Development of bread products containing *Chordaria cladosiphon* (Mozuku) and its bioactive extract fucoidan

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Abstract

Many seaweeds such as *Chordaria cladosiphon*, commonly known as mozuku, have been shown to contain several health promoting components such as dietary fibres, antioxidants, and a range of bioactive compounds. Mozuku is an edible brown seaweed which constitutes an important part of the diet of native Okinawans who enjoy long lives and consume the seaweed mixed with various seafoods and vegetables. Brown seaweeds and their isolated compounds, specifically a long-chain polysaccharide known as fucoidan, are reported to retard the formation and growth of various cancer cells in humans as well as having anticoagulation, antiviral and immunological activities. The present study developed wheat and gluten-free bread formulations containing mozuku powder in order to introduce its potential health effects into a staple food product thus making it more accessible to a wider range of consumers.

Nutritional analysis of mozuku powder was determined by proximate analysis, fatty acid analysis and amino acid analysis, particle size distribution of the powder was also determined. Mozuku powder was added to modified wheat bread and developed gluten-free bread formulations with adjustments in levels of added salt. The effects of mozuku powder inclusion on bread quality were assessed by measuring changes in bread quality parameters, with standard methods being used to determine texture characteristics, crust and crumb colour, specific volume and water activity. Samples of gluten-free and wheat bread were evaluated by consumer sensory panellists for appearance, texture, aroma, taste, and overall acceptability using the 9-point hedonic scale. Microbial stability of wheat bread was determined by enumeration of total aerobic plate counts and, yeast and mould counts over the course of 3 days.

Particle size distribution of mozuku powder showed that 90% of particle by weight were less than 500μm in diameter and that only 10% were less than 90μm. At these particle sizes, mozuku flakes were observable in the finished loaves. Nutritional analysis of mozuku powder contained (w/w, wet basis) 46.9% ash, 30.4% dietary fibre, 19.0% sodium, 5.4% protein, 4.7% available carbohydrates, 1.0% fat, 0.13% sugar and an overall energy content of 209.1 kJ/100 g. The most prevalent amino acids in mozuku powder were aspartate (0.59 mg/100 mg), glutamate (0.55 mg/100 mg), and leucine (0.42 mg/100 mg). Of the fatty acids, palmitic (69%) and oleic acid (13%) were present in highest concentrations however due to the total fat content of 1% they are unlikely to contribute to overall health.

Addition of mozuku powder to both gluten-free and wheat bread formulations with adjusted salt levels, reduced specific volume and breadcrumb lightness of the products without affecting water activity. However, inclusion of the seaweed powder in formulations increased redness/yellowness in the bread crumb. There were no significant differences ($P<0.05$) in textural changes between wheat bread containing 1 and 2 % mozuku powder. However, wheat bread containing 2 % mozuku powder was characterised by decreased cohesiveness with no perceived changes in hardness, chewiness, resilience and springiness. Wheat bread containing 1% and 2% mozuku powder were well accepted by consumer sensory panellists receiving mean scores of 6.8 and 6.4 in overall acceptability on the 9-point hedonic scale. The addition of mozuku powder to wheat bread at 1% and 2% did not affect the microbial stability of the loaves during storage at 20°C for 3 days. With respect to gluten-free bread formulations, mozuku powder (up to 3%) did not affect texture ($P<0.05$), however, the inclusion of 4% mozuku powder in gluten-free bread increased hardness, chewiness and resilience. The gluten-free formulation containing a concentration of 2.5% mozuku powder was selected for consumer sensory trials due to its favourable quality results and received a mean score of 6.4 in overall acceptability on the 9-point hedonic scale thus indicating the product was well-liked by consumer sensory panellists.

In this study, wheat bread and gluten-free bread containing variable levels of mozuku powder were successfully developed. Wheat bread containing 1% and 2% mozuku powder and gluten-free bread containing 2.5% mozuku powder had desirable textural characteristics, were well liked by consumer sensory panellists, and would be suitable for use in clinical trials.

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List of Abbreviations

1% M	Wheat Bread With 1% Mozuku Powder
1% RSM	Wheat Bread With 1% Mozuku Powder And Reduced Salt
2% M	Wheat Bread With 2% Mozuku Powder
2% RSM	Wheat Bread With 2% Mozuku Powder And Reduced Salt
*a**	Red-Green Axis
AACC	American Association Of Cereal Chemists
ANOVA	Analysis Of Variance
a_w	Water Activity
*b**	Blue-Yellow Axis
CD	Celiac Disease
CFU	Colony Forming Unit
CIE	Commission Internationale De L'éclairage
CMC	Carboxymethyl Cellulose
CMYK	Cyan, Magenta, Yellow, Black Model
EPA	Eicosapentaenoic Acid
FFA	Free Fatty Acids
FSANZ	Food Standards Australia New Zealand
GCF	Grade Colour Figure
GFB	Gluten Free Bread
HPLC	High-Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
*L**	Lightness (Value) Axis
M	Mozuku
NaCl	Table Salt
PCA	Plate Count Agar
RDI	Recommended Daily Intake
RGB	Red, Green, Blue Colour Profile
SV	Specific Volume
U.S. FDA	United States Food And Drug Administration
YGC	Yeast Glucose Chloramphenicol

1 Introduction

Chordaria (C.) cladosiphon, commonly known as mozuku is a brown macroalgae found in several regions of the southern hemisphere including the coastal waters of the Tongan Islands and the waters surrounding the islands of Japan (Guiry, 2016a; Nagaoka et al., 1999). Mozuku contains high levels of dietary fibre, minerals and the bioactive compounds, fucoxanthin and fucoidan (Mabeau & Fleurence, 1993). Previous research has shown that fucoidan, a high molecular weight sulphated polysaccharide, exhibits a number of biological activities including anticancer, immunomodulatory, anti-inflammatory and antioxidant activities (Elizondo-Gonzalez et al., 2012; Li, Lu, Wei, & Zhao, 2008). Consumption of mozuku has also been reported to control symptoms of type-II diabetes (Kim, Yoon, & Lee, 2012).

Access of mozuku (and therefore fucoidan) to consumers is limited as mozuku is available in the New Zealand market in few forms. Only one source referring to the presence of mozuku as an ingredient in a western food product could be found; a paleo cracker produced by Venerdi Ltd. (2016) is listed to contain mozuku powder on their website. Thus, this thesis aimed to incorporate *C. cladosiphon* into formulations of standard white bread and gluten-free bread to increase the accessibility of mozuku and fucoidan to consumers (Siro, Kapolna, Kapolna, & Lugasi, 2008).

Bread is one of the most commonly consumed staple foods in the world and the process for bread-making has been known for over 6,000 years (Kahlon & Chiu, 2014). Bakery products are an integral part of many cuisines and provide a vital source of easily digestible, high-energy carbohydrate (Coulston et al., 1984). The Joint FAO/WHO recommendations state that 55-70% of total energy intake should consist of carbohydrates and bread, as well as other baked cereals, are primary sources of carbohydrates (WHO, 2003). In addition to the calorific content, bread also contains other nutritional components such as dietary fibres, vitamins (B1, B2, and B6) and minerals (sodium, potassium, chloride) (Vogel & Ganzle, 2009). However, for an increasing number of consumers, the consumption of standard white bread causes several digestion-related health issues, of which the presence of gluten is the primary cause. Gluten-related disorders may be caused by the ingestion of gluten by individuals with genetic and/or immunological predisposition to these conditions (Sapone et al., 2012). Of these health conditions, coeliac disease, wheat allergies and non-coeliac gluten sensitivity are the widest spread and best understood (Catassi et al., 2007; Ostblom, Wickman, van Hage, & Lilja, 2008; Sapone et al., 2012; Sapone et al., 2010). The prevalence of coeliac disease is estimated at 1% in the US, Europe and New Zealand (Fasano et al., 2003; Sporea, 2003). According

to a study conducted in Sweden the prevalence of wheat allergies in children is estimated at 5% (Ostblom et al. (2008). Gluten-free bakery products are gaining popularity not only due to increases in the number of diagnoses of health complications related to the consumption of wheat containing products, but also due to the nutritional benefits of gluten-free diets (Sapone et al., 2012). Standard white bread has a glycaemic index of about 100 which is comparable to ingesting raw glucose (Wolever et al., 1985), and therefore the consumption of large quantities of bread may increase the likelihood of developing type-II diabetes (Willett, Manson, & Liu, 2002). Gluten-free bread is baked using composite flours which consist of ingredients including legume flours, psyllium and guar gum, such ingredients have been shown to be beneficial to health (Alencar, Steel, Alvim, de Morais, & Bolini, 2015; Alvarez-Jubete, Arendt, & Gallagher, 2010b).

The incorporation of mozuku into wheat and gluten-free bread formulations is likely to pose several challenges. In any baked bread product the inclusion of additional ingredients may affect the physico-chemical characteristics of the product (Cauvain, 2012). The textural quality of bread is dependent on components such as gluten and other various hydrocolloids which form structural matrices (Vivas, 2013). The presence of long-chain polysaccharides, fibres and other components may interrupt these matrices thereby affecting texture and shelf life (Vivas, 2013). Additionally, the high presence of minerals (i.e. NaCl) in the mozuku powder may interfere with the growth and fermentation of yeast, thus leading to altered CO_2 production (Cauvain & Young, 2009). Therefore, formulations, dough preparation procedures and baking conditions may need to be adjusted to optimise the textural characteristics of the final product (Cauvain & Young, 2009).

To our knowledge, there are no published articles on the incorporation of mozuku in bakery products or other foods despite an increasing trend in the consumption of seaweed in western markets (Chan, Ho, & Phang, 2006; MacArtain, Gill, Brooks, Campbell, & Rowland, 2007; Rebours et al., 2014). There is therefore a real opportunity in the market for the development of food products containing mozuku and fucoidan.

1.1 Aim

The aim of this study was to develop gluten-free and wheat bread formulations containing powdered seaweed, *Chordaria cladosiphon* commonly known as mozuku.

1.2 Specific objectives

The experiments were conducted in eight phases with the following specific objectives:

1. To conduct a comprehensive literature review of wheat and gluten-free bread making, seaweed as a food product, and the health effects associated with fucoidan.

2. To conduct the following nutritional analysis of mozuku powder:
    - Fatty acid analysis
    - Amino acid analysis
    - Proximate analysis (carbohydrate, protein, fat, ash, sugar, fibre, sodium, moisture, energy)

3. To investigate particle size distribution of mozuku powder

4. To characterise the effect of adding mozuku powder on the quality parameters of a standard white bread recipe

5. To develop a white bread formulation containing mozuku powder

6. To develop a gluten-free bread formulation containing mozuku powder

7. To conduct consumer evaluations of the developed wheat and gluten-free bread products

8. To determine the effect of mozuku powder on the microbial stability of the developed wheat bread formulations

2 Literature Review

2.1 Introduction

Seaweeds are defined taxonomically as macroalgae, which are macroscopic, multicellular marine algae, which can be separated into four different classifications: brown algae (phaeophyta), red algae (rhodophyta), green algae (chlorophyta), and blue-green algae (cyanophyta). The seaweed used in this project is known by a number of names including, mozuku (Japanese), limu tanga'u (Tongan) and angels hair seaweed (English) depending on where it has been sourced and where it will be sold. There are two main species of mozuku, the first originates from the Okinawan region of Japan and is known as *Cladosiphon okamuranus* (Guiry, 2016b). The second species of mozuku originates from New Zealand, the Chatham Islands and the Polynesian Islands and is likely to belong to the species C*hordaria cladosiphon* (Guiry, 2016a). However, reports covering mozuku industries and resources in Tonga generally refer to the seaweed simply as *Cladosiphon sp*. (Fisheries Division, 2012). This project utilises powdered *Chordaria c.,* which is sourced from Tonga and which will be referred to as mozuku in this thesis.

2.2 Mozuku

2.2.1 Introduction

Mozuku (*Cladosiphon sp.*), a dark brown edible seaweed is most commonly consumed in Japan where it is often served with rice vinegar as a starter or palate refresher (Figure 2.1). In Japan, mozuku is harvested in the spring with an estimated 20,000 tons being produced by the economy of Okinawa (Fontana, 2016).

Figure 2.1 Freshly harvested Okinawan mozuku

Retrieved 25th January 2017; from http://okinawaclip.com/en/detail/458

Mozuku (*Cladosiphon chordaria*) develops in two generations each year (Fisheries Division, 2012). The first generation is a microscopic gametophyte generation that develops between mid-summer and late-autumn each year (Fisheries Division, 2012). This gives rise to the macroscopic sporophyte generation which begins its development during winter when it blooms, reaches maturity in spring, and is finally harvested once a sea water temperature of 22°C has been reached (Fisheries Division, 2012). Mozuku found in Tonga grows at water depths of up to 5m at high tide, generally, in the

vicinity of the island of Tongatapu, where it is grown commercially in rows on an artificial substrate, or naturally on widely dispersed beds of the sea-grass *Halodule uninervis* (Fisheries Division, 2012; Lovell, 1996). After the seaweed has been harvested, it is frozen and stored in containers until it is shipped to New Zealand where it undergoes rinsing, freeze drying and milling (A. Smith, personal communication, May, 10th 2016). The raw mozuku may also be processed to extract the components fucoidan and fucoxanthin which are sold as health supplements due to their beneficial health properties (Fitton, Stringer, & Karpiniec, 2015; Ostraff, 2006; Xia et al., 2013).

Fucoidans are a class of polysaccharides found within brown macroalgae (e.g. mozuku, kelp and wakame) which contain high levels of L-fucose and sulphate ester groups and have been shown to possess strong bioactive properties (Li et al., 2008). Most of the research conducted into the bioactive properties of mozuku primarily focuses on its sulphated polysaccharide fucoidan as well as the accessory pigment fucoxanthin (Ale, Mikkelsen, & Meyer, 2011; Maeda, 2015). Studies have demonstrated the biological activity of fucoidan includes antitumoral, anti-inflammatory, and antioxidative effects (Li et al., 2008).

2.2.2 Current seaweed market

Data collected from the Food and Agriculture Organization of the United Nations (FAO) has shown that the total traded volume of seaweed has increased five-fold since 1984 (Burg et al., 2013). The most recent reports state that in 2014 the global seaweed market was valued at over $7.79bn NZD (FAO, 2016). Asia produces the vast majority of seaweed related products with China and Japan being the largest contributors (Burg et al., 2013). China's demand for raw seaweed has outstripped its domestic supply thus relying on imports from countries such as Korea (Burg et al., 2013). In comparison, the consumption rate of seaweed by Western consumers is minimal, for example, the entirety of Europe consumes 70 tonnes whereas Japan consumes 97,000 tonnes (dry product) (Darcy-Vrillon, 1993).

Marketing of seaweeds has increased in the past few years with campaigns promoting the concept of seaweeds as health superfoods (Nehal, 2014). Marketing ideas such as rebranding seaweeds as sea-vegetables, in order to change the perception of marine algae as "weeds" and remove the negative connotations that are associated with this term, have also been suggested (Xu, 2001). However, marketing alone is unlikely to substantially increase seaweed consumption in western markets as even the most health conscious consumers will not compromise on taste as the critical factor. Additional

research into the understanding of how seaweed components interact with food ingredients and the development of new products containing seaweed must be untaken to improve consumer acceptance of seaweed containing products (Verbeke, 2006).

2.2.3 Food standards and safety

2.2.3.1 Food standards

Regulations surrounding the use of seaweeds in food products in New Zealand and Australia are most concerned with the levels of contaminants and toxicants present. Schedule 19 of the Australian and New Zealand Food Standards Code sets out maximum levels of metal contaminants; non-metal contaminants; natural toxicants; and average and maximum levels of mercury. Schedule 19 also specifically states that for seaweed products, calculations of these toxicants must be carried out at 85% hydration ("Parliamentary questions," 2013).

Certain species of brown algae have obtained GRAS (generally regarding as safe) status in the USA. The U.S. FDA has listed multiple species of brown algae as being acceptable for use in food products as spices, seasonings, and flavourings, however, species belonging to the genus *Cladosiphon* are not mentioned in this list (U.S. Food and Drug Administration, 2016a). Fucoidan from *Undaria pinnatifida* has also obtained GRAS status and is approved for use in baked goods (bread, cake, noodles), soups, snack foods, imitation dairy products, and seasonings and flavours at usage levels of up to 30 milligrams per serving (U.S. Food and Drug Administration, 2016b).

Europe considers seaweeds as a novel food and currently 21 macroalgae and 3 microalgae are authorized for use in foods and condiments, however there is no mention of *Cladosiphon sp.* (Fleurence et al., 2012; Mabeau & Fleurence, 1993). Generally, brown algae are considered safe to consume as long as the ingredients and products they form do not exceed the maximum allowed levels of toxic minerals and bacteria (CEVA, 2014; Official Journal of the European Union, 2008).

2.2.3.1 Food Safety

Brown seaweed is considered safe for adults when consumed in moderation, although there have been cases where pregnant women, breastfeeding women and their children have become ill following excessive consumption due to high levels of iodine intake (Nishiyama et al., 2004). In these cases,

the mothers were consuming brown seaweed products several times a day, and children, especially infants, are more susceptible to iodine intake due to their low body weight (Food Standards Australia New Zealand, 2011). Occasional consumption of seaweed containing a high iodine content, e.g. once a week, does not present a risk, as the body rapidly excretes excess amounts (Food Standards Australia New Zealand, 2011).

A safety evaluation investigating the effects of excess consumption of fucoidan (4 g daily for 2 weeks) from brown seaweed (mozuku) in 20 healthy volunteers, showed no abnormalities in gastrointestinal function and faecal states (Abe et al., 2013).

2.2.4 Nutritional content

In general, all macroalgae are excellent sources of fibre, minerals and phytonutrients (MacArtain et al., 2007). However, macroalgae in its raw state contains a large percentage of water, ranging from 70% through to 90%, with the moisture content depending on species (Indergaard & Minsaas, 1991). Therefore, drying the seaweed is beneficial for improving nutritional density, as well as product preservation and may be easily rehydrated for use.

2.2.3.1 Polysaccharides

There are numerous forms of carbohydrates found in mozuku, with most being long chain polysaccharides which are not readily broken down during digestion (Kusaykin et al., 2008). Fucoidan makes up the bulk of the carbohydrates found in raw mozuku with percentages ranging from 1 - 3% w/w (wet basis) (Tako, Yoza, & Tohma, 2000). Mozuku also contains alginate, which has been shown to have beneficial effects on textural parameters in gluten-free bread formulations (Tako et al., 2000), however, the level of alginate in mozuku (0.1% w/w, wet basis) is unlikely to be sufficient to impact bread quality (Rosell & Rojas, 2001).

The two types of polysaccharides present in seaweed are classified as either structural (cell-wall polysaccharides) or storage molecules. Table 2.1 shows the structural and storage polysaccharides of the three edible seaweed types. Many uses for both types of polysaccharides have been found in a number of industries (Rosell & Rojas, 2001), for example, alginates, agars and carrageenans have textural and stabilizing properties which are relevant to the food-safe hydrocolloid industry (FAO,

2004) while celluloses and starches may be used as bulking agents or chemically modified into water binding agents such as carboxymethyl cellulose (CMC) (Marshall, Goff, & Hartel, 2003).

Table 2.1 Various polysaccharides found in seaweed species

Seaweed type	Cell-wall polysaccharide	Storage polysaccharide
Brown seaweed	Alginate (guluronic acid, mannuronic acid,); Fucans (sulphated fucose)	Laminarin (glucose)
Red seaweed	Carrageenans (galactose, sulphate); Agar (galactose): Cellulose; Xylan	Floridean starch (glucose)
Green seaweed	Cellulose; Xylan; Mannan; Glucuronoxylorhamnan (sulphated)	Starch

Source: Mabeau and Fleurence (1993)

2.2.3.2 Proteins

The mean crude protein content in seaweed dry matter is within a range of 10-20% in brown seaweed, and 20-50% in red and green seaweed species (Indergaard & Minsaas, 1991). Proteins in macroalgae have been shown to contain all essential amino acids, especially glycine, alanine, arginine, proline, glutamic and aspartic acids, however, variations in concentrations of these amino acids are known to occur (Černá, 2011; Galland-Irmouli et al., 1999). In addition, specific seaweed species may serve as a source of taurine and other rare non-protein amino acids such as chondrine (Fleurence, 1999; Holdt & Kraan, 2011) (Madgwick, Ralph, Shannon, & Simes, 1970).

2.2.3.4 Lipids

The average lipid content of seaweeds is low (0.2% to 4%, dry matter) and varies depending on seasonal environmental conditions (Patarra, Leite, Pereira, Baptista, & Neto, 2012). Studies have suggested that ω-3 marine fatty acids, particularly EPA (C20:5 n-3), are the predominant fatty acids in seaweeds (Holdt and Kraan, 2011). However, the only species containing sufficient EPA to confer health benefits following consumption are those belonging to the genera *Palmaria* and *Sargassum*. Species of *Ulva* contain relatively high amounts of oleic acid (Ginneken, Helsper, Visser, Keulen, & Brandenburg, 2011) which has been shown to reduce blood pressure, increase fat burning and reduce the risk of inflammatory conditions (Sales-Campos, Souza, Peghini, da Silva, & Cardoso, 2013).

Overall, the low lipid content in most seaweeds means that only consumption of specific species such as *Palmaria* are likely to contribute health benefits (Burg et al., 2013).

2.2.3.5 Vitamins and antioxidants

Seaweeds have been shown to contain both water- and fat-soluble vitamins and pigments including carotenoids, chlorophylls and phycobiliproteins. Vitamins present in many seaweeds include β-carotene (provitamin A activity) and tocopherol (vitamin E) (Mabeau & Fleurence, 1993). The vitamin profile of seaweeds varies according to species (Table 2.2), season, algal growth stage and environmental growth conditions (Burg et al., 2013).

Table 2.2 Vitamin contents of various red, green and brown seaweed species.

Source: MacArtain et al. (2007)

Mozuku is a potential rich source of beneficial non-enzymatic antioxidants (Kristinsson, 2014), such as phenolic compounds, which are considered one of the most important classes of natural antioxidants (Kuang, 2013). Phenolic compounds found in brown algae include phlorotannins, which may have strong therapeutic properties (anticancer, antioxidative, antibacterial, anti-allergic, anti-diabetes, anti-aging, anti-inflammatory and anti-HIV activities) (Heiras-Palazuelos et al., 2013). One specific phlorotannin, fucoxanthin, present in mozuku has been shown to exhibit strong antioxidant properties (Xia et al., 2013).

The levels of vitamins and antioxidants present in raw seaweeds are sufficient to contribute to the Recommended Daily Intake (RDI). However, additional research is required into seaweeds integrated into food products to determine how their vitamin and antioxidant contents are affected by various processing conditions (Kuda, Tsunekawa, Goto, & Araki, 2005).

2.2.3.6 Minerals

Seaweeds are rich sources of minerals, containing on average between 10% – 50% ash content (dry matter) roughly equalling 5 to 10 times higher than land vegetable products (Burg et al., 2013). Their high mineral content is due to their environment and ability to integrate the surrounding compounds into their cellular structure (Vinogradov, 1953). Brown seaweeds accumulate many elements and have been found to be a good source of iodine, copper, magnesium, and iron (McCance, Widdowson, & Holland, 1993; Ministry of Health, 2004). Kombu is one example of a brown seaweed with an excellent source of magnesium and as already mentioned most seaweeds contain an abundance of iodine, an element important in many metabolic functions (Garrow, James, & Ralph, 1997; Ministry of Health, 2004). There is some concern relating to the presence of certain trace elements found in seaweed (Rose et al., 2007), therefore arsenic and other heavy metals must be tested for to ensure their concentrations are below toxic levels (Rose et al., 2007). However, for the large majority of seaweeds grown naturally, heavy metals are below food safety limits (Rose et al., 2007).

