalent interactions (Bloksma, 1990; Delcour & Hoseney, 2010; Verbauwhede et al., 2020). Air cells incorporated during mixing act as nuclei for bubbles later on (Delcour & Hoseney, 2010; Sadot et al., 2017). During dough formation, flour particles become hydrated, which activates enzymes like amylase and protease, and oxidation reactions occur which strengthen the dough (Delcour & Hoseney, 2010). There are several processing factors that affect the properties of the dough, like temperature, mixing time and mixing speed/ technique.

An increase in volume occurs as the yeast activity increases and the pH in the dough decreases from around 6.0 to 5.0 caused by production of CO_2, and its dissolution into the aqueous phase (Delcour & Hoseney, 2010). The amount of yeast, NaCl and sugar, as well as the temperature can affect fermentation in dough. Chemical changes in baking start with breaking of hydrogen bonds above 45°C, and exposure of hydrophobic areas (Verbauwhede et al., 2020). With an increase in the temperature hydrophobic interactions become stronger, which promotes protein aggregation. With further increase in temperature (>90°C) the gliadins become involved in the gluten network through SH-SS exchange reactions. These processes are accompanied by dough expansion (Delcour & Hoseney, 2010), denaturation of proteins (Delcour & Hoseney, 2010), gelatinization of starch (Purlis, 2011), formation of a crust (Purlis, 2011), inactivation of yeast and enzymatic activities (Delcour & Hoseney, 2010) and formation of flavour through the Maillard reaction (Capuano et al., 2008). These reactions are affected by temperature, baking time and the humidity in the baking chamber (Therdthai et al., 2002).

The most used bread baking methods are straight-dough, sponge-and-dough and the Chorleywood method (Mondal & Datta, 2008). Straight-dough making is considered to be the simplest procedure (Delcour & Hoseney, 2010). First all the ingredients are mixed, then the dough ferments before it is divided into loaf-sized pieces. Further, the dough proofs, which is a secondary fermentation, and lastly it is baked. The characteristics of a bread baked using this method involve less flavour compared to the two other bread baking methods, a coarser structure, and it being time sensitive when fermenting. The sponge-and-dough procedure is a

somewhat more complex method but has the advantages of being more tolerant to variations in fermentation and process time, as well as leading to a more intense bread flavour (Delcour & Hoseney, 2010). First, about two thirds of the flour, parts of the water and yeast are mixed together to form a loose dough, referred to as "sponge" (Cavanagh et al., 2010). It is fermented for several hours (typically 1-4 h), before it is combined with the rest of the ingredients after which is referred to as a dough. This dough proofs for 20-40 minutes, referred to as "floor time" (Delcour & Hoseney, 2010). The last steps are the same as for straight-dough: dividing, moulding, proofing and baking. This style gives a soft bread with a fine cell structure. In contrast to the more labour-intensive sponge-and-dough procedure, the Chorleywood method is shorter as all the ingredients are blended and mixed under partial vacuum, where no fermentation is needed. While this may come at the expenses of flavour development, it makes the process more economical, and the partial vacuum is beneficial as the small bubbles created expand under reduced pressure and can further be subdivided into more bubbles throughout the mixing process (Delcour & Hoseney, 2010).

2.2.4 Analysis of gluten proteins and their effect on dough and bread properties

2.2.4.1 Farinograph

The farinograph is one of the most used instruments for wheat quality evaluation and was invented in 1912 by Jenö von Hankóczy (Wrigley et al., 2022). Later the farinograph was commercially developed in collaboration with Carl Wilhelm Brabender and until this day, the company Brabender is the leading producer of this equipment. The instrument tests the dough properties during mixing and can be used for different purposes, like examining composite flour, the effect of different baking ingredients, reconstituted type flour systems and the effect of different components, but was originally developed for wheat quality control, in particular for refined wheat flour (Faridi, 1985).

The farinograph parts of importance include a mixing bowl, two Z type kneaders, a lever system, and a thermostat (Faridi, 1985). Temperature-controlled water is circulating in a jacket around the bowl during dough mixing, to achieve 30°C in the mixing bowl (Faridi, 1985) (Pojić & Torbica, 2011). Different bowl sizes (10, 50 and 300 g) are available (Pojić & Torbica, 2011). Parameters obtained from farinograph analysis include dough stability (DS), water absorption (WA) and dough development time (DDT) (Lallemand, 2018). The instrument measures the resistance to mixing of a dough that is typically made from water and

flour, i.e., NaCl and yeast are excluded. The resistance is recorded as the dough develops and eventually breaks down. The shape of the curve indicates the strength of the flour. Further, the stability time of the dough is the interval between the arrival time, i.e., when 500 Brabender Units (BU) are reached, and the departure time, i.e., when the value falls under 500 BU. A higher stability time indicates that the flour is more tolerant to mixing. WA of the flour is determined as the amount of water required to reach 500 BU (Lallemand, 2018), so the dough has a fixed consistency (Faridi, 1985). It is the main components of flour, (damaged) starch and gluten (as well as arabinoxylans, in particular in flour of higher extraction), that primarily influence WA (Pojić & Torbica, 2011).

2.2.4.2 Extensograph

The main principles of extensional techniques include testing dough after mixing and resting (Pojić & Torbica, 2011). After resting, the shaped dough is subjected to large deformations until it ruptures. It is the gliadins that determine extensibility, which is therefore heavily dependent on the protein quality of the flour. Different extensional techniques have been developed, including the Brabender extensograph, Alveograph and Kieffer Rig dough and gluten extensibility rig. The Brabender extensograph and Kieffer dough and gluten extensibility rig are uniaxial extension methods, meaning that the dough is stretched in one direction. The Alveograph is on the other hand a biaxal extension method, i.e., the dough is then stretched in two opposing directions which may mimic the stretching of gas bubbles better (Delcour & Hoseney, 2010).

The Brabender extensograph provides information about the extensibility and the resistance to extension of dough (Pojić & Torbica, 2011). It is an internationally accepted method (ISO 5530-2, ICC 114/1, AACC54-10), where doughs containing 2% NaCl (flour basis) firstly are prepared in the farinograph. Cylindrically shaped dough pieces are made and rested for a fixed period of time, i.e., 45, 90 and/or 135 minutes. Lastly, a hook is passed through the dough pieces until they rupture. As illustrated in Figure 2, there are four parameters recorded by the Brabender extensograph: maximum resistance to extension (R_{max}), dough extensibility, the ratio of resistance to extensibility and the area under the curve (Pojić & Torbica, 2011). R_{max} gives an indication of the elasticity of a dough, whereas the extensibility represents the plastic properties (Irkes, 2023). The ratio of resistance to extensibility gives indications of the gluten properties and baking volume (Pojić & Torbica, 2011). The area under the curve is

expressed in cm^2 and proportional to the energy required to stretch the dough to its rupture point.

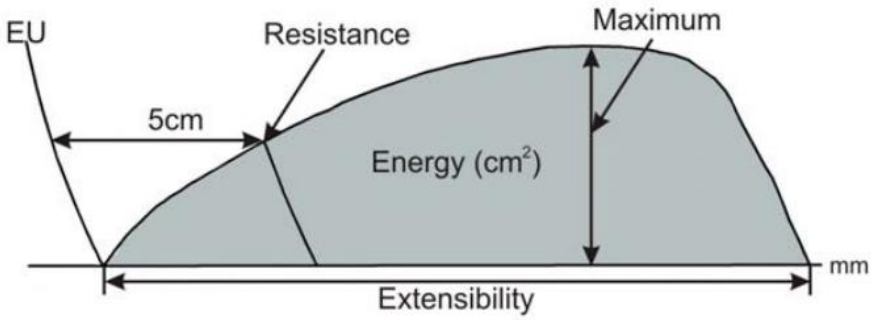

Figure 2. Extensograph curve with parameters R_{max}, extensibility, the ratio of resistance to extensibility and the area under the curve. Figure from (Pojić & Torbica, 2011).

2.2.4.3 Stickiness analysis

Stickiness in dough is of particular concern in industrial bread production and dough machinability, where it can result in operational problems, loss of product quality and costly disruptions to production schedules (Adhikari et al., 2001; Huang & Hoseney, 1999). Therefore, to investigate the stickiness of doughs is particularly important in the cereal industry. To achieve correct measurement of dough stickiness the adhesive force between the probe and the surface of the dough needs to be measured (Chen & Hoseney, 1995). In 1995, Chen and Hoseney (1995) developed the SMS/Chen–Hoseney Dough Stickiness Rig method to determine dough stickiness by using the Texture Analyser. A probe made of plexiglass is used, because of its low surface energy to achieve a clean separation at the probe-dough interface (Hoseney & Smewing, 1999). A flat dough surface is achieved by using a cell, also allowing for control of the contact area or interface area between the dough and the probe and the compression force applied on the dough (Chen & Hoseney, 1995). As a result of dough being a pressure-sensitive adhesive material, the compression force needs to be constant for each test or the unit area of the dough surfaces changes, and the results will no longer be valid.

Three parameters are recorded: stickiness (g), work of adhesion (g.s) and dough cohesiveness (mm). Stickiness is measured in force g and is the peak of the curve. High g values represent a sticky dough. According to Chen and Hoseney (1995) the average values for very sticky doughs are 230 g, 129 g for sticky doughs and 52 g for non-sticky doughs. Work of adhesion is the area under the curve and dough cohesiveness is the distance in mm from the start of the

curve to the end. Besides a high g value, a material is perceived as being sticky when the cohesive force is low, and the adhesive force is high (Hoseney & Smewing, 1999). Factors that affect the dough stickiness include the protein composition, concentration of NaCl, amount of water and enzyme levels (Chen & Hoseney, 1995). Further, the stickiness of a dough is related to the strength of the dough because a stronger and more elastic dough can overcome the force of adhesion and separate from the probe (Tebben & Li, 2019).

2.2.4.4 Chromatography of gluten proteins

Ahuja (2003) defined chromatography as "*a physical method of separation in which components to be separated are distributed in two phases, one of which does not move (appropriately called the stationary phase) and the other that moves through it in a definite direction (commonly described as the mobile phase)"*. There is a wide range of chromatographic methods frequently used to analyse gluten protein profiles and interactions including size exclusion high performance liquid chromatography (SE-HPLC), which separates molecules in solution by their size and the shape (Kuktaite et al., 2000; Yang et al., 2015). To achieve separation of the gluten proteins in SE-HPLC, extraction of the wheat flour proteins is needed. SDS is an anionic detergent that is used to separate the SDS-extractable proteins by molecular-weight, by disrupting the non-covalent interactions in the native proteins. The SDS-unextractable proteins are sonicated to achieve a complete extraction, where the proteins become soluble, as only dissolved molecules can be separated by HPLC (Singh et al 1990).

Figure 3 shows chromatograms of SDS-extractable (a) and SDS-unextractable proteins (b) extracted from dough with whole wheat flour and refined wheat flour. Peak F1* (unextractable) and F1 (extractable) are containing lager polymeric proteins and F2 contains smaller polymeric proteins, whereas F3 contains larger monomeric proteins and F4 smaller monomeric proteins (Singh et al., 1990). The percentage of unextractable polymeric proteins (%UPP) corresponds to the proportion of unextractable polymeric protein in the total polymeric proteins (%UPP=F1*/(F1*+F1)×100).

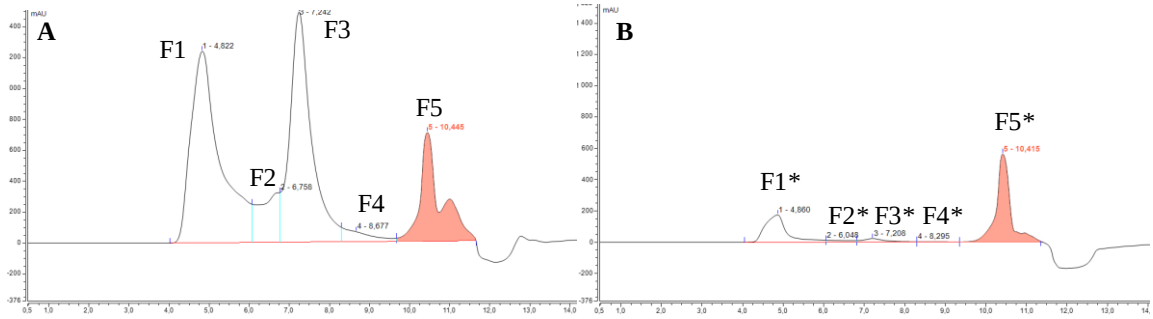

Figure 3. Size-exclusion HPLC Chromatogram illustrating **(A**) SDS-extractable proteins and (**B)** SDS-unextractable proteins. F1/F1*= larger polymeric proteins, F2/F2*= smaller polymeric proteins, F3/F3*= larger monomeric proteins F4/F4*= smaller monomeric proteins, F5/F5*=albumins and globulins.

2.2.4.5 Analytical methods to investigate bread properties

Texture, volume, and colour are all important quality parameters for bread. Crumb firmness (force, g) and structure are measured using a Texture Analyser and image segmentation (i.e., C- Cell), respectively (Angioloni & Collar, 2009; Scanlon & Zghal, 2001). Crumb firmness and structure provide, among other things, information on the baking quality of the flour used, the effect of wheat and other non-wheat components in bread, as well as the conditions during fermentation and baking (Angioloni & Collar, 2009; Scanlon & Zghal, 2001). High porosity (higher number of cells) and a fine regular gas cell structure indicate good quality of the crumb (Angioloni & Collar, 2009). Specific volume is measured to compare volume in bread with different weight. The volume of the bread (mL) is divided by the weight (g). This parameter gives an indication of the gluten content and quality of the flour (Khalid et al., 2017), as the protein determines the cell wall thickness (Delcour & Hoseney, 2010). A high specific volume is a desired quality trait in bread. Lastly, colour measurements of the crumb and crust is relevant, as the colour is the first sensation that consumer perceive (Angioloni & Collar, 2009). The results from colour measurements are presented following the CIELAB system (Qazi et al., 2021). *L** is representing the scale of whiteness, from 0 black to 100 white, whereas *a** extends from a negative value which represent green hue to a positive value which represent a red hue (-60 to 60), *b** scale from a negative blue (-60) to a positive yellow (60).

2.2.5 The technological functions of sodium in bread

NaCl influences dough rheology, dough stickiness and fermentation and thereby greatly affects dough quality (Silow et al., 2016). Consequently, it impacts characteristics like texture, volume, flavour, colour, and shelf life (Belz et al., 2012; Silow et al., 2016). The underlying chemical and biochemical effects include the impact on the gluten structure during dough mixing, inhibition of the yeast during dough fermentation and reduction of water activity to increase shelf life (Belz et al., 2012; Silow et al., 2016). These three technological functions will be further discussed in detail below.

It has been suggested that the presence of NaCl delays hydration of the gluten proteins and development of the gluten matrix (Silow et al., 2016). NaCl is thought to shield the charges on the surface of the amino acids and reduce the water-binding capacity of gluten, which may delay protein hydration and enhance protein aggregation. A delay in the hydration decreases the WA of the flour, increases the mixing time and dough strength (Avramenko et al., 2018), as well as DDT, dough resistance, elasticity and extensibility (Belz et al., 2012; McCann & Day, 2013). Overall, an increase in the bread height and improvement of overall bread quality are consequences (Silow et al., 2016). By reducing repulsion among proteins, NaCl facilitates interactions between gluten proteins, especially through hydrogen bonds, which are important for the formation of the fibrous gluten network (Tuhumury et al., 2016).

The influence sodium has on the formation of gluten matrix and structure affects the rheological properties of dough. Despite the influence of sodium on dough rheology, reducing NaCl levels from 1.2 to 0.3% (flour basis) did not significantly affect the rheological properties and bread making performance of wheat doughs in some studies (Lynch et al., 2009; Ooms & Delcour, 2019), while other authors have shown that a reduction in NaCl can lead to a significant increase of stickiness in the dough (Silow et al., 2016). Moreover, Belz et al. (2012) stated that a NaCl reduction from 1.2% to 0.6% significantly increased the storage modules G`, which reflects the elasticity of a material, but there were no significant differences in G", which is used to measure the viscosity of a material (Belz et al., 2012).

Sodium also modulates yeast metabolism and thus CO_2 production (Silow et al., 2016). When reducing the NaCl levels in the bread, an increase in gas production occurs (Belz et al., 2012), due to the electrochemical potential of sodium and chloride ions and a decrease in the osmotic pressure on the membrane of the yeast (Silow et al., 2016). While a decrease in sodium in dough increases the total volume of released gas, but the combination of a weaker gluten network and increased gas production results in loss of CO_2 from the dough and thereby a

decrease in the specific loaf volume (Belz et al., 2012). A decrease in the loaf volume also affects the crumb, where bread without NaCl has fewer number of larger air cells and an increased cell to total area ratio.

Bread colour is affected by the Maillard reaction between reducing sugars and amino acids, which occurs at temperatures over 120°C, resulting in a myriad of different compounds, including the melanonids that are responsible for browning (Capuano et al., 2008). NaCl affects the Maillard reaction because it has a plasticising effect during heating that enhances the mobility of the reactants (Silow et al., 2016). Further, in bread with lower or no NaCl the yeast metabolizes the available sugars, and a reduction of free reducing sugars occurs, which results in a lighter crust colour due to less browning (Belz et al., 2012).

NaCl is responsible for aroma and flavour in bread, as well as it has flavour enhancing (sweetness) and masking effects (bitterness). It mainly influences the perception of salt taste, in which the presence of Cl^- has effects on the receptor cells (Albarracín et al., 2011). NaCl was further shown to enhance aromatic notes, but it can also supress flavours, including bitterness (Elias et al., 2020). Pflaum et al. (2013) showed that a reduction from 1.3% to 1% NaCl (flour basis) could be detected by sensory panellists, but not between the samples with higher NaCl concentrations employed in that study. This could indicate that NaCl levels <1% can be a critical concentration for detection of salty taste (Silow et al., 2016). Lynch et al. (2009) showed significantly differences in salty taste in bread with 1.2% and 0% NaCl, but bread with 0.6% and 0.3% NaCl was closely placed in the sensorial space (Lynch et al., 2009).

2.3 Sodium chloride reduction in bread

2.3.1 Recommendations

Nordic Nutrition Recommendations state that NaCl intake should not exceed 6 grams per day for adults, which corresponds to 2.3 grams per day of sodium (Nordic Council of Ministers, 2013). In 2004 *Nordic Nutrition Recommendations* advised for a gradual reduction to 5 grams NaCl per day in the long term (Nordic Council of Ministers, 2004). This value is in line with the recommendations by the World Health Organization (World Health Organization, 2012). In 2023 new recommendations will be presented by the Nordic Councils of Ministers and recommendations for further NaCl reduction are expected.

Keyhole labelling is a volunteer labelling scheme in the Nordic countries to promote a healthy diet that was established based on "Nøkkelhullforskriftene" in 2009 with the aim to make it easier for the consumer to make healthier purchasing choices (Helsedirektoratet, 2021). Further, it is intended to stimulate the food industry to develop more nutritious food products. For different products, there are specific requirements for sugar, salt, fat, or dietary fibre content. In order for bread to be marked with the keyhole, the requirements for the product are that the dietary fiber content is above 5 g/100 g and the fat, sugar and NaCl content are below 7, 5, and 1g/100g, respectively (Helse- og omsorgsdepartementet, 2021). In general, the low NaCl content in food products is an important criterion for the food producer to be able to mark their product packaging with the keyhole logo (Helsedirektoratet, 2021).

The background for the recommendations when it comes to NaCl intake is the effect sodium has on the development of lifestyle diseases, such as high blood pressure and the risk of cardiovascular disease, stroke and coronary heart disease (World Health Organization, 2012). He and MacGregor (2010) state that elevated blood pressure is responsible for over 60% of all strokes and around 50% of all coronary disease. Karppanen and Mervaala (2006) state that reduction in the daily sodium intake by 35-40% could be expected to result in a blood pressure reduction 2.5 times greater than what's achieved if 10% of the population were to use blood pressure reducing medication. Doctors should therefore recommend products with the keyhole label to patients with elevated and high blood pressure, as a measure to reduce the blood pressure without medications (Christophersen & Alm, 2011). Moreover, dietary NaCl intake is positively associated with the risk of gastric cancer although a direct causal relationship has not yet been established (Ge et al., 2012). Silow et al. (2016) states that the physiological requirement ranges between 200-500 mg sodium per day whereas average NaCl intakes are around 9-12 grams per day (World Health Organization, 2012).

2.3.2 Sodium chloride reduction

The different strategies for NaCl reduction and sodium replacement encompass a gradual reduction of NaCl, NaCl replacers, taste enhancer and taste contrast to enhance NaCl perception (Silow et al., 2016). Gradual reduction of NaCl in food products has been adopted by the industry, often without advertising it (Inguglia et al., 2017). By reducing the sodium content in foods that consumers buy regularly and repeatedly, their taste receptors can adapt to the reduction (He & MacGregor, 2009). Lawless and Heymann (2010) defined adaptation as a decrease in responsiveness during condition of constant stimulation. The specific taste

receptors become more sensitive to lower NaCl concentrations as the NaCl intake falls (He & MacGregor, 2009). The result of this strategy is that the consumers experience the same intensity of the salty taste even though the concentrations are lower (Inguglia et al., 2017).

NaCl substitutes includes potassium chloride (KCl), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$) and ammonium chloride (NH_4Cl). Examples of taste enhancer are phosphate- and lactate salts (Ruusunen & Puolanne, 2005), yeast extract (Inguglia et al., 2017; Vinitha et al., 2022), amino acids (Vinitha et al., 2022), monosodium glutamate (Vinitha et al., 2022), herbs and spices (Cepanec et al., 2017), and different edible macroalgae (Inguglia et al., 2017; Vinitha et al., 2022). The function of taste enhancers is that they increase the intensity of the salty taste perception, (Vinitha et al., 2022) and they are often mixed with inorganic NaCl substitutes to mask their undesirable taste (Cepanec et al., 2017).

Which NaCl substitutes to use is dependent on the food product and its formulation (Inguglia et al., 2017). KCl is considered to be the most used substitute (Cepanec et al., 2017; Tan et al., 2022), as it has a similar structure and functionality as NaCl, but it gives an undesirable aftertaste (acrid, bitter and metallic) and a slightly less intensive taste (Tan et al., 2022). Cepanec et al. (2017) have shown that to avoid unwanted flavours and to secure overall consumer acceptance of NaCl reduced food products it is effective to use a mixture of KCl (30-45%) and NaCl (50%), and inclusion of one or more taste enhancer (5-20%) (Cepanec et al., 2017).

Studies on NaCl reduction and substitution usually include a sensory analysis, in particular descriptive analysis (DA) as it is considered as the gold standard (Lawless & Heymann, 2010). This method gives a complete sensory description of the product and is regarded as useful when a detailed specification of sensory attributes is desired. It is a very powerful tool in product development as it can assess both the suitability of a prototype as well as how close an introduction is to the target product. This method is also used for comparison of sensory differences among products on the market, in shelf-life testing and quality assurance. When using DA, a trained panel of 8-12 participants is required. Such a panel is calibrated to each attribute to be evaluated (Lawless & Heymann, 2010). DA is divided into 4 stages: brainstorming, pre-test, main test, and statistical analysis of the results. Brainstorming is a process where the panellists develop their own language for the sensory properties of the product and a list of all the attributes is made. These attributes should be assessed in both the pre- and main test. After the brainstorming, the two most different test samples are served to

the panellists in the pre-test, and they rank the intensity of all selected attributes decided in the brainstorming, usually one a scale from 1-9. The reason for doing a pre-test is to check that all the panellists are calibrated, are agreed on the intensity and understand the meaning of the different attributes. After the pre-test has been completed, the list of attributes is adjusted if needed, and is used in the main test (Lawless & Heymann, 2010). In DA it is recommended that the number of samples is a minimum of 5 and maximum of 12. It is most common to analyse 8 product samples, in several replicates. The samples are served in a randomized order with a three-digit code, to reduce the occurrence of bias. Each panellist sits in separate sensory booth.

3. Materials and Methods

3.1 Experimental plan

Analysis was conducted on a total of 12 breads (Figure 4). Four of the breads were controls (C1, C2, C3A, C3B), whereas three of those were NaCl reduced at different levels (C2, C3A, C3B). Eight of breads were made with fermented and unfermented *S. latissima* and *A. esculenta*. The two lowest levels of NaCl reduction (C3A and C3B) contained different amounts of macroalgae, based on the sodium content of the macroalgae, to achieve the same concentration of sodium as for control bread C2 (0.24 g Na/100 g bread). The sodium content fermented algae powder was lower compared to nonfermented algae, as well as *S. latissima* had a higher sodium content compared to *A. esculenta* (Appendix 1).

Figure 4. The content of sodium chloride, sodium and macroalgae per 100 g of bread, in control breads and breads containing macroalgae.

First, analysis was conducted at the laboratory to investigate the effect of NaCl reduction and addition of macroalgae on rheological and chemical properties on dough. Further, a small-scale-baking trial was carried out on all breads to select samples for baking on a large scale and sensory analysis that involved a descriptive analysis of bread conducted by a trained sensory panel at Nofima Ås.

3.2 Moisture content in flour

The moisture content in the flour was measured with the moisture balance analyser Sartorius Thermo Control (Sartorius, Göttingen, Germany). Flour (5 g ±2 g) was placed on aluminium dishes and exposed to infrared heating until constant weight. The moisture content was calculated as the weight difference before and after the heating and as a percentage based on the initial weight of the flour sample.

3.3 Farinograph analysis

A Farinograph-TS (Brabender, Duisburg, Germany) was used to analyse WA, DDT and DS. The dough consisted of 66.6% whole wheat flour (Sammalt hvete fin, Lantmännen Cerealia AS, Oslo, Norway) and 33.3% refined wheat flour of strong protein quality (Hvetemel standard, Lantmännen Cerealia AS, Oslo, Norway). Flour was substituted with different amounts of macroalgae (Orkla AS, Oslo, Norway), as outlined in Table 2. Characterization of the algae is presented in Appendix 1. The weight of the flour in samples was adjusted to 14% moisture basis. The refined wheat flour had a moisture content of 13.9% while the whole wheat flour contained 12.7%. In samples containing macroalgae, the amount of macroalgae was subtracted from the amount of flour (i.e., 2*X g macroalgae subtracted from 197 g whole wheat flour, 1* X g macroalgae subtracted from 99.9 g refined wheat flour).

Table 2. The amount of whole wheat flour, wheat flour and macroalgae in grams in each sample. C1, C3A and C3B refers to sodium chloride content of 1.5%, 0.87% and 0.75% per 100 g flour, respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Samples	Whole wheat flour (g)	Wheat flour (g)	Macroalgae (g)	Total (g)
Control	197	99.9	-	296.90
C3A+SL	195.22	99.01	2.67	296.90
C3A+FSL	195.06	98.94	2.91	296.91
C3A+AE	193.90	98.30	4.71	296.91
C3A+FAE	193.44	98.12	5.33	296.89
C3B+SL	193.66	98.23	5.00	296.89
C3B+FSL	193.36	98.08	5.45	296.89
C3B+AE	191.10	96.99	8.82	296.91
C3B+FAE	190.30	96.60	9.99	296.89

The samples were mixed with a spoon before performing analysis in the farinograph. Water absorption was determined, and analysis were carried out at 30°C 68 rpm for 20 min, according to ISO 5530-1- standard. Two replicates of each sample were carried out. Figure 5 A and B present the farinograph used in the analysis and the dough after mixing, respectively.

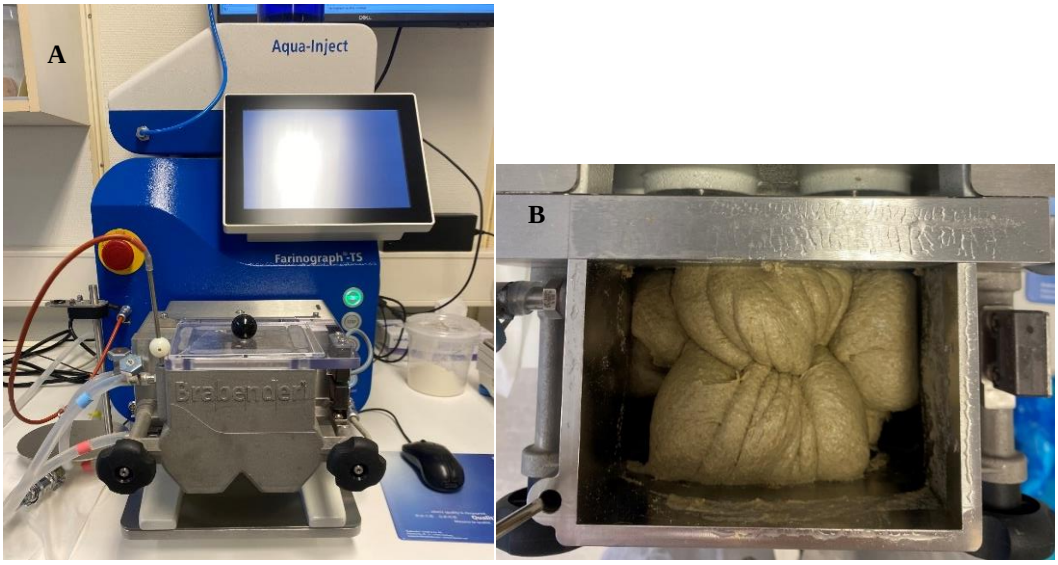

Figure 5. (A) Farinograph and (B) dough with fermented *A. esculenta* after 20 minutes of mixing.

3.4 Extensograph analysis

Extensional tests to were performed using an Extensograph-E (Brabender) to measure resistance to extension and extensibility. Doughs were prepared in a Farinograph-TS (Brabender) operated at 126 rpm with a bowl temperature of 22°C. NaCl was added upon the calculated flour and macroalgae, presented in Table 3. Water addition was according to WA (determined as described in 2.2) minus 1.5% due to high dough stickiness. Mixing was continued to a total energy input of 12 Wh/kg.

Table 3. Composition of dough samples evaluated in the farinograph and extensograph. C1, C2, C3A and C3B refers to sodium chloride content of 1.5 g, 1.0 g, 0.87 g and 0,75 g, per 100 g flour mix (14% moisture basis) respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Dough samples	Whole wheat flour (g)	Wheat flour (g)	NaCl (g)	Macroalgae (g)	Macroalgae (%/ 100 g flour)	Total (g)
C1	197	99.9	4.50	-	-	301.40
C2	197	99.9	3.00	-	-	299.90
C3A	197	99.9	2.61	-	-	299.51
C3B	197	99.9	2.25	-	-	299.15
C3A+SL	195.22	99.01	2.61	2.67	0.89	299.51
C3A+FSL	195.06	98.94	2.61	2.91	0.97	299.52
C3A+AE	193.90	98.30	2.61	4.71	1.57	299.52
C3A+FAE	193.44	98.12	2.61	5.33	1.78	296.50
C3B+SL	193.66	98.23	2.25	5.00	1.67	299.14
C3B+FSL	193.36	98.08	2.25	5.45	1.82	299.14
C3B+AE	191.10	96.99	2.25	8.82	2.94	299.16
C3B+FAE	190.30	96.60	2.25	9.99	3.33	299.14

Two independent replicates of each sample were prepared in the farinograph. The dough was further divided into two 150±0.2 g pieces. The extension test was performed according to AACC method 54.10, with modifications. Samples that only contained NaCl were shaped in the balling and rolling units in the Extensograph-TS while samples that included macroalgae were shaped by hand. First by rounding into a ball in the Extensograph-TS, then by rolling into a cylinder (5 times back and forth). This was carried out because these were too sticky to be made in the Extensograph-TS. The dough was allowed to rest for 45 and 90 minutes at 30°C in high humidity before it was stretched. Figure 6 shows the instrument used in the analysis and how the measurements of the dough were carried out.

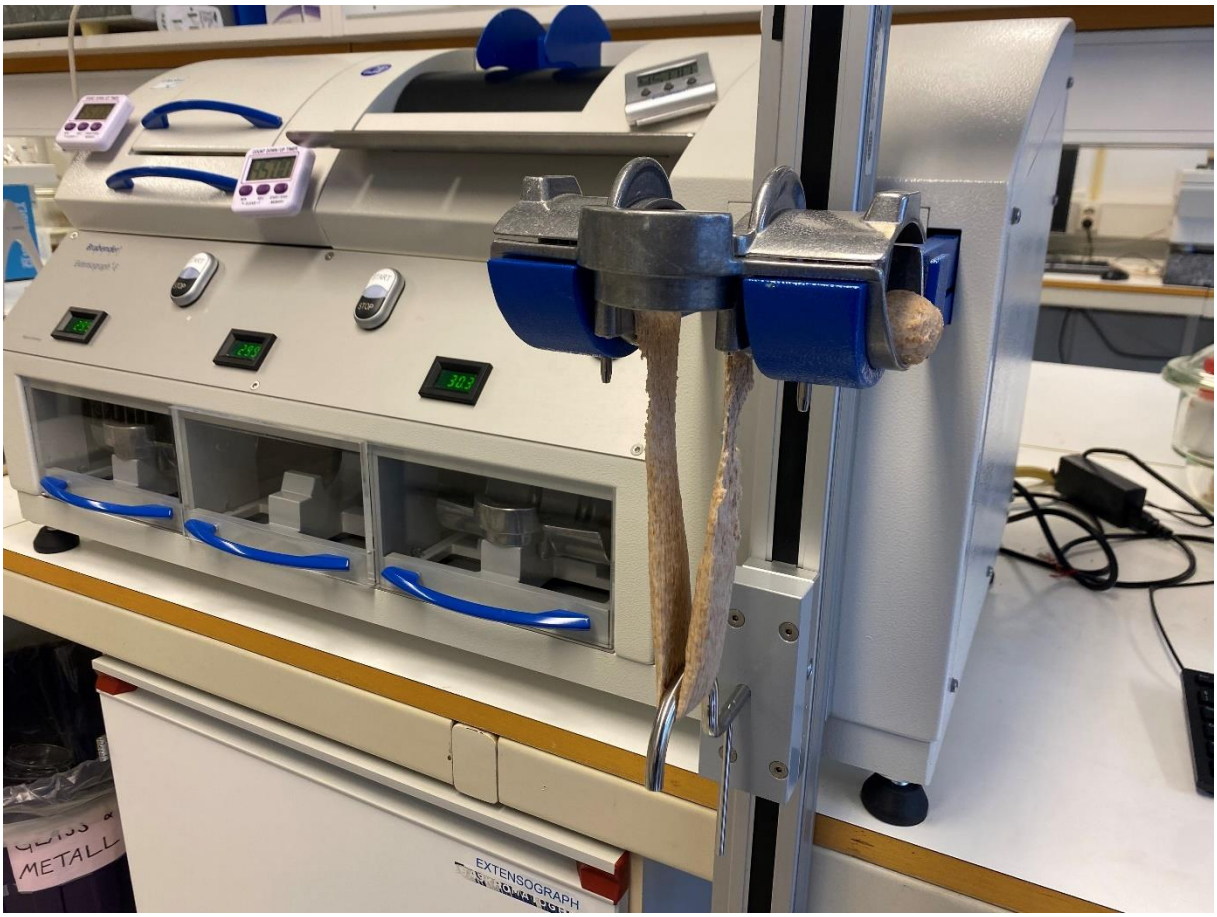

Figure 6. Brabender Extensograph-E used for extensional analysis of dough.

3.5 SMS/Chen-Hoseney Dough Stickiness Rig

The SMS/Chen–Hoseney Dough Stickiness Rig was used to measure the following indices: stickiness (g), work of adhesion (g.sec) and dough cohesiveness (mm). The dough was prepared using a farinograph, with a 50 g mixing bowl instead of the 300 g mixing bowl. Recipes were adjusted accordingly, by dividing all amounts listed in Table 3 by 6. Dough was

mixed as described for the extensograph (3.4) but with water addition according to WA (no subtractions as for extensograph).

Dough stickiness was analyzed using a TA.XTplusC Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a 500 g load cell. A SMS/Chen–Hoseney Dough Stickiness Rig and 25 mm perspex cylinder probe were used for the analysis as described by Huang and Hoseney (1999), with modifications. The contact time between the probe and sample was changed from 0.1 to 1 s to increase reproducibility.