2.2.3.7 Fibre

The fibre content of various seaweeds has been found to be as high as or higher than other available vegetable sources (Table 2.3) (MacArtain et al., 2007). Based on the RDI of 30g of fibre for adult males one 8g serving of seaweed is able to provide up to 10% of a person's daily fibre requirement. The majority of dietary fibre found in seaweed species is in the form of alginates, carrageenans and agars (Mabeau & Fleurence, 1993). Seaweed fibres such as alginates have been shown to provide beneficial effects on gut health, including: aiding water binding, faecal bulking and decreased transit time (Brownlee et al., 2005).

Table 2.3 Fibre and carbohydrate nutritional values from various seaweeds and food products

Source: MacArtain et al. (2007)

2.3 Fucoidan

2.3.1 Introduction

Fucoidans form a class of fucose-rich sulphated polysaccharides most commonly found in brown marine algae, various echinoderms (Berteau & Mulloy, 2003) and more recently, in seagrasses (Kannan, Arumugam, & Anantharaman, 2013). The major source of fucoidans are from the edible brown seaweeds mozuku (*Cladosiphon okamuranus*), kombu (*Laminaria japonica*), tengusa (*Gelidium crinale*), and wakame (*Undaria pinnatifida*), (Berteau & Mulloy, 2003). Research into fucoidan is still in its early stages but the data that is available has indicated that it may have the potential to be used in a wide range of therapeutic treatments, as well as a functional food ingredient (Ale et al., 2011; Ermakova, Kusaykin, Trincone, & Tatiana, 2015; Kwak, 2014).

2.3.2 Structure and stability

Fucoidan is a polysaccharide the structure of which varies between species. It is characterised by its total molecular weight, relative ratios of sulphate ester groups and L-fucose sugars as well as the

presence of branching residues (Ale et al., 2011). For example, the structure of fucoidan from *Fucus vesiculosus* is comparatively simple, containing mostly fucose and sulphate esters (Kusaykin et al., 2008), whereas fucoidan sourced from other seaweed species may contain a more complex chemical structure incorporating compounds such as: monosaccharides (glucose, galactose, xylose, mannose etc.) uronic acids, proteins and acetyl groups interspersed throughout the fucose backbone (Li et al., 2008). Fucoidan structure may also vary by including: alternating 3- and 4-linked α-L-fucopyranose 2-sulphate residues (Bilan et al., 2002); random acetylation and undersulphation at several repeating units (Bilan et al., 2004), and highly branched chains at C-4 positions (Adhikari et al., 2006; Andrade et al., 2004; Bilan, Grachev, Shashkov, Nifantiev, & Usov, 2006; Chizhov et al., 1999; Hussein, Abdelaziz, & Salem, 1980a, 1980b). Figure 2.2 shows the macromolecular structure of fucoidan from the brown Seaweed *Turbinaria ornata* and indicates the degree of branching present in this species' fucoidan, other species may be more or less branched.

Figure 2.2 Molecular model of fucoidan extracted from the brown Seaweed *Turbinaria ornata*

Source: Thanh, Tran, Yuguchi, Bui, and Nguyen (2013)

The understanding of the structural backbone of the fucoidan polysaccharide has changed much over the past forty years, initially it was believed the main component of fucoidan was 1,2-α-fucose linkages (Conchie & Percival, 1950). However, further research revealed the true structure to be primarily a polymer with α-(1-3) linked fucose molecules, sulphate groups substituted at the C-4 position and fucose braches occurring every 2-3 units at the C-2 position (Patankar, Oehninger, Barnett, Williams, & Clark, 1993). A study of *Cladosiphon okamuranus* showed that fucoidan from this species consisted of 1→3-linked α-fucopyranose with a half sulphate substitution at the C-4

positions, α-glucoronic acid residues being linked at the C-2 position and a portion of the fucose residues being O-acetylated (Nagaoka et al., 1999).

The beneficial health effects (Section 2.3.4) of fucoidan have been found to be directly related to its structure (Ale et al., 2011) in particular the average polysaccharide length and degree of sulphation are of importance and are in part determined by the individual constituents of the overall structure (Cho, Han, & You, 2011; Kim, Rioux, & Turgeon, 2015). The structures of fucoidan from three brown seaweed species (*Fucus serratus*, *Ascophyllum nodosum*, and *Fucus evanescens*) can be seen in Figure 2.3 and while all share key structural similarities, differences occur in location and degree of sulphation and the presence of branching residues, these differences may alter potential health effects and therefore research should be focused into determining the functional role of each structural component.

Figure 2.3 Base chemical structure of fucoidan from three brown algae species

Source: Ale et al. (2011)

2.3.2.1 Thermal Stability

As the beneficial health properties of fucoidan are structure dependant, there is concern that detrimental changes may occur during the bread making process compromising potential health benefits. It is therefore important to determine the thermal stability of fucoidan from different species, and to date only limited research has been conducted in this area. The results of one study into the thermal stability, anti-oxidative potential, and bioactivity of fucoidan and lamarin added to raw and cooked pork products indicate that the biological activity of fucoidan may in some cases be negatively affected by exposure to high temperatures (>70 °C), this decrease in bioactivity was mitigated in fucoidan that had been chemically modified to increase the degree of sulphation present (Moroney, O'Grady, Lordan, Stanton, & Kerry, 2015). The antioxidant activity of fucoidan also decreased significantly ($p < 0.05$) after cooking, however, this reduction was counteracted when lamarin, another seaweed polysaccharide, was used in combination with fucoidan (Moroney et al., 2015). Overall these results indicate that the bioactive properties of fucoidan may be protected from the effects of heating by the use of algal polysaccharides or increased sulphation (Moroney et al., 2015).

2.3.2.2 Enzymatic interactions

Fucoidans have been shown to be resistant to enzymatic degradation, with current research indicating they are only able to be digested by a class of marine enzymes known as fucoidanases (Silchenko et al., 2013).

Fucoidans have been shown to inhibit certain enzymes to varying degrees; for example studies into diabetes therapies indicate fucoidan inhibits α-amylases, α-glucosidases and attenuates their hyperglycaemic effects (Kim, Rioux, & Turgeon, 2014; Kim et al., 2015; Lakshmana Senthil et al., 2014; Lakshmana Senthil et al., 2015; Tundis, Loizzo, & Menichini, 2010). It is possible that fucoidan may also inhibit the activity of naturally occurring and added amylases in bakery products, which are essential in the breakdown of starches to enhance crumb textural properties and retard staling (Hug-Iten, Escher, & Conde-Petit, 2001). The inhibition of enzymes in bread may be detrimental to its quality as they are commonly used throughout the industry to improve many quality parameters related to bread making (Maarel, Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2001). However, additional research is required to establish whether the inhibition of enzymes such as amylases and proteases by fucoidan would significantly affect bread quality.

2.3.3 Digestion and absorption

A crucial part in investigating the health effects of any food compound relies on understanding how the digestive system processes it. A study into the digestibility of fucoidan conducted at the Auckland University of Technology (Cao, 2015),, utilized two *in vitro* digestion models, a static and a dynamic model, which showed that the digestion of fucoidan resulted in significant increases in the number of reducing sugars and monosaccharides; including glucose, galactose, xylose, mannose and fucose, thus indicating the breakdown of the fucoidan structure (Cao, 2015). Digestion was also shown to significantly decrease the level of sulphur containing compounds found on the structure of fucoidan, which may be undesirable due to positive health effects being attributed to the presence of these groups (Haroun-Bouhedja, Ellouali, Sinquin, & Boisson-Vidal, 2000). Higher molecular weight fucoidans, which are likely to be less susceptible to digestion confer greater health promoting qualities, and therefore may be more suitable in food products (Cao, 2015).

In order for fucoidan to have a systemic bioactive effect it must be absorbed into the bloodstream, one study has shown that following oral consumption fucoidan is absorbed apparently unchanged into the blood and excreted in urine in a degraded form (Tokita, Nakajima, Mochida, Iha, & Nagamine, 2010). An additional study in which healthy rats were fed a 0.2% fucoidan chow, showed that fucoidan uptake occurred at high levels in intestinal cells and at lower levels into the bloodstream. Liver cells were also shown to uptake fucoidan. These two studies have demonstrated the *in vivo* absorption of fucoidan in both an animal and a human model.

2.3.4 Health effects

The reported health effects relating to the intake of fucoidan vary greatly, and research into the therapeutic and dietary uses of fucoidan has increased in recent years (Fitton et al., 2015), with significant health benefits being observed following administration of fucoidan via multiple methods (topical, intravenous and oral) (Fitton, 2011). The following sections outline the most notable and researched health effects of fucoidan and the mechanisms behind some of these health effects are summarised in Table 2.4.

Table 2.4 Overview of biological activities of fucoidans from a range of brown seaweeds

Source	Health benefit	Effect	Reference
Ecklonia cava	Anti-coagulant	Inhibiting biological activity of serine proteases II, and VII	(Athukorala, Jung, Vasanthan, & Jeon, 2006)
Undaria pinnatifida	Anti-virus	Blocking HSV-1 and HSV-2 replication Protecting mice from infection with HSV-1	(Lee, Hayashi, Hashimoto, Nakano, & Hayashi, 2004) (Cooper et al., 2002)
Dictyota mertensii, Lobophora variegate, Spatoglossum schroederi, and Fucus vesiculosus		Inhibiting HIV reverse transcriptase	(Queiroz et al., 2008)
Ecklonia cava	Anti-inflammation	Suppressing inflammatory response in LPS-stimulated RAW 264.7 cells	(Kang et al., 2011)
Fucus vesiculosus		Suppressing inflammatory response in LPS-induced microglia cells	(Park, Han, et al., 2011)
Undaria pinnatifida	Anti-allergy	Augmenting Th1 cell response in normal BALB/c mice and inhibiting Th2 cell response Reducing IgE level in mice serum	(Maruyama, Tamauchi, Hashimoto, & Nakano, 2005)
Laminaria japonica	Antioxidant	Scavenging superoxide radical and hypochlorous acid Preventing the increase of lipid peroxide in serum, liver, and spleen of diabetic mice	(Zhao, Xue, Cai, Wang, & Fang, 2005) (Li et al., 2002)
Focus vesiculosus	Anti-obesity	Inhibiting fat accumulation through the regulation of lipolysis in 3T3-L1 adipocytes	(Park, Jung, & Roh, 2011)
Cladosiphon okamuranus	Anti-tumor	Inducing apoptosis via caspase-3 and -7 activation-dependent pathways	(Teruya, Konishi, Uechi, Tamaki, & Tako, 2007)
Cladosiphon okamuranus	Gastric protection	Inhibiting the growth of stomach cancer cells without any effects on normal cells	(Kawamoto et al., 2006; Shibata et al., 2000)
Fucus vesiculosus	Against hyperoxaluria	Preventing the increased excretion of calcium oxalate monohydrate crystals	(Veena, Josephine, Preetha, & Varalakshmi, 2007)

Source: (Vo & Kim, 2013)

2.3.4.1 Anticoagulation effects

Fucoidans have a wide variety of biological activities, but their potent anticoagulant action is by far the most widely studied (Shanmugam & Mody, 2000). The anticoagulant activity of algal and invertebrate sulphated fucoidans is likely mediated by antithrombin and/or heparin cofactor II (Church, Meade, Treanor, & Whinna, 1989; Mourão, 2004). Mourão (2004) showed that regular, linear sulphated α-L-fucans and sulphated α-L-galactans express anticoagulant activity, which is dependent on the pattern of sulphation and monosaccharide composition. Overall, fucoidans may have potential applications as natural anticoagulants in functional foods and as a part of medicinal therapies (Cheng & Wang, 2003).

2.3.4.2 Antiviral effects

Fucoidan's from multiple brown seaweeds have been demonstrated to possess antiviral activity against both human and non-human viral agents (Elizondo-Gonzalez et al., 2012; Hayashi, Lee, Nakano, & Hayashi, 2013; Trejo-Avila et al., 2014). *Cladosiphon okamuranus* fucoidan has been investigated as a natural antiviral agent against Newcastle Disease Virus (NDV), a serious, contagious viral infection affecting many poultry (Elizondo-Gonzalez et al., 2012). Results from an *in vitro* study indicated that NDV activity was significantly inhibited by the application of fucoidan solution (2.5mg / mL) to the cell cultures, with a demonstrated 48% decrease in viral infection rates (Elizondo-Gonzalez et al., 2012). Fucoidan from *Cladosiphon sp.* has also been shown to reduce Canine Distemper Virus (CDV) activity and replication during the initial stages of infection, most likely by inhibiting CDV-mediated cell fusion. An *in vivo* mouse model study into the antiviral effects of orally administered fucoidan from *Undaria pinnatifida* against influenza A showed that fucoidan increased antibody production in mucosa linings and blood thus decreasing weight loss and prolonging survival (Hayashi et al., 2013).

2.3.4.3 Effects on cancer and tumour growth

A substantial amount of research has been conducted into the use of fucoidan in treating and preventing tumour and cancer cell growth (Boo et al., 2011; Lee, Kim, & Kim, 2012; Sarkar & Li, 2004) with particular focus into colon cancers, due to the ability of fucoidan to directly interact with

cells in the digestive tract (Han, Lee, & Lee, 2015). A study into the effects of orally administered fucoidan (5 g/kg bodyweight) extracted from *Cladosiphon okamuranus* in a (colon 26)-tumour-bearing mouse model (Azuma et al., 2012), indicated that fucoidan suppresses tumour growth and prolongs survival times in tumour bearing mice. Other mouse and *in vitro* studies support these promising results indicating that fucoidan may have beneficial effects at inhibiting human colon cell growth (Fitton et al., 2015). However, intake levels of 5 g/kg bodyweight daily is equivalent to a 60 kg person consuming 300 g of fucoidan per day; these levels are excessive considering the average daily consumption of seaweed in Japan is estimated to be 5.3g per person (Matsumura, 2001). Future clinical trials involving lower intake levels are needed to establish fucoidan's overall efficacy against tumour and cancer cell growth at reasonable daily consumption rates.

2.3.4.4 Effects on digestion and diabetes

Fucoidans from a range of species have been shown to positively influence conditions such as obesity and diabetes, with studies also showing the potential for orally administered fucoidan to improve overall gut health (Kim & Vo, 2013; Maeda, 2015). Fucoidan and fucoxanthins isolated from *Saccharina japonica* and *Sargasum fulvellum* have been shown to promote the recovery of blood glucose and support insulin production in type 2-diabetics, two key parameters essential for the stabilisation of blood glucose levels (Wang, Fu, & Han, 2014). Fucoidan extracted from *Undaria pinnatifida*, is able to repress the differentiation of adipose cells by inhibiting key adipocyte biomarkers, inflammatory cytokines and overproduction of reactive oxygen species (Kim & Lee, 2012), therefore indicating the potential of fucoidan for use in the control of obesity. Boars orally administered *Laminaria*-derived fucoidan showed increased levels of beneficial gut bacteria such as Lactobacilli spp. indicating that fucoidan may provide dietary means to improve gut health in pigs (Lynch, Sweeney, Callan, O'Sullivan, & O'Doherty, 2010), however additional research is required in order to determine whether humans are similarly effected (Lynch et al., 2010).

2.3.4.5 Anti-inflammatory effects

Fucoidans from a wide range of brown algae have been shown to have anti-inflammatory effects (Cumashi et al., 2007). Administration of fucoidans reduces the severity of the symptoms and presence of key biomarkers in inflammatory conditions such as aneurysm, colitis and pancreatitis

(Fitton et al., 2015). An animal model involving a simulated blood clot in an aortic aneurysm, showed that intraperitoneally administered fucoidan lessened the swelling of the artery by reducing the number of inflammatory neutrophils accessing the area, thus reducing the risk of the mice developing a ruptured aorta (Alsac et al., 2013; Fitton, 2011). A recent mouse model study has shown the inhibitory effects of orally administered fucoidan on the symptoms of colitis, an inflammatory condition affecting the inner lining of the colon (Lean, Eri, Fitton, Patel, & Gueven, 2015). *Fucus vesiculosus* fucoidan intake in colitis-affected mice was associated with increased body weight retention and reduced diarrhoea, colon weight, faecal blood loss, and spleen weight, indicating decreased inflammation and therefore diminished pathological symptoms (Lean et al., 2015). Intravenous delivery of fucoidan from *Fucus vesiculosus* has also been shown to reduce the severity of acute pancreatitis in mice; the results indicate fucoidan is able to significantly attenuate pancreatitis-related histological changes by decreasing neutrophil infiltration and systemic inflammation (Carvalho et al., 2014). The mechanisms of these health related effects may be due the ability of fucoidan to bind to purified and membrane-exposed P- (found in platelet and endothelial cells) and L-selectins (found in lymphocytes), inhibiting the recruitment of leukocytes into inflamed tissues and thus reducing the severity of inflammation related symptoms (Heinzelmann, Polk, & Miller, 1998).

2.4 Bread making

2.4.1 Introduction

Bread making originated as early as 2,000 BC and has been recorded in engravings from Ancient Egypt (Delwen, 1994), the basic recipe of bread has essentially remained the same since then, being a combination of flour and water along with a number of possible ingredients, including fat, salt, yeast and sugar. Technological advances in bread making include ingredient optimisation, large-scale batch processing and automation technologies (Decock & Cappelle, 2005). Ingredients such as wheat have been refined to the optimal composition for bread making by selective breeding of wheat strains, flour bleaching and flour standardisation. Changes in processes have focussed on improving the development of the gluten network, reducing fermentation times, increasing batch sizes and utilising a variety of additives to improve bread loaf characteristics and extend shelf life (Cauvain, 2012)

2.4.2 Nutrition

Bread is a staple food in many cultures around the world, making up a large portion of many peoples' daily diet, and helping provide the required nutrients for the development and maintenance of general well-being (Barrett, 1975). The bulk of the nutritional content in white bread originates from wheat flour (*Triticum aestivum*), however, many of the fibres, vitamins, complex carbohydrates and other beneficial nutrients contained in wheat germ and bran are removed in the production of white wheat flour (Dhingra & Jood, 2002) with the nutrients retained being: B vitamins, niacin, riboflavin and thiamine (Cauvain, 2012). Whole-meal and whole-grain breads which contain wheat germ and bran possess larger quantities of proteins, complex carbohydrates, fibres, vitamins and minerals (Jones, 2006) although, wholemeal wheat is still deficient in two essential amino acids, lysine and threonine (Sharma, Sekhon, & Nagi, 1999), therefore, supplementation of wheat flour with other inexpensive staple ingredients such as cereals and pulses helps improve the nutritional quality of all wheat products (Dhingra & Jood, 2002). The nutritional value of bread may be increased via the addition of rye (*Secale cereale*), barley (*Hordeum vulgare*) and/or oats (*Avena sativa*) (Dewettinck et al., 2008) or via direct fortification with nutrients such as folic acid. The differences in nutritional value between New Zealand wheat flours is shown in Table 2.5.

Table 2.5 Nutritional properties of New Zealand white wheat flours (mg per 100g)

	Retail Flour White	Retail Flour Wholemeal	South Island Bread Flour White	North Island Bread Flour White	South Island Biscuit Flour	North Island Biscuit Flour
Protein	11740	12360	12030	11510	8660	8610
Calcium	20.8	35.9	22.8	19	18.6	16.9
Iron	1.45	3.25	1.24	1.03	1.1	0.93
Potassium	194.8	406.7	183.1	194.6	191.7	176.3
Total dietary fibre	3140	12040	3250	3750	3230	3100
Thiamin	27	45	-	-	-	-
Riboflavin	9	15	-	-	-	-
Niacin	132	220	-	-	-	-
Vitamin B6	16	45	-	-	-	-

Retrieved 10th October 2016; from 'http://www.bakeinfo.co.nz/Facts/Nutrition/Nutritional-properties-of-flour'

2.4.3 Bread types

2.4.3.1 White bread

White bread is popular around the world due to its clean taste and light mouthfeel, it is made from white flour, which is wheat flour that contains only the endosperm, with the bran and germ layers removed (Cauvain & Young, 2007). The primary recipe for white bread includes white flour, water, yeast, salt and lipids, with commercial white bread loaves including additives aimed to enhance many aspects of a loaf's quality. Bread additives such as mono- and di-glycerides, preservatives, dough conditioners, emulsifiers and enzymes are able to improve a range bread making parameters (Cauvain & Young, 2007) including shelf life extension, sensory characteristics and decreases in processing times (Cauvain & Young, 2007).

2.4.3.2 Wholegrain bread

Wholegrain bread is made using whole-meal bread flour (Heaton, Emmett, & Odonnell, 1988), often in combination with an assortment of other cereal grains or culinary seeds (Cauvain & Young, 2007), such as brown linseeds, sunflower seeds, pumpkin seeds, millet seeds, golden linseed, poppy seeds and sesame seeds, which impart their own flavours and contribute nutrients to the loaf (Djuricic, Marinkovic, Dodevska, Ciric, & Sohajic, 2015). However, the inclusion of seeds and wholegrains may negatively affect bread quality parameters such as softness and loaf volume, requiring adjustments in the formulation (Cauvain & Young, 2007).

2.4.3.3 Gluten-free bread

Gluten-free breads differ significantly from wheat-based breads as they lack the important structural component, gluten. Gluten-free products may be made from various flours and starches so long as they lack the protein components of gluten (gliadin and glutenin). Common flours used in gluten-free breads include: brown and white rice, buckwheat, chia, maize, corn, potato, rye, sorghum, soya and tapioca (Ziobro, Korus, Witczak, & Juszczak, 2012).

The need for gluten-free bread has arisen due to people suffering from coeliac disease (CD) and wheat allergies, in both cases the presence of wheat proteins elicits negative immune responses (Hamer, 2005). CD is an autoimmune disorder in which sufferers cannot tolerate the consumption of gluten as

it damages the inner lining of their small intestine and hinders the absorption of nutrients (Sporea, 2003). When celiac sufferers consume gluten, their immune system inappropriately responds by causing inflammation of the small intestine and damaging the fragile microstructures present (Green & Cellier, 2007). Damage caused to the villi prevents the proper absorption of nutrients and may lead to further complications such as diarrhoea, abdominal distension, anaemia, and failure to thrive (Shiner, 1956). A person suffering from CD should not consume most grains, pastas, and cereals, as well as many processed foods, however they are still able to consume a well-balanced diet consisting of a wide variety of foods and wheat alternatives such as: potato, rice, soy, amaranth, quinoa, buckwheat, and bean flour. Currently, the prevalence of CD is approximately 1% in the US, Europe and New Zealand (Fasano et al., 2003; Sporea, 2003) while the prevalence of wheat allergies in children is reported to be 5% (Ostblom et al., 2008). Individuals suffering from wheat allergies must avoid wheat, but may consume certain gluten containing products such as barley, rye and oats.

2.4.3 Trends in the bakery industry

2.4.3.1 Gluten-free market

The gluten-free market has undergone large expansion in recent years with global sales of gluten-free foods doubling between 2008 and 2013 (Gallagher, 2009; Mishra, Devi, & Jha, 2015), and the increase in the gluten-free food market shows no signs of stopping (Transparency Market Research, 2015), with recent data predicting the total value of the gluten-free food segment will rise to $4.89 billion by 2021, growing at a compound annual growth rate of 7.7 percent. The relative success of the gluten-free market may be due to increasing incidence of CD (Kasarda, 2013) and an increased demand from non-celiac consumers who perceive that a gluten-free diet may help in treatment of disorders such as autism, chronic fatigue, schizophrenia, attention deficit disorder, multiple sclerosis, migraine and fertility problems (Ferguson, Holmes, & Cooke, 1982; Jackson, Eaton, Cascella, Fasano, & Kelly, 2012; Shor et al., 2009; Whiteley et al., 2013). Consumers in France perceive that obesity is a symptom of gluten intolerance, which in turn has propelled the idea among consumers that a gluten free diet could help to reduce weight (Transparency Market Research, 2015). Additionally, celebrity endorsement of gluten-free and wheat-free products as part of a healthy weight loss regimen has further increased consumer interest in gluten-free products (Glutenull, 2014).

Europe is the largest regional market for gluten-free products in both revenue and volume with North America being the fastest growing market for gluten free food (Transparency Market Research, 2015). Recent data indicates approximately 15-25% of American parents actively purchase gluten-free

products for their children (Mandala & Kapsokefalou, 2011), and that the number of USA consumers who purchase gluten-free products in general is 20% (Lee, Ng, Zivin, & Green, 2007). Consumers who purchase gluten-free products have been found to do so for a number of reasons, 65% of consumers purchase gluten-free products because they consider them to be a healthier alternative, 27% for weight-loss, 7% for inflammation or gluten sensitivity and 4% to combat depression, a number of consumers purchase for a combination of these reasons (Quinteros-Fernandez, 2015).

As demand for gluten-free products increases food companies have been expanding their product portfolios to include a wider range of gluten-free foods and beverages, and improving their sensory characteristics, thus attempting to overcome the negative bias previously associated with such products (Leffler et al., 2008; Wee, 2011).

2.4.4 Bread components

2.4.4.1 Ingredients common to wheat and gluten-free breads

Water

Water is one of the main ingredients of wheat and gluten-free bread, it solubilises, rehydrates and allows for interactions between other bread ingredients, thus playing an important role in the major physical (e.g. expansion of bubbles) and chemical changes (e.g. starch gelatinisation) that occur during bread making (Wagner, Lucas, Le Ray, & Trystram, 2007). Water content and its distribution ultimately lead to important textural properties such as crumb softness, crust crispness and impacts on shelf-life (Wagner et al., 2007). Bread dough containing an insufficient amount of water is likely to be excessively firm and difficult to mould, the resulting loaves often have decreased volume and poor external characteristics (Cauvain & Young, 2009). In contrast, excessive water content results in soft doughs that are unable to retain gas from fermentation leading to bread loaves with poor crumb texture (Cauvain & Young, 2009).

The microbial and physical shelf life of a particular bread product is heavily affected by its water activity and water content. Water activity and content heavily influences the extent to which microbes are able to grow and thrive while also playing a role in the physical changes that occur during storage. During storage moisture migrates from the crumb to the crust resulting in a reduction in crumb softness, crust crispiness, and during starch retrogradation water is expelled leading to product staling (Abuajah, Ogbonna, & Osuji, 2015).

Salt

Salt has a number of functions in bread doughs such as flavour enhancement, modulation of yeast growth and fermentation rate, development of crust colour and preservation by limiting the growth of undesirable microbes during fermentation (Vivas, 2013). In wheat doughs specifically, salt helps achieve an easily workable dough, by promoting interactions between gluten proteins (Danno & Hoseney, 1982). Salt is typically added as a brine to ensure its homogenous and rapid dissolution throughout the dough, thus preventing areas of high salt concentration which result in reduced fermentation and areas of high saltiness within the crumb.

Lipids

Lipids contribute to the final products structure, flavour and palatability. In both wheat and gluten free breads, lipids have an important role in gas cell stabilization, however, this is more significant in gluten-free products due to the lack of gluten. During baking, fat crystals melt and the fat-liquid interface of the absorbed lipids provides a source of interfacial material that facilitates bubble formations, bread loaf expansion and prevents structural collapse (Czernohorsky & R, 2010; Vivas, 2013). With the rise in health conscious consumers, the baking industry has moved towards using healthier ingredients such as fractionated or inter-esterified oils to replace partially or fully hydrogenated fats (Imran & Nadeem, 2015; Vieira, McClements, & Decker, 2015).

Yeast

Yeast or baker's yeast (*Saccharomyces cerevisiae*) is a living organism which belongs to the fungi kingdom and functions as a leavening agent in bread by transforming sugars into carbon dioxide and ethanol during fermentation. Carbon dioxide produced during fermentation is trapped in the dough, causing it to 'rise' and thus increasing its overall volume while most of the alcohol produced during fermentation evaporates during the baking process (Logan & Distefano, 1998). Yeast also produces compounds which contribute to the development of bread flavour and aroma, the production of which are time-dependent with shorter fermentation times resulting in breads with reduced yeast flavour characters (Vivas, 2013).

Yeast is available in various forms including active-dried, instant and rapid-rise. Active-dried yeast is different from instant or rapid-rise yeast as the granules are coated in a protective layer which extends its shelf-life, however, due to this protective coating, active-dried yeast requires rehydration

prior to being mixed with flour (Ali, Shehzad, Khan, Shabbir, & Amjid, 2012). Instant and rapid-rise yeasts have much smaller granule sizes that lack a protective coating and thus can be sufficiently rehydrated during mixing without the additional rehydration step (Potter, 2010).

Improvers

Bread improvers are ingredients added to wheat bread and gluten-free bread formulations to 'improve' the resulting loaf's quality. Different flour formulations require different combinations and ratios of improvers and exact improver quantities and type are often closely guarded trade secrets. The main categories are as follows:

- Oxidising agents are added to improve potential gas retention of the dough; the result being a larger loaf with an improved crumb texture (Joye, Lagrain, & Delcour, 2009; Ranum, 1992). However, certain oxidising agents are banned in some regions of the world due to health concerns (e.g. potassium bromate) (Loft et al., 1998).
- Reducing agents are used in low concentrations to facilitate gliadin–glutenin crosslinking during bread making (Lagrain, Brijs, & Delcour, 2006). The addition of reducing agents weakens the dough allowing it to be processed more easily (Joye et al., 2009).
- Emulsifiers are food additives that encourage the suspension of lipids in water or vice versa, in bakery products they improve crumb texture and act as stabilisers which increase shelf life (Stampfli & Nersten, 1995).
- Enzymes fulfil and replace the roles of oxidising agents in bread and have become more prevalent as limitations on the use of oxidising agents have increased. Commonly added enzyme classes include alpha-amylases, hemicellulases and lipases (Kulp & Lorenz, 2003).

Sugars

The amount and type of sugar added to bread formulations is largely regarded as a stylistic choice, primarily affecting flavours, sweetness and crust colour. New Zealand and UK breads (along with many other countries) use very little (typically sucrose or dextrose) or no added sugar, while many American brands such as 'Wonder Bread' are relatively high in sugar (commonly from high fructose corn syrup) and possess a distinct sweetness (Cauvain, 2012). Added sugar increases caramelisation and Maillard reactions at higher temperatures, resulting in increased browning of the crust (Cauvain, 2012). Added sugar also affects yeast metabolism, which requires sugar for fermentation, however,

most flours provide the necessary quantities to produce well leavened breads without the need for added sugar. In fact, excess sugar may inhibit yeast fermentation (Cauvain, 2012).

2.4.4.2 Wheat-bread specific ingredients

Wheat flour

Wheat flour makes up the bulk of the three essential ingredients required when making wheat-based bread products (the other two being yeast and water). Wheat flour composition and quality is affected by many factors including; wheat variety, agricultural practices, growing conditions and milling practices (Cauvain & Young, 2009). A study of over 300 wheat varieties showed high variations between wet gluten content, dough stability, farinograph quality values and sedimentation values, with each of these properties influencing bread quality (Yang, Wu, Zhu, Ren, & Liu, 2014). The location in which the wheat is grown also plays an important role, as environmental factors such as rainfall, soil composition, sun exposure, humidity and disease pressure can all help determine final flour composition (Ball, Owens, & McCracken, 2013), for example, early sown wheat cultivars exposed to lower temperatures under rain-fed conditions have been shown to result in higher protein contents (Singh, Gupta, & Kaur, 2012). While the above mentioned factors are important in determining a flour's final composition, millers are able to control protein and nutrient levels by mixing flours of varying compositions (Cauvain & Young, 2009).

One of the most important compositional factors of flour is the quantity and quality of the gluten-forming proteins, generally the higher the protein quantity of flour the better it is able to trap carbon dioxide and water vapour resulting in a larger loaf volume with a softer texture. Protein quality is more ambiguous than quantity and is assessed via dough rheological tests, although, at present these tests are limited in their ability to predict bread quality (Cauvain & Young, 2009).

The level of bran present in wheat flour influences bread quality parameters with higher bran levels resulting in reduced crumb softness and decreased final loaf volumes due to bran diluting and disrupting the gluten matrix (Cauvain, 2012). There are three methods available for the analysis of bran content including grade colour figure (GCF), ash analysis or Branscan valuing (Cauvain, 2012).

The natural enzyme content of wheat flour is an important factor in bread making and is also dependent on wheat variety, agricultural practices and growing conditions. The enzymes of most concern are the alpha-amylases: this collection of enzymes are capable of breaking down damaged

starch granules into dextrins and, with the aid of beta-amylase, into maltose, thus influencing the gelatinisation rate of starches which is critical in bread making (Cauvain, 2012).

As previously mentioned, milling practices play a key role in flour quality and therefore in the resulting bread quality. When wheat grains are milled, the microscopic starch granules present are damaged to varying extents, this is important as damaged starch granules more readily absorb water gaining greater exposure to enzymes which break down starch into fermentable sugars (Stauffer, 2007). Water absorption by starches also influences the water available to gluten proteins and affects the formation of the essential gluten matrix, potentially limiting the formations of a strong elastic dough (Stauffer, 2007).

Gluten

Gluten is one of the most important constituents in wheat flour, with flours graded according to their gluten protein content (Brown, 2004). Gluten is a water insoluble protein conglomerate consisting of two cereal proteins; gliadin and glutenin (Wieser, 2007), these proteins are found at varying concentrations in wheat and related grains such as barley and rye (Zeltner, Glomb, & Maede, 2009). In the presence of water, gliadin and glutenin bond via disulphide linkages to form gluten (Figure 2.4) (Bache & Donald, 1998).

Gliadin and glutenin are both important contributors to the rheological properties of dough, though their functions are different (Gobbetti & Gänzle, 2013). Gliadins are less cohesive and have less elasticity than glutenins, and contribute mainly to the viscosity and extensibility of the dough (Gobbetti & Gänzle, 2013). In contrast, glutenins are both cohesive and elastic, therefore being mainly responsible for dough strength and elasticity (Gobbetti & Gänzle, 2013). Both proteins contribute to the viscoelastic properties needed to allow the formation of a thin, gas-retaining film and an extensible protein-starch matrix which facilitates increased dough volumes and softer crumb textures (Vivas, 2013).

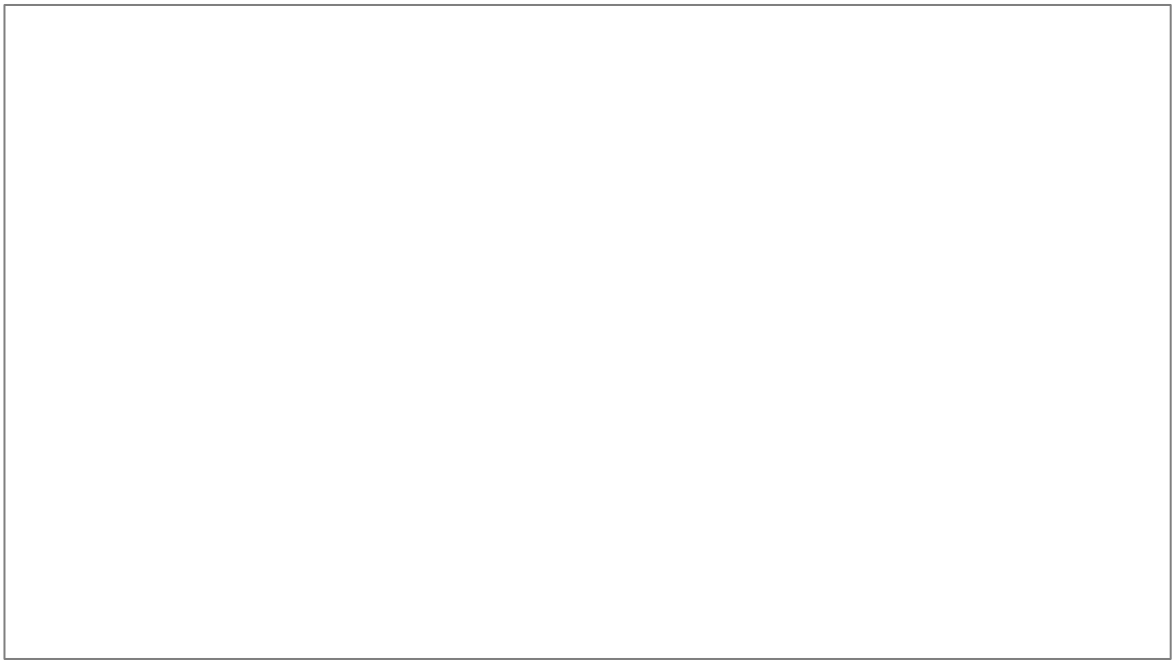


Figure 2.4 A structural model for wheat gluten showing the interactions between glutenin and gliadin subunits via disulphide bonds

Source: Lamacchia, Camarca, Picascia, Di Luccia, and Gianfrani (2014)

The development of the gluten matrix must be closely controlled as over, or under-development may result in partially aggregated gluten proteins and a stiff inelastic dough that is unable to trap gas effectively (Figure 2.5) (Amend & Belitz, 1990). Examples of factors which may affect the strength and development of a dough's gluten matrix include:

- Water content: Protein hydration is critical for the formation of a strong gluten network and the amount of water added will determine how quickly and to what extent the gluten network will form. Too little water prevents gluten formation while too much water may dilute the dough so much that protein interactions are reduced (de la Hera, Rosell, & Gomez, 2014).
- Temperature: Temperatures up to 35-40^{o}C increase gluten network formation due to increased reaction kinetics, allowing for faster gluten hydration and an increase in the interactions between gliadin and glutenin over a set time (Cuq, Boutrot, Redl, & Lullien-Pellerin, 2000).

- Sodium chloride: Sodium chloride has the effect of slowing and controlling enzyme action as well as the rate of fermentation. Sodium chloride also strengthens gluten and result in a higher volume and finer crumb texture (Danno & Hoseney, 1982).
- Mixing: Mixing influences the extent of protein interaction occurring and thus the chance of crosslink formation, and in large-scale commercial bakeries this is maximized by very high-speed mixing for relatively short periods of time (Gomez, Ferrero, Calvelo, Anon, & Puppo, 2011)
- Lipids, emulsifiers and sugars: Lipids and emulsifiers tend to coat the proteins involved in gluten development and prevent both hydration and protein interactions (Blaszczak, Fornal, & Ramy, 2004), thus slowing gluten development (Blaszczak et al., 2004). Sugar is strongly hydrophilic, thus competing for water and reducing the accessibility of proteins to hydration causing reduced gluten-network development and therefore a weaker dough (Amend & Belitz, 1990).
- Minerals: Minerals may either enhance or hinder the development of the gluten matrix depending on which minerals are present. Magnesium salts have been shown to improve bread quality possibly by facilitating the formation of cross-linkages between gluten molecules, (Charlton, MacGregor, Vorster, Levitt, & Steyn, 2007; Ranhortra & Winterringer, 1982). In contrast, calcium salts are known to reduce overall bread volume by reducing the strength of the gluten matrix (Roach, Lai, & Hoseney, 1992).
- Other factors: Wheat variety affects the initial gluten protein composition (He & Hoseney, 1992); certain enzymes may be used to encourage gluten protein interactions (Altuna, Ribotta, & Tadini, 2016); and water pH may affect the stabilisation and interactions between gluten proteins (Thiele, Grassl, & Ganzle, 2004).

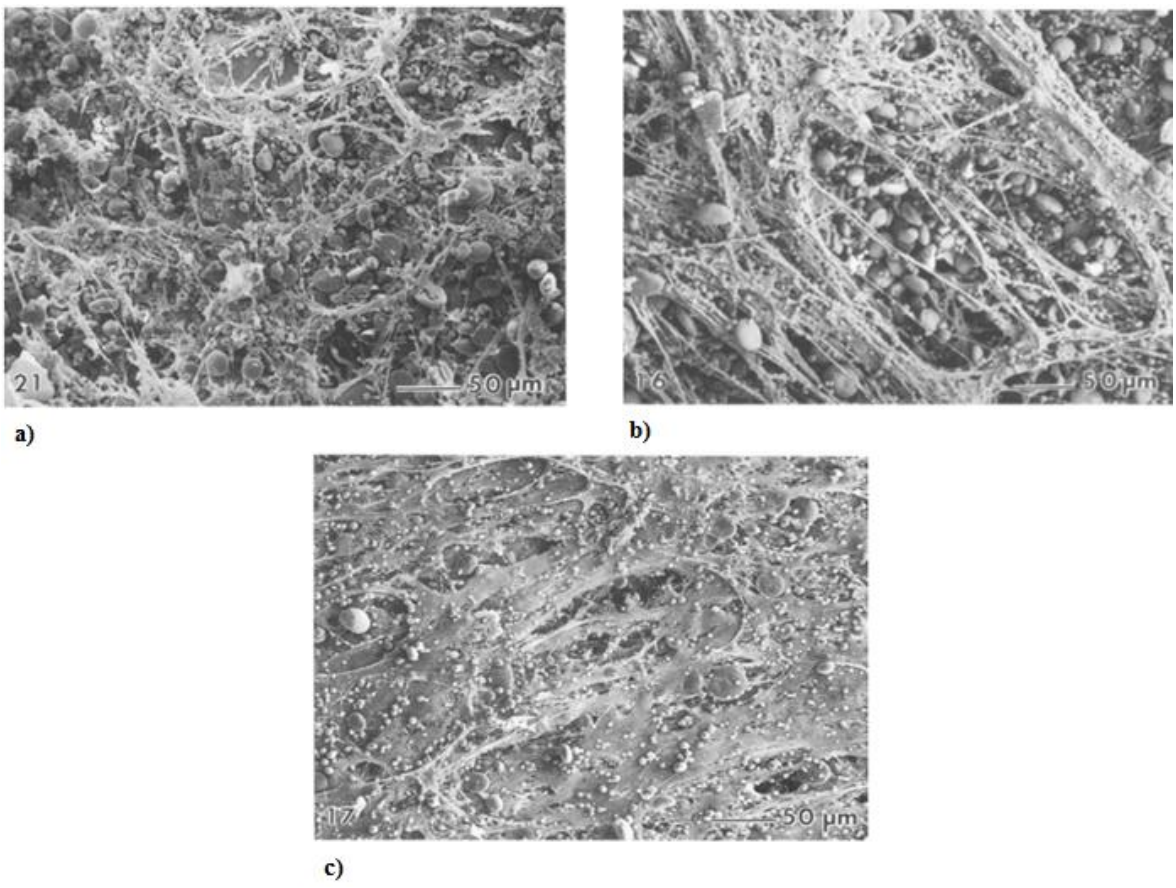


Figure 2.5 a) Underworked dough showing stretching and partial formation of gluten protein. b) Overworked dough showing stretching and partial formation of gluten protein aggregates. c) Optimally worked dough showing the cohesive formation of gluten protein aggregates.

Source: Amend and Belitz (1990).

2.4.4.3 Gluten-free bread specific ingredients

For gluten-free breads, the lack of gluten results in a viscous batter rather than mouldable doughs and therefore the inclusion of various gums, starches, and types of protein are required to emulate the viscoelastic properties of gluten (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007; Vivas, 2013). The absence of gluten also impairs the water holding capacity of the breads, often resulting in breads consisting of a dry crumb which stales quickly (Vivas, 2013).

Starch and modified starch

Starches are naturally occurring hydrocolloids found in plant matter which possess functional properties as thickeners, gel forming agents and filler ingredients, thus playing an important role in establishing the structure and mechanical properties of the food product. In gluten-free breads the inclusion of starch improves batter consistency during mixing and enhances the softness of the crumb (Sciarini, Ribotta, Leon, & Perez, 2010). To obtain these favourable effects, adequate starch hydration and gelatinisation is crucial during bread making (Chaisawang & Suphantharika, 2006). The increase in viscosity improves the ability of the batter to trap carbon dioxide thus improving the overall volume and texture of the loaf. Recent studies into the incorporation of starches in gluten-free bread formulations have indicated that maize, rice, potato or tapioca starch appear to be the most suitable for the development of gluten-free bread (Vivas, 2013; Gallagher, Gormely, & Arendt, 2004).

Tapioca starch is a useful gluten-free starch ingredient as it provides excellent thickening properties while contributing very little flavour or aromatic characteristics and produces loaves with improved volume and texture (Milde, Ramallo, & Puppo, 2012).

Modified starches are also used to improve the structure of gluten-free bread loaves and have specific advantages over natural starches (Chiu & Solarek, 2009). Starches are labelled as modified starch if they have been chemically, physical or enzymatically altered to improve functionality in certain situations. Improved functionality includes: resistance to high temperature, improved storage life, resistance to cooling and freezing (Jobling, 2004), increased control over food viscosity and increased crumb stability which retards retrogradation, thus extending shelf-life (Ziobro et al., 2012). Pre-gelatinized, hydroxylpropyl modified starches are used in the production of a range of gluten-free foods due to their ability to rapidly form slurries and pastes of high viscosity (Abdel-Aal, 2009).

Hydrocolloids

Hydrocolloids are high molecular weight compounds which promote the stabilization of emulsions, suspensions and foams, and may also be capable of gel formation (Rosell & Rojas, 2001). Bread formulations incorporate hydrocolloids as multifunctional additives, fulfilling roles such as fat replacers, water binders, texturisers, adhesives and emulsion stabilisers. In addition, they aid in the prevention of ice recrystallization and improve organoleptic properties (Dickinson, 2009). Hydrocolloid functionality depends on their source, extraction process, chemical modifications, amount included, and their interactions with other food polymers and ingredients (Vivas, 2013).

Hydrocolloids with thickening and stabilizing properties such as arabic gum, carboxymethyl cellulose (CMC), hydroxpropyl methyl cellulose (HPMC), guar gum and xanthan gum are suitable for use in gluten-free breads as they help provide a structural matrix to aid in the trapping of carbon dioxide released by the leavening agents (Velazquez, Sanchez, Osella, & Santiago, 2012). HPMC has been used to soften bread crumb, increase bread volume, improve sensory characteristics and extend shelf-life in a wide range of gluten-free bread products (Gallagher, Gormely, & Arendt, 2004; Vivas, 2013). Added hydrocolloids act as gluten replacers resulting in the formation of an elastic dough-like batter with stable air cells. This results in improved baking performances from starches and gluten-free flours (BeMiller, 2008). Additional interactions between the gums and starches present in the gluten-free formulation may lead to improved rheological and textural properties ultimately contributing to a product with high acceptability and stability (Alvarez-Jubete, Arendt, & Gallagher, 2010a). Other hydrocolloids which may be suitable for gluten-free baking include: carrageenan, alginate, locust bean gum, or a mixture of these gums.