The dough prepared in the farinograph was first transferred to a 120 mL specimen container followed by being transferred to the chamber, and the cell lid was screwed on. The remaining dough was placed in the cup in a warming cabinet at a temperature of 30°C for 45 minutes. The dough was analyzed at 0 minutes and after 45 minutes. Six replicates were performed on each independently prepared dough sample (average of replicates were further used for statistical analysis). About 1 mm of dough was extruded before each measurement (Figure 7B), which rested for 30±5 seconds with a small lid taped with a piece of moistened cotton before it was analyzed. A blade was used to wipe the extruded dough off the surface before the next measurement. The results (stickiness, work of adhesion and cohesiveness) from the analysis were processed in Exponent Connect. Figure 7 shows the chamber and lid, and a TA.XTplusC Texture Analyser (A) and SMS/Chen–Hoseney Dough Stickiness Rig used in the stickiness analysis (B).

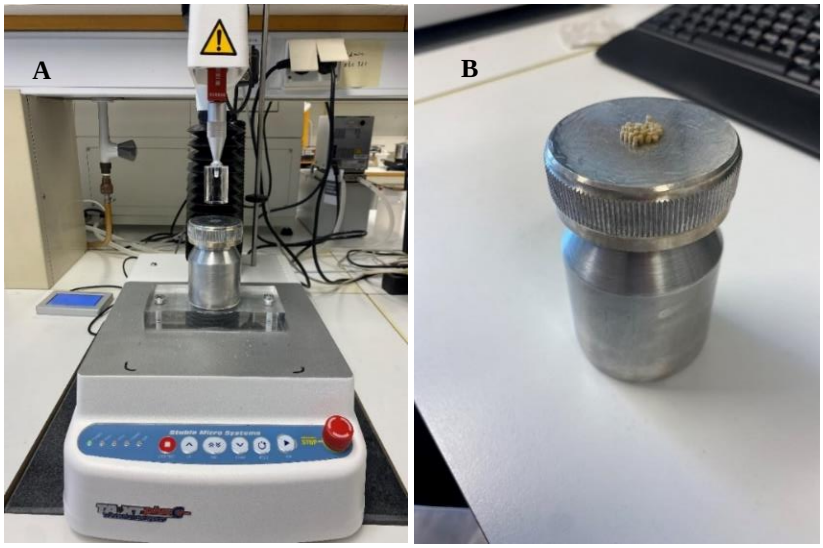

Figure 7. (A) TA.XTplusC Texture Analyser and SMS/Chen–Hoseney Dough Stickiness Rig, and (B) Chamber and lid used in the stickiness analysis.

3.6 SE-HPLC

The size distribution of gluten proteins was analyzed by SE-HPLC in dough samples (5±0.2g) that were prepared for the extensional test described in section 3.4. Samples were put into a 50 mL plastic centrifuge tube and placed in a cabinet at 30°C. After 90 minutes the samples were transferred to a freezer (-18°C). The frozen dough samples were freeze-dried and manually crushed using a mortar. SE-HPLC was conducted according to Singh et al. (1990). The proteins from the dough were extracted by a two- step extraction procedure. A 0.1 M phosphate extraction buffer with 1% SDS with pH 6.9 was added (1.5 mL) to 15 mg freeze dried dough sample. In the first step the samples were vortexed followed by shaking in a Termomixer F1.5 for 30 minutes at 75°C to inactivate enzymes in the sample. Further, the samples were centrifuged (15 minutes, 1300 rpm) and supernatant was recovered (SDS-extractable proteins).

In the second step phosphate buffer was added to pellet and the samples were sonicated using Q55 Sonicator (Qsonica Sonicators, Connecticut, USA) the samples were centrifuged (15 minutes, 1300 rpm) to separate the supernatant. Lastly, the two different fractions were filtered through Millipore Millex-HV PVDF 0.45 µm filter (Merck Millipore, Burlington, Massachusetts, USA) into glass vials and sealed with crimp cap.

The extracted proteins were separated on a Bio-Step^{TM} 5µm SEC-s4000 500 Å column (Phenomenex, California, USA) on a Dionex Ultimate 3000 (UHPLC^{+}) (Thermo Fisher Scientific, California, USA) chromatography, and a UV detector set to 214 nm was used for detection. The injection volume of sample was 10 µl. The eluent consisted of 30% acetonitrile with 0.05% trifluoracetic acid and had a flow rate of 0.4 mL/ min.

3.7 pH in macroalgae sample and dough

The pH of 5% w/v macroalgae solution (0.5 grams of macroalgae sample (Figure 8) stirred for 10 minutes in 10mL distilled water, n=2) was measured for all macroalgae samples using a PHM210 MeterLab Standard calibrated pH meter (Radiometer analytic, Lyon, France).

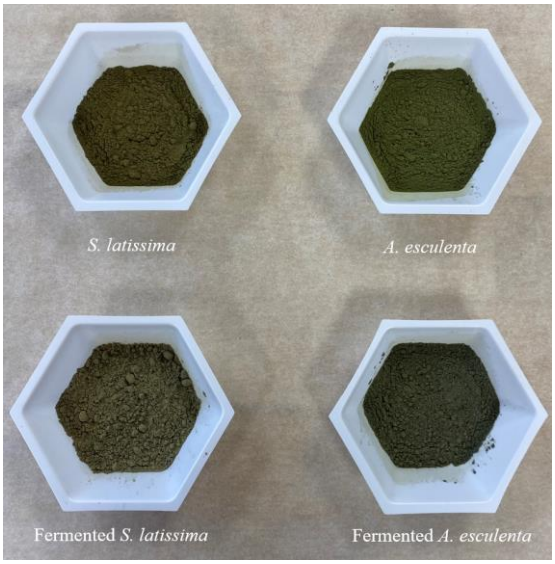

Figure 8. Algae powder of *S. latissima* and *A. esculenta* with and without fermentation.

The pH was measured in dough (3 grams of dough and 5 mL distilled water in 50 mL plastic centrifuge tubes first stirred by Multi OSU-20 and then by hand until the dough was dispersed) with the same pH meter as used for macroalgae sample. One measurement of each dough, including two replicates of each sample, was conducted.

3.8 Baking

3.8.1 Small-scale baking

A small-scale straight dough baking experiment was carried out to determine the baking performance and to choose breads for the sensory analysis. The contents of refined and whole wheat, NaCl and macroalgae are specified in Table 3. Water addition was according to farinograph WA and 3 g dry yeast, and 4.5 g rapeseed oil were used per dough. All doughs were prepared in duplicate and in randomized order (Figure 9A). Doughs were mixed in a DoughLab (Perten, Stockholm, Sweden). (Figure 9B) using a 300 g bowl at 126 rpm to a total energy input of 12 Wh/kg. The bowl temperature was set to be 23°C, to achieve a final dough temperature of 27°C.

The dough was shaped by hand and rested for 30 minutes at 32°C and RH 75% in a bowl (Lillinord, Odder, Denmark). After resting, the dough was divided into 3 pieces of 150g each. The dough pieces were shaped by Dough rounder R10 (FriulCo, Maniago, Italy) two times and placed on a baking tray. Then the dough proofed at 32°C and RH 75% for 45 minutes. The breads were baked for 20 minutes at 210°C (oven temperature was set to 240°C) with 10

seconds of steam in a rotating oven (Revent type 626 G EL IAC, Revent international, Vesby, Sweden). After baking, the breads rested for a minimum of 60 minutes before further analyses.

Volume and shape ratio (height/width) was measured using a TexVol BVM-6630 Series Analyser (Perten, Stockholm, Sweden). Bread weights were measured using a scale. Further, a 2.5 cm slice was cut from the middle of bread with a spacer and knife. A picture of each bread was taken with C-cell Color (Calibre, Warrington, UK) and crumb firmness was measured using the same Texture Analyser as for stickiness, equipped with a 5 kg load cell according to AACC Method 74–09. Lastly, the color (hue and brightness) of the bread was measured with Chroma Meter CR-400 (Konica Minolita, Chiyoda, Japan).

Figure 9. (A) Sample material used in dough and (B) DoughLab used for dough mixing.

3.8.2 Large-scale baking

Based on the small-scale baking, seven breads were selected for large-scale baking and sensory analysis. Three controls (C1, C2, C3A) and NaCl reduction level C3A with all four macroalgae types were chosen. The amount of wheat flour, whole wheat flour, NaCl and macroalgae in the different samples as presented in Table 3 was multiplied by 10, and 30 g dry yeast and 45 g rapeseed oil was added to all the samples. The amount of water was calculated based on farinograph WA and a flour weight of 3000 g. The amount of the material used for large-scale baking is presented in Table 4.

Table 4. Amount of whole wheat and refined flour, macroalgae, NaCl, rapeseed oil, yeast and water in dough used for large-scale baking. C1, C2 and C3A refers to sodium chloride content of 1.5 g, 1.0 g and 0.87 g per 100 g flour mix (14% moisture basis), respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Dough samples	Whole wheat flour (g)	Wheat flour (g)	Macroalgae (g)	NaCl (g)	Rapeseed oil (g)	Yeast (g)	Water (g)
C1	1970.0	990.0	0	45.0	45	30	2040
C2	1970.0	990.0	0	30.0	45	30	2040
C3A	1970.0	990.0	0	26.1	45	30	2040
C3A+SL	1952.2	990.1	26.67	26.1	45	30	2124
C3A+FSL	1950.6	989.4	29.09	26.1	45	30	2130
C3A+AE	1939.0	983.0	47.06	26.1	45	30	2130
C3A+FAE	1934.4	981.2	53.33	26.1	45	30	2196

The doughs were prepared and baked in a randomized order, and one replicate of each dough was made. Diosna SP12 (Diosna, Osnabruck, Germany) was used to mix the doughs. The doughs were first mixed at 30 Hz for 240 seconds. After 240 seconds the mixing speed was increased to 40 Hz and the dough were mixed until the dough temperature was 27°C. The dough was transferred to a plastic box without lid and rested for 30 minutes at 32°C and RH 75% (Lillinord). After resting, the dough was divided into 9 pieces of 550 g each. The dough pieces were shaped to bread by hand and placed on a baking tray. Then the dough proofed at 32°C and RH 75% for 45 minutes. The oven was pre-heated at 240°C which was reduced to 210°C when baking the bread. The bread was baked in a rotating oven (Revent type 626 G EL IAC, Revent international) for 30 minutes with 10 seconds of steam. After baking, the breads rested for a minimum of 60 minutes before further analyses of the bread. Measurements of volume, bread/ height ratio, crumb firmness, pictures and color were conducted as described in chapter 3.8.1. Restrictions in sample size prevented statistical analysis of the result.

3.9 Sensory analysis

A quantitative descriptive analysis was conducted according to ISO 13299:2016 by a panel consisting of trained assessors, on 7 breads (C1, C2, C3A and NaCl reduction level C3A with all four macroalgae). The trained panel consisted of 11 assessors selected by their abilities to recognize smell and taste that meet the requirements in ISO 8586:2012. Before the main test was the panel calibrated through a trial with samples C3A and C3A+FAE and trained to use the selected attributes and the intensity of these. They evaluated 23 sensory attributes, listed in Table 5. Explanation of attributes are presented in Appendix 10. The intensity of each attribute was ranked on a scale from 1-9. Bread samples were served in a randomized order with respect to samples, assessor, and replicate, with a three-digit code. The breads samples were assessed in two replicates. A total of 14 samples was served in five serving sessions. Software used in the sensory analysis was EyeQUestion (Logic8 BV, Utrecht, Holland) and EyeOpenR (Logic8 BV, Utrecht, Holland).

Table 5. Attributes evaluated during the descriptive analysis on whole wheat bread, with and without algae.

	Sensory attributes
Appearance	Colour hue, colour intensity, whiteness, poring
Odour	Grain, roasted, algae, drawer, rancid
Taste/ flavour	Total flavour intensity, sour flavour, sweet taste, salty taste, bitter taste, raw flavour, grain flavour, algae flavour, cloying flavour, rancid flavour, metallic flavour
Texture	Juiciness, chew resistance, tackiness

3.10 Statistical analysis

All dough and bread samples were analysed using one-way analysis of variance (ANOVA), followed by Tukey`s Honestly Significant Difference test. General linear models were used to study the effect of the species of macroalgae, fermentation of macroalgae and NaCl reduction level, and the interactions between the three factors. The statistical analysis was carried out using Minitab Statistical Software, version 21.1 (Minitab, Inc. State College, PA, USA). A significance level of $\alpha<0.05$ was used.

ANOVA-tests using F-tests were used to analyse the results, and Tukeys multiple comparison test was performed on significant attributes from the F-test, to determine which of the samples were different from each other. Excel 365 (Microsoft Corporation, Redmond, WA, USA) was

used to perform the tests. A significance level of $\alpha<0.05$ was used. Also, a Principal Component Analysis (PCA) was conducted on the results from the sensory analysis.

4. Results

4.1 Dough characteristics

4.1.1 Farinograph analysis

Results from farinograph analysis presented in Table 6. Significant differences in DDT, WA and DS were observed ($p<0.001$).

Table 6. Dough development time (DDT), water absorption (WA) and dough stability (DS) in dough (n=2). Different letters denote significant differences among means ($p<0.05$). Control refers to sodium chloride content of 1.5%/100 g flour. C3A and C3B refers to sodium chloride content of 0.87%/100 g flour and 0.75%/100 g flour, respectively. *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Dough sample	DDT (s)	WA (%)	DS (s)
Control	731 ± 16^{a}	67.3 ± 0.0^{e}	964 ± 33^{a}
C3A+SL	696 ± 16^{ab}	69.8 ± 0.0^{d}	705 ± 0^{c}
C3A+FSL	652 ± 4^{bcd}	69.8 ± 0.1^{d}	797 ± 5^{b}
C3A+AE	666 ± 16^{abc}	70.5 ± 0.1^{d}	744 ± 15^{bc}
C3A+FAE	599 ± 25^{cd}	72.4 ± 0.3^{b}	591 ± 6^{d}
C3B+SL	626 ± 29^{bcd}	71.4 ± 0.0^{c}	619 ± 11^{d}
C3B+FSL	664 ± 4^{abc}	71.4 ± 0.1^{c}	639 ± 11^{d}
C3B+AE	620 ± 34^{bcd}	72.6 ± 0.6^{b}	635 ± 6^{d}
C3B+FAE	585 ± 25^{d}	76.6 ± 0.1^{a}	489 ± 1^{e}

The highest WA was observed for the control dough without macroalgae, whereas WA significantly increased ($p<0.001$) with increased amounts of macroalgae in dough (Table 6). The dough with the highest amounts of algae i.e., C3B+FAE (3.3% algae/100 g flour) showed the highest WA. Comparing dough with the same algae type (i.e., algae in dough C3A *vs* C3B), it can be observed that higher algae levels resulted in significantly higher WA. The results from the general linear model (Appendix 2) observed that doughs with NaCl reduction level C3A with algae had a significant lower WA ($p<0.001$), as well as a significant higher WA ($p<0.001$) for doughs with *A. esculenta*, and that use of fermented macroalgae led to significantly higher WA ($p<0.001$) compared to unfermented macroalgae.

Furthermore, the highest DDT and DS was found in control dough without macroalgae. C3B+FAE had a significantly lower DS than all other samples ($p<0.001$), and a significantly lower DDT ($p<0.001$) than C3A+SL, C3A+AE, and C3B+FSL. The results further showed that doughs with the same type of macroalgae (i.e., algae in dough C3A *vs* C3B) resulted in a significantly lower DS, which indicated that higher levels of algae resulted in a significantly lower DS. Due to high variability, only few differences among the DDT of samples were significant. However, the results from the general linear model (Appendix 2) indicated

significant differences in all evaluated factors for DDT and DS. Samples with *S. latissima* had a significantly higher DDT ($p<0.01$) and DS ($p<0.001$) compared to doughs with *A. esculenta*, doughs with NaCl reduction level C3A with algae had significantly higher DS ($p<0.001$) and DDT ($p<0.05$) than C3B, and lastly the use of fermented macroalgae led to significantly lower DDT ($p<0.05$) and DS ($p<0.001$) compared to unfermented macroalgae.

4.1.2 Extensograph analysis

Figure 10A and B present the results for extensibility and R_{max} after 45 and 90 minutes of resting (n=2 independently prepared dough samples). Numerical values can be found in Appendix 3 and 4.

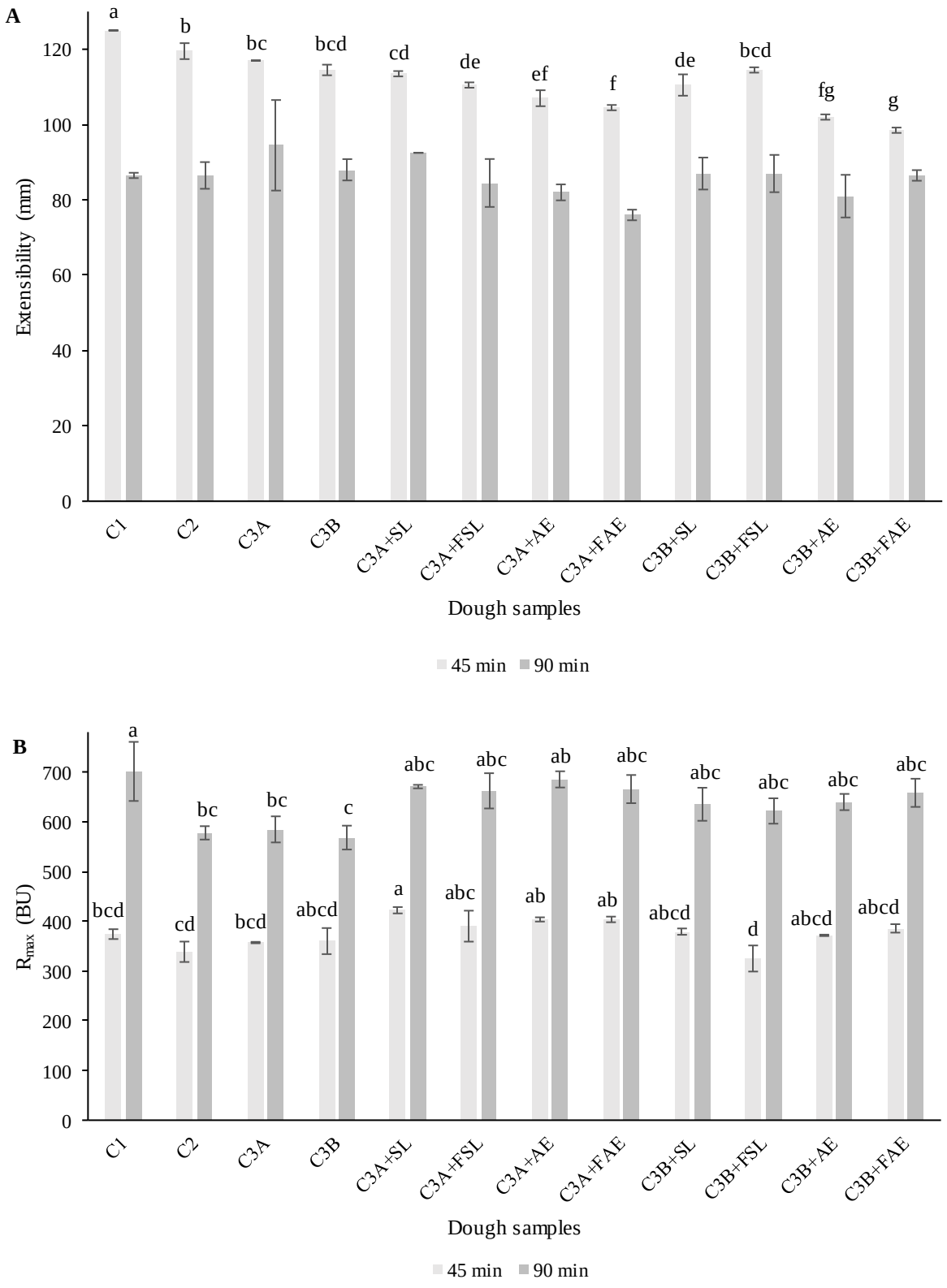

Figure 10. Extensibility (mm) (A) and Rmax (B) in dough samples (n=2) after 45 and 90 minutes of resting time. C1, C2, C3A and C3B refers to sodium chloride content of 1.5%, 1.0%, 0.87% and 0,75%/100 g flour, respectively. Error bars represent half of the range, while different letters denote significant differences among means ($p<0.05$). There were no significant differences in extensibility after 90 min. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

After 45 minutes (Figure 10A), extensibility of algae samples significantly differed ($p<0.001$) from controls C1 and C2, as well as their respective controls C3A and C3B (except C3A+SL, C3B+FSL). Incorporation of fermented *A. esculenta* into dough resulted in significantly lower extensibility than fermented and unfermented *S. latissima* at the same NaCl level (i.e., comparing with C3A or C3B), and C3A+AE. Doughs with fermented *A. esculenta* contained a higher amount of alga, compared to the ones with fermented *S. latissima* and unfermented *S. latissima* and *A. esculenta* (Table 3). Samples with the same algae type but different NaCl amounts were in most cases not significantly different from each other, only dough with fermented *A. esculenta* resulted in significantly lower extensibility in C3B vs C3A breads. The factors (species of macroalgae and NaCl reduction level) and their interactions significantly affected the extensibility (Appendix 4). The differences were likely driven by the decrease in extensibility samples with added *A. esculenta* ($p<0.001$), and with lower NaCl contents ($p<0.05$).

In contrast to the values after 45 minutes, the extensibility in dough samples was not significantly different after 90 minutes of resting (Figure 10A). However, an effect of algae (higher extensibility in samples with *S. latissima)* and interaction between algae and NaCl reduction were observed in the results from the general linear model (Appendix 4).

Figure 10B shows that there were no significant differences in R_{max} between the control doughs after 45 minutes of resting, but after 90 minutes, the NaCl reduced controls had a significant lower Rmax compared to C1 ($p<0.01$). Furthermore, C3A+SL was significantly higher ($p<0.001$) than the control doughs (except for C3B) and C3B+FSL after 45 minutes of resting, and R_{max} for C2 was significantly lower compared to C3A+ FAE and C3A+AE. The general linear model showed that interactions between type of algae and fermentation of macroalgae significantly reduced R_{max} ($p<0.01$), and that the NaCl reduction level C3B ($p<0.001$) and use of fermentation of macroalgae ($p<0.05$) significantly decreased R_{max} in dough samples with macroalgae after 45 minutes of resting.

Similar to the extensibility after 90 minutes (Figure 10A), few significant differences ($p<0.01$) were detected in R_{max} after 90 minutes of resting (Figure 10B). The control with reduced NaCl (C2, C3A and C3B) had significantly lower Rmax than C1, but no differences between C1 and doughs with macroalgae were observed. There were no significant differences between the samples with macroalgae, however reduction in NaCl (i.e., from C3A to C3B) had a significant effect on R_{max} ($p<0.05$) (Appendix 4). Thus, a reduction in NaCl significantly decreased the R_{max} after 90 minutes of resting.

4.1.3 Stickiness

Results from SMS/Chen–Hoseney Dough Stickiness Rig experiments are presented in figure 11, 12 and 13. Corresponding numerical values are in Appendix 5.

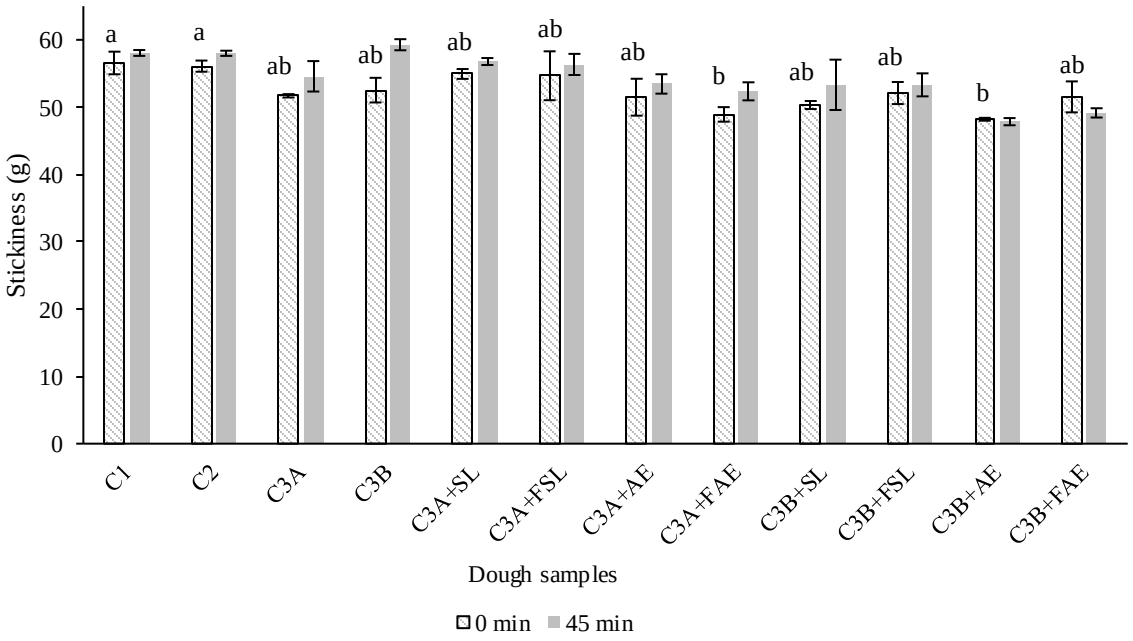

Figure 11. Stickiness (g) in dough samples (n=2) after 0 minutes and 45 minutes of resting time. C1, C2, C3A and C3B refers to sodium chloride content of 1.5%, 1.0%, 0.87% and 0,75%/100 g flour, respectively. Error bars represent half of the range, while different letters denote significant differences among means ($p<0.05$) between dough samples after 0 minutes. There were no significant differences in stickiness between samples at 45 min. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Few significant differences in stickiness were detected, and samples only differed significantly from each other immediately after their preparation (0 min) ($p<0.01$), but not after 45 minutes of resting. More specifically, the stickiness of C3A+FAE and C3B+AE was significantly lower than C1 and C2 ($p<0.01$). Results from the general linear model showed that dough with *S. latissima* had a higher stickiness compared to *A. esculenta* ($p<0.05$), and a significant effect in interactions between NaCl reduction level and fermented vs fermented macroalgae ($p<0.05$).

Despite the lack of significant differences between dough samples with the one-way ANOVA after 45 minutes of resting, a significant higher stickiness was observed in samples with *S. latissima* compared to *A. esculenta* ($p<0.001$) and in samples with NaCl reduction level C3A compared to C3B ($p<0.001$) (Appendix 6). Also, a significant effect of interactions between NaCl reduction level and fermented vs not fermented algae was detected ($p<0.05$).

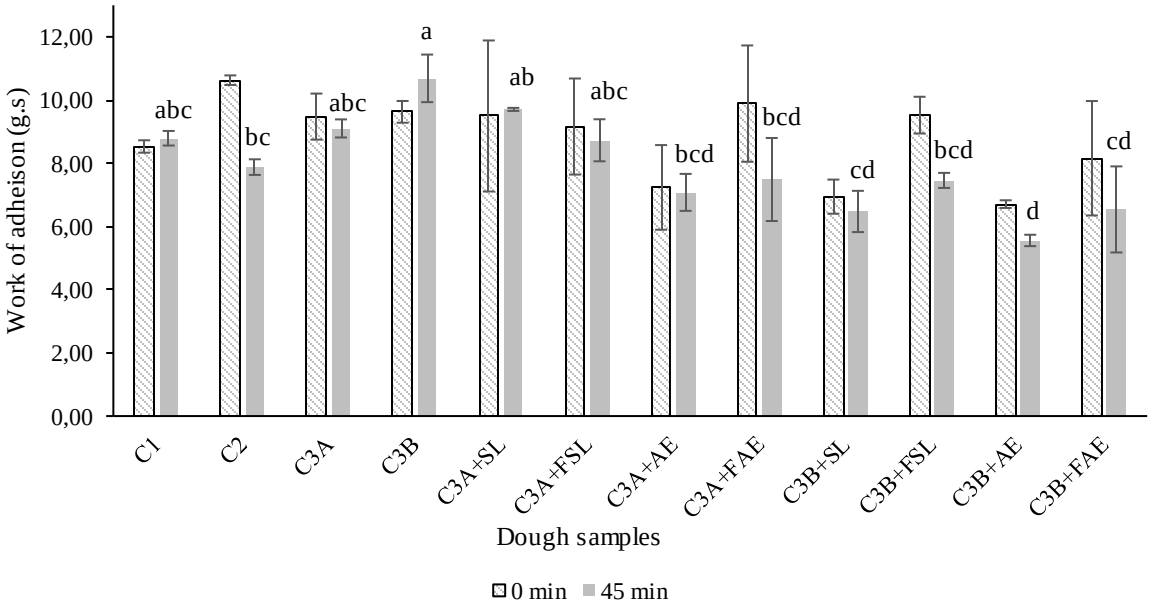

Figure 12. Work of adhesion (g.s) in dough samples (n=2) after 0 and 45 minutes of resting time. C1, C2, C3A and C3B refers to sodium chloride content of 1.5%, 1.0%, 0.87% and 0,75%/100 g flour, respectively. Error bars represent half of the range, while different letters denote significant differences among means ($p < 0.05$). There were no significant differences in work of adhesion between samples at 0 min. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Significant differences in work of adhesion (g.s) between dough samples were not found after 0 minutes, but some were present ($p<0.001$) after 45 minutes of resting. However, the results from the general linear model showed a significant effect of fermentation of macroalgae ($p<0.05$), where fermented macroalgae in dough increased the work of adhesion compared to the non-fermented algae after 0 minutes of resting time (Appendix 6). After 45 minutes of resting were C3B significantly different from C2, whereas no other differences were detected between the controls. Furthermore, C3B had a significantly higher work of adhesion than C3A+AE, C3A+FAE and all samples with NaCl reduction level C3B with algae. Furthermore, C3A+SL had the second highest work of adhesion, which was significantly higher than C3B+SL, C3B+AE and C3B+FAE. C3B+AE's work of adhesion was significantly lower compared to C1, C3A, C3A+SL and C3A+SL. Samples with *S. latissima* ($p<0.01$) and NaCl reduction level C3A ($p<0.001$) had a significantly higher work of adhesion (Appendix 6). No significant interactions were found among any of the factors.

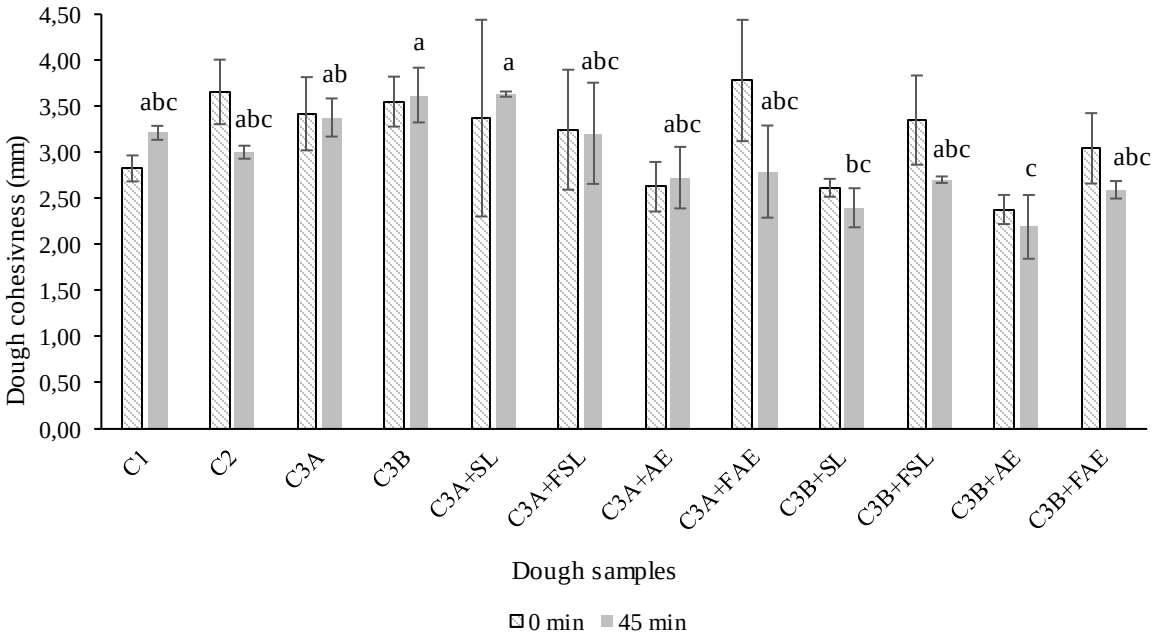

Figure 13. Dough cohesiveness/distance (mm) in dough samples (n=2) after 0 and 45 minutes of resting time. C1, C2, C3A and C3B refers to sodium chloride content of 1.5%, 1.0%, 0.87% and 0,75%/100 g flour, respectively. Error bars represent half of the range, while different letters denote significant differences among means ($p < 0.05$). AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

For dough cohesiveness, significant differences were also found after 45 minutes resting time ($p<0.01$), but not at 0 minutes. There were no significant differences between the NaCl reduced controls without algae. However, the cohesiveness in C3A, C3B and C3A-SL was significantly higher than in C3B+ SL and C3B+AE. A significant effect of NaCl reduction level was found in the general linear model, where samples with NaCl reduction level C3A had a higher cohesiveness ($p<0.001$) (Appendix 6). Moreover, NaCl reduction level in the present design was correlated to the amount of incorporated algae. Finally, an effect of macroalgae species was detected, where dough with *A. esculenta* had a significantly lower dough cohesiveness compared to *S. latissima*. However, none of the interactions (species of macroalgae, NaCl reduction level and fermented vs unfermented macroalgae) between the factors were significant.

4.1.4 Monomeric and polymeric gluten proteins

The percentage of unextractable polymeric proteins (UPP%) and the ratio between monomeric and polymeric proteins in dough samples were calculated based on the results from SE-HPLC and are presented in table 7.

Table 7. Percentage of unextractable polymeric proteins in total polymeric proteins (%UPP) and ratio between polymeric and monomeric proteins. The different letters denote significant differences among means ($p < 0.05$). C1, C2, C3A and C3B refers to sodium chloride content of 1.5%, 1.0%, 0.87% and 0,75%/100 g flour, respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Dough sample	%UPP	Pol:mon
C1	10.1±1.3	1.21 ± 0.19^{ab}
C2	8.5±0.0	1.30 ± 0.06^{a}
C3A	9.0±0.7	1.30 ± 0.00^{a}
C3B	9.7±0.3	1.29 ± 0.00^{a}
C3A+SL	10.0±0.5	1.13 ± 0.02^{abc}
C3A+FSL	9.5±1.2	1.15 ± 0.02^{abc}
C3A+AE	10.2±1.3	1.08 ± 0.00^{abcd}
C3A+FAE	8.2±2.2	1.01 ± 0.06^{bcd}
C3B+SL	9.5±0.4	0.92 ± 0.03^{cde}
C3B+FSL	8.2±2.5	0.87 ± 0.01^{de}
C3B+AE	9.1±1.3	0.77 ± 0.05^{ef}
C3B+FAE	6.5±0.2	0.54 ± 0.03^{f}

The dough samples did not differ significantly in %UPP, but in ratio between polymeric and monomeric proteins ($p<0.001$). All C3B samples with algae had significantly lower ratios than controls without algae (C3B) and the ratio between polymeric and monomeric protein decreased with an increased amount of macroalgae in dough. With the exception of dough with unfermented *S. latissima*, the ratio was significantly different when NaCl levels were reduced and thus algae inclusion levels increased. Within algae samples at the same NaCl level (i.e., within C3A or C3B), no significant differences were present in C3A samples, while C3B+FAE had a significantly lower ratio than C3B+SL and C3B+FSL. For dough samples with algae a significant effect of species ($p<0.001$), NaCl reduction level ($p<0.001$) and use of fermented *vs* unfermented algae ($p<0.05$) were observed, and all interactions were also significant (Appendix 7). Thus, dough with unfermented and fermented *A. esculenta* as well as fermented *S. latissima* with reduced NaCl had lower amounts of polymeric proteins.

Figure 14 presents HPLC-curves for the SDS-extractable (Figure 14A) and SDS-unextractable (Figure 14B) proteins in three different dough samples (C1, C3A+SL and C3A+FSL). The curve was divided into 5 peaks. Peak 1 and 2 represent the polymeric proteins, peak 3 and 4 the monomeric proteins and peak 5 the albumins, and globulins.