Cereal flours

In gluten-free breads, cereal flours and starches must not contain gluten in any form, therefore cereals other than wheat, barley and rye must be utilised. The most desirable base flours are those which impart little impact on the sensory profile of the final product, with additional ingredients being added to enhance nutrition, flavour and texture if desired (Gujral & Rosell, 2004). Each of the following cereal flours will require the addition of hydrocolloids such as those described in the previous sections to act as gluten replacers (Gujral & Rosell, 2004).

- Maize flour is a commonly used wheat alternative in gluten-free bread formulations, it acts as a filler ingredient and when used in combination with additional fibres and bulking agents is able to produce a loaf of high sensory quality (Mariotti, Lucisano, Pagani, & Perry, 2009).
- Oats are another cereal commonly used in gluten-free bread, their function is to improve the nutritional quality of these starch-based breads that would otherwise lack fibre, vitamins and micronutrients (Vivas, 2013).
- Sorghum is a cereal grain recommended as a safe food alternative to wheat for celiac patients and may be used as a base flour in the production of gluten-free breads (Schober, Messerschmidt, Bean, Park, & Arendt, 2005).

- Rice flour possesses a light taste, hypoallergenic properties, easily digestible carbohydrates and low levels of sodium (Mandala & Kapsokefalou, 2011), and is therefore a suitable cereal flour to replace wheat flour in gluten-free products.

Other possible gluten-free bread ingredients

Pseudocereals resemble true cereal crops in both function and composition, like cereals, they produce starch-rich seeds that are also processed into flours, examples include: amaranth, quinoa, and buckwheat (Alvarez-Jubete et al., 2010a). There is an increasing trend in the use of pseudocereals in gluten-free bread formulations as they impart additional nutritional value to the product such as higher levels of protein, fibre, vitamins, minerals and healthy oils (Alencar et al., 2015). Aside from the added nutritional value, certain pseudocereals such as white quinoa flour have been shown to have functional properties increasing the volume of gluten-free bread by 33% when compared to rice/maize flour (Elgeti et al., 2014).

Animal protein sources and particularly dairy and egg proteins, are widely used in the bakery industry for many purposes. Gluten-free bread formulations which contain egg form a structural network which is comparable to the gluten matrix in standard white bread (Crockett, Ie, & Vodovotz, 2011). In addition, added egg proteins improve the shelf life of the loaves over control formulations (Moore, Schober, Dockery, & Arendt, 2004). Dairy proteins added to gluten-free bread formulations encourage crust browning, delay the staling process and increase moisture retention due to their increased water holding and network forming capacities (Gallagher, Gormely, & Arendt, 2004).

Legumes can be a good supplement for cereal-based foods due to their high protein content, (18-25%) which complements the nutritional value of cereal proteins (Tharanathan & Mahadevamma, 2003). Legumes also provide a good source of slow release carbohydrates and dietary fibre (Tharanathan & Mahadevamma, 2003). The addition of soya flour to gluten-free bread improves the structural properties of the loaf by increasing the consistency of batter, specific loaf volume and crumb fineness (Gallagher et al., 2004).

2.4.5 The science and technology of bread making

2.4.5.1 Yeast activation & sugar salt solution preparation

Creating an active yeast suspension is the first step in bread making. Yeast may be purchased in a number of forms, the most common being active-dried yeast which is the most resilient form available. However, active-dried yeast must be reactivated prior to mixing to ensure an even distribution of live cells and thus a uniform gas cell structure. Yeast can survive and thrive in a relatively wide temperature range but for fast activation the water temperature should ideally be between 30^{o}C and 35^{o}C. Typically, a portion of the water to be used in the recipe is poured into a vessel with the yeast, covered and set aside for at least 10 minutes to allow the yeast to activate.

2.4.5.2 Mixing and kneading

Mixing is the first significant step in the production of any bread product. When the ingredients are combined they form a quasi-homogenous mixture which develops into a three dimensional dough matrix (Autio & Laurikainen, 1997). Mixing causes physio-chemical changes to occur in the ingredients which leads to the development of an elastic dough, these changes begin with the solubilisation, hydration and homogenisation of ingredients and their components.

Proper mixing results in the hydration of, and interaction between the gluten precursor proteins glutenin and gliadin. Inadequately mixed doughs have a rough appearance, contain compacted protein masses and tear easily due to their low extensibility which results in them being unable to effectively trap gasses (Autio & Laurikainen, 1997). Doughs which are over-mixed become firm, difficult to handle and tear easily, they are also unable to effectively trap gasses (Cauvain, 2012). It is not necessary to mix and knead gluten-free doughs and batters to the same extent as standard wheat bread doughs as they do not require the development of a gluten matrix, rather optimum hydration of hydrocolloids and the homogenous dispersion of ingredients is the primary concern (Cauvain, 2012; Vivas, 2013).

In traditional wheat bread making kneading is a step which occurs after mixing and each fermentation rest period (Mondal & Datta, 2008), with typical bread recipes requiring three kneading steps and two rest periods prior to the final proofing stage (Buehler, 2006). The first kneading stage is primarily to encourage the development of the gluten network, with subsequent stages aiming to evenly distribute the gas cells being produced by yeast fermentation (Stauffer, 2007). The gluten network will be

optimally developed via kneading when the dough transforms from a rough 'shaggy' appearance that tears easily, to a smoother more elastic appearance and feel (Cauvain, 2012). The windowpane test, which is a technique used by bakers to determine when a dough has been adequately kneaded (Campbell Todd, personal Communication, 25 February, 2016, Bakels NZ), is carried out by flattening and stretching the dough with the fingers until it is translucent, if this is not able to be achieved without the dough tearing it has not passed the test and requires further kneading (Limongi, Simões, & Demiate, 2012).

2.4.6.3 Fermentation

Fermentation is a broad term for the bulk growth of micro-organisms on/in a medium that results in the production of a wide range of compounds (Stear, 1990). In bread, fermentation is carried out by baker's yeast, *Saccharomyces cerevisiae,* or in the case of naturally fermented sourdoughs, lactic acid bacteria and naturally occurring yeast species (Stear, 1990). The most important reason for the addition of yeast to bread is its function as a leavening agent via the production of carbon dioxide, with yeast strains for bread baking being selected in part due to their ability to produce CO_2 (Cauvain & Young, 2007).

The major factors in bread fermentation are the fermentation temperatures, number of fermentative stages and their length. Temperature influences and is influenced by the rate of fermentation. External heat from a proofing oven and internal heat as a by-product of biological activity of yeast increases the rate of growth and fermentation of the yeast biomass (Stear, 1990), however, depending on the strain, yeast cells are only able to survive and reproduce up to temperatures as high as 45^{o}C (Cauvain, 2012). The optimum temperature range for yeast growth and fermentation is also strain-dependent, with temperatures of 27 - 32^{o}C generally being acceptable, temperatures lower than this range may result in reduced fermentative capacities (Cauvain, 2012).

The total fermentation time from mixing to just prior to baking increases the quantities of compounds produced by the yeast, such as CO_2, organic acids, aroma compounds and enzymes, of these the most important is CO_2. Fermentation times must be carefully selected to produce optimal levels of CO_2 as insufficient or excessive amounts may result in a loaf possessing poor textural qualities (low loaf volume, hard crumb textures, and unattractive external loaf characters).

2.4.6.4 Dividing and shaping

Prior to proofing, the dough is divided and scaled at a certain weight into discrete units which are then moulded into their final shape. Dough shaping is a stylistic choice and may be carried out in a number of ways, for example dough may be shaped in baking tins for a symmetrical loaf or into complicated weaves and braided patterns. The selected scale weight and shape of the loaves will influence its baking requirements, in general more spherical, heavier loaves require longer baking times at lower temperatures to ensure the cold point is adequately cooked without burning the crust (Cauvain, 2012).

2.4.6.5 Proofing

Proofing is the final phase of fermentation which occurs prior to baking and after the shaping stage. Temperatures of proofing (between 25-45 ^{o}C) and humidity (at least 65%) must be monitored closely during this stage as the external layer of the dough is at risk of drying out, which may result in cracks developing in the crust of the finished loaf (Mondal & Datta, 2008). Proofing times and temperatures vary depending on the bread product being produced and must be optimised for the specific recipe being followed (Therdthai, Zhou, & Adamczak, 2002) to avoid over- or under-proofing. Under-proofed products will fail to rise to their full potential and result in a loaf possessing a dense crumb texture due to the lack of CO_2 production (Cauvain & Young, 2007). Over-proofed doughs rise too much, resulting in a weakened dough structure that may collapse during baking, it is associated with a number of faults including; oversized and unevenly distributed gas cells, air pockets below the crust, an overly fluffy crumb texture, and a dense deflated loaf (Cauvain, 2012).

2.4.6.6 Baking

Baking converts unpalatable dough into an edible product with improved organoleptic and nutritive properties via biological, physical and chemical reactions such as the formation of a stable porous structure, increases in volume, protein denaturation, starch gelatinization, water evaporation and crust formation (Figoni, 2008; Mondal & Datta, 2008). The baking process also destroys most living microbes thus increasing the products shelf life (Therdthai et al., 2002).

Times and temperatures must be monitored and closely controlled during baking and both depend upon a number of variables including: oven capacity, physical dimensions of the dough and the type of bread being baked (both the formula used and desired loaf properties) (Cauvain, 2012; Therdthai et al., 2002). For standard white loaves baking times vary between 20 and 25 minutes, with oven temperatures beginning at 200°C, before rising to between 220°C and 235°C (Figoni, 2008). Steaming the oven prior to, or just after insertion of the dough is performed in order to raise the relative humidity of the oven to 100%, thus reducing the likelihood of the crust forming too quickly and developing cracks during oven spring (Cauvain, 2012). The baking process is divided into three main phases determined by loaf temperatures:

1. Oven spring: the enzymatically active zone, internal loaf temperatures are between 30 and 60/70 °C
2. Gelatinisation of starches: temperatures between 55 and 60 °C to no higher than 90 °C
3. Browning and aroma formation: temperatures above 100 °C (Quail, McMaster, Tomlinson, & Wootton, 1990)

Oven spring is the final increase in loaf volume which occurs in the first several minutes of baking and occurs for a number of reasons, including the expansion of the already present gas cells after exposure to heat, conversion of liquid water to water vapour and temporary increases in fermentation rate (Figoni, 2008). Following oven spring as the temperature of the loaf continues to rise, proteins begin to denature and starches gelatinise, while proteins and sugars on the developing crust undergo Maillard and caramelisation reactions to develop colour, aroma and flavour compounds (Rada-Mendoza, Garcia-Banos, Villamiel, & Olano, 2004). Internal crumb temperature should never exceed 100°C, the boiling point of water (Haegens, 2014). During the initial third of the baking time, the crust typically reaches temperatures of around 150°C, ultimately reaching 180°C or higher by the end of the baking time.

2.4.6.7 Cooling and packaging

Bread is cooled after baking in order to stop the cooking process prior to packaging and reduces the amount of condensation forming within the packaging material, thus reducing the growth of spoilage organisms (Edwards, 2007). Rapid cooling is important to minimise losses in moisture content, which helps maintain loaf quality and also minimises weight loss (Piazza & Masi, 1995). Bread loaves must

be cooled to below 35°C before slicing and wrapping can occur without causing damage to the loaf (He & Hoseney, 1990).

Packaging is vital in increasing a products shelf life, reducing microbial growth and preventing contamination of the product from outside substances. All packaging materials must minimise or control moisture loss by providing a functional barrier and be able to withstand transportation and storage (Giannou, Kessoglou, & Tzia, 2003). There are a diverse range of materials available for bread packaging such as modern antimicrobial materials and modified atmosphere packaging. Typically, commercial wheat bread packing consists of unsealed polyethylene bags which allows moisture to leave the product and packaging, thus minimising internal package humidity and reducing the growth of microbes (Hotchkiss & Appendini, 1998).

2.4.6 Gluten-free bread production

2.4.6.1 Introduction

The production of gluten-free bread differs significantly from the production of wheat bread and although a number of the same stages are carried out, times and conditions differ considerably (Figure 2.6). Gluten-free bread often has liquid batters, making it difficult to follow a traditional wheat bread production process. Mixing and proofing times are also shorter than wheat bread due to the lack of an elastic dough structure, while baking is often performed at lower temperatures for longer periods of time (Moore et al., 2004)

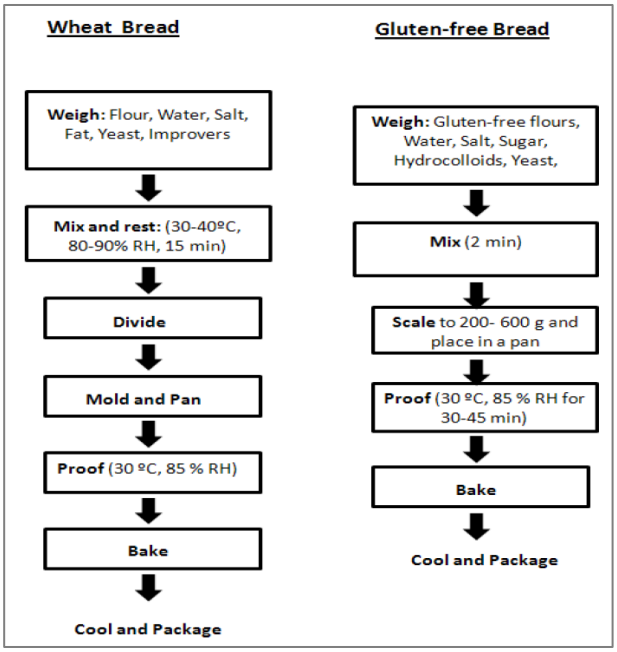


Figure 2.6 Wheat and gluten-free baking processes

Source: Ardent et al. (2008).

2.4.6.2 Basic gluten-free formulation

The selection of a wheat flour alternative is one of the most important steps in gluten-free bread formulation. Common flours used to replace wheat include corn, soybean, rice and potato. Rice flour in particular is an excellent base ingredient to use in the production of gluten-free bread as it possesses suitable sensory and nutritional properties, most notably being colourless and having a neutral flavour profile (Gujral & Rosell, 2004).

Starches are often included in gluten-free bread formulations in order to improve batter consistency during mixing, crust texture and crumb softness. Modified tapioca starch is an ideal starch to use in gluten-free bread formulation due to its functional rheological properties, neutral sensorial aspects, desirable viscoelastic and moisture retention properties during baking which contribute towards increasing overall bread quality (Takizawa, Silva, Konkel, & Demiate, 2004).

Gluten-free formulations also require polymeric substances, such as hydrocolloids, that contain viscoelastic properties which provide structure and aid in gas retention (Milde et al., 2012). Gluten-free breads consisting of the hydrocolloid hydroxypropyl methylcellulose (HPMC) with a base of rice flour have been shown to produce loaves with excellent sensory attributes and loaf characteristics

(Cato, Rafael, Gan, & Small, 2004; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). The use of carboxy-methylcellulose (CMC), xanthan and guar gum in gluten-free breads has also been shown to increase bread volume and soften crumb textures (Sabanis & Tzia, 2010). Lazaridou (2007), reported that the addition of 1% carboxymethylcellulose (CMC) significantly increased the bread volume of gluten-free bread based on rice flour, corn starch and sodium caseinate. The addition of 1% and 1.5%, HPMC and guar gum respectively to gluten-free bread formulations have resulted in larger loaf volumes, increased crumb softness, and richer crust colour compared to control samples. Shelf life was also reported to be increased in breads containing HPMC and guar gum due to the improved moisture-retention capabilities of the resulting loaves (Sabanis & Tzia, 2010). Sensory evaluation by a trained panel revealed a preference for bread containing 1.5% HPMC when compared to xanthan, κ-carrageenan and guar gum (at 1%, 1.5% and 2%) due to its impact on loaf volume, appearance and softness characteristics (Sabanis & Tzia, 2010).

2.4.7 Shelf life and spoilage

The micro-organisms responsible for microbial spoilage in bakery products are fungi, yeast and bacteria (Cauvain & Young, 2009). The growth of these spoilage microbes and therefore, the stability of the food product is dependent on intrinsic and extrinsic factors (Steele, 2004). Intrinsic factors are characteristics of the food itself which include: initial microbial load, physical and chemical properties such as pH, sugar content, water content and activity, acidity, and preservative content (Jay, 2000). The water activity at which micro-organisms grow in various foods is shown in Table 2.6. Extrinsic factors are those that refer to the environment surrounding the food, they include atmospheric conditions (temperature, humidity, gas contents) and factors relating to product contamination by microbial organisms (FDA, 2001) such as processing, packaging, and handling of the product post-packaging (Steele, 2004).

Table 2.6 Water activities at which various pathogenic and spoilage microorganisms grow

Microorganisms grow at this a_w and above	Food examples
0.95 Pseudomonas, *Escherichia, Proteus, Shigella, Klebsiella, Bacillus, Clostridium perfringens*, some yeasts	Highly perishable foods (fresh and canned fruits, vegetables, meat, fish), milk, cooked sausages, breads, foods with up to 4 oz sucrose or 7% NaCl
0.91 Salmonella, Vibrio parabaemolyticus, C. botulinum, Lactobacillus, some molds	Some cheese (Cheddar, Swiss, Provolone), cured meat, fruit juice concentrates with 55% sucrose or 12% NaCl
0.87 Many yeasts, Candida, Torulopsis, Hansenula micrococcus	Fermented sausage, sponge cakes, dry cheese, margarine, foods with 65% sucrose or 15% NaCl
0.80 Most molds, most *Saccharomyces* spp., *Debaryomyces, Staphylococcus aureus*	Most fruit juice concentrates, condensed milk, syrup, flour, high-sugar cakes, pulses containing 15-17% moisture
0.75 Most halophilic bacteria, Mycotoxigenic aspergilli	Jam, marmalade, glace fruits, marzipan, marshmallows
0.60 Osmophilic yeasts, few molds 0.50 0.40	Dried fruits with 15-20% moisture, caramel, toffee, honey Noodles with 12% moisture, spices with 10% moisture Whole egg powder with 5% moisture
0.03	Whole milk powder with 2-3% moisture, dehydrated soups

Source: Beuchat (1981)

Fungi involved in bread spoilage include the filamentous fungi *Rhizopus and Mucor spp*., *Penicillium spp., Eurotium spp, Aspergillus spp*. and *Monilia (Neurospora) sitophilia* (Saranraj & Geetha, 2012). *Rhizopus stolonifer* is the most common bread mould and is often referred to as the 'bread mould' (Jay, 2000). One of the most important concerns regarding spoilage in bakery products is the production of fungal mycotoxins, which in severe cases, may lead to nervous system failure and death (Saranraj & Geetha, 2012).

Yeast spoilage is also common in bakery products and is caused by either fermentative or filamentous yeasts (Legan & Voysey, 1991). Fermentative spoilage is most often the result of bakers' yeast (*Saccharomyces cerevisiae*) and occurs due to fermentation of the remaining sugars found in the product. The spoilage they cause is typically displayed via the production of 'alcoholic' or 'estery' off-odours which vary based on which yeast is causing spoilage (Legan & Voysey, 1991).

Filamentous yeasts are known for forming a white, spreading growth on the surface of the bread which is often referred to using the misnomer 'chalk mould' despite it being classified as yeast (Legan & Voysey, 1991). *Pichia butonii* is the most common of these yeasts and has the ability to grow very fast while being more resistant than other organisms to various preservatives and disinfectants (Legan & Voysey, 1991).

Microbes belonging to the genus *Bacillus* are responsible for the majority of bacterial spoilage of bread (Bailey & Vonholy, 1993). *Bacillus subtilis* is the most common cause of ropiness, however *B. lichenformis, B. megaterium* and *B. cereus* have also been known to be associated with ropy bread (Bailey & Vonholy, 1993; Kirschner & Vonholy, 1989; Rosenkvist & Hansen, 1995; Sorokulova et al., 2003; Thompson, Waites, & Dodd, 1998). Signs of spoilage caused by bacteria can show up as quickly as 12-24 h after the loaf has left the oven and its presence initially manifests itself as a sweet fruity odour reminiscent of rotting pineapple (Bailey & Vonholy, 1993; Kirschner & Vonholy, 1989; Thompson et al., 1998) or rotting melons (Rosenkvist & Hansen, 1995). *Bacillus* bacterial spores are relatively heat resistant and may survive the baking process or be introduced during post baking processes (Kirschner & Vonholy, 1989; Rosenkvist & Hansen, 1995).

2.6 Analysis techniques

Techniques for assessing bread quality fall into three broad categories: internal characteristics, external characteristics and texture/eating quality (Cauvain, 2012). External characteristics include specific volume, dimensions, appearance and crust colour. Internal characteristics are limited to the sizes, number and distribution of gas cells in the crumb (crumb grain), crumb colour and any major quality defects, such as unwanted holes or dense patches visible in a cross-section of the product. Texture and eating qualities include factors such as crumb firmness, chewiness and resilience which may be evaluated by a texture analyser. However, eating quality relies entirely on subjective analysis and therefore is typically carried out via consumer sensory evaluations.

The majority of analyses conducted are instrumental in nature as they produce objective results that may be obtained quickly and with a high degree of reproducibility (Cauvain, 2012). Sensory testing, is just as vital in the product development process and is used to gain an insight into consumer opinion on the developed formulations (Cauvain, 2012).

2.6.1 Texture

Texture is one of the most important quality factors in bread, it plays an important role in determining consumer preferences and product acceptability due to consumers associating bread texture with freshness and quality (Cauvain, 2012). Methods of texture analyses fall into two categories, sensory testing and instrumental testing. Sensory testing benefits from the relatability and ease of interpretation of results, however, it is expensive and time consuming to hire/train reliable panellists, therefore it is often limited to the final analysis (Winopa, Drobny, & Schneider-Häder, 2015). Instrumental analysis is often preferable during the initial and main product formulation stages due to its reliability, repeatability, and fast turnaround time, a typical output of a textural profile analysis is shown in Figure 2.7 (Abbott & Lu, 2004). The downside of instrumental testing methods are that it is more difficult to correlate their results with real world meaning, although, as more research is conducted in this field the association between instrumental data and sensory perception becomes clearer (Winopa et al., 2015).

Multiple instruments have been developed to aid bakers in understanding the correlation between texture in both the dough, and final loaf, and the attributes sought after by consumers (Hoye & Ross, 2011). A texture analyser is an advanced tool capable of testing a product for a wide range of force variables. Figure 2.7 shows an example of a cylinder probe attached to a texture analyser commonly used to determine crumb texture. The texture analyser is able to be set to a number of different testing parameters including, penetration distance, force exerted by the probe, probe travel speed and multiple compression and expansions (Brady & Mayer, 1985). Samples may be subjected to a number of controlled conditions in either the form of stress (force per unit area) or strain (deformation relative to initial size) (Abbott & Lu, 2004). Stresses are classified as either normal stresses (perpendicular to the sample) or shear stress where the force is applied at an angle. Testing for texture in food products is mostly focussed on assessing normal stresses, for example when testing for crispiness in potato chips or crumb softness in breads, while testing shear forces is relevant to a narrower range of products including cheeses, snack bars and chewing gum (Abbott & Lu, 2004). Additionally, stresses may be classed as either tensile (force acting to pull or separate a sample) or compressive (a force acting to push and squeeze a sample) (Adhikaria, Howesb, Bhandaric, & Truongc, 2001). Of these two stresses, compression testing is more common in food texture analysis as it more closely imitates mastication (Adhikaria et al., 2001). Compression testing of bread is able to concurrently measure hardness, chewiness, cohesiveness, springiness and resilience as defined in Table 2.7.