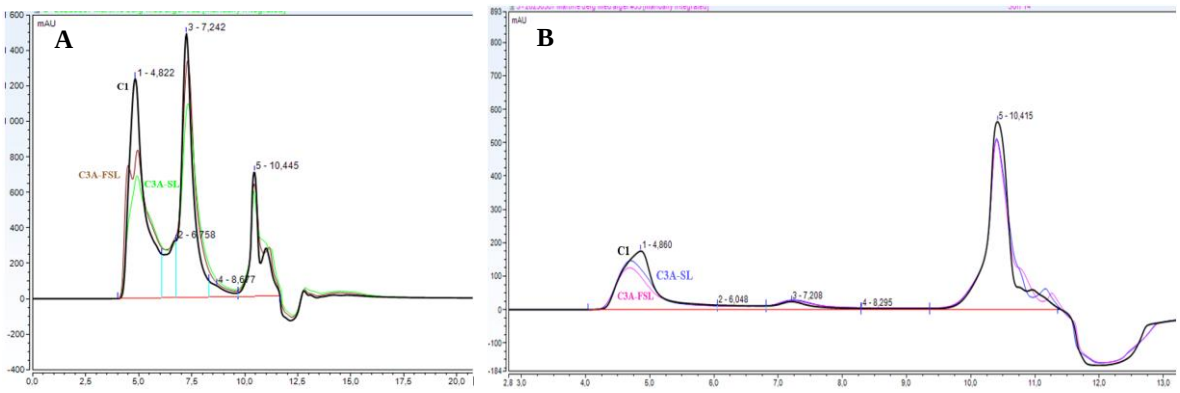

Figure 14. Size exclusion HPLC curves (A) for SDS-extractable proteins and (B) SDS-unextractable proteins. C1 and C3A refer to sodium chloride content 1.5% per 100 g flour and 0.87% per 100 g flour, respectively. SL, *Saccharina latissima*; F, fermented.

Peaks 1 and 2 were lower for the dough samples with macroalgae (C3A+SL, C3A+FSL) compared to the control sample (C1), which indicates that there were fewer SDS-extractable polymeric gluten proteins present in the doughs with macroalgae. Further, a peak appeared split for the dough sample with fermented macroalgae. Small differences are shown in the presence of the monomeric proteins (peak 3 and 4) between the samples. This indicates that the differences in the total of SDS-extractable monomeric proteins were small between the samples. The peaks that represent SDS-unextractable proteins showed small differences between the three samples.

4.1.5 pH in macroalgae powder and dough

pH was measured in macroalgae powder (Table 8) and in all doughs (Table 9) to investigate if fermented *A. esculenta* and *S. latissima* affects the pH in powder and dough, and if it further influences the breadmaking properties.

Table 8. pH in macroalgae powders (n=2). Letters denote significant differences among means ($p<0.05$). AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Macroalgae powder	pH
Saccharina latissima	6.23 ± 0.00^{b}
Fermented *Saccharina latissima*	4.13 ± 0.00^{c}
Alaria esculenta	6.52 ± 0.00^{a}
Fermented *Alaria esculenta*	3.80 ± 0.00^{d}

The pH in the different macroalgae powders was significantly different from each other ($p<0.001$). Fermented *A. esculenta* and *S. latissima* had a notable lower mean compared to the unfermented macroalgae.

Table 9. pH in dough samples (n=2). Letters denote significant differences among means ($p<0.05$). C1, C2, C3A and C3B refers to sodium chloride content of 1.5%, 1.0%, 0.87% and 0.75% per 100 g flour, respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Dough sample	pH
C1	5.88 ± 0.03^{bc}
C2	5.99 ± 0.06^{ab}
C3A	6.03 ± 0.00^{a}
C3B	5.98 ± 0.01^{abc}
C3A+SL	6.02 ± 0.02^{a}
C3A+FSL	5.86 ± 0.04^{c}
C3A+AE	5.95 ± 0.03^{abc}
C3A+FAE	5.57 ± 0.01^{e}
C3B+SL	5.99 ± 0.01^{ab}
C3B+FSL	5.70 ± 0.02^{d}
C3B+AE	5.97 ± 0.00^{abc}
C3B+FAE	5.57 ± 0.02^{e}

The pH of dough samples without algae ranged from 5.88 to 6.03. Except for C3A+FSL, all dough with fermented algae had significantly lower pH than all samples without algae or with unfermented algae and ranged from 5.57 to 5.86. C3A+FSL also had significantly lower pH than C2, C3A, C3+SL and C3B+SL. Dough with unfermented algae ranged between 5.95 and 6.02. An effect of macroalgae species ($p<0.001$), NaCl reduction level ($p<0.01$), fermentation of macroalgae ($p<0.001$) on pH in dough with macroalgae, as well as all interactions between them were significant (Appendix 8).

4.2 Baking

4.2.1 Small-scale baking

Results from the small-scale baking is presented in Table 10. Specific volume, ratio (height/width), colour measurements, number and area of cells and holes in bread (n=2 independently baked breads) were measured.

Table 10. Specific volume, ratio (height/width), *L**, *a**, *b**, crumb firmness, number and area of cells, and number and area of holes of bread from small scale baking (n=2). Different letters signify significant differences among means ($p<0.05$). C1, C2, C3A and C3B refers to sodium chloride content of 1.5%, 1.0%, 0.87% and 0,75%/100 g flour, respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Sample name	Specific volume (mL/g)	H/B Ratio	*L**(*)	*a**(*)	*b**(*)	Crumb firmness (g)	Number of cells	Area of cells (%)**	Number of holes	Area of holes (%)**
C1	3.76±0.07^{abcd}	0.72±0.03	63±1.90	3.7±0.3	20.1±0.6	519±8	2784±38	52.7±0.7	1.49±0.41	1.69±0.00
C2	3.61±0.18^{abcde}	0.71±0.03	60±1.51	3.7±0.3	19.3±0.6	576±93	2760±43	52.7±1.3	1.24±0.59	1.56±1.09
C3A	4.11±0.13^{a}	0.70±0.00	60±1.84	3.5±0.4	19.0±0.5	472±68	2653±167	54.5±0.9	0.94±0.56	2.57±0.75
C3B	3.99±0.28^{ab}	0.70±0.01	63±19.28	3.4±1.0	20.0±0.8	565±135	4132±1807	53.9±2.0	1.89±0.47	3.66±0.04
C3A+SL	3.82±0.11^{abc}	0.68±0.02	55±11.66	1.0±0.3	24.9±7.6	653±167	2616±98	54.5±0.4	1.48±0.76	3.05±0.59
C3A+FSL	3.62±0.24^{abcde}	0.70±0.00	57±2.05	2.8±0.3	22.0±0.8	655±189	2697±26	53.6±0.7	1.38±0.48	3.05±0.28
C3A+AE	3.51±0.01^{bcde}	0.70±0.02	51±2.37	1.3±0.4	25.3±0.7	638±89	2717±58	53.3±0.2	0.79±0.62	1.71±1.17
C3A+FAE	3.33±0.19^{cde}	0.71±0.03	52±1.20	2.2±0.4	22.6±0.6	653±75	2779±205	53.1±0.3	1.278±0.53	1.56±0.37
C3B+SL	3.58±0.02^{abcde}	0.71±0.02	52±1.75	0.2±0.2	27.1±8.4	654±33	2781±128	53.6±0.6	2.09±1.00	3.61±0.61
C3B+FSL	3.49±0.08^{bcde}	0.72±0.04	55±2.63	3.3±3.2	22.8±0.8	717±16	2685±208	53.8±0.5	1.75±0.41	3.92±2.48
C3B+AE	3.19±0.10^{de}	0.73±0.00	46±2.30	0.9±0.5	26.5±0.3	750±0.5	2651±123	53.0±0.1	1.49±0.77	2.07±1.51
C3B+FAE	3.07±0.00^{e}	0.71±0.01	48±1.52	1.8±0.2	22.6±0.4	857±155	2506±4	52.9±1.0	1.75±0.41	2.97±1.42

*n=1 bread, mean and SD for 3 technical replicates. ** Area of cells: the total area of cells as a percentage of the slice area. Area of holes: The area of holes as a percentage if the slice area.

Breads prepared on a small-scale differed significantly in terms of specific volume ($p<0.001$) but not in any other parameter. NaCl-reduced samples with algae, C3A+FAE, C3A+AE, C3B+FSL, C3B+AE, and C3B+FAE, had a significantly lower specific volume than bread without algae (C3A). Further, C3A+FAE, C3B+AE and C3B+FAE had a significant lower specific volume then NaCl reduced control C3B. Also, C3A+SL was higher in volume than C3B+AE and C3B+FAE. In contrast, all factors (macroalgae species ($p<0.001$), NaCl reduction ($p<0.05$) and fermentation ($p<0.004$)) had a significant effect on specific volume in bread with macroalgae (Appendix 9). None of the interactions between factors were however significant. The results showed a clear tendency of reduced specific volume with higher algae inclusion levels.

For colour measurement, only breads from second small-scale baking test were analysed, and no statistical analysis was conducted. A decrease in brightness (L^*) with increased amounts of macroalgae was found, a general higher a^* (red hue) for control samples and small differences in mean for b^* (yellow hue). The cell areas were not significantly different between the breads but results from the general linear model showed an effect of macroalgae ($p<0.05$), more specifically a significant increase in the area of cells in samples with *S. latissima* compared to *A. esculenta* (Appendix 9). However, only small differences in the means were present.

Pictures of bread slices are taken with C-cell Colour. In Figure 15, one technical replicate of each type of bread is presented, to illustrate the differences in colour contrast and crumb structure.

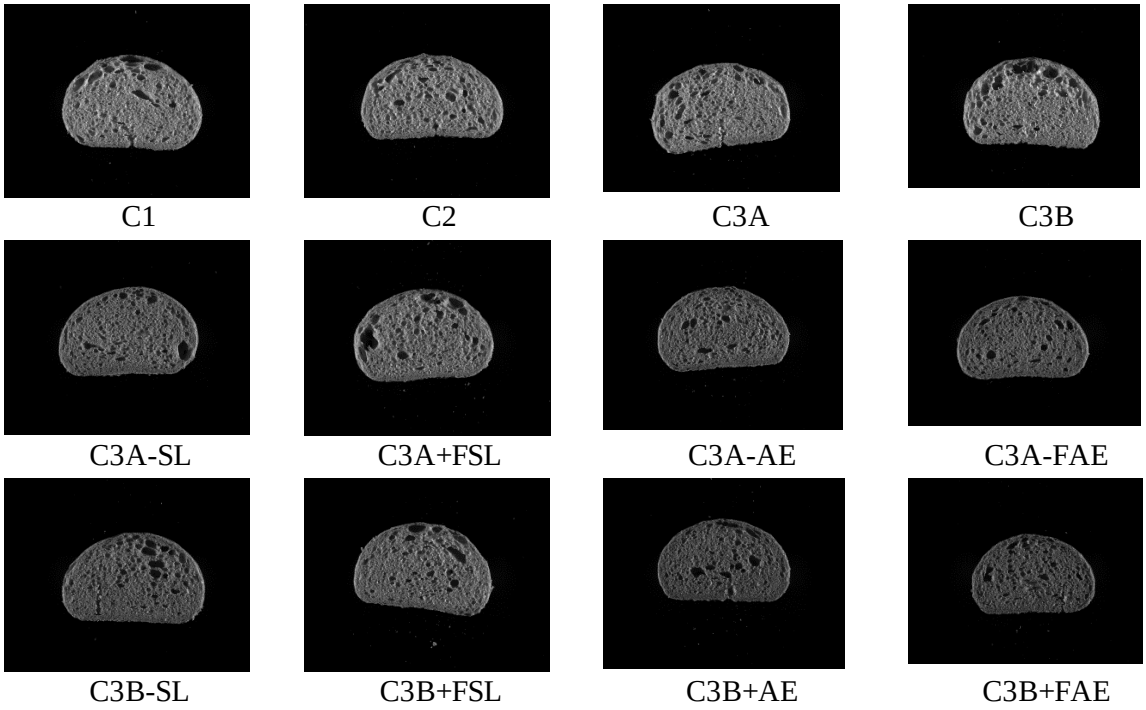

Figure 15. C-cell picture of bread. C1, C2, C3A and C3B refers to sodium chloride content of 1.5%, 1.0%, 0.87% and 0,75%/100 g flour, respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Figure 15 shows clear differences between the breads in colour contrast, and smaller differences in crumb structure. Bread with unfermented *S. latissima*, fermented and unfermented *A. esculenta* had a darker contrast, compared to the controls and bread with fermented *S. latissima.* In general, an even crumb was formed for all samples, which also was confirmed by the area of cells and holes (Table 10).

4.2.2 Large-scale baking

Large-scale baking was conducted on seven breads (n=1). The parameters analysed specific volume, H/B ratio, colour, crumb firmness, number and area of cells and holes (Table 11).

Table 11. Specific volume, H/B ratio, *L**, *a**, *b**, crumb firmness, number and area of cells, and number and area of holes of from large-scale baking (n=1). C1, C2, C3A and C3B refers to sodium chloride content of 1.5 g, 1.0 g, 0.87 g and 0,75 g, respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Dough sample	Specific volume (mL/g)	H/B Ratio	*L**(*)	*a** (*)	*b**(*)	Crumb firmness (g)	Number of cells	Area of cells (%)**	Number of holes	Area of holes (%)**
C1	3.32	0.78±0.01	63±2	3.7±0.3	19.4±0.7	409±41	3499	2.59	51.7	3.1
C2	3.55	0.72±0.002	64±2	3.6±0.4	19.3±0.7	342±267	3253	1.74	52.0	1.8
C3A	3.42	0.79±0.03	64±1	3.6±0.3	19.2±0.5	368±35	3639	2.59	52.1	3.0
C3A+SL	3.31	0.80±0.02	58±2	1.0±0.3	23.9±0.8	387±20	3470	3.39	52.5	4.0
C3A+FSL	3.39	0.74±0.03	56±3.	2.5±0.2	21.3±0.7	365±20	3600	1.81	52.6	2.5
C3A+AE	3.39	0.73±0.03	54±2	1.5±0.2	25.2±0.3	417±56	3468	1.71	52.6	2.5
C3A+FAE	3.37	0.79±0.06	54±2	2.6±0.3	22.3±0.5	427±38	3601	2.99	52.5	2.8

*n=1 bread, mean and SD for 4 technical replicates. ** Area of cells: the total area of cells as a percentage of the slice area. Area of holes: The area of holes as a percentage if the slice area.

Small differences in specific volume and H/B ratio were observed between the breads baked in the large-scale baking, whereas the specific volume was slightly lower, and H/B was slightly higher than for the small-scale baking. Crumb firmness, number of cells and area of holes was lowest in C2. There were small variations in mean for number of cells and holes for the remaining breads, but the area of cells and holes was clearly highest for C3A+SL. The colour of the bread was noticeably affected by the presences of macroalgae. Brightness (L^*) and a^* was lower in bread with algae (greener hue compared to controls), whereas b^* was higher. Bread with unfermented macroalgae had a lower a^* than bread with fermented, whereas bread with fermented macroalgae had a lower b^*. Lastly, the variation in crumb firmness was considerably large between the breads. The highest was in bread with fermented and unfermented *A. esculenta*, and lowest in C2. The energy input during mixing ranged from 11.44 to 16.58 Wh/kg.

4.3 Sensory analysis

Table 12 and PCA plot (Figure 16) presents the result from the sensory analysis conducted by the trained sensory panel at Nofima Ås.

Table 12. Mean descriptive ratings and p-values for attributes evaluated in descriptive analysis of seven breads (n=2) with and without macroalgae. Different letters denote significant differences among means ($p < 0.05$). C1, C2 and C3A refers to sodium chloride content of 1.5 g, 1.0 g and 0.87 g, respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

	C1	C2	C3A	C3A+SL	C3A+FSL	C3A+AE	C3A+FAE	P-value
Colour hue	6.38^{a}	6.30^{a}	6.15^{a}	4.64^{b}	5.48^{ab}	4.77^{b}	4.60^{b}	<0.001
Colour strength	5.23^{a}	4.98^{ab}	4.90^{ab}	4.78^{ab}	4.64^{b}	4.56^{b}	4.65^{b}	0.001
Whiteness	4.74^{bc}	4.77^{b}	5.29^{a}	4.25^{cd}	4.56^{bc}	3.77^{de}	3.69e	<0.001
Poring	4.77^{ab}	5.34^{ab}	4.53^{b}	5.47^{a}	5.30^{ab}	5.49^{a}	4.96^{ab}	0.004
Cereal odour	5.12^{a}	5.30^{a}	5.09^{a}	3.65^{b}	3.98^{b}	3.31^{b}	3.54^{b}	<0.001
Roasted odour	4.11^{ab}	4.18^{a}	4.23^{a}	3.00^{c}	3.09^{bc}	3.14^{bc}	2.65^{c}	<0.001
Algae odour	1.30^{c}	1.30^{c}	1.20^{c}	5.47^{ab}	4.37^{b}	5.48^{ab}	6.18^{a}	<0.001
Drawer odour	2.70^{a}	3.25^{a}	3.10^{a}	2.97^{a}	3.21^{a}	2.72^{a}	2.80^{a}	0.761
Rancid smell	1.34^{bcd}	1.25^{c}	1.17^{c}	2.80^{ab}	2.18^{bc}	2.75^{ab}	3.35^{a}	<0.001
Total flavour intensity	5.05^{a}	4.51^{cd}	4.38^{d}	6.23^{a}	5.07^{bcd}	5.41^{abc}	5.80^{ab}	<0.001
Sour flavour	3.12^{a}	3.17^{a}	3.06^{a}	1.60^{b}	1.90^{b}	1.33^{b}	1.53^{b}	<0.001
Sweet taste	2.78^{a}	2.84^{a}	2.95^{a}	2.49^{a}	2.84^{a}	2.58^{a}	2.75^{a}	0.288
Salty taste	4.03^{a}	3.20^{bc}	2.95^{c}	3.99^{a}	3.61^{abc}	3.97^{a}	3.90^{ab}	<0.001
Bitter taste	3.61^{cd}	3.36^{d}	3.14^{d}	4.97^{a}	4.23^{bc}	4.51^{ab}	4.83^{ab}	<0.001
Raw flavour	2.48^{a}	2.38^{a}	2.59^{a}	3.26^{a}	2.53^{a}	3.00^{a}	2.75^{a}	0.195
Grain flavour	4.96^{a}	4.97^{a}	5.10^{a}	3.08^{b}	3.37^{b}	3.13^{b}	2.85^{b}	<0.001
Algae flavour	1.59^{c}	1.50^{c}	1.29^{c}	6.22^{ab}	4.99^{b}	6.05^{ab}	7.00^{a}	<0.001
Cloying flavour	1.78^{b}	1.95^{b}	1.65^{b}	5.30^{a}	4.43^{a}	4.87^{a}	5.85^{a}	<0.001
Rancid flavour	1.51^{c}	1.32^{c}	1.25^{c}	3.05^{ab}	2.77^{b}	3.01^{ab}	4.05^{a}	<0.001
Metallic flavour	2.28^{c}	1.69^{c}	1.74^{c}	4.05^{ab}	3.86^{b}	4.18^{ab}	4.83^{a}	<0.001
Juiciness	4.57^{a}	4.38^{a}	4.34^{a}	4.50^{a}	4.47^{a}	4.60^{a}	4.69^{a}	0.750
Chewing resistance	4.75^{ab}	4.71^{ab}	4.36^{b}	4.90^{ab}	4.87^{ab}	5.03^{ab}	5.14^{a}	0.054
Tackiness	4.77^{a}	4.54^{a}	4.79^{a}	4.87^{a}	4.59^{a}	4.84^{a}	4.94^{a}	0.283

Significant differences in all attributes except from drawer odour, sweet taste, raw flavour, juiciness, chewing resistance and tackiness were detected by the panellists. The control breads were evaluated as possessing a significantly more intense cereal odour and flavour, and sour taste, compared to the breads with macroalgae. On the other side, bread with macroalgae had a significantly more intense algae flavour, cloying, rancid and metallic flavour and algae odour, compared to the controls. However, bread with fermented *S. latissima* was perceived to have a significantly lesser intense rancid and metallic flavour, and algae odour, compared to bread with fermented *A. esculenta*. Both salt- reduced control (C2 and C3A) were evaluated as significantly less salty than the full NaCl control C1. Furthermore, C3A had a significantly less intense salty taste compared to C3A+SL, C3A+AE and C3A+FAE. None of the macroalgae-containing samples were perceived as different in saltiness from C1.