Figure 2.7 Texture analysis of white bread with 36mm cylinder probe attachment

Retrieved 6th December 2016; from http://www.foodtechcorp.com/aacc-cylinder-probe

Table 2.7 Texture parameters used in the texture profile analysis of bread

Parameter	Sensory definition	Instrumental definition
Hardness (g)	Force required to compress food between the molars. Also known as the force necessary to attain a given deformation.	Peak force of the compression cycle
Chewiness (g)	The energy required to chew a solid food to the point required for swallowing	The product of hardness, cohesiveness and springiness
Cohesiveness*	The strength of internal bonds making up the body of the product	The area of work during the second compression divided by the area of work during the first compression
Springiness*	The amount a product springs back to its original shape following compression	Sample height after the first compression divided by initial height
Resilience*	Measurement of how well a sample resists deformation	It is calculated by dividing the upstroke energy of the first compression by the downstroke energy of the first compression.

Note: * Cohesiveness, Springiness and Resilience are dimensionless parameters

Source: (Bourne, 1978; Szczesniak, Brandt, & Friedman, 1963)

2.6.2 Colour

A food product's appearance consists of a number of basic visual attributes including colour, gloss, opacity, visual structure, visual texture and perceived flavour (Imram, 1999). Colour is the most well-studied of the visual attributes (Imram, 1999). Colour, along with the other visual aspects of a product is often the first parameter a consumer will notice, therefore its role in consumer acceptance is one of the most important (Imram, 1999). The simplest method of colour measurement is via visual inspection, whereby a researcher notes down a product's colour as accurately as possible. Whilst this method is fast, cheap, and easy, it is also unreliable, prone to human error and extremely subjective. Other, more accurate, methods utilise a colourimeter to accurately measure the colour of a product (Leon, Mery, Pdreschi, & Leon, 2006).

There are numerous systems which exist to measure and quantify colour including the Red, Green, Blue (RGB) colour model, the Cyan, Magenta, Yellow, Black (CMYK) colour model, the Pantone colour space, the natural colour space, and the CIE Lab colour space. Each has their advantages and disadvantages, and the most applicable in the scientific setting is CIE Lab colour space (Caivano & Pilar Buera, 2012).

The CIE *L*a*b** colour space, as shown in Figure 2.8, utilises three values which equate to coordinates in the corresponding colour space. The "*L**" axis indicates the lightness, with 100 being pure white light, 0 being pure black. The "*a**" axis is the red-green axis, negative values are green, positive values are red. Similarly the "*b**" axis is the blue-yellow axis, negative values are blue and positives values are yellow. For both the "*a**" and "*b**" scales a value of zero indicates no colour from that axis (Jha, 2010; Kress-Rogers & Brimelow, 2001). In the measurement of bread colour, the crust and the crumb are distinguished. The crust is noticeably darker due to higher temperature exposure causing Maillard browning (Pour-Damanab, Jafary, & Rafiee, 2012).

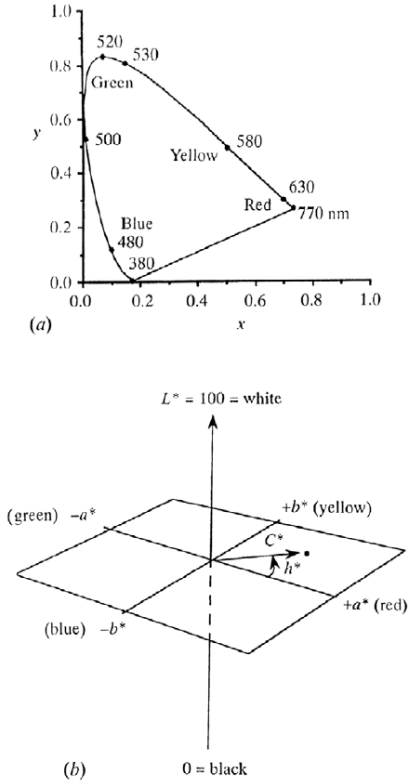


Figure 2.8 Colour diagrams: (a) CIE 1931 chromaticity diagram showing non-uniformity of spacing of red, yellow and blue unique hues; (b) CIELAB uniform diagram showing relationship of red/green (*a**./ÿ) and yellow/blue.

Source: MacDougall (2002)

2.6.3 Loaf volume

Loaf volume is influenced by a number of parameters including ratios and variety of ingredients present (Lorenz & Coulter, 1991; Nakamura, Suzuki, & Ohtsubo, 2009; Zhang et al., 2007), mixing processes (Ktenioudaki, Butler, & Gallagher, 2010), baking conditions (Faridi & Rubenthaler, 1984) and fermentation schedules (Aplevicz, Ogliari, & Sant'Anna, 2013). These parameters affect the dough's ability to produce and retain gasses; mostly prominently by interacting with leavening agents and the elastic protein polymer gluten (Amend & Belitz, 1990). Ingredients which disrupt or enhance the formation of a strong gluten matrix will influence final bread volume.

The two main methods of determining bread loaf volume are the use of a laser-based 3-dimensional scanner, and the more traditional method of seed displacement (AACC International, 2001; Smewing, 2016). Laser volume analysis allows for rapid 3-dimensional digitisation of products and automatic

calculation of several detailed dimension related parameters, albeit at a high initial equipment cost (Smewing, 2016). Volumetric bread loaf measurement by rapeseed displacement involves measuring the volume of seeds displaced by the addition of the test loaf to a container of a known size (AACC International, 2001).

2.6.6 Sensory evaluation

Sensory evaluation involves the measurement, analysis and interpretation of participant responses to characteristics of foods as they are perceived by human senses (sight, smell, taste, touch, and hearing) (Meilgaard, Civille, & Carr, 1991). Numerous sensory evaluation methods are available and are divided into three categories based on the information desired: descriptive tests, discrimination tests and affective tests. The selection of the type of sensory test and specific method is determined by the required information and specific properties of the food being tested (Brady & Mayer, 1985).

Affective tests are used for measuring the liking and preference for a product with paired comparison and the 9-point hedonic ratings being two commonly used methods (Verbeke, 2006). Paired comparison tests determine the preference between two samples, while the 9-point hedonic scale is able to measure the extent to which consumers like and accept the product (Lawless & Heymann, 2010). The 9-point scale is useful as it is easily understood method by consumers and has been demonstrated to be reliable and valid for the purposes of assessing product acceptability (Rosas-Nexticapa, Angulo, & O'Mahony, 2005). The 9-point scale utilises 9 verbal categories the consumer is able to select for each question, the responses range from ‘dislike extremely’ to ‘like extremely’ (Lawless & Heymann, 2010). For statistical analysis and data representation the verbal categories are converted into their corresponding numerical value, 1 for dislike extremely’ and 9 for ‘like extremely’. Standard statistical analysis such as ANOVA is then able to be carried out to provide valuable information pertaining to differences in appearance, texture, aroma, taste, and overall acceptability between products (Lawless & Heymann, 2010; Villanueva, Petenate, & Da Silva, 2005).

2.6.4 Moisture and water activity

Water is extremely important in bread making as its presence throughout a loaf largely determines the microbial shelf life and bread textural qualities such as dryness, hardness, chewiness and crispiness. Water activity (a_w) is a key characteristic in the determination of the shelf life of bread and

is defined as the ratio between the vapour pressures of pure water and the product being analysed with pure water having a water activity of 1 (Troller & Christian, 1978). The water activity of various breads ranges between 0.80 – 0.98 (Troller & Christian, 1978) and is most commonly measured instrumentally using a water activity meter (Rahman, 2007).

2.6.6 Microbial growth

As discussed in Section 2.4.7 due to their relatively high water activities (~0.95) bakery products are susceptible to a range of pathogenic and spoilage micro-organisms (yeasts, fungi and bacteria). The number of microbes present in a food sample may be analysed via the use of plate count methods which involve diluting colony-forming units (CFUs) with a diluent solution (e.g. sterile peptone water), plating them onto a number of possible growth media, incubating the plates at a constant temperature for a set period of time and counting the plates that contain between 30 and 300 colonies (Atlas, 2004). The growth media used determines which species of micro-organisms will be able to grow on the plates, with two of the main types of media being non-selective and selective media (Jay, 2000). Non-selective media have a basic nutrient composition which contain all the elements that most bacteria need for growth and does not inhibit any particular species, common formulations consists of peptone, yeast extract, glucose, salt and agar (Atlas, 2004). The Aerobic Plate Count method uses plate count agar (a non-selective media) and is one of the most commonly used methods in determining the total viable cell count of a food product (Blackburn, 2006). Selective media differ from nutrient media, as in addition to the base nutrient composition they also contain compounds which inhibit the growth of certain types of organisms (Atlas, 2004). The inclusion of the broad spectrum antibiotic chloramphenicol in yeast-glucose-chloramphenicol (YGC) agar is one example of a media able to selectively prevent the growth of bacteria, thereby allowing colonies of fungi to develop and be enumerated (Atlas, 2004), it is particularly useful in the determination of yeasts and mould contamination in bakery products (Nakhchian, Yazdi, Mortazavi, & Mohebbi, 2014). In order for food products to be deemed safe to be consumed in New Zealand, CFUs per sample must not exceed levels listed in the Microbiological Reference Criteria for Food (Ministry of Health, 1995), for example in ready-to-eat foods such as bread, no more than two out of five samples may exceed CFUs of 10^5 as determined by the Aerobic Plate Count method (Ministry of Health, 1995).

2.7 Summary

In summary, mozuku powder contains a number of key constituents which, when included in a bread formulation may have a negative or positive effect on a loaf's quality parameters, for example a high mineral content (typical in seaweed products) may decrease yeast fermentation, thus reducing CO_2 production and therefore final loaf volume. However, the presence of long-chain polysaccharides and dietary fibres in mozuku powder may enhance certain bread characteristics such as chewiness and softness by reducing the amount of water lost during baking. The underlying chemical and biological processes that occur during bread making are affected by many factors, and therefore the addition of a novel ingredient such as mozuku powder is likely to alter the development of a loaf's key structural bread components whilst also influencing its sensorial profile. The solute concentration and water availability are two important factors in a bread dough, which affect the hydration and function of components (starches, gluten, hydrocolloids and yeasts) that influence the physical and sensorial characteristics of the bread loaf. The inclusion of mozuku powder in bread is also likely to impact its nutritional content by introducing additional minerals, dietary fibres and the sulphated polysaccharide, fucoidan, which has been demonstrated by a number of studies to have a range of bioactive properties including antitumoral, anti-inflammatory, and antioxidative effects. Future research is required in order to determine whether fucoidans health effects will be retained in bread following the bread-making process. This study aims to clarify the effects mozuku powder has on the physical and sensory properties of white-wheat and gluten-free bread formulations by analysing a number of important bread quality parameters and conducting consumer sensory evaluations.

3 Materials and Methods

3.1 Introduction

In the manufacture of bread, recipe formulation, dough preparation, and baking conditions are important in the development of products with desirable physical, chemical and sensory qualities (Therdthai & Zhou, 2003). This chapter describes the experimental design, formulation of two types of breads, characterisation of the produced products and the methods of analyses. The study was conducted in three stages.

The first stage involved an analysis of nutrient composition of mozuku powder and its mesh size. The nutritional analyses included proximate analysis (moisture, ash, protein, fat, carbohydrate, total dietary fibre), sugar, sodium, energy content, fatty acids and amino acids.

The effect of mozuku powder in a standard wheat bread formulation was investigated in stage two. Standard wheat breads were formulated containing different levels of mozuku. Samples of baked wheat bread containing mozuku were analysed for physical characteristics (texture, colour, volume, water activity, bake loss) and evaluated by consumer sensory evaluation. The samples were also analysed for total aerobic plate count and yeasts and moulds.

Stage three involved the development of a base gluten-free formulation with added mozuku powder. Similar analyses conducted in stage two were also applied to products produced in stage three. The base formulation then had mozuku added at various inclusion rates with adjustments to salt levels. The gluten-free formulations with added mozuku were again compared to a commercial gluten-free loaf and one formulation proceeded to consumer sensory evaluation.

3.2 Ingredients used in bread formulations

Freeze dried mozuku powder was supplied by Glenorie International (Auckland, New Zealand). The fucoidan content of the dried mozuku powder was specified by the supplier to be 22.7% (Appendix C). Ingredients for wheat bread listed in Table 3.1a were purchased from a local supermarket chain (Pak n' Save, a subsidiary of Foodstuffs North Island Limited, Auckland, New Zealand). Gluten-free

ingredients and hydrocolloids in Table 3.1b were supplied by Venerdi Limited (Auckland, New Zealand). The nutritional compositions of the ingredients were obtained from the nutrition information panel and are listed in Appendix C.

Table 3.1a Ingredient composition of wheat bread

Ingredient	% Flour (w/w)
Flour	100
Water	66
Yeast	5
Salt	Variable
Sugar	5
Soy oil	3
Mozuku powder	Variable

Table 3.1b Ingredient composition of gluten-free bread

Ingredient	% Flour (w/w)
Rice flour	Variable
Modified tapioca starch	Variable
HPMC	Variable
Guar gum	Variable
Xanthan	Variable
CMC	Variable
Soy oil	12
Sugar	6
Salt	Variable
Yeast	Variable
Water	Variable
Mozuku powder	Variable

Note: HPMC = Hydroxypropyl Methyl Cellulose

3.1 Analysis of mozuku powder

3.1.1 Nutritional analysis

Nutritional content of mozuku powder was analysed at the Nutrition Laboratory, Massey University Palmerston North, New Zealand using reference and standard methods listed in Table 3.2. Analyses of the powder comprised of the determination of: 16 amino acids, 38 fatty acids, proximate analysis (moisture, ash, protein, fat, carbohydrate, total dietary fibre), sugar, sodium, and energy content (Appendix C).

Table 3.2 Analytical methods used in the analysis of the nutritional content of mozuku powder

Nutritional Component	Method	Reference
Amino acids	High-performance liquid chromatography	(Rutherfurd & Moughan, 1998)
Ash	Incineration of sample and weighing of remnants	(AOAC International, 2012a)
Dietary fibre	Megazyme enzymatic and chemical analysis	(AOAC International, 2012b)
Energy	By calculation according to Schedule 11 of the Food Standards Code	(FSANZ, 2015)
Fatty acids	Gas-liquid chromatography analysis of fatty acid methyl esters	(Sukhija & Palmquist, 1988)
Moisture	Weight loss on drying	(AOAC International, 1999)
Nitrogen	Leco CN analyser followed by the calculation of crude protein with a 6.25 multiplication factor	(AOAC International, 2005)
Sodium	Inductively coupled plasma emission spectroscopy	(AOAC International, 2007)
Sugars	Phenol sulfuric acid assay	(Hall, Hoover, Jennings, & Webster, 1999)
Total fats	Mojonnier gravimetric acid hydrolysis	(AOAC International, 1977)

3.1.2 Mesh size analysis of mozuku powder

The mesh size of freeze-dried mozuku powder was measured using a set of eight sieves on a sieve shaker (Octagon D200, London, UK) following the ASTM standard C136 - 01 (ASTM International, 2007). Completion of sieving was determined following the recommendations of the National Institute of Sieving Technology (Jillavenkatesa, Dapkunas, & Lum, 2001). The aperture of the sieves ranged from 45 μm to 710 μm. Each empty sieve was weighed on a Sartorius top-pan balance (CP4202s, Goettingen, Germany) and then assembled into a stack of decreasing aperture size with

largest aperture on the top and a collection plate on the bottom. A sample of mozuku powder (200 g) was weighed into the top sieve, covered with a lid and the stack of sieves placed on the sieve shaker. The sieve shaker was set to vibrate on medium intensity for 10 min. After shaking, the lid was removed and each sieve (with powder particles) was weighed. The lid was replaced and all sieves shaken for an additional 5 min. Sieves (with powder particles), were weighed again and the difference in powder particle weight was calculated. The sieving process was repeated in 5 min increments until the difference between the weights of the particles retained in each sieve was less than 1%. The results were plotted in a graph to indicate the size distribution of the powder (Advantech Manufacturing, 2001; HORIBA Instruments, 2016).

3.2 Production of wheat bread with added mozuku powder

3.2.1 Approach

3.2.1.1 Experimental design

Figure 3.1 overviews the four main stages involved in the development of wheat bread containing mozuku and its analysis (loaf quality testing, consumer evaluation and microbial stability testing). The four main stages comprised of: base formulation, addition of mozuku, consumer sensory evaluation and testing for microbial stability during storage.

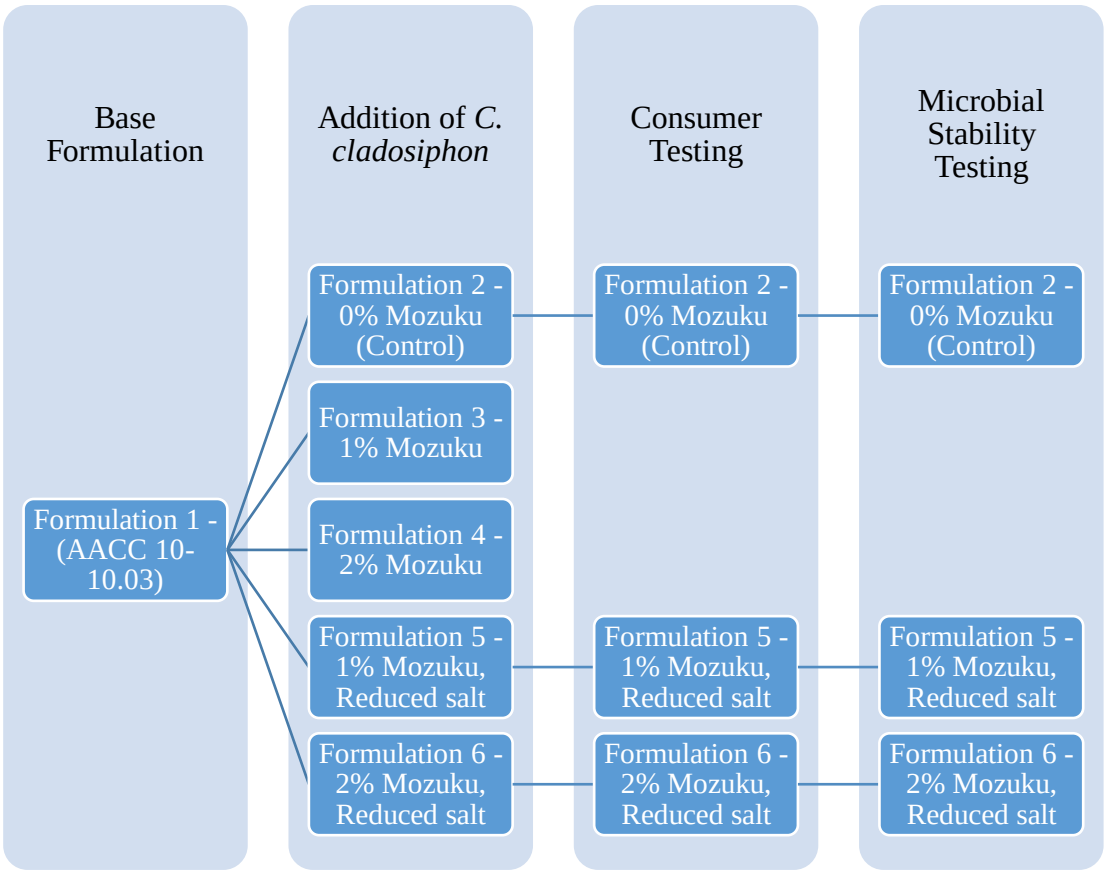


Figure 3.1 Overview of the experimental procedures used in the development and testing of wheat bread containing mozuku powder

3.2.1.1 Base formulation

The method for the production of a standard wheat bread loaf was based on the standard AACC International method 10-10.03 (AACC International, 1999a). The standard did not state amounts of water required to be added to the bread formulation. Therefore, the amount of water used in the formulation was obtained from previous studies (Phillips, 2000; Scott & Wing, 1999). Table 3.3 shows the wheat bread formulations prepared according to procedures described in Section 3.2.3.

Table 3.3 Base formulation for standard wheat bread

Ingredient	% Flour (w/w)	Weight (g)
Flour	100	300
Yeast	5	15
Salt	1.5	4.5
Sugar	5	15
Soybean oil	3	9
Water	66	198

3.2.1.2 Addition of mozuku powder to standard white wheat bread

In stage 2, mozuku was added at 0% (control), 1% and 2% (w/w baker's percentage). Selected concentrations of added mozuku were based primarily on cost – ideally, to keep the concentration low so that the final product remained affordable for low income populations. At the same time, the final product with mozuku should contain additional nutritional benefits over a standard wheat loaf while retaining acceptable bread quality parameters. Formulations 2, 3 and 4 as shown in Appendix A, were produced following procedures described in Section 3.2.3 and tested according to the bread quality parameter tests set out in Section 3.4.

3.2.1.2 Adjustment of sodium chloride concentration in wheat bread formulation containing mozuku

Formulations 5 and 6 were formulated with added mozuku and adjusted salt concentrations by removing 0.5% table salt for every 1% mozuku added (baker's percentages). Wheat bread formulations 5 and 6 as shown in Appendix A, were produced following procedures described in Section 3.2.3 and tested according to the procedures set out in Section 3.4.

3.2.3 Production of wheat bread

3.2.3.1 Dough mixing

All the ingredients were weighed using a Sartorius top pan balance (CP4202s, Goettingen, Germany) according to each formulation (Appendix A). Two 100 mL portions of potable water (30°C) were weighed into two 500 mL plastic beakers, sugar and salt were added to one beaker of warm water

(30°C) and stirred with a spoon until dissolved. The active dried yeast was added to the remaining beaker, stirred and allowed to activate for 10 min. Flour and mozuku powder were weighed (Sartorius CP4202s, Goettingen, Germany) into an 8-L Delta Planetary Mixing bowl (Delta 8L Planetary Mixer, Delta Faucet, New Zealand) and a well was formed in the centre of the flour to contain the liquids. The sugar-salt solution, activated yeast solution, remaining water and soybean oil were then added to the pocket in the flour. To mix the ingredients, a dough hook was attached to a planetary mixer (Delta 8L Planetary Mixer, Delta Faucet, New Zealand) and used to mix the dough to an optimum dough consistency as determined by the windowpane test. During the windowpane test a small sample of dough (30 – 50 g) was removed by hand and rolled into a ball. The rolled dough was then held between the thumb and forefingers and gently spread into a thin translucent membrane as shown in Figure 3.2, the sample was then return to the dough. If the dough did not tear during spreading, the dough had been sufficiently kneaded (Campbell Todd, personal Communication, 25 February, 2016, Bakels NZ). Optimum mixing time for dough development using a Delta 8L Planetary Mixer, (Delta Faucet, New Zealand) was determined to be 13 min at setting 1, followed by 1 min at setting 2.

Figure 3.2 Windowpane testing: Progressive spreading of dough to a thin translucent membrane

Retrieved: 12th December, 2016 from: http://flour.co.uk/images/blog-posts/212.jpg

3.2.3.2 Dough fermentation, proofing and shaping

After mixing, the dough was transferred to a lightly greased stainless steel bowl capable of accommodating a volume increase of at least 300%. The bowl with mixed dough was covered with plastic food wrap (Gilmours Cling Wrap 380 mm x 300 M, Gilmours Ltd, NZ) and the dough was left to rise at ambient temperature (20°C) for 50 min. The dough was then removed from the bowl and placed onto a clean stainless steel bench that had been lightly dusted (sprinkled) with flour. The dough was 'punched down' using an open palm and a wooden rolling pin to flatten it to a thickness of about 5 mm (AACC International, 1999a). The flattened dough was then folded in half twice and placed back into the stainless steel bowl, covered with plastic food wrap and left to rise for an additional 25 min at ambient temperature, following which the dough was removed from the bowl and the punch down step was repeated. The dough was then placed back into the bowl for a further 13 min, and then removed for shaping to the size of the bread pan (220 x 120 x 60 mm). The shape dough was placed 'seam down' into a lightly greased 500 g bread pan and covered with aluminium foil (Gilmours foil 440 mm x 90 M, Gilmours Ltd, NZ). The dough was then allowed to proof at ambient temperature for 35 min, thus completing bread dough preparation.

3.2.3.3 Baking, cooling and packaging

A convection oven (Turbofan 32Max, Moffat Pty Ltd, New Zealand) was preheated to 200°C and then steamed for 10 seconds prior to baking (Le-bail, Dessev, Leray, Lucas, & Mariani, 2011). Following removal of the aluminium foil covering the bread pans, the bread pans containing the dough were baked for 25 min. The bread loaves were removed from the bread pans and placed onto cooling racks to cool for 3 hours at ambient temperatures (20°C) before being packaged. Each loaf was packaged in a perforated polyethylene bread bag (350 x 445 mm) and stored in a slightly aerated container at ambient room temperature (20°C) out of sunlight prior to bread quality testing.

3.2.3.4 Analyses of wheat bread

Samples of wheat bread were analysed using standard methods described in section 3.4, 3.5 and 3.6. The samples were analysed for colour, texture, and water activity, and bread volume was determined. Consumer sensory evaluation was carried out on the products using a 9 point hedonic scale. Total

plate counts and, yeasts and moulds were also analysed. All analysis took place within 12 hours of baking, except in the case of the microbial stability testing which took place after 1, 2 or 3 days of storage.

3.3 Development and production of gluten-free bread with added mozuku powder

3.3.1 Experimental design

The experimental design used in the development and analysis of gluten-free bread containing mozuku was conducted in three main stages: base formulation, addition of mozuku and consumer sensory evaluation. Figure 3.3 shows the progression of the experiments.

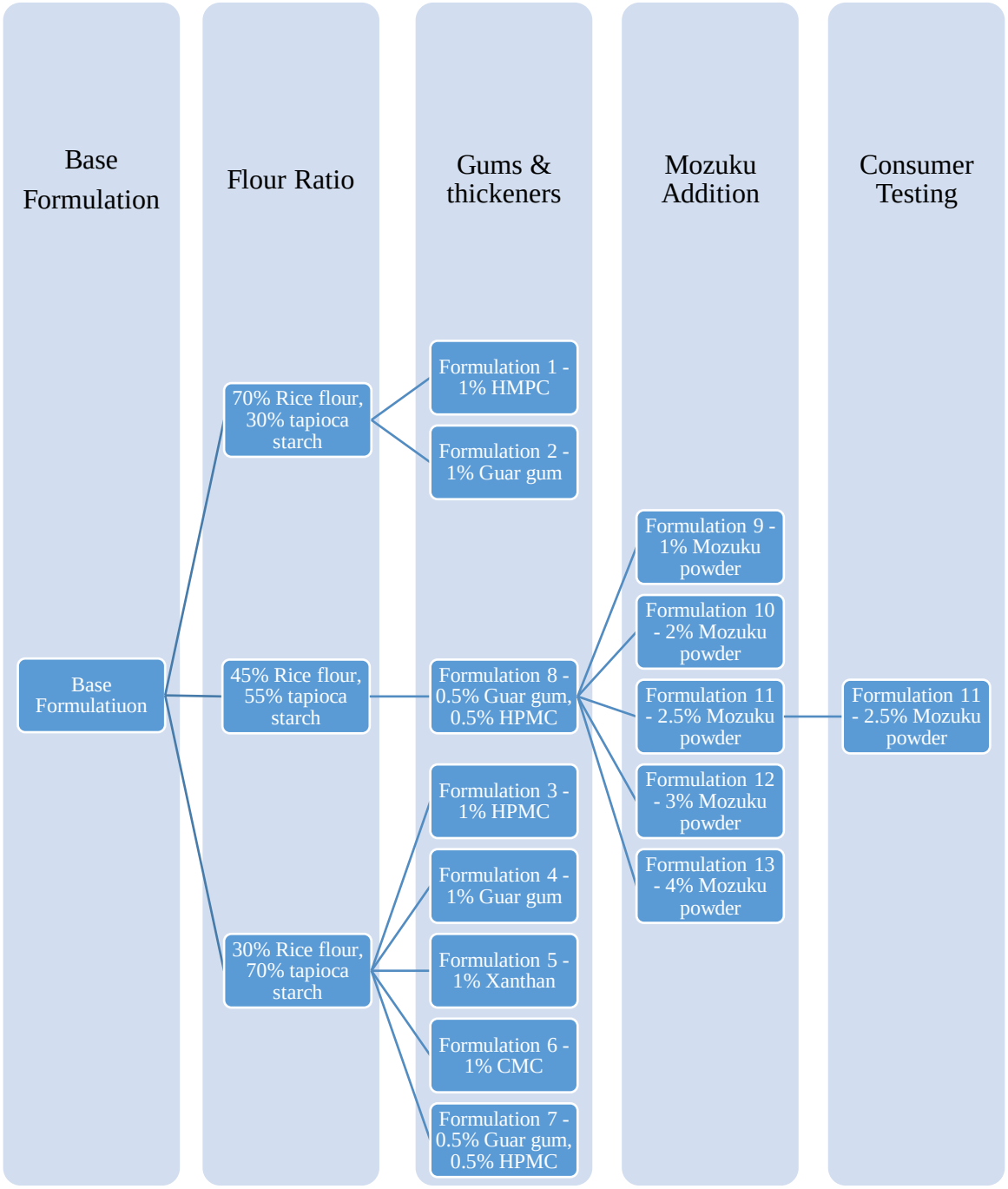


Figure 3.3 Overview of experimental procedures used in the development and testing of the gluten free bread containing mozuku

3.3.1.1 Base formulation

The aim of this stage was to develop a basic gluten-free bread formulation suitable for as a control and as a base to which mozuku powder could be added. Eight formulations were developed for testing based on previous studies reviewed in Section 2.4. Formulations varied in ratios of rice flour to modified tapioca starch as well as the types and quantities of hydrocolloids added (HPMC, xanthan, CMC, and guar gum). An overview of each formulation is shown in Table 3.4. A list of ingredients present in each gluten-free formulation is located in Appendix A. The best formulation was determined by comparing GF bread developed in this study to a commercial gluten free loaf (Vogel's Gluten-Free and Dairy Free Bread, Goodman Fielder New Zealand Ltd, Auckland, New Zealand). Formulation 8 was considered the best and was therefore used for experiments containing mozuku.

Table 3.4 Base formulations of gluten-free bread

	% flour		Thickener added per 100g of flour (g)			
Formulation	Rice flour	Tapioca starch	HPMC	Guar gum	Xanthan gum	CMC
1	70	30	1	1	0	0
2	70	30	0	0	0	0
3	30	70	1	0	0	0
4	30	70	0	1	0	0
5	30	70	0	0	1	0
6	30	70	0	0	0	1
7	30	70	0.5	0.5	0	0
8	45	55	0.5	0.5	0	0

3.3.1.2 Addition of mozuku powder to gluten-free bread formulation

In this stage Mozuku was added (0 – 4% w/w flour) to formulation 8 selected in the previous section. Added salt levels were adjusted and yeast concentrations were increased to 3.5% (bakers' percentage) to improve loaf volume and texture (Lai, Davis, & Hoseney, 1989).

3.3.2 Production of gluten-free bread

3.3.2.1 Preparation of batter and proofing

Ingredients were weighed using a Sartorius top pan balance (CP4202s, Goettingen, Germany) according to each formulation (Appendix A). One hundred mL (100 mL) potable water (30°C) was weighed into a 500-mL plastic beaker. Active dried baker's yeast was added to the beaker with water, stirred and allowed to activate for 10 min. The remaining ingredients were then were weighed and transferred into an 8-L Delta Planetary mixing bowl (Delta Food Equipment, Port Coquitlam, Canada) and mixed using a beater attachment for 1 min at medium speed, followed by 2 min at high speed. The batter was scaled at 600g and placed in a lightly greased bread pan (22 x 12 x 6 cm), covered with aluminium foil and proofed for 50 min at ambient temperature (20 °C).

3.3.2.2 Baking, cooling and packaging

The convection oven (Turbofan 32Max, Moffat Pty Ltd, New Zealand) was preheated to 190°C and steamed for 10 seconds prior to baking (Le-bail et al., 2011). The aluminium foil covering the bread pans was removed and the gluten-free batter was baked for 45 min. Following baking the loaves were removed from the bread pans and placed on cooling racks to cool for at least 3 hours at ambient temperature (20 °C). After cooling, bread loaves were packaged in perforated polyethylene bread bags (350 x 445 mm). The loaves were stored in a slightly aerated container at ambient temperature (20°C) out of sunlight, and all bread quality tests were carried out within 12 hours

3.3.2.3 Analyses of gluten-free bread

Samples of gluten-free bread were analysed using standard methods described in Sections 3.4 and 3.5. The samples were analysed for colour, texture, and water activity, and bread volume was determined. Consumer sensory evaluation was carried out on the products using the 1-9 point hedonic scale.

3.4 Tests conducted on wheat and gluten-free breads

3.4.3 Determination of specific volume

Bread loaf volume was determined based on the rapeseed displacement method (AACC International, 2001). Loaves were weighed after cooling (3 hours), using a Sartorius top pan balance (CP4202s, Goettingen, Germany). For testing, a loaf was placed into a sturdy plastic container of known volume (30cm x 30cm x20cm) large enough to encompass it completely. The container with the loaf was then placed on a large stainless steel tray (for catching wayward seeds). The container was then filled to excess with rapeseeds using a wide-mouth funnel. A straight edge was used to level the surface of the seeds in the container. The seeds were then carefully dispensed out of the container into a 1L graduated cylinder and the volume was recorded. The loaf volume was then calculated as the difference between seed volume and container volume. Specific volumes were calculated by dividing the loaf volumes by loaf weights (AACC International, 2001).

3.4.1 Texture profile analysis –Double Compression Test

The texture profile analysis of bread samples was conducted using a Stable Micro Systems, Texture Analyser (Stable Micro Systems Surrey, United Kingdom). The texture analyser was fitted with a 31mm aluminium cylindrical probe and the procedure was based on the AACC International Method 74-09.01 (AACC International, 1995). An additional compression phase was included to measure the springiness, resilience and chewiness of bread samples. Each sample loaf was cut into slices of 25mm thickness using a bread knife. Three slices approximately equidistance apart along the loaf were selected for analysis. Samples were placed under the centre of the probe and double compression testing was carried out to determine the following texture attributes: hardness, cohesiveness, springiness, adhesiveness, and chewiness.

3.4.2 Analysis of crust and crumb colour

Analysis of colour was conducted on bread loaves using a Minolta CR-300 Chroma meter (Konica Minolta, Osaka, Japan) based on the methods of Angioloni and Collar (2009). The equipment was used following manufacturer's instructions and the data was recorded in the CIE Lab colour space (Jha, 2010). The device was calibrated using a white reference tile with known colour value (L^* =

97.59, a^* = -5.00, b^* = +6.76) by selecting the calibration option on the device, placing it against the tile and running the analysis. Measurements of crust colour were obtained at three selected positions approximately equidistance apart on the top surface of the loaf. Crumb colour measurements were taken in the centre of three chosen slices for each sample loaf.

3.4.4 Determination of water activity

Analysis of water activity of the bread crumb was carried out using an Aqualab water activity meter (Decagon Devices, Pullman, USA), using the method of Czuchajowska, Pomeranz, and Jeffers (1989) with minor modification. Prior to measurement, the device was calibrated with SAL-T standards (humidity 90%). For each loaf, three slices (6-7 mm thick), were cut from the bread. From each slice, a cylinder of crumb approximately 3cm in diameter was removed from the centre using a knife and placed into a sample dish (40 mm diameter x 12 mm deep) supplied with the equipment. The dish was inserted into the device and the water activity was measured.

3.5 Consumer sensory evaluation

3.5.1 Introduction

The aim of the consumer sensory evaluation was to determine the level of likeness of baked bread samples containing variable concentrations of Mozuku powder. Separate consumer sensory evaluations were conducted for both wheat and gluten-free bread types. Bread samples containing Mozuku were evaluated by consumer sensory panellists for texture, aroma, taste, appearance and overall acceptability (Janz, 2003).

3.5.1.1 Sensory evaluation of wheat and gluten-free breads

Sensory testing procedures were based on the methods of Resurreccion (1998) and Meilgaard et al. (1991). The sensory evaluations were carried out at Massey University's sensory laboratory (Albany, New Zealand), in a climate-controlled sensory evaluation laboratory equipped with separately partitioned booths. Consumer sensory participants were randomly recruited from around the campus by email, display boards and posters (Appendix B). This project was evaluated by peer review and

judged to be ethically of low risk, consequently, it was not reviewed the University's Human Ethics Committees (Appendix B). Loaves were baked a day prior to sensory evaluation using formulations found in Appendix A. Wheat bread was baked following procedures set out in Section 3.2.3, while gluten free bread was baked according to methods described in Section 3.3.2. Each bread formulation was assigned a random three digit number. Prior to sensory evaluation, each loaf was sliced into 1.25 cm thick slices and each slice was cut into quarters. Samples were placed in coded disposable plastic containers and presented to participants with distilled water (for palate cleansing). Participants were asked to taste each sample and complete a questionnaire consisting of six questions per sample (Appendix C). Samples of bread were evaluated for appearance, texture, aroma, taste, and overall acceptability on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). In addition, for the wheat bread samples, consumer panellists were requested to indicate their preferred sample of the three presented and for the gluten free bread sample, panellists were also asked to respond to a question on purchase intent.

3.6 Microbial stability

Analysis of microbial growth in wheat bread was based on AACC International method, 42-11.01 (AACC International, 1999b) and Isong, Akpan, Udota, and Barber (2013). Microbial analysis took place over the course of three days. On days one, two and three each bread loaf was tested in duplicate for total aerobic plate count and, yeasts and moulds.

On day zero (the day prior to the first analysis), 18 loaves were prepared, six loafs of each wheat bread formulation (formulations 2, 5 and 6 see Appendix A) were baked according to procedures set out in Section 3.2.3, labelled and stored at ambient temperatures (20°C). On each day of analysis, two loaves per formulations were randomly removed from storage for microbial testing. A breadcrumb sample was removed from the centre of each loaf and weighed using a top pan balance to 10±0.01 g (PB 1502, Mettler Toledo, Columbus, USA). Samples were then placed into separate stomacher bags (LABPLAS, Quebec, Canada) with 90 mL of 0.1% peptone solution (Merck, Darmstadt, Germany) and homogenized for 60 sec to give a 10^{-1} dilution. Tenfold serial dilutions were then prepared up to 10^{-6} using 0.1% peptone solution. Aliquots of 1 mL of each dilution were plated onto 12-15 mL of warm (45 - 48°C) (Harley & Prescott, 1996) plate count agar (PCA) (BD Diagnostics, Sparks, MD, USA) and yeast extract glucose chloramphenicol agar (YGC) pour plates (Merck, Darmstadt, Germany). The plates with PCA were incubated (IM 1000, Clayson Laboratory Apparatus Pty Ltd, Australia) for 48 hours at 35°C (AACC International, 1999b) while the plates with YGC were

incubated at 25°C (IM 1000, Clayson Laboratory Apparatus Pty Ltd, Australia) for 3 days (Tournas, Stack, Mislivec, Koch, & Bandler, 1998). After incubation, only plates with between 30 and 300 colonies were counted and recorded. Results were expressed as colony forming units per gram of bread crumb (CFU / g). All materials and equipment used for microbiological analyses were sterile.

3.7 Analysis of data

Data analyses were conducted using Minitab version 16 (Minitab Inc., PA, USA) and Microsoft Excel version 14.0.0 (Santa, CA, USA). Data was analysed by one way analysis of variance (ANOVA) with a reliability of 95% ($P<0.05$). A post hoc analysis using Tukey's multiple comparisons test was conducted where applicable to separate samples into statistically significant groups. Descriptive statistics were also used to analysis data from the mesh size analysis, microbial analysis and sensory data for gluten-free bread.

4 Results and Discussion

The results and discussion in this chapter are divided into the three following sections: mozuku powder analysis (nutritional and mesh size analysis), investigation into the effects of mozuku powder on a standard wheat bread recipe with and without an adjustment in salt levels, and the development and incorporation of mozuku powder into a gluten-free bread formulation.

4.1 Analysis of mozuku powder

4.1.1 Analysis of nutritional content

4.1.1.1 Energy, sugar, sodium and proximate analysis

Proximate analysis (moisture, ash, protein, fat, total carbohydrate, and fibre) and tests for energy, sugar and sodium content were conducted at the Massey University Nutrition Laboratory (Palmerston North, NZ), the results of these analyses are shown in Table 4.1.

Table 4.1 Macronutrient analysis of freeze-dried mozuku powder

	Moisture %	Ash %	Protein %	Fat %	Available Carb %	TDF %	Total Carb %	Energy KJ/100 g	Sugar g/100 g	Sodium g/100g
Dry Basis	13	53.1	6.1	1.1	5.3	34.4	39.7	209.1	0.15	21.5
Wet Basis (As is)	11.5	46.9	5.4	1.0	4.7	30.4	35.1	209.1	0.13	19

Note: TDF = Total Dietary Fibre

Results from the moisture analysis indicated that the mozuku powder ingredient used in this thesis contained a moisture content of 11.5%, which is within the recommended range (<35%) to prevent quality deterioration during periods of storage (Tiroba, 2006).

The analysed mozuku powder (Table 4.1) contained an ash content of 53.1% (dry basis) which is relatively high when compared to other brown seaweeds such as *Undaria pinnatifida* (26.58%) (Smith, Summers, & Wong, 2010), however it is still within the range of ash contents for those shown

in Table 4.2 (7.72 – 55.11 %). The ash percentage of a food product indicates its mineral content and may include potassium, sodium, calcium, magnesium, aluminium, iron, copper, manganese, zinc, arsenic, iodine, fluorine and any other elements present after the material has been ignited at a high temperature (typically above 500 °C). Due to the high ash content of the tested mozuku powder, it is recommended that a mineral assay be carried out to determine the exact quantities of minerals present, as certain minerals such as calcium and magnesium have been shown to have an effect on bread quality parameters (Roach et al., 1992).

Table 4.2 Proximate chemical analysis of different seaweed species reported by various authors on a dry matter basis

Species	Protein	Carbohydrate	Lipid	Fiber	Ash	Moisture
Ulva rigida[a]	6.40	18.10	0.30		52.00	
Gelidium pristoides[a]	11.80	43.10	0.90		14.00	
Caulerpa racemosa[b]	3.98	3.60		1.36	55.11	
Sargassum filipendula[b]	8.72	3.73		6.57	44.29	
Gracilaria cornea[b]	5.47	36.29		5.21	29.06	
Ulva lactuca[c]	7.06	14.60	1.64		55.40	10.60
Hypnea japonica[c]	19.00	4.28	1.42			9.95
Porphyra tenera[d]	34.20	40.70	0.70	4.80	8.70	
Enteromorpha sp.[e]	9.45				36.38	9.00
Gracilaria cervicornis[f]	22.96	63.12	0.43	5.65	7.72	14.33
Sargassum vulgare[f]	15.76	67.80	0.45	7.73	14.20	14.66

Values are given as percent of dry matter.
[a] Foster and Hodgson (1998).
[b] Robledo and Freile-Pelegrin (1997).
[c] Wong and Cheung (2000).
[d] Arasaki and Arasaki (1983).
[e] Aguilera-Morales et al. (2005).

Source: Marinho-Soriano, Fonseca, Carneiro, and Moreira (2006)

The results from the nitrogen analysis show that the protein content of the mozuku powder was 6.1% (dry basis), which is on the lower end of the reported range of protein contents for seaweeds (4-47%) (Ito & Hori, 1989; Marinho-Soriano et al., 2006). The protein content of dried seaweeds varies greatly between species, as can be seen in Table 4.2 which displays the protein content of a number of seaweed species with protein levels ranging from as low as 3.98% for *Caulerpa racemose* to as high at 34.20% in *Porphyra tenera* (Marinho-Soriano et al., 2006).

The total carbohydrate content of the mozuku powder was 39.7% (dry basis), this falls within the reported carbohydrate content range of the seaweed species shown in Table 4.2 (3.6 % - 67.8 %). Studies have shown that there is an inverse relationship between the protein and carbohydrate content

present in marine algae (Marinho-Soriano et al., 2006), our results appear to support this, as relative to other seaweed species, the analysed mozuku's protein content is relatively low while its carbohydrate content is much higher.

The lipid content of the mozuku powder (1.1%) is comparable to the lipid content of other seaweeds found in Table 4.2 (0.30 % – 1.64 %) and is also in agreement with a study which showed that wild and cultured *C. okamuranus* had a lipid content (dry basis) of 0.9 and 1.2 % respectively (Saito, Xue, Yamashiro, Moromizato, & Itabashi, 2010).

The total dietary fibre content (30.4%) of the tested mozuku powder exceeds the other species shown in Table 4.2, which may be due to the high fucoidan content in mozuku powders (Appendix C) as fucoidan is classified as a dietary fibre (Rupérez & Saura-Calixto, 2001). Dietary fibre increases the nutritional value of bread while also altering the rheological properties of dough and the quality and sensorial properties of bread (Gómez, Ronda, Blanco, Caballero, & Apesteguía, 2003). Previous studies have shown that increasing dietary fibre in bread formulations increases water absorption in the dough and bread loaf shelf life (Gómez et al., 2003).

Results from the sodium content analysis show the mozuku powder contains 19% sodium, which is relatively high for a food ingredient, as pure table salt (NaCl) has a sodium content of 39.22%. The reason for the high sodium level is likely to be due to salt-water residues remaining after processing and thus being present on the external surface of seaweed material. The processing methods used for this particular mozuku powder focus on maximising the quality and yield of fucoidan present in the dried product, as a consequence the rinsing and washing of the raw seaweed is minimised in order to preserve the fucoidan found on the exterior surface of the plant (Eluvakkal, Sivakumar, & Arunkumar, 2010). When used as an ingredient in bakery products the sodium content of the mozuku powder may be offset by reducing added NaCl. However, the high sodium content does restrict the quantity of seaweed able to be added as there is a limit to the amount of NaCl able to be removed from formulations and generally only small quantities of sodium are included in bread due to its negative effects on fermentation.