Significant differences in colour hue were perceived between the control breads and the bread with algae, except for bread with fermented *S. latissima*. A more intense colour hue was detected in the bread without macroalgae. In colour intensity, significant differences were only observed between bread with 1.5% NaCl (C1) and bread with fermented *S. latissima*, *A. esculenta* and fermented *A. esculenta*. The breads with the two unfermented macroalgae scored significantly higher for the attribute poring compared to the control C3A.Total flavour intensity significantly decreased with NaCl reduction from C1 to C2 and C3A, whereas C3A differed significantly from C3A+Sl, C3A+AE and C3A+FAE. For bitter taste, bread with macroalgae (C3A-SL, C3A+AE, C3A+FAE) had a significantly more intense taste compared to the controls C2 and C3A. Lastly, for the texture attributes only chewing resistance was evaluated as significantly different, between C3A and C3A+FAE.

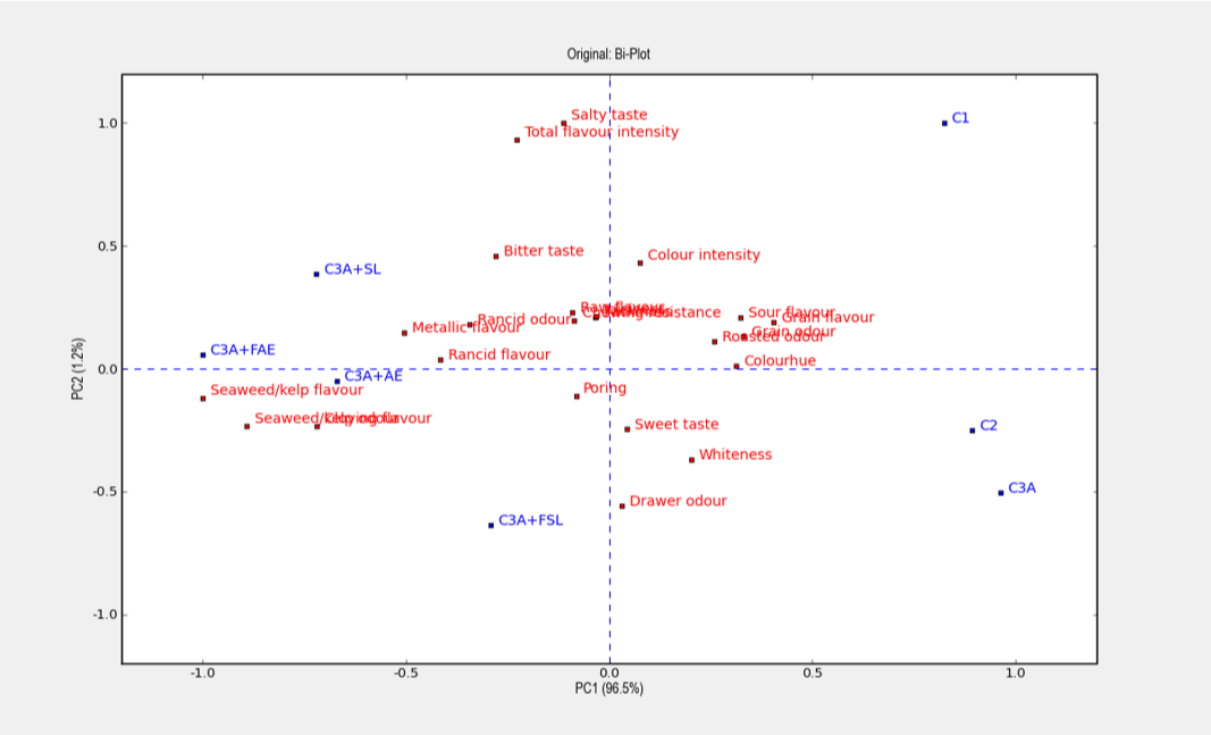

Figure 16. Principal component analysis of the matrix of mean sensory attribute ratings across bread samples. Both attributes and samples are shown in the space represented by principal components 1 and 2. C1, C2 and C3A refers to sodium chloride content of 1.5 g, 1.0 g and 0.87 g, respectively. AE, *Alaria esculenta*; SL, *Saccharina latissima*; F, fermented.

Figure 16 presents a PCA biplot of attributes and breads characterized in the descriptive analysis. PC 1 and PC 2, accounted for 96.5% and 1.2% of variance, respectively. PC1 separated all control breads from those with macro algae, while PC2 differentiated between control C1 vs C2 and C3A. Except for C3A+FSL, bread with macroalgae, were characterized by algae flavour and odour, rancid odour and flavour, metallic and cloying taste. Thus, there were differences for the breads with fermented and unfermented *S. latissima*. The control breads were characterized by sour taste, cereal odour and flavour, and colour hue.

5. Discussion

5.1 Effect of macroalgae and sodium chloride reduction on dough properties

Incorporation of non-traditional ingredients into the dough matrix is a technological challenge and can negatively affect the physical and rheological properties of wheat dough, and the quality of final products (Graça et al., 2018). This has been observed with incorporation of micro- and macroalgae (Arufe et al., 2018; Graça et al., 2018; Mamat et al., 2014; Onyango et al., 2021; Qazi et al., 2021). Moreover, reduced NaCl levels have been demonstrated to lead to weaker dough systems and higher stickiness (Silow et al., 2016). This is related to the disturbance of the dough structure, mainly the gluten network (Graça et al., 2018; Silow et al., 2016). As the sodium content in the dough samples used in this study were matched, some of the differences between the samples were presumably a result of the macroalgae addition.

WA increased in a dose-dependent manner with macroalgae incorporation, which agrees with other studies on algae in bread (Mamat et al., 2014; Onyango et al., 2021; Qazi et al., 2021). The increase in WA was explained by algae hydrocolloids competing with other constituents for water, due to their hydroxyl groups forming hydrogen bonds with water (El-Baz et al., 2017; Mamat et al., 2014; Qazi et al., 2021). This is supported by data from Arufe et al. (2018) who detected higher water retention capacity in algae powder compared to wheat flour. The dough with 0.75% NaCl/100 g flour and fermented *A. esculenta* required more macroalgae powder to achieve a matched sodium content. Some studies suggested that the farinograph properties of bread and pasta dough with micro- or macroalgae were influenced by the amount of algae incorporated (El-Baz et al., 2017; Mamat et al., 2014; Onyango et al., 2021; Qazi et al., 2021).

Concomitantly, a significant decrease in DDT (except for C3A+SL and C3B+FSL) and DS between the control sample and the doughs with macroalgae were observed. However, literature results on DDT and DS are somewhat conflicting. An increase in DDT and DS was observed with increased amounts of *Kappaphycus alvarezzi* and *Eucheuma denticulatum* (Mamat et al., 2014; Onyango et al., 2021), whereas a decrease in the DDT with increased microalgae in pasta dough and bread dough was detected (El-Baz et al., 2017; Qazi et al., 2021). El-Baz et al. (2017) also observed a reduction in DS. An explanation for these results was not discussed by these authors. The reduction in DDT and DS observed in our study could be related to the decreased ratio between polymeric and monomeric proteins, as reduction of NaCl and addition of algae can influence the size of polymers, protein aggregates

and interaction between gluten proteins. For the control dough, DDT and DS were significantly higher compared to doughs with macroalgae (except for DDT in C3A+SL), whereas a reduction in DS occurred as DDT decreased, with increased amounts of macroalgae in dough. It has also been suggested that by replacing wheat flour with other ingredients, a dilution and weakening of the gluten structure occurs (Rieder et al., 2012).

Differences in farinograph outputs between the control dough and doughs with macroalgae, as well as a significant effect of NaCl reduction level between doughs with macroalgae were detected. The results are in line with other studies on NaCl reduction that reported an effect on farinograph parameters (Avramenko et al., 2018; Belz et al., 2012; McCann & Day, 2013; Silow et al., 2016). It has been suggested that NaCl delays protein hydration due to the competition of sodium and chloride ions with proteins for water, which tends to decrease the flour's WA (Beck et al., 2012). This is a result of electrostatic shielding by NaCl of the charged amino acid at the surface which further reduces the electrostatic repulsion between proteins and promotes their interactions (Avramenko et al., 2018; Belz et al., 2012; McCann & Day, 2013; Silow et al., 2016). This leads to an increase in DDT and DS (Belz et al., 2012).

The extensograph results are in the range of previous studies on micro- and macroalgae addition for bread dough with regards to extensibility, whereas R_{max} was lower in our study as observed previously (Onyango et al., 2021; Qazi et al., 2021), which may relate to different raw materials as well as algae addition levels. Qazi et al. (2021) used refined wheat flour and 4-16% wheat flour replacement level of algae, whereas in the current study 66% whole wheat flour and 0.9 to 3.3% flour replacement levels were used. NaCl reduction and the addition of macroalgae showed an effect on R_{max} and extensibility, where both parameters exerted a weakening effect, as observed in other studies (Kim et al., 2023; Onyango et al., 2021; Qazi et al., 2021). Qazi et al. (2021) stated that it was a result of gluten dilution and weakening of dough structure as flour was substituted with algae. R_{max} was on the other hand significantly higher for C3A+SL than the controls C1, C2 and C3A after 45 minutes. It could be a result of the different mineral composition between doughs without algae and doughs with *S. latissima*. Algae provides dough with a higher content of minerals (Kumar et al., 2008), in which all ions affect the charged sidechains of the amino acids (Avramenko et al., 2020). There may also have been other factors that influenced the increase in R_{max} for dough with *S. latissima*, which is not yet explained and therefore requires further studies.

The results also indicated that a reduction of NaCl (I.e., 1%, 0.87% and 0.75%/100 g flour) had a weakening effect on extensibility and R_{max} in doughs without algae. Both parameters were significantly higher for C1 compared to all other controls (i.e., C2, C3A and C3B). However, Lynch et al. (2009) did not find significant differences in R_{max} and extensibility between doughs with 1.2%, 0.6% or 0.3% NaCl/100 g flour, only between doughs with 0% and 1.2%/100 g flour. Nor were significant differences present in R_{max} and extensibility between doughs with 1.5%, 1%, 0.5% and 0% NaCl/100 g flour in a study conducted by (Beck et al., 2012), only for doughs above 1.5% NaCl/100 g flour. Avramenko et al. (2018) observed on the other hand an increased resistance to extension and extensibility with increased levels of NaCl (0-4%) in different wheat cultivars using the Kieffer rig analysis. Further, Tanaka et al. (1967) also observed an increase in resistance to extension at pH 5.8 in dough with increased levels of NaCl (0%, 1% and 3%/100 g flour) and a higher extensibility in dough with 1% compared to 0%. These studies and most of the other published work on sodium reduction have used refined wheat flour (Beck et al., 2012; Diler et al., 2016; McCann & Day, 2013), while in our study 66% whole wheat flour was used. Experience from the industry is that higher proportion of whole grain flour are more prone to the negative effects of NaCl reduction. In general, systems with weaker gluten networks are more impacted by NaCl reduction (Avramenko et al., 2020), which could indicate that dough with whole grains may also be more affected as the network is already diluted and hindered in its formation by the fiber molecules (Hemdane et al., 2016). It could therefore be that studies conducted with refined wheat have found less impact of NaCl reduction on dough parameters. Both farinograph and extensograph measurements are adapted to refined wheat flour, as well as a NaCl content of 2% for the extensograph (Faridi, 1985; Pojić & Torbica, 2011). The results from the extensograph measurements may have been influenced by dough pieces with algae having been rolled out by hand, as the first dough samples with algae made in the farinograph were too sticky for the extensograph to handle. This was also observed by Qazi et al. (2021) in dough with microalgae.

Stickiness results showed that doughs with fermented *A. esculenta* (NaCl reduction level C3A) and unfermented *A. esculenta* (NaCl reduction level C3B) had a significantly lower stickiness compared to the controls C1 and C2 after 0 minutes. Stickiness is a complex phenomenon and poorly understood (Avramenko et al., 2018). It is impacted by the differences in protein composition and quality, level of hydration, enzymatic activity, water-soluble carbohydrates and processing (Avramenko et al., 2018; Grausgruber et al., 2003).

Stickiness has been associated with increased water mobility within a dough matrix (Avramenko et al., 2018), which is why stronger flours are generally more tolerant to NaCl reduction, as a stronger network can better stabilize water against flow (Avramenko et al., 2020; McCann & Day, 2013). The stickiness phenomenon manifests in dough systems with low cohesive forces (as there are few interactions between dough constituents), which is characteristic for weak gluten networks. While there were some significant differences in stickiness among samples, they were of low magnitude and all dough samples would be categorized as non-sticky following the classification from Chen and Hoseney (1995).

While Beck et al. (2012) reported a decrease in dough stickiness with decreasing NaCl concentration from 4 to 0% NaCl, several studies have reported an increase in stickiness with decreased levels of NaCl (Diler et al., 2016; Silow et al., 2016).This can be explained by the weakening of the gluten network when reducing NaCl (Nahar et al., 2019). NaCl reduction resulted in increased stickiness in this study which agree with some of the previous work (Diler et al., 2016; Silow et al., 2016). According to the findings in the study by Mamat et al. (2014), addition of macroalgae in dough decreased stickiness, work of adhesion and cohesiveness. Thus, only a minimal effect of incorporation of algae was present in this study. While statistical analysis with the general linear model indicated significantly lower stickiness and cohesiveness for samples with *A. esculent* than for *S. latissima*, is it uncertain what causes the differences between the two species. A possible explanation could be the higher amounts of *A. esculenta* at both NaCl reduction levels compared to *S. latissima*.

However, the low instrumentally determined stickiness for algae-containing dough may have been influenced by sample preparation. It was difficult to create a perfectly even dough surface when using the stickiness cell. This was presumably because of the presence of bran in the whole wheat flour used in all dough samples (with and without algae), which may have resulted in incomplete contact between the probe and sample. Also, the small dough pieces transferred to the stickiness cell could affect the results. Therefore, implementation of another stickiness rig and more sample material (500g dough pieces) with a knife-like attachment could be relevant. Further work is also needed to assess the relationship between macroalgae incorporation, NaCl levels and dough stickiness, ideally on a sample set that can entirely be prepared in the extensograph. Future studies could also investigate the relationships between stickiness and the water status, for example by conducting thermogravimetric analysis (Avramenko et al., 2018) or nuclear magnetic resonance experiments (Hopkins et al., 2019).

Few studies have addressed the effect of macroalgae addition in dough and bread properties. As mentioned earlier, incorporation of non-traditional ingredients can disturb the dough structure, which may be affected by particle size (Graça et al., 2018). This has been demonstrated for cereal bran addition too and it is therefore assumed that besides gluten dilution, steric hindrance contributes to weaker gluten networks (Hemdane et al., 2016). Moreover, particle size influences the surface area, which may affect the interaction with water, primarily due to hydrophilic groups on the particle surfaces. Although the structure and particle size of cereal bran is different from algae (Arufe et al., 2018; Hemdane et al., 2016), Arufe et al. (2018) showed that addition of algae particles to bread had a similar effect as bran. However, with the exception of Qazi et al. (2021), most studies on algae in cereal-based products did not report the particle size of the evaluated material. The particle size of the algae powder used in the doughs did vary between the fermented and unfermented *S. latissima* and *A. esculenta*. The results on dough properties do however present a trend with increased amounts of alga powder, not between the fermented and the unfermented powders. Based on this, it can be hypothesized that it is the quantity of the incorporated algae powder and not the particle size of the powders that primarily affected dough properties in this study. Further studies are needed to assess the relationship between particle size distribution of algae powders and dough properties.