4.1.1.2 Amino acid analysis

An amino acid analysis was conducted on mozuku powder in order to assess whether its inclusion in breads would fortify them with substantial levels of individual amino acids. The results shown in Figure 4.1 indicate the highest levels of the tested amino acids were aspartic acid, glutamic acid,

alanine and leucine. Other studies have shown that the most abundant amino acids found within seaweed proteins are aspartic acid, glutamic acid and alanine, but also glycine (Černá, 2011). Certain species of seaweed such as *Porphyra tenera* have been shown to be particularly rich in protein and amino acids, with proteins contents ranging from 33 - 47% of dry matter (DM), these levels are comparable to foods typically considered high in protein such as eggs (49.6% DM) and milk (13.8% DM) (USDA, 2016). However, results show the mozuku powder's protein content to be comparatively lower at 6.1% which indicates it is unlikely to fortify bread with substantial levels of amino acids at the estimated inclusion rate of 5g of mozuku powder/loaf. If the main objectives were to increase a bread loaf's protein and amino acid content then, the addition of red seaweeds such as *P. tenera* would be more suitable (Černá, 2011).

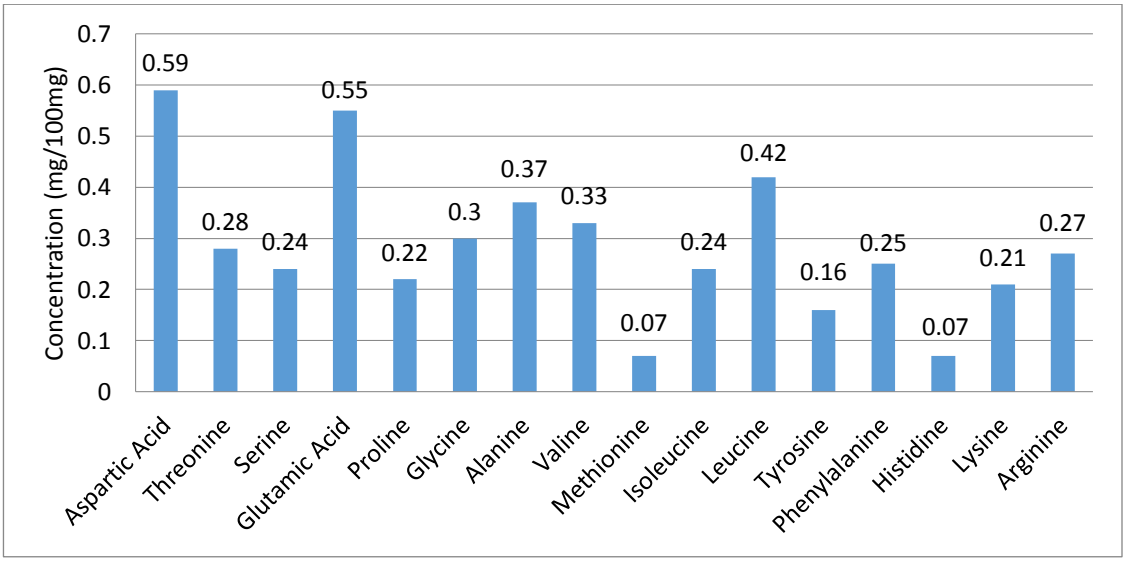


Figure 4.1 Amino acid content of freeze-dried mozuku powder (wet basis)

4.1.1.3 Fatty acid profile analysis

Free fatty acids (FFA) were measured in mozuku powder by analysing the fatty acid methyl ester content using gas-chromatography in order to investigate whether mozuku powder provided FFAs at sufficient levels to positively impact human health. The results of the analysis showed that in total, nine fatty acids were identified in the mozuku powder (Figure 4.2), with C16:0 (palmitic acid) being the most abundant, accounting for 68.8% of all fatty acids. Other studies have also shown that palmitic acid is the most abundant FFA in a variety of seaweed species, such as canned *Saccorhiza polyschides*

(42.14%) and *Himanthalia elongate* (36.73%); dried *H. elongate* (32.53%), *Laminaria ochroleuca* (28.51%) and *Palmaria sp.* (45.44%) (Sanchez-Machado, Lopez-Cervantes, Lopez-Hernandez, & Paseiro-Losada, 2004). The mozuku powder also contained the essential fatty acids C18:2ω6 (linoleic acid) and C18:3ω3 (linolenic acid) which, when consumed in high enough quantities have been shown to possess beneficial bioactive effects on human health (Simopoulos, 1999). A study by Saito et al. (2010) into the fatty acid composition of wild mozuku grown in Okinawa, Japan (*C. okamuranus*) reported lower ratios of C16:0 (20.5 % compared to 68.8 %) and C18:1ω9 fatty acids (8.1 % compared to 12.5 %) when compared to the results obtained in this thesis, this difference may be due to genetic and/or environmental factors as the mozuku powder used in this thesis grew wild in the Tongan Islands (Nelson, Phleger, & Nichols, 2002). Overall, due to the low lipid concentration (1.1g / 100g) of the mozuku powder any fatty acids present are unlikely to benefit health by any substantial amount. Based on an inclusion rate of 5g of mozuku powder per loaf, only 55mg of lipids would be added and according to the Institute of Medicine (2002) the recommended daily intake for adults 19-50 years of age is 17g (male) / 12g (female) for linoleic and 1.6g (males) / 1.1g (female) for linolenic acid.

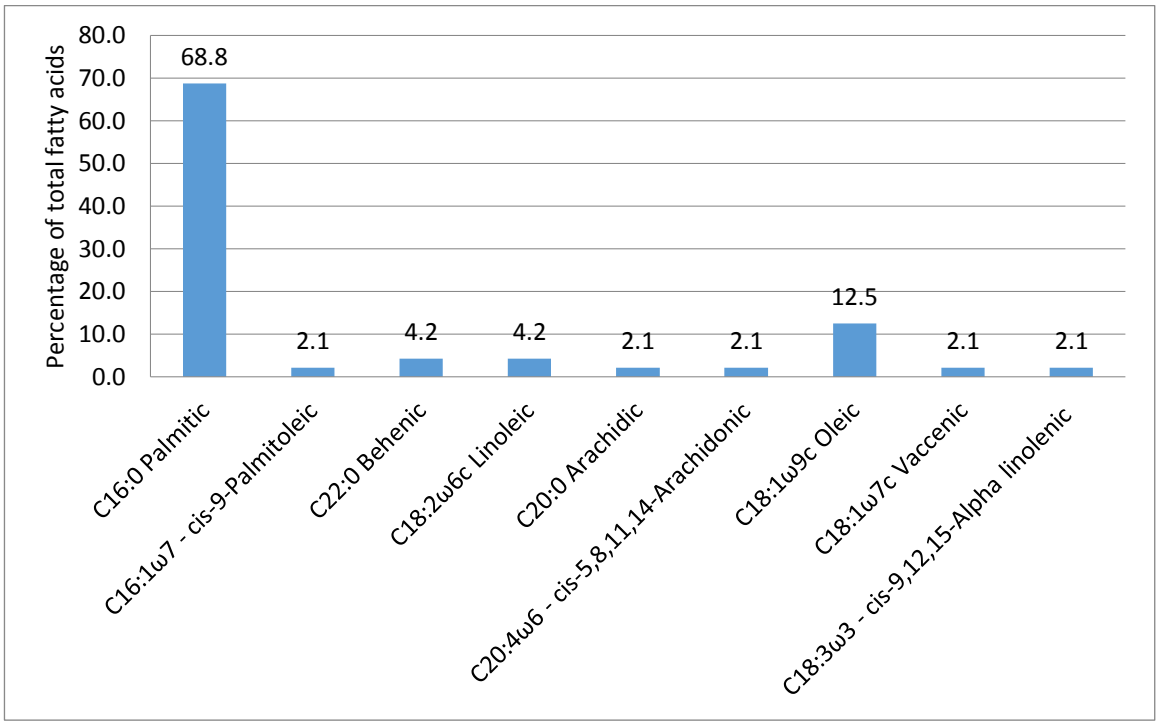


Figure 4.2 Levels of detected free-fatty acids as a percentage of total fatty acids in freeze dried mozuku powder

4.1.2 Mesh size analysis

The distribution of the mozuku powder's particle sizes as determined by mesh size analysis is shown in Figure 4.3 and displays the presence of two peaks, one in the 63-90 µm range and another smaller peak in the 355-500 µm range. A distribution consisting of two peaks is referred to as being bimodal, and may arise from a process involving the breakup of large particles, multiple sources of particles, or the presence of variable growth mechanisms in the material (HORIBA Instruments, 2016; Mukherjee, Sahu, Sen, & Sahu, 2011). The breakup of larger particles is the most likely mechanism responsible for the bimodality in this distribution, as an intact mozuku specimen possesses several biological structures (Lin, Chang, & Kuo, 2005) that each break apart in different ways during milling, thus resulting in particles tending towards specific sizes depending on which biological structure they originated from. The distribution of the mozuku powder shown in Figure 4.3 may be defined as D10 90 µm, D50 250 µm, and D90 500 µm, these values indicate at least 10% of the distribution is not greater than 90µm, 50% of the distribution is not more than 250 µm and 90% of the distribution is not more than 500 µm (HORIBA Instruments, 2016). In contrast, previous studies have shown that 89-98% of flour particle sizes are within a range of 10-300 µm (Hareland, 1994). Therefore, milling the mozuku powder to below a mesh size of 300 µm is likely to reduce the presence of visible mozuku flakes in the finished product.

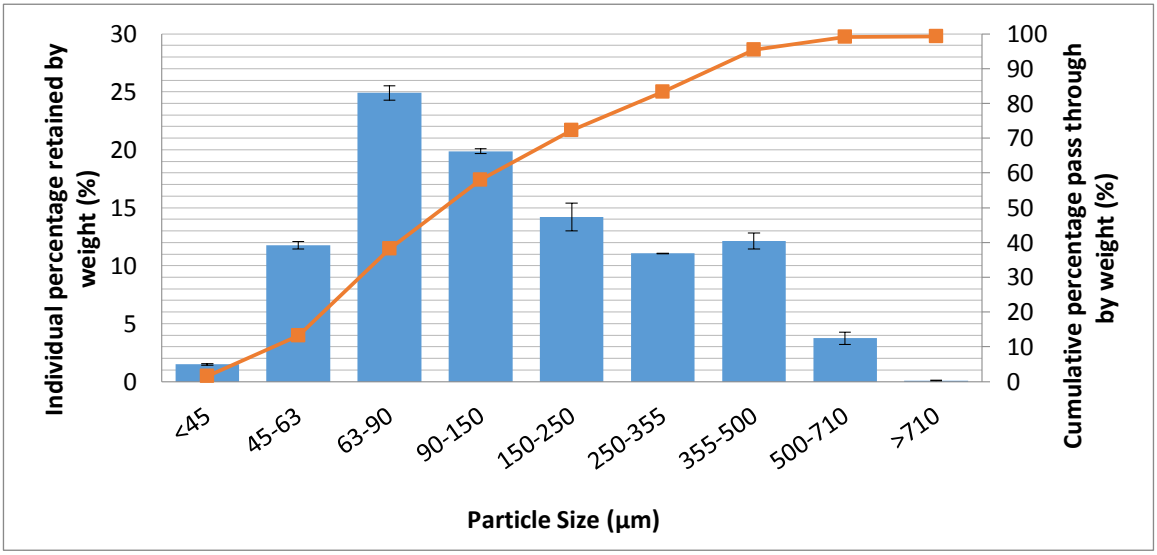


Figure 4.3 Particle size distribution of mozuku powder by weight with error bars indicating standard deviation, and the line indicating cumulative weight percentage

4.2 Wheat bread

4.2.1 Product formulation

This section describes bread quality parameters (texture, loaf volume, loaf colour and water activity) that were analysed in a standard white wheat bread formulation (AACC International, 1999a) containing mozuku powder at levels of 0%, 1% and 2%. Additional formulations were developed and analysed which included adjustments in the levels of salt (NaCl) in order to balance its content as mozuku powder concentrations were increased, these adjustments were calculated based on the salt and ash contents of mozuku powder as shown in Section 4.1.1.

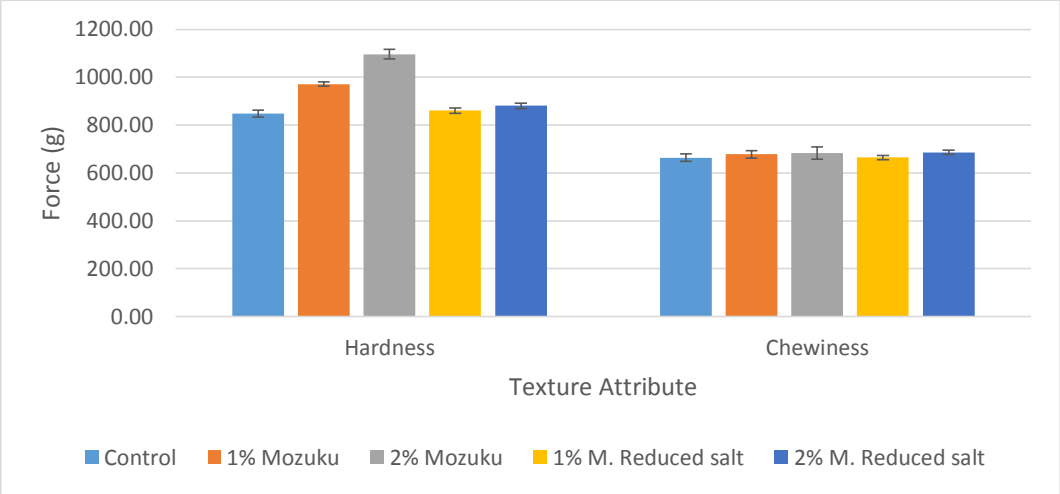
4.2.2.1 Loaf observations

The addition of mozuku powder, both with and without a reduction in salt, resulted in differences in loaf appearances with perhaps the most obvious visual difference being the visible presence of small flecks of dark brown mozuku particles in both the crust and crumb in all formulations containing mozuku powder. Depending on the individual baker and the expectations of the loaf the visible presence of these mozuku particles may or may not be regarded as a fault. If the mozuku powder were to be incorporated into a white bread recipe as seamlessly as possible without affecting its visual appearance, then the presence of these particles may be problematic. Alternatively, the presence of visible mozuku particles in wholegrain or whole-meal loaves would be of less concern due to other whole-grains and seeds already being present. One possible way to reduce the visible presence of mozuku particles in the finished loaf would be to mill the powder to a finer grade. The mesh size analysis of the mozuku powder (Section 4.1.2), showed that 15.9% of particles were larger than a mesh size of 355 µm, in comparison 96% of flour particles are within the range of 10 – 300 µm (Hareland, 1994), therefore milling the mozuku powder to below a mesh size of 300 µm would likely reduce the visible presence of mozuku particles. Aside from the visible seaweed particles, loaves containing mozuku appeared noticeably darker in colour, which was investigated in more depth in Section 4.2.2.4.

4.2.2.2 Texture analysis

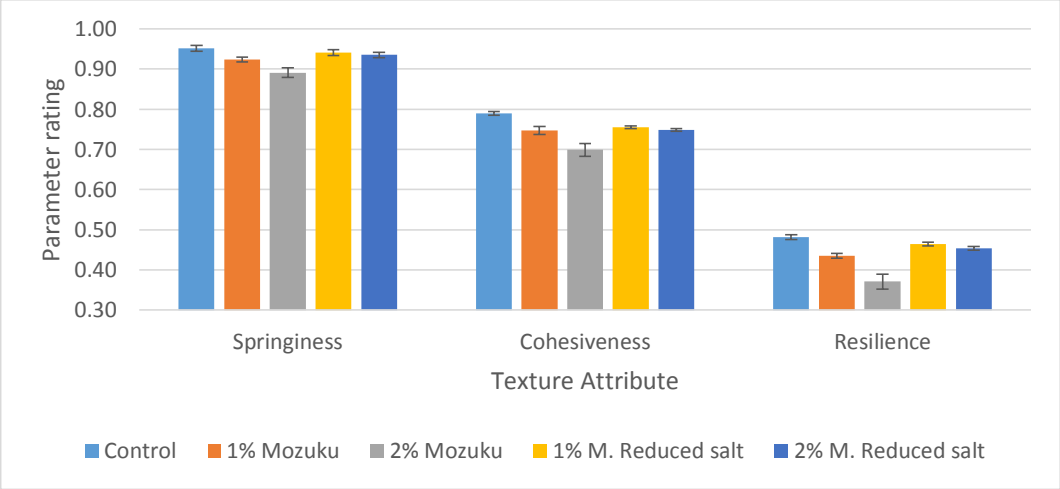
Texture analysis of five wheat bread formulations: control, 1% mozuku (1% M), 2% mozuku (2% M), 1% reduced salt mozuku (1% RSM) and 2% reduced salt mozuku (2% RSM) are shown in Figures 4.4 and 4.5. Crumb hardness increased significantly ($P<0.05$) and progressively with increasing amounts of mozuku powder in formulations where NaCl levels were not adjusted. However, no significant differences in hardness were observed between formulations containing mozuku powder and adjusted NaCl levels. Chewiness was not significantly affected by the inclusion of mozuku powder regardless of whether the NaCl levels were adjusted. Springiness was found to decrease as mozuku concentrations increased in all formulations, however, this reduction was only significant for the 2% M formulation. Cohesiveness was also reduced as mozuku concentrations increased, with significant differences from the control being present in the 1% M, 2% M and 2% RSM formulations. Additionally, resilience decreased as the mozuku inclusion rate increased, although this was only significant in the formulations without NaCl adjustments. The decrease in textural quality in 1% M and 2% M as compared to the control is most likely due to the increased solute concentrations (most notably NaCl) that were introduced by the mozuku powder as in formulations where NaCl level were adjusted many of the negative textural affects were reversed.

High solute levels in bread formulations have been shown to negatively affect the function of important bread ingredients; for example, high solute levels inhibit yeast fermentation thus reducing its leavening capacity, resulting in bread with a denser, less desirable crumb texture. However, in this study the addition of mozuku powder still resulted in a significant reduction in cohesiveness in the 2% RSM loaf, this reduction may be due to the presence of other minerals in the mozuku powder that were not adjusted for such as calcium, which has been shown to negatively impact bread quality (Roach et al., 1992). Another possible reason for the decreased cohesiveness in 2% RSM may be due to the additional dietary fibre in the mozuku powder. Dietary fibres from a range of sources have been shown to negatively impact certain bread quality parameters by physically interfering with the gluten matrix in the dough (Gómez et al., 2003). However, the negative effects of dietary fibres on quality parameters generally only affect sensory quality at concentrations of above 2%, and the mozuku powder only adds 0.61% fibre for the 2% RSM formulation.



Note: M. = Mozuku

Figure 4.4 Texture results for hardness and chewiness. Error bars indicate standard error. Testing profile parameters: Full-scale force of 5 kg, compression speed: 100mm/min, to 25% compression (6.3mm for a 25mm slice), time interval between compressions: 5sec

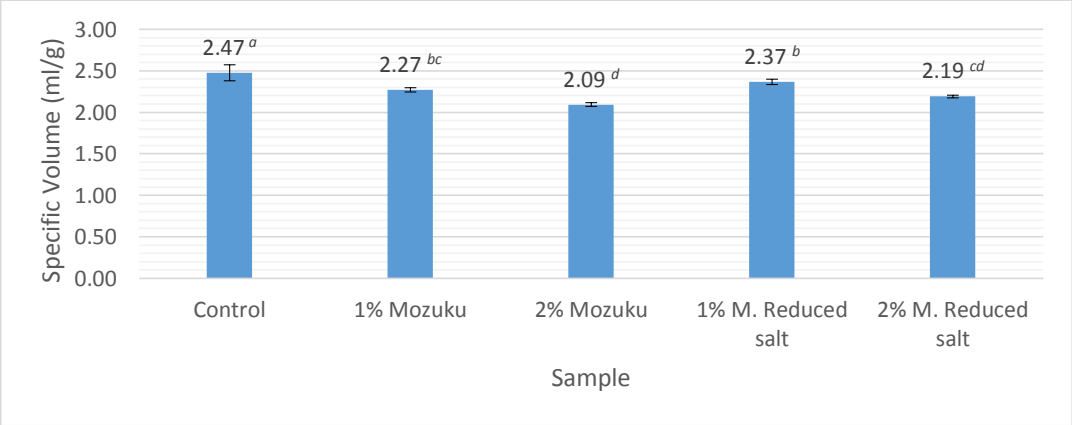


Note: M. = Mozuku

Figure 4.5 Texture results for springiness, cohesiveness and resilience. Error bars indicate standard error. Testing profile parameters: Full-scale force of 5 kg, compression speed: 100mm/min, to 25% compression (6.3mm for a 25mm slice), time interval between compressions: 5sec

4.2.2.3 Specific loaf volume

Figure 4.6 shows the specific loaf volumes of the five wheat bread formulations (control, 1% M, 2% M, 1% RSM, 2% RSM). The control loaf had a specific volume of 2.47 mL/g which was significantly ($P<0.05$) higher than all other formulations, while the 2% M formulation had the lowest specific volume at 2.09mL/g. Results indicated that as mozuku concentrations increased specific volumes decreased with this effect being greatest for the formulations without adjusted NaCl levels. A significant difference ($P<0.05$) was present between the 1% and 2% samples of both the standard and adjusted NaCl formulations, thus indicating that the presence of mozuku regardless of salt adjustment reduces specific loaf volume. The presence of minerals and compounds other than NaCl in the mozuku powder may explain the decrease in specific loaf volume in the NaCl adjusted samples, for example, calcium, which is relatively abundant in many seaweed species (MacArtain et al., 2007) has been shown to significantly reduce the specific volume of wheat bread (Roach et al., 1992) . The presence of dietary fibre in the mozuku powder may also explain the reduced specific volumes as the inclusion of bran and other dietary fibres in bread have been shown to result in decreased dough strengths, loaf volumes, impaired crumb structures and a reduced crumb softness due to the structural distortion of gas cells (Gan, Galliard, Ellis, Angold, & Vaughan, 1992; Gómez et al., 2003; Sami Hemdane et al., 2015; Sivam, Sun-Waterhouse, Quek, & Perera, 2010).



Note: M. = Mozuku

Figure 4.6 Specific volume measurements of wheat bread formulations with error bars indicating standard deviation and different letters indicating significantly different groups (Tukey method, $P<0.05$)

4.2.2.4 Crust and crumb colour analysis

Crust colour

A colour analysis was carried out on the crusts of the five wheat bread formulations (control, 1% M, 2% M, 1% RSM, 2% RSM) and results are shown in Table 4.3. The *L** and *a** colour parameters for the crust colour of wheat bread did not differ significantly between formulations. However, the *b** parameter which corresponds to a decreased yellowness in the sample had significantly decreased mean values for both the 2% M and 2% RSM when compared against the control formulation. Adjustments in the NaCl levels were not found to affect crust colour (P>0.05).