5.2 Effect of macroalgae and sodium chloride reduction on bread properties

The study showed that bread with NaCl content of 0.87% per 100g flour (C3A) and 0.75% per 100g flour (C3B) had the highest specific volume, which could be a result of enhanced gas production that occurs when reducing the NaCl content (Belz et al., 2012). Lynch et al. (2009), on the other hand, did not report any significant differences in specific volume in NaCl reduced bread (0%-1.2%), only a trend towards an increase at reduced level of NaCl. Moreover, they used 100% refined wheat flour, whereas bread made in this study consisted of 66% wholewheat flour. The presence of wheat bran in bread leads to reduced end-product quality compared to refined flour-based products, including a decrease in bread loaf volume (Hemdane et al., 2016). The polymeric/monomeric ratio was also significantly higher in the control doughs, compared to bread with algae (i.e., C3B+FSL, C3B+AE and C3B+FAE). Polymeric glutenins are responsible for the elasticity and strength in dough and allow the gas cell walls to expand without rapid gas loss (Delcour & Hoseney, 2010). This leads to a higher specific volume compared to bread where the size of polymers and proteins aggregates are

reduced. Holmes and Hoseney (1987) suggested that the pH in dough also influenced bread volume, where the volume in bread increased from pH 4.65 to 6.15. As the pH in our dough samples ranged only between 5.57-6.03, it is less likely that the pH affected the bread volume.

Furthermore, significantly lower specific volumes in bread with the highest macroalgae level (3.3%/100 g flour) were observed. Also, the results showed a notable decrease in mean value for specific volume with increased amounts of macroalgae. This is in line with the weakened dough properties observed in the farinograph and extensograph. Qazi et al. (2021) also observed an effect of microalgae on volume, where a significant decrease in specific volume occurred when 4% flour or more was substituted with *Tetraselmis chuii.* Mamat et al. (2014) also observed a significant decrease in volume with increased amounts of *K. alvarezii.* The authors hypothesized that the increased water absorption by the hydrocolloids could suppress the amount of generated steam, secondly the disruption of the gluten network by macroalgae (Mamat et al., 2014). Graça et al. (2018) suggested that microalgae content over 3.0 g/100 g flour can result in a phase separation of the algae and disruption of the gluten matrix, which affects the dough structure after fermentation and further leads to a reduction in the specific volume. These can be the explanations for why breads with the highest amounts of algae had a notable lower mean specific volume compared to the controls and bread with lower amounts of algae.

No significant difference was found in crumb firmness or C-cell measurements between the breads in the current study. Lynch et al. (2009) stated that NaCl strengthens and improves the gluten network which leads to an even crumb structure in bread. However, the relationship is complex. Ambrosewicz-Walacik et al. (2016) tested the effect of NaCl levels on crumb firmness in breads prepared from refined, whole meal wheat and rye. In systems containing yeast, lower NaCl levels significantly reduced firmness in refined wheat bread and whole meal rye bread but increased it in whole meal wheat bread and refined rye bread. A possible explanation for why was not established by the authors. The lack of significant difference among bread firmness in our study may have been due to large variations between replicates, as well as opposing influences exerted by sodium reduction and algae incorporation. Several studies showed an increase in crumb firmness with increased amounts of algae (incorporation at substitution levels from 1-16%) in bread with *K. alvarezii* (Mamat et al., 2014), *T. chuii* (Qazi et al., 2021), *F. vesiculosus* (Arufe et al., 2018) and *Chlorella vulgaris* (Graça et al., 2018).

Furthermore, there were some differences regarding the specific volume, ratio, crumb firmness and crumb structure between the breads made in small-scale and large-scale. First of all, the sample material for the first three breads (C3A-FAE, C3A and C1) were weighed the day before the large-scale baking trial, which resulted in room temperature flour. Moreover, the flour used for the four other samples was taken out of the refrigerator the same day as the baking trail. Because of a lower starting temperature on the dough, an increase in energy input occurred in these sample, as more energy was needed to achieve a dough temperature of 27°C. The energy input for doughs made in the DoughLab during the small-scale baking was the same for all doughs (12 Wh/kg). However, very little differences in the baking performance of strong wheat flour and wheat flour barley mix (40% barley) were previously observed in the specific energy input with a range from 11.5 to 16 Wh/kg (unpublished data). On the other hand, energy input can influence bread quality parameters. Also, the differences between the samples within the large-scale baking and between the two different baking trails (small- and large scale) could be a result of other factors including dough weight, different mixing machines and the use of machine vs hand to form the breads.

Colour measurements show the same trends for the breads made in the small- and large-scale baking. In both cases a decrease in crumb brightness occurred with increased amounts of macroalgae, which is in line with the observations by Mamat et al. (2014) and Qazi et al. (2021). The reduction in brightness can be explained by the presence of pigments in algae i.e., chlorophylls (a and c) and carotenoids (Mautner, 1954). Furthermore, *a**-values were lower in bread with macroalgae, especially with unfermented *A. esculenta* and *S. latissima*. Arufe et al. (2018) and Amoriello et al. (2021) also detected a significantly lower *a** with increased amounts of algae, as a result of the green colour of the powder added. Higher *b**-values were observed in breads with unfermented algae from both the large- and small-scale baking. Lee et al. (2010) and Mamat et al. (2014) demonstrated that bread gets more yellow with increased amounts of algae.

5.3 Effect of macroalgae and sodium chloride reduction in bread on sensory properties

Sensory properties were impacted by the NaCl level, the addition of algae as well as the type of algae. There were significant differences between the control breads and the breads with *S. latissima* and *A. esculenta*, both in flavour, odour, texture and appearance. Firstly, significantly lower intensities of salty taste were detected by the panellists for C1 and the two

NaCl reduced controls (i.e., C2 and C3A). The results from the sensory analysis further indicated that the presence of algae in bread can increase the perception of salty taste in NaCl reduced bread. However, the differences between C2 and C3A, and fermented *S. latissima* were not significant. Bruhn et al. (2019) showed that heat-treatment and fermentation of *S. latissima* caused a reduction in the saltiness and umami flavour compared to fresh *S. latissima.* The loss of saltiness and umami taste in fermented *S. latissima* was described by Bruhn et al. (2019) as a result of loss of the mineral's sodium (15%) and magnesium (21%), in which are responsible for salty taste. Loss of minerals in fermented *S. latissima* could be the reason why bread with fermented *S. latissima* has similar saltiness as C2 and C3A.

An increase in consumer's saltiness perception in breads with brown macroalgae (6% and 8% *C. crispus* and *A. nodosum)* has also been observed by Lamont and McSweeney (2021). However, salty flavor negatively affected the liking of bread with *C. crispus.* On the other hand, Gorman et al. (2023) showed that the use of 4% macroalgae powder (unspecified species) could reduce the NaCl content by 20% in bread without negatively affect the consumer acceptability, whereas a 30%, 40% and 50% reduction gave a high percentage of "not salty enough" responses by the consumers. Neither of these studies reported the intrinsic sodium content of the macroalgae, moreover the sodium content was not consistent between samples (Gorman et al., 2023; Lamont & McSweeney, 2021). In our study, we took the intrinsic sodium content in algae into account and prepared breads with and without algae with consistent sodium level. Further studies should how consumers evaluate the saltiness perception in bread with algae and matched sodium levels, to determine the effect of using algae for NaCl reduction in bread.

Although macroalgae increased the perception of saltiness in NaCl-reduced bread, the sensory panellists observed distinct algae flavour and odour. The flavour and odour and sour taste of macroalgae was significantly more intense in all breads with macroalgae compared to the controls. Taste of macroalgae has been shown to negatively affect consumer acceptances in different foods like yoghurt (Robertson et al., 2016), muffins (Mamat et al., 2018) and bread (Lamont & McSweeney, 2021). Mamat et al. (2018) found a decrease in acceptance by consumers with increased amounts of *K. alvarezii* in muffins, due to strong algae taste and fishy odour. In bread with 2 and 4% *A. nodosum* and *C. crispus* no seafood taste was detected by the consumers, but with increased amounts of macroalgae (6% and 8%) a moderate to strong seafood taste as well as a strong aftertaste was reported (Lamont & McSweeney, 2021). Cloying, rancid and metallic flavour in bread with algae were also detected by the

panellist which was significantly more intense than in the controls. Macroalgae consist of different volatile compounds, in which 76 volatile compounds (belonging to 9 chemical groups) was identified in blanched and dehydrated *A. esculenta* (Zhu et al., 2022) and 127 in dehydrated *S. latissima* (López-Pérez et al., 2017). The presence of some specific volatile compounds are the obstacles for the consumption of products with algae as it gives products an unpleasant off-flavour (Nie et al., 2022).

The results from the colour measurements were in partial agreement with the results from the sensory analysis. Controls were perceived significantly brighter by the panellist compared to bread with macroalgae (except for FSL), in which the colour hue increased with increased amounts of algae. However, in breads made in the large-scale baking trial, the *L** value was slightly higher in bread with *S. latissima* compared to fermented *S. latissima*. On the other hand, the pictures taken by the C-cell at the small-scale baking trial showed a brighter colour hue for fermented *S. latissima* compared to the other breads with macroalgae, like perceived by the panellists. Further, the colour strength was significantly different between C1 and bread with fermented *S. latissima*, unfermented and unfermented *A. esculenta*. In whiteness, there were significant differences between C3A and all other breads, as well as between bread with and without macroalgae. Bread with fermented *A. esculenta* had the lowest mean for whiteness, which shows the impact of increased macroalgae content on the colour of the bread. Mamat et al. (2018) observed a significantly higher liking in the colour of muffins that contained 2% algae powder than 10%, where the muffin with 10% algae had a significantly lower *a** value (Mamat et al., 2018). This may indicate that greener colour of bread negatively affects the liking by consumers.

While C-cell analysis from the small-scale baking did not show any significant differences between crumb structure (amounts of cells and holes, area of cells and holes), significant differences were found in the sensory analysis between C3A and bread with unfermented *S. latissima* and *A. esculenta* in poring. To the best of our knowledge, other studies have not evaluated differences in crumb structure in breads with incorporation of algae. However, Garzon et al. (2021) analysed (unfermented) microalgae-enriched bread samples with different addition levels in which dough was prepared either by yeast fermentation, sourdough preparation or chemical acidification. The porosity (%) (comparing the area of the pixels of the bread image) increased with increasing amounts of microalgae (Garzon et al., 2021). Also, the breads in the study by Garzon et al. (2021) displayed that a higher porosity as well as bigger gas cells had a more open crumb structure, probably due to the dough double

coalescence, likely derived from the weakening of the gluten matrix. Contrary, the results from the large-scale baking showed a higher number of cells in C3A, compared to the breads with unfermented algae, which indicates that the poring was higher in this bread. As mentioned earlier, NaCl reduction affects the crumb structure (Lynch et al., 2009).

C3A also had a significantly lower chewing resistance than bread with fermented *A. esculenta*. No other significant differences were observed among breads in the textural attribute's juiciness and tackiness, which suggests that at the algae amount used in our study (<3.3%/100 g flour), did not affect the textural attributes to the same extent as taste, odour and appearance. Lamont and McSweeney (2021) however found a more dry, hard and dense texture in bread with higher amounts of algae (6% *C. crispus* and 8% *A. nodosum)*. This was also observed by Gorman et al. (2023), in bread with NaCl reduction of 40 and 50%. Authors stated that occurrence of dryness in the breads was a result of increased fibre and hydrocolloid content (Lamont & McSweeney, 2021). The water retention capacity has been shown to be higher in algae compared to wheat flour (Arufe et al., 2018). Dryness and denseness were attributes that led to poor consumer acceptance in both studies (Gorman et al., 2023; Lamont & McSweeney, 2021) However, the authors did not optimize WA (Gorman et al., 2023; Lamont & McSweeney, 2021). This can be a part of the reasons why they found more dry, hard and dense texture in their bread compared to the result from our quantitative descriptive analysis.

The PCA plot suggests that fermentation influenced the sensory properties of *S. latissima* larger to *A. esculenta*. While breads with *A. esculenta* are located closely together on the plot, breads with *S. latissima* were further apart, and especially separated by PC2. Bread with fermented *S. latissima* was characterized by less macroalgae odour and flavour, less bitter taste, and less rancid and metallic flavour. Given that fermentation extends the shelf-life of algae and facilitates their handling by manufacturers (Blikra et al., 2021; Hurtado et al., 2022), fermented *S. latissima* can be a promising macroalgae product for applications such as bread.

6. Conclusion and further perspectives

This thesis studied the effect of Norwegian brown macroalgae *S. latissima* and *A. esculenta* as potential NaCl replacers in bread that contained whole wheat. Both macroalgae and NaCl reduction levels negatively affected rheological properties of dough. Increased levels of macroalgae and reduced levels of NaCl increased WA, but decreased DDT, DS, R_{max}, extensibility and stickiness. A significant reduction in specific volume with increased levels of algae and reduction of NaCl was also observed. However, no significant differences were found in bread quality parameters such as volume and H/B. Only a noticeable reduction in brightness and an increase in green colour of the breads were detected. From a sensory point of view, macroalgae have a potential to provide salty taste and increased flavour intensity. However, it gave a distinct algae flavour and odour which can influence consumer acceptance negatively. Fermented *S. latissima* received lower scores for certain undesirable sensory characteristics and had a more favourable sensory profile than the other breads, and could therefore be a good candidate for incorporation of algae to food products.

In conclusion, this study added to the growing body of research that suggests macroalgae as suitable NaCl replacers. Moreover, this study was the first to evaluate the effect of fermented *vs* unfermented macroalgae in bread with whole wheat flour. From our results it can be concluded that addition levels are crucial, and that fermentation of macroalgae may not only enhance their shelf life but also influence their functionality as an ingredient. Further studies should investigate the optimum level of algae that is both accepted by consumers and has minimal negative impacts on rheological properties of dough and bread quality, as the reduced DDT, DS and viscoelastic properties, stickiness, specific volume and colour observed in our study affect the bread production-and quality. From our results, incorporation of fermented *S. latissima* is recommended over fermented *A. esculenta* (as well as their unfermented counterparts) as it caused fewer off-flavours. It would also be appropriate to investigate whether there are other types of Norwegian algae species that are more suitable to incorporate into bread in terms of their flavour profile, as well as whether there are other processing methods of algae that achieve a more desirable flavour and chemical composition adapted to bread. Lastly, further studies on consumer acceptability and liking should be carried out, as well as analyses of the nutritional composition of the bread to evaluate if relatively low amounts of macroalgae, as used in our study, can contribute to the intake of essential nutrients to bread in addition to reducing the NaCl content.

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Appendix

Appendix 1

Table 1. Chemical composition and particle size of *Saccharina latissima*, fermented *Saccharina latissima*, *Alaria esculenta* and fermented *Alaria esculenta*. Particle size was measured with laser diffraction. Dx(50) and Dx(90) signifies that 50 and 90% of the samples had a particle size less than that value.

Nutritional content (g/100 g)	Saccharina latissima	Fermented Saccharina latissima	Alaria esculenta	Fermented Alaria esculenta
Moisture	4.2	7	3.3	4.57
Ash	36	24.5		21.6
Carbohydrate				
Fiber				
Protein	17.7	10.6	17.2	12.2
Fatty acids		1.6		0.9
Salt				
Sodium	6	5.5	3.4	3
Potassium		1	5.5	2.5
Iodine (µg/g)		340	530	770
Calcium (mg/kg DW)		21667	20333	25333
Magnesium (mg/kg DW)		11667	8500	25400
Phosphorous (mg/kg DW)		1167	5233	1800
Cumulative particle size distribution % (µm)				
Dx(50)	37.6	38.7	36.5	36.4
Dx(90)	150	130	161	132

Appendix 2

Table 2. Results from the general linear model on farinograph parameters for dough (n=2) with macroalgae. The effect of macroalgae species, NaCl reduction and fermented vs unfermented algae, and the interactions between them were studied. P-value <0.05 represent a significant effect of the factors and interactions.

	DDT (s)					WA (%)					DS (s)				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	6899.2	6899.22	18.78	0.003	1	21.6794	21.6794	417.74	0	1	0	0	484.67	0
NaCl reduction	1	3965.8	3965.78	10.79	0.011	1	21.6628	21.6628	417.42	0	1	0	0	846.11	0
Fermented/Not fermented	1	3164.8	3164.85	8.61	0.019	1	7.3525	7.3525	141.68	0	1	0	0	294.58	0
Alger*NaCl reduction	1	27.4	27.41	0.07	0.792	1	1.451	1.451	27.96	0.001	1	0	0	9.66	0.015
Alger*Fermented/Not fermented	1	1536.7	1536.72	4.18	0.075	1	7.6572	7.6572	147.55	0	1	0	0	779.83	0
NaCl reduction*Fermented/Not fermented	1	4212.6	4212.55	11.47	0.01	1	0.6129	0.6129	11.81	0.009	1	0	0	48.09	0
Algae*NaCl reduction*Fermented/Not fermented	1	865.6	865.58	2.36	0.163	1	0.5297	0.5297	10.21	0.013	1	0	0	2.99	0.122
Error	8	2939.3	367.42			8	0.4152	0.0519			8	0	0		
Total	15	23611.4				15	61.3608				15	0			

Appendix 3

Table 3. Extensibility and R_{max} in dough (n=2) after 45 and 90 minutes of resting. Different letters indicate significant differences among means ($p<0.05$). C1, C2, C3A and C3B refers to NaCl content of 1.5 g, 1.0 g, 0.87 g and 0,75 g, respectively. AE, *Alaria Esculenta*; SL, *Saccharina Latissima*; F, fermented; KI, potassium iodine.

	45 minutes		90 minutes	
Dough sample	**Extensibility (mm)**	**R_{max} (BU)**	**Extensibility (mm)**	**R_{max} (BU)**
C1	125.0±0.0^{a}	374.0±9.9^{bcd}	86.5±0.7	701.0±59.4^{a}
C2	119.5±2.1^{b}	338.5±20.5^{cd}	86.5±3.5	577.5±13.4^{bc}
C3A	117.0±0.0^{bc}	357.0±1.4^{bcd}	94.5±12.0	584.5±26.2^{bc}
C3B	114.5±0.7^{bcd}	360.0±5.7^{abcd}	88.0±1.4	568.0±28.3^{c}
C3A+SL	113.5±2.1^{cd}	422.0±4.2^{a}	92.5±2.1	670.5±16.3^{abc}
C3A+FSL	110.5±0.7^{de}	390.0±31.1^{abc}	84.5±6.4	662.0±335.4^{abc}
C3A+AE	107.0±1.4^{ef}	403.5±26.2^{ab}	82.0±2.8	685.0±24.0^{ab}
C3A+FAE	104.5±0.7^{f}	403.5±6.4^{ab}	76.0±0.0	665.5±3.5^{abc}
C3B+SL	110.5±0.7^{de}	379.0±8.5^{abcd}	87.0±1.4	635.0±28.3^{abc}
C3B+FSL	114.5±0.7^{bcd}	325.0±1.4^{d}	87.0±5.7	621.5±16.3^{abc}
C3B+AE	102.0±2.8^{fg}	371.5±6.4^{abcd}	81.0±4.2	639.5±33.2^{abc}
C3B+FAE	98.5±0.71^{g}	385.5±26.2^{abcd}	86.5±5.0	658.0±25.5^{abc}
P- value	<0.001	<0.002	<0.125	<0.008

Appendix 4

Table 4. Results from the general linear model on extensograph parameters for dough (n=2) with macroalgae. The effect of macroalgae species, NaCl reduction and fermented vs unfermented algae, and the interactions between them were studied. P-value <0.05 represent a significant effect of the factors and interactions.

	Extensibility 45 min					R_{max} 45 min				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	339,844	339,844	163,71	0	1	0	0	3,89	0,084
Salt reduction	1	20,013	20,013	9,64	0,015	1	0	0	24,59	0,001
Fermented/Not fermented	1	4,839	4,839	2,33	0,165	1	0	0	6,45	0,035
Algae*Salt reduction	1	31,123	31,123	14,99	0,005	1	0	0	4,38	0,07
Alger*Fermented/Not fermented	1	10,923	10,923	5,26	0,051	1	0	0	11,56	0,009
Salt reduction*Fermented/ Not fermented	1	12,279	12,279	5,91	0,041	1	0	0	0,77	0,405
Algae*Salt reduction*Fermented/Not fermented	1	17,265	17,265	8,32	0,02	1	0	0	2,61	0,145
Error	8	16,608	2,076			8	0	0		
Total	15	452,895				15	0			
	Extensibility 90 min					R_{max} **90 min**				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	0	0	10,65	0,011	1	1467656100	1467656100	1,4	0,271
Salt reduction	1	0	0	1,11	0,322	1	7069614561	7069614561	6,73	0,032
Fermented/Not fermented	1	0	0	1,33	0,281	1	238795209	238795209	0,23	0,646
Algae*Salt reduction	1	0	0	2,5	0,153	1	209409841	209409841	0,2	0,667
Algae*Fermented/Not fermented	1	0	0	0,56	0,477	1	159037321	159037321	0,15	0,707
Salt reduction*Fermented/Not fermented	1	0	0	6,33	0,036	1	483912004	483912004	0,46	0,516
Algae*Salt reduction*Fermented/Not fermented	1	0	0	0,54	0,483	1	806276025	806276025	0,77	0,406
Error	8	0	0			8	8401872570	1050234071		
Total	15	0				15	1,8837E+10			

Appendix 5

Table 5. Stickiness, work of adhesion and cohesiveness in dough (n=2) after 0 and 45 minutes of resting. Different letters indicate significant differences among means ($p<0.05$). C1, C2, C3A and C3B refers to NaCl content of 1.5 g, 1.0 g, 0.87 g and 0,75 g, respectively. AE, *Alaria Esculenta*; SL, *Saccharina Latissima*; F, fermented; KI, potassium iodine.

	Stickiness (g)		**Work of adhesion (g.s)**		**Cohesiveness (mm)**	
Dough sample	**0 min**	**45 min**	**0 min**	**45 min**	**0 min**	**45 min**
C1	56.56±1.68^{a}	58.05±0.43^{a}	8.53±0.20	8.79±0.23^{abc}	2.83±0.14	3.21±0.08^{abc}
C2	56.09±0.828^{a}	58.00±0.39^{a}	10.63±0.15	7.88±10.25^{bcd}	3.66±0.35	3.00±0.07^{abc}
C3A	51.69±0.25^{ab}	54.58±2.26^{ab}	9.48±0.73	9,10±0.29^{abc}	3.42±0.38	3.38±0.21^{ab}
C3B	52.53±1.08^{ab}	59.27±1.34^{a}	9.63±1.84	10.69±1.31^{a}	3.55±0.66	3.62±0.50^{a}
C3A+SL	54.93±2.74^{ab}	56.78±1.44^{ab}	9.50±1.34	9.70±0.59^{ab}	3.37±0.27	3.63±0.33^{a}
C3A+FSL	54.66±3.63^{ab}	56.35±1.57^{ab}	9.16±1.52	8.73±0.67^{abc}	3.25±0.65	3.21±0.55^{abc}
C3A+AE	51.49±1.83^{ab}	53.45±0.82^{ab}	7.24±0.34	7.08±0.75^{bcd}	2.63±0.27	2.73±0.21^{abc}
C3A+FAE	48.92±0.730^{b}	52.35±0.52^{ab}	9.89±2.39	7.49±0.05^{bcd}	3.78±1.07	2.79±0.03^{abc}
C3B+SL	50.32±2.30^{ab}	53.31±0.69^{ab}	6.94±1.81	6.48±1.36^{cd}	2.62±0.38	2.40±0.09^{bc}
C3B+FSL	52.10±0.21^{ab}	53.31±0.53^{ab}	9.52±0.58	7.46±0.18^{bcd}	3.35±0.16	2.71±0.35^{abc}
C3B+AE	48.20±1.08^{b}	47.84±3.74^{b}	6.71±0.54	5.56±0.65^{d}	2.38±0.10	2.19±0.21^{c}
C3B+FAE	51.53±1.64^{ab}	49.15±1.71^{ab}	8.16±0.58	6.54±0.24^{cd}	3.05±0.48	2.60±0.04^{abc}
P-value	<0.007	<0.071	<0.102	<0.001	<0.182	<0.004

Appendix 6

Table 6. Results from the general linear model that studied the effect of the factors macroalgae species, NaCl reduction and fermented vs unfermented algae, and the interactions between them , on stickiness, work of adhesion and cohesiveness in doughs with macroalgae (n=2). P-value <0.05 represent a significant effect of the factors and interactions.

	Stickiness 0 min					Work of adhesion 0 min					Cohesiveness 0 min				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	33,005	33,0048	9,95	0,013	1	0,03493	0,03493	1,46	0,261	1	0,001812	0,001812	1,83	0,213
Salt reduction	1	12,707	12,7067	3,83	0,086	1	0,071727	0,071727	3	0,121	1	0,003217	0,003217	3,25	0,109
Fermented/ Not fermented	1	1,697	1,6967	0,51	0,495	1	0,155933	0,155933	6,53	0,034	1	0,008313	0,008313	8,4	0,02
Algae*Salt reduction	1	7,382	7,3823	2,23	0,174	1	0,000146	0,000146	0,01	0,94	1	0,000038	0,000038	0,04	0,85
Algae*Fermented/ Not fermented	1	0,111	0,1107	0,03	0,86	1	0,009862	0,009862	0,41	0,538	1	0,001744	0,001744	1,76	0,221
Salt reduction*Fermented/ Not fermented	1	19,105	19,1051	5,76	0,043	1	0,018124	0,018124	0,76	0,409	1	0,001225	0,001225	1,24	0,298
Algae*Salt reduction*Fermented/ Not fermented	1	4,612	4,612	1,39	0,272	1	0,055907	0,055907	2,34	0,165	1	0,001479	0,001479	1,5	0,256
Error	8	26,527	3,3159			8	0,191096	0,023887			8	0,007913	0,000989		
Total	15	105,146				15	0,537726				15	0,025739			

	Stickiness 45 min					Work of adhesion 45 min					Cohesiveness 45 min				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	334,662	334,662	37,75	0	1	1975,81	1975,81	20,84	0,002	1	0,00884	0,00884	7,25	0,027
Salt reduction	1	283,713	283,713	32	0	1	2745,4	2745,4	28,96	0,001	1	0,02459	0,02459	20,18	0,002
Fermented/ Not fermented	1	8,432	8,432	0,95	0,358	1	37,01	37,01	0,39	0,55	1	0,001932	0,001932	1,59	0,244
Algae*Salt reduction	1	10,999	10,999	1,24	0,298	1	394,37	394,37	4,16	0,076	1	0,001618	0,001618	1,33	0,282
Algae*Fermented/ Not fermented	1	15,2	15,2	1,71	0,227	1	126,87	126,87	1,34	0,281	1	0,00157	0,00157	1,29	0,289
Salt reduction*Fermented/ Not fermented	1	34,072	34,072	3,84	0,086	1	338,14	338,14	3,57	0,096	1	0,005335	0,005335	4,38	0,07
Algae*Salt reduction*Fermented/ Not fermented	1	23,733	23,733	2,68	0,14	1	152,44	152,44	1,61	0,24	1	0,000124	0,000124	0,1	0,758
Error	8	70,931	8,866			8	758,51	94,81			8	0,00975	0,001219		
Total	15	781,742				15	6528,56				15	0,05376			

Appendix 7

Table 7. Results from the general linear model which studied the effect of macroalgae species, NaCl reduction and fermented vs unfermented algae, and the interactions between on %UPP and polymeric:monomeric proteins. P-value <0.05 represent a significant effect of the factors and interactions.

	%UPP					Polymeric: monomeric				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	674,2	674,25	1,12	0,321	1	0,32067	0,32067	97,04	0
Salt reduction	1	1555,7	1555,71	2,58	0,147	1	1,35774	1,35774	410,89	0
Fermented/ Not fermented	1	2738,8	2738,75	4,54	0,066	1	0,0565	0,0565	17,1	0,003
Algae*Salt reduction	1	68,2	68,15	0,11	0,745	1	0,02229	0,02229	6,75	0,032
Algae*Fermented/ Not fermented	1	562,9	562,86	0,93	0,362	1	0,0405	0,0405	12,25	0,008
Salt reduction*Fermented/ Not fermented	1	57,1	57,11	0,09	0,766	1	0,02057	0,02057	6,22	0,037
Algae*Salt reduction*Fermented/ Not fermented	1	7,1	7,13	0,01	0,916	1	0,0002	0,0002	0,06	0,812
Error	8	4824,5	603,06			8	0,02644	0,0033		
Total	15	10488,4				15	1,8449			

Appendix 8

Table 8. Results from the general linear model on pH for dough (n=2) with macroalgae. The effect of macroalgae species, NaCl reduction and fermented vs unfermented algae, and the interactions between them were studied. P-value <0.05 represent a significant effect of the factors and interactions.

	pH				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	0,000008	0,000008	109,07	0
Salt reduction	1	0,000001	0,000001	11,05	0,01
Fermented/ Not fermented	1	0,00004	0,00004	571,43	0
Algae*Salt reduction	1	0,000001	0,000001	16,05	0,004
Algae*Fermented/ Not fermented	1	0,000003	0,000003	50,08	0
Salt reduction*Fermented/ Not fermented	1	0,000001	0,000001	10,1	0,013
Algae*Salt reduction*Fermented/ Not fermented	1	0	0	4,22	0,074
Error	8	0,000001	0		
Total	15	0,000054			

Appendix 9

Table 9. Results from the general linear model on bread quality parameters measured in breads with macroalgae (n=2) at the small-scale baking. The effect of macroalgae species, NaCl reduction and fermented vs unfermented algae, and the interactions between them were studied. P-value <0.05 represent a significant effect of the factors and interactions.

	Specific volume					Ratio				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	0,000214	0,000214	6,1	0,039	1	0,009987	0,009987	0,69	0,43
Salt reduction	1	0,001257	0,001257	35,91	0	1	0,047623	0,047623	3,3	0,107
Fermented/ Not fermented	1	0,000544	0,000544	15,55	0,004	1	0,000481	0,000481	0,03	0,86
Algae*Salt reduction	1	0,000006	0,000006	0,16	0,696	1	0,005517	0,005517	0,38	0,554
Algae*Fermented/ Not fermented	1	0,000007	0,000007	0,21	0,656	1	0,010376	0,010376	0,72	0,421
Salt reduction*Fermented/ Not fermented	1	0,00008	0,00008	2,27	0,17	1	0,014415	0,014415	1	0,347
Algae*Salt reduction*Fermented/ Not fermented	1	0,000002	0,000002	0,05	0,835	1	0,002944	0,002944	0,2	0,663
Error	8	0,00028	0,000035			8	0,115458	0,014432		
Total	15	0,002389				15	0,206801			

	Crumb firmness					Number of cells				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	11869	11869,5	0,93	0,364	1	0	0	0,31	0,592
Salt reduction	1	35989	35989,1	2,81	0,132	1	0	0	0,68	0,433
Fermented/ Not fermented	1	8834	8834,4	0,69	0,43	1	0	0	0,23	0,644
Algae*Salt reduction	1	15666	15665,9	1,22	0,301	1	0	0	3,81	0,087
Algae*Fermented/ Not fermented	1	814	814,3	0,06	0,807	1	0	0	0,15	0,713
Salt reduction*Fermented/ Not fermented	1	5752	5751,5	0,45	0,522	1	0	0	2,48	0,154
Algae*Salt reduction*Fermented/ Not fermented	1	230	230,3	0,02	0,897	1	0	0	0,01	0,929
Error	8	102465	12808,1			8	0	0		
Total	15	181620				15	0			

	Area of cells					Number of holes				
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Algae	1	2,54536	2,54536	8,76	0,018	1	0,11222	0,112222	1,53	0,251
Salt reduction	1	0,37324	0,37324	1,28	0,29	1	0,24249	0,242489	3,3	0,107
Fermented/ Not fermented	1	0,34625	0,34625	1,19	0,307	1	0,04363	0,043628	0,59	0,463
Algae*Salt reduction	1	0,05481	0,05481	0,19	0,676	1	0,0036	0,003596	0,05	0,83
Algae*Fermented/ Not fermented	1	0,06265	0,06265	0,22	0,655	1	0,0335	0,033502	0,46	0,518
Salt reduction*Fermented/ Not fermented	1	0,49192	0,49192	1,69	0,229	1	0,00106	0,001065	0,01	0,907
Algae*Salt reduction*Fermented/ Not fermented	1	0,29136	0,29136	1	0,346	1	0,01377	0,01377	0,19	0,676
Error	8	2,32427	0,29053			8	0,58759	0,073449		
Total	15	6,48986				15	1,03786			

Source	Area of holes				
Algae	DF	Adj SS	Adj MS	F-Value	P-Value
Salt reduction	1	0,74253	0,74253	5,02	0,055
Fermented/ Not fermented	1	0,207	0,207	1,4	0,271
Algae*Salt reduction	1	0,02594	0,02594	0,18	0,686
Algae*Fermented/ Not fermented	1	0,01451	0,01451	0,1	0,762
Salt reduction*Fermented/ Not fermented	1	0,01654	0,01654	0,11	0,747
Algae*Salt reduction*Fermented/ Not fermented	1	0,03241	0,03241	0,22	0,652
Error	1	0,02374	0,02374	0,16	0,699
Total	8	1,18325	0,14791		
	15	2,24593			

Appendix 10

Table 10. Description of the sensory attributes evaluated in the Quantitative Descriptive Analysis. Appearance, odour, taste/flavour and texture were evaluated by the trained sensory panel.

ASSESSMENT OF BREAD	
Explanation of attributes	
APPEARANCE - crust (top only)	
Colourhue	Colour evaluated on the surface according to the NCS-system
	No intensity = yellowG 90 Y
	Distinct intensity = yellow/red Y 40 R
Colour intensity	Colour evaluated on the surface according to the NCS-system
	No intensity = no colour intensity
	Distinct intensity = clear colour intensity
Whiteness	Colour evaluated on the surface according to the NCS-system
	No intensity = no whiteness, black
	Distinct intensity = clear whiteness
APPEARANCE - crumbly	
Poring	"Cavities / channels in the crumb, refers to Dahlmans porosity table"
	No intensity = little porosity (ref. picture no.1)
	Distinct intensity = clear poring (ref. picture no.8)
ODOUR - crust+crymbly	
Grain odour	Related to the odour of grains (eg barley, oats, wheat, rye)
	No intensity = no grain odour
	Distinct intensity = clear grain odour
Roasted odour	Related to a odour of roasted
	No intensity = no roasted odour
	Distinct intensity = clear roasted odour
Seaweed/kelp odour	Related to the odour of seaweed and kelp
	No intensity = no kelp odour
	Distinct intensity = clear kelp odour
Drawer odour	Related to a drawer odour
	No intensity = no drawer odour
	Distinc tintensity = clear drawer odour
Rancid odour	Related to the odour of oxidized fatty substances (grass, hay, stearin, paint)
	No intensity = no rancid odour
	Distinct intensity = clear rancid odour

FLAVOUR/TASTE - crumbly	
Total flavour intensity	The strength of all flavours in the sample
	No intensity = no flavour
	Distinct intensity = clear flavour
Sour flavour	Related to a fresh, balanced flavour
	No intensity = no sour flavour
	Distinct intensity = clear sour flavour
Sweet taste	Related to the basic taste sweet
	No intensity = no sweet taste
	Distinct intensity = clear sweet taste
Salty taste	Describes the basic taste salty (sodium chloride)
	No intensity = no salty taste
	Distinct intensity = clear salty taste
Bitter taste	Describes all the taste/flavours for bitter
	No intensity = no bitter taste
	Distinct intensity = clear bitter taste
Raw flavour	A raw taste related to undercooked flour etc. grain
	No intensity = no raw flavour
	Distinct intensity = clear raw flavour
Grain flavour	Related to the flavour of grains (eg barley, oats, wheat, rye)
	No intensity = no grain flavour
	Distinct intensity = clear grain flavour

Seaweed/kelp flavour	Related to the flavour of seaweed and kelp
	No intensity = no kelp flavour
	Distinct intensity = clear kelp flavour
Cloying flavour	An unfresh and sickening flavour
	No intensity = no cloying flavour
	Distinct intensity = clear cloying flavour
Rancid flavour	Related to the flavour of oxidized fatty substances (grass, hay, stearin, paint
	No intensity = no rancid flavour
	Distinct intensity = clear rancid flavour
Metalic flavour	Flavour of metallic (ferrous sulphate)
	No intensity = no metallic flavour
	Distinct intensity = clear metallic flavour
TEXTURE - crumbly	
Juiceness	Surface textural property that describes liquid absorbed or released from
	a product. Perception of water after 6-7 chews
	No intensity = no juiciness
	Distinct intensity = clear juiciness
Chewing resistance	Mechanical texture property related to the time and number of chewings required to comminute the sample for swallowing
	No intensity = no chewing resistance (tender)
	Distinct intensity = clear chewing resistance (tough)
Tackiness	Mechanical textural property related to the force required to remove a substance that adheres to the mouth or to a substrate
	No intensity = no stickiness
	Distinct intensity = clear stickiness

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