Table 4.3 Crust colour expressed in *L**, *a**, *b** and RGB values of white wheat bread loaves containing mozuku powder with different letters indicating significantly different groups (Tukey method, P<0.05)

Formulation	L		a*		b*		RGB
Control	58.98	± 2.92 [a]	12.90	± 1.78 [a]	35.39	± 0.81 [a]	
1% Mozuku	57.26	± 2.37 [a]	11.70	± 2.22 [a]	34.47	± 1.29 [a]	
2% Mozuku	57.07	± 1.93 [a]	11.64	± 1.53 [a]	33.25	± 1.28 [b]	
1% M. Reduced salt	57.83	± 2.51 [a]	12.65	± 1.63 [a]	34.71	± 0.74 [a]	
2% M. Reduced salt	57.99	± 0.68 [a]	12.23	± 1.57 [a]	33.20	± 0.93 [b]	

Note: RGB = Red, Green, Blue colour profile, *L** = lightness axis, *a** = red-green axis, *b** = yellow-blue axis, M. = Mozuku

Crumb colour

The results from the crumb colour analysis on the five wheat bread formulations (control, 1% M, 2% M, 1% RSM, 2% RSM) are shown in Table 4.4. For both the *L** and *b** values significant differences (p<0.05) in measurements were present between formulations with differing mozuku concentrations regardless of salt levels. The *L** value was highest in the control formulation and decreased as mozuku concentrations increased, this is in agreement with the visual observations in Section 4.2.2.1 where the loaves that contained mozuku powder appeared noticeably darker. The *b** value was lowest in the

control formulations and increased with higher mozuku inclusion, thus indicating that mozuku powder increased the yellowness of the crumb. This result is in contrast to the decrease in yellowness observed in the crust with higher mozuku powder concentrations. Similarly, as mozuku powder concentrations increased *a** values were also shown to significantly increase ($p<0.05$) thus indicating increases in redness, although no significant difference was observed between the 1% RSM and 2% RSM formulations. In general regardless of salt adjustments, formulations containing mozuku powder had increased crumb yellowness and redness with reduced lightness when compared to the control.

Table 4.4 Crumb colour expressed in *L**, *a**, *b** and RGB values of white wheat bread formulations containing mozuku with different letters indicating significantly different groups (Tukey method, $P<0.05$)

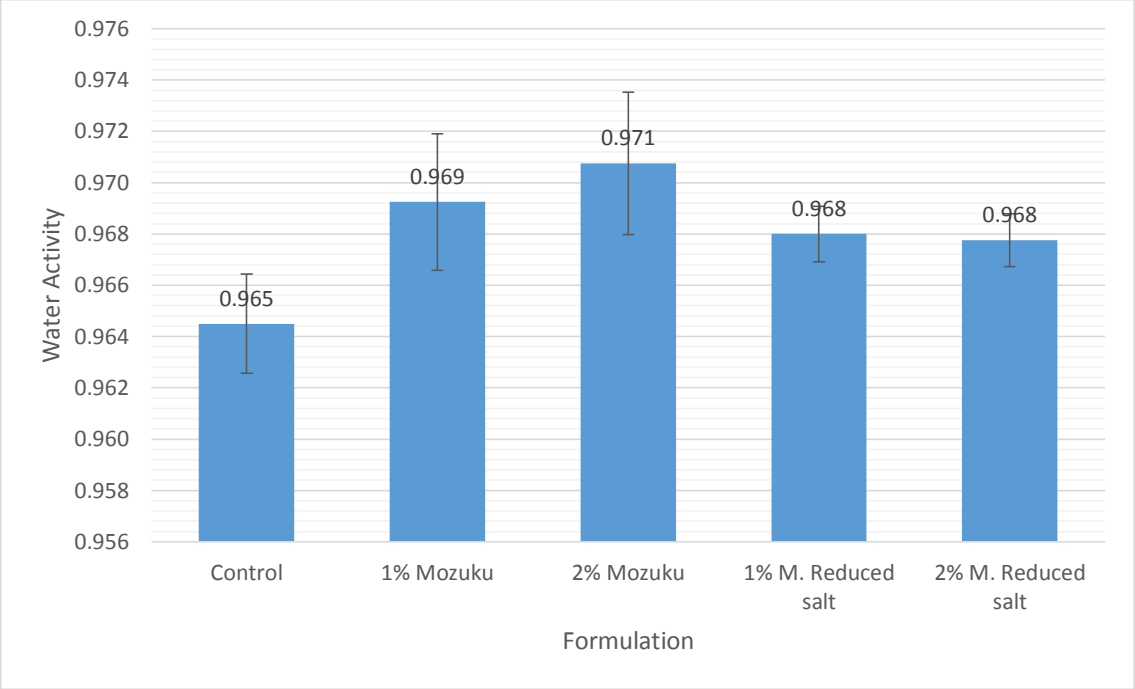
Formulation	L		a*		b*		RGB
Control	72.57	± 1.64 [a]	-2.65	± 0.06 [d]	17.36	± 0.61 [c]	
1% Mozuku	69.59	± 1.20 [b]	-2.03	± 0.15 [c]	18.43	± 0.39 [b]	
2% Mozuku	64.98	± 2.09 [c]	-1.72	± 0.21 [a]	19.63	± 0.53 [a]	
1% M. Reduced salt	69.91	± 1.18 [b]	-1.98	± 0.12 [bc]	18.77	± 0.59 [b]	
2% M. Reduced salt	66.01	± 1.43 [c]	-1.82	± 0.12 [ab]	19.63	± 0.36 [a]	

Note: RGB = Red, Green, Blue colour profile, *L** = lightness axis, *a** = red-green axis, *b** = yellow-blue axis, M. = Mozuku

4.2.2.5 Water Activity

Figure 4.7 shows the water activities (a_w) of each wheat bread formulation (control, 1% M, 2% M, 1% RSM, 2% RSM) with no significant differences being found between them. This is in contrast to what was expected, which was a decrease in a_w as mozuku powder levels increased due to the presence of additional solutes. However, the presence of other compounds in the mozuku powder such as fucoidan and dietary fibres may have reduced the loss of water during baking and cooling, thus resulting in a higher a_w (Barbosa-Canovas, Fontana, & Schmidt, 2007) than expected. Additional

research is required to further understand the mechanism behind the influence that mozuku had on the a_w in bread.



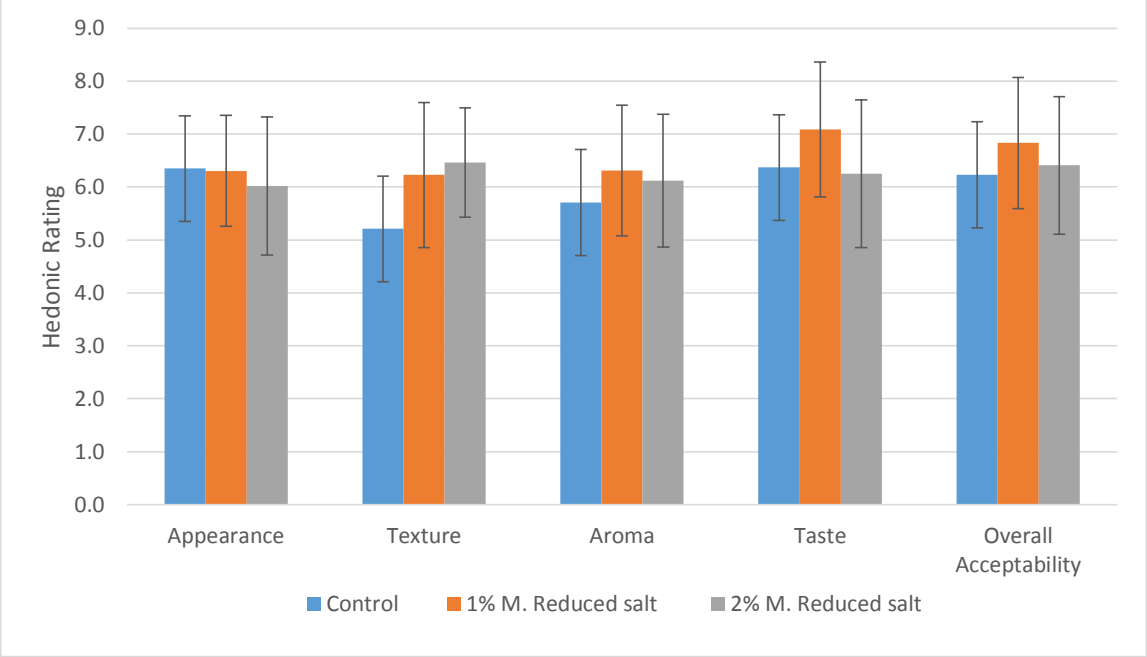
Note: M. = Mozuku

Figure 4.7 Water activity of white bread formulations with error bars indicating standard error

4.2.2 Consumer evaluation

Consumer evaluation was carried out using only the reduced salt formulations and the control (control, 1% RSM, 2% RSM) due to the results of the texture analysis, which showed increased hardness in formulations without adjusted salt levels results and preliminary tasting evaluations which indicated excessive saltiness in the un-adjusted mozuku formulations (T. Mutukumira, personal communication, July 20, 2016). The results of the consumer sensory evaluation are shown in Figure 4.8 and cross sectional images of the loaves used in the study are displayed in Figure 4.9. Each formulation was assessed by consumers in the parameters of appearance, texture, taste, aroma, and overall acceptability, with results showing that no statistically significant differences were present between formulations for any of the sensory factors. Therefore, the results indicated that the inclusion

of up to 2% mozuku powder (with NaCl adjustment) in wheat bread did not negatively impact consumer sensory perception of the breads ($P<0.05$); this may be due to the concentrations of mozuku powder used, at higher concentrations sensory differences may become more apparent. Results from the final question regarding which sample the consumer preferred showed that no single formulation was preferred over any other, however future consumer sensory evaluations into mozuku bread should include additional participants in order to increase data precision.



Note: M. = Mozuku

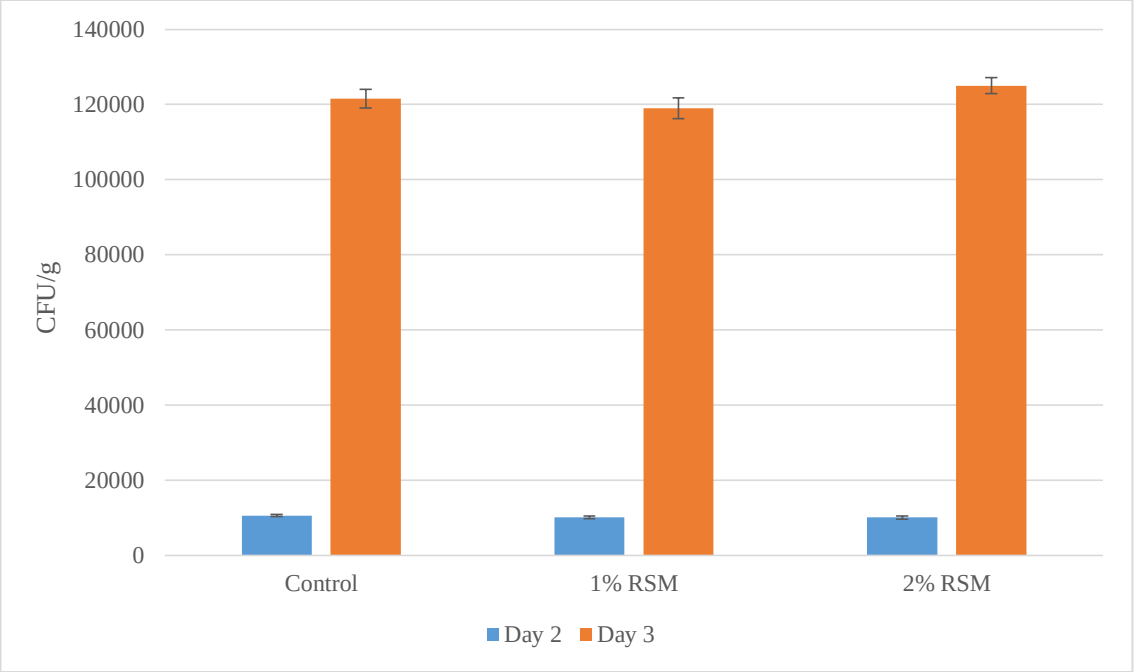
Figure 4.8 Average consumer (n=40) sensory evaluations results of three wheat bread formulations with error bars indicating standard deviation

Figure 4.9 Cross sectional slices of wheat bread formulation used in sensory evaluation. Formulations top to bottom: Control, 1% Reduced Salt Mozuku and 2% Reduced Salt Mozuku

4.2.3 Microbial stability

A three-day microbial stability test was conducted on three wheat bread formulations (control, 1% RSM, 2% RSM) in order to assess whether the inclusion of mozuku powder in bread formulations had any effect on microbial growth (total aerobic plate count, yeasts and moulds count). Figure 4.10 shows the results of the microbial analysis conducted using PCA to determine total aerobic plate count, these results indicate the presence of general bacterial populations in a food sample as PCA is not selective for any particular species. Results show no significant differences in CFU/g between each formulation on each day, thus indicating differences in formulation had no effect on microbial growth. Day 1 showed 0 CFU/g being present on each dilution plate for every sample of each formulation therefore indicating they were within an acceptable range for total aerobic plate counts for ready-to-eat foods ($<10^4$ CFU/g) (Ministry of Health, 1995). For day 2, the CFU/g of all samples ranged from 10100 to 10600 thus surpassing a value of 10^4 CFU/g and therefore would be deemed marginally acceptable according to the Microbiological Reference Criteria for Food (Ministry of Health, 1995). All three formulations exceeded the maximum allowed limit of CFU/g of 10^5 by day 3 as laid out in the Microbiological Reference Criteria for Food (Ministry of Health, 1995) and would be rejected due to high bacterial populations. Each of the formulations tested lacked preservatives which are typically used in commercial bread products (such as calcium acetate and sodium acetate), this may explain the high microbial populations present by day 3 (Saltmarsh, 2013).

Analysis using YGC agar resulted in no yeast or fungal growth over the three days of testing, and no mould growth was visibly present on any of the storage samples. The similarities in microbial populations between the three formulations (control, 1% RSM, 2% RSM) is most likely due to each possessing relatively similar water activities of between 0.965 and 0.968 as water activity is a key factor in determining bacterial and fungal growth (Czuchajowska et al., 1989). Overall, the results indicated the inclusion of mozuku powder in wheat bread did not increase the presence or growth of microbial organisms in the finished loaf. Future research on the microbial impact of mozuku powder should be conducted over a longer period of time with additional replicate samples. Additionally, commercial loaves which incorporate mozuku powder are likely to contain preservatives and therefore, future research into whether the functionality of such preservatives is altered by the presence of mozuku powder may be required.



Note: RSM = Reduced-salt mozuku formulations

Figure 4.10 CFU/g of three wheat bread formulations using PCA on the second and third days of storage with error bars indicating standard error, day one results are not shown as no microbial growth was detected at that time point

4.3 Gluten-free bread

4.3.1 Base formulation

4.3.1.1 Initial formulation and observations

The development of a gluten-free (GF) bread base formulation involved the comparison of eight formulations to a store-bought reference GF loaf, with each of the eight formulations varying in quantities of rice flour, modified tapioca starch, and inclusion of hydrocolloids. The following attributes were analysed and compared: appearance, texture, crust and crumb colour. Figures 4.11 and 4.12 show the cross sectional slices of each gluten-free formulation and a reference loaf, which will be discussed in terms of visual appearance, texture, colour and water activity in the following sections.

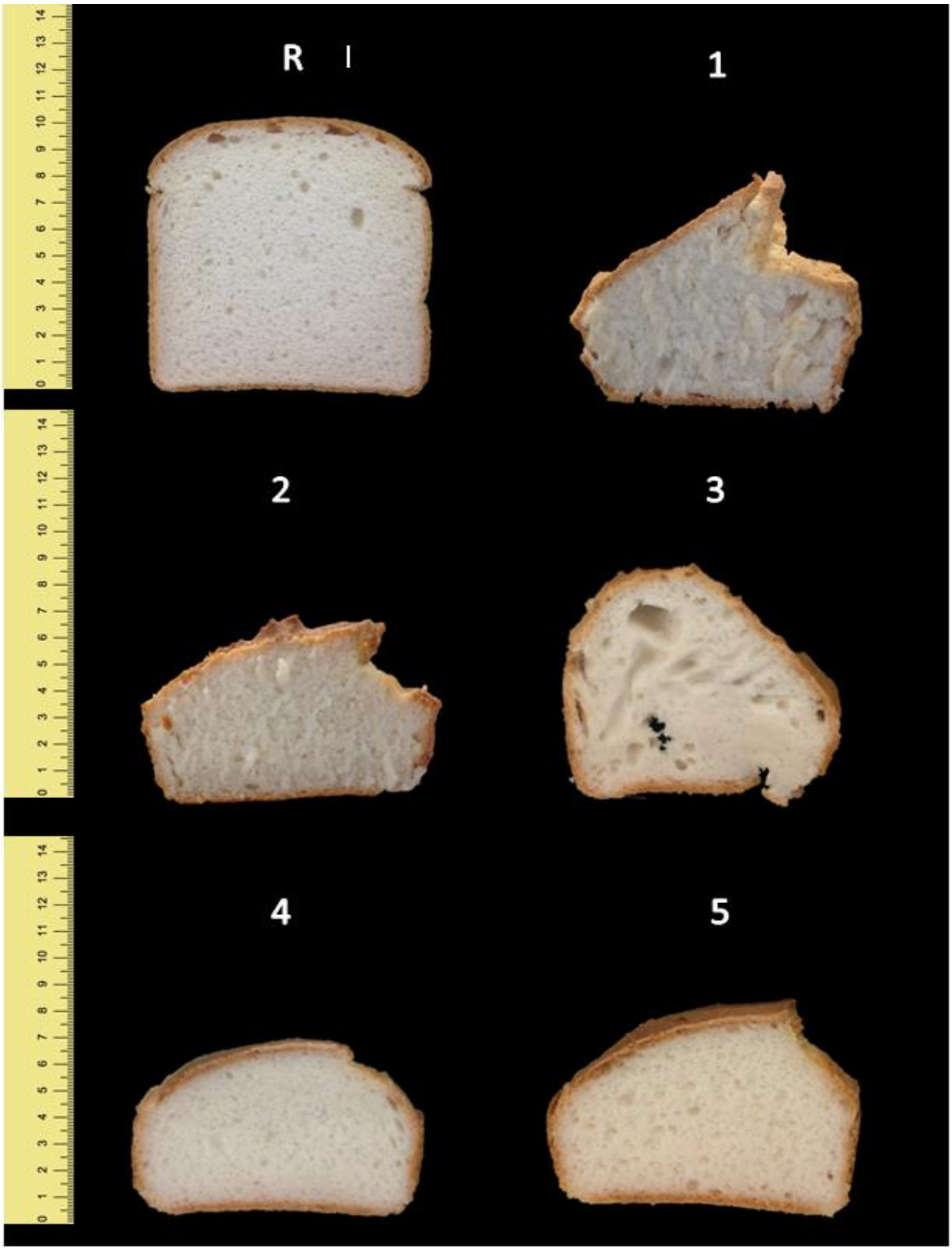


Figure 4.11 Crumb texture and height of gluten-free bread formulation

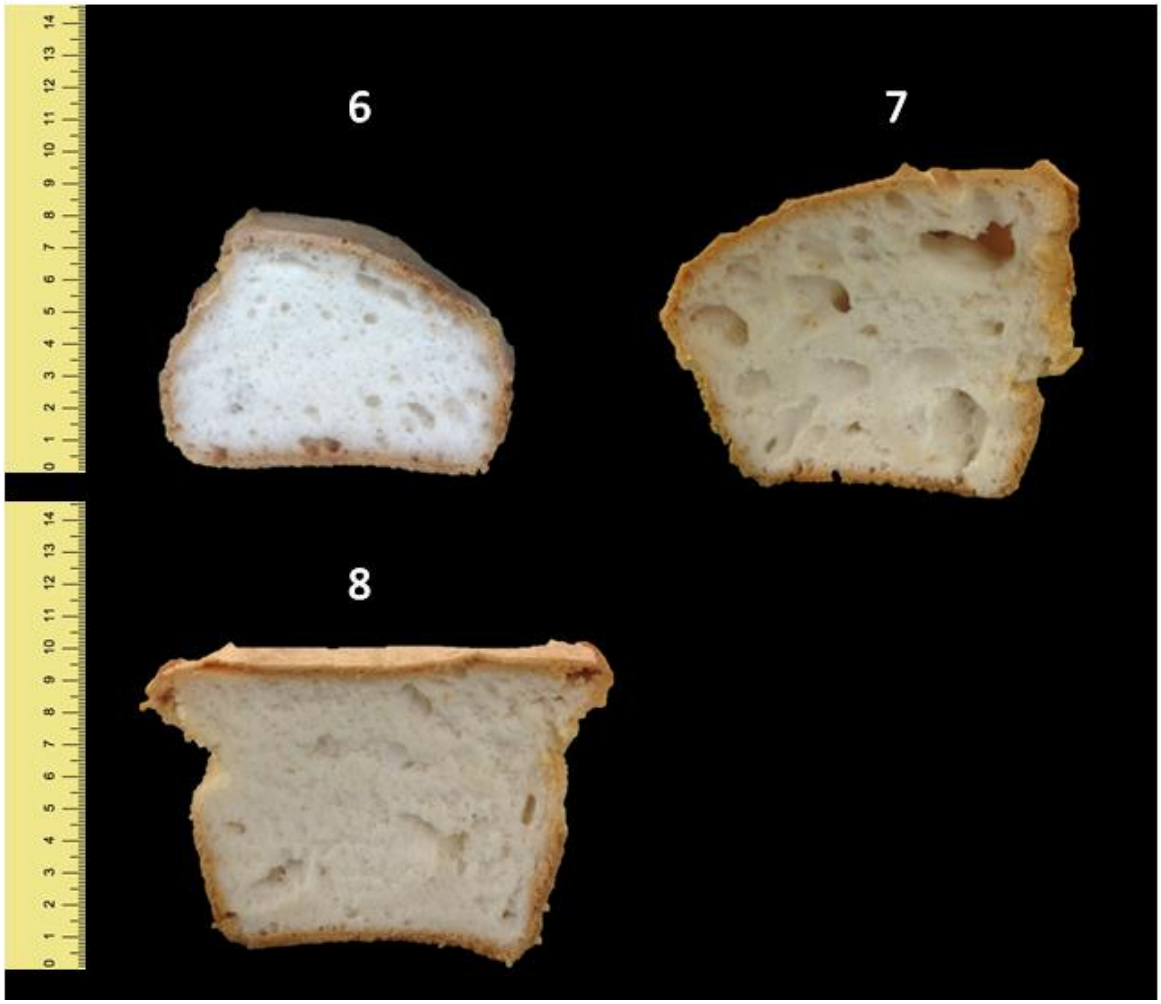


Figure 4.12 Crumb texture and height of gluten-free bread formulation

4.3.1.2 Texture analysis

The different bread loaves (formulations 1-8, and the reference loaf) were analysed on the textural attributes of hardness, chewiness, springiness, cohesiveness, and resilience (Figures 4.13 and 4.14).

Formulations 1 and 2

Formulation 1 and 2 contained 70% rice flour to 30% tapioca starch and varied in hydrocolloid type: HPMC (formulation 1) and guar gum (formulation 2). Formulation 2 showed the highest hardness and chewiness followed by formulation 1 which may be due to rapid staling occurring in formulation 2. The differences in hydrocolloids used in formulations 1 and 2 may have inhibited the rate of staling to differing extents (Anton & Artfield, 2009). During staling, bread loses its freshness and the crust toughens, becomes firmer and less elastic (Arendt, Morrissey, Moore, & Bello, 2003). Later formulations possessed lower flour to starch ratios and investigated the effects of a wider range of hydrocolloids in an attempt to reduce the high hardness and chewiness ratings of formulations 1 and 2.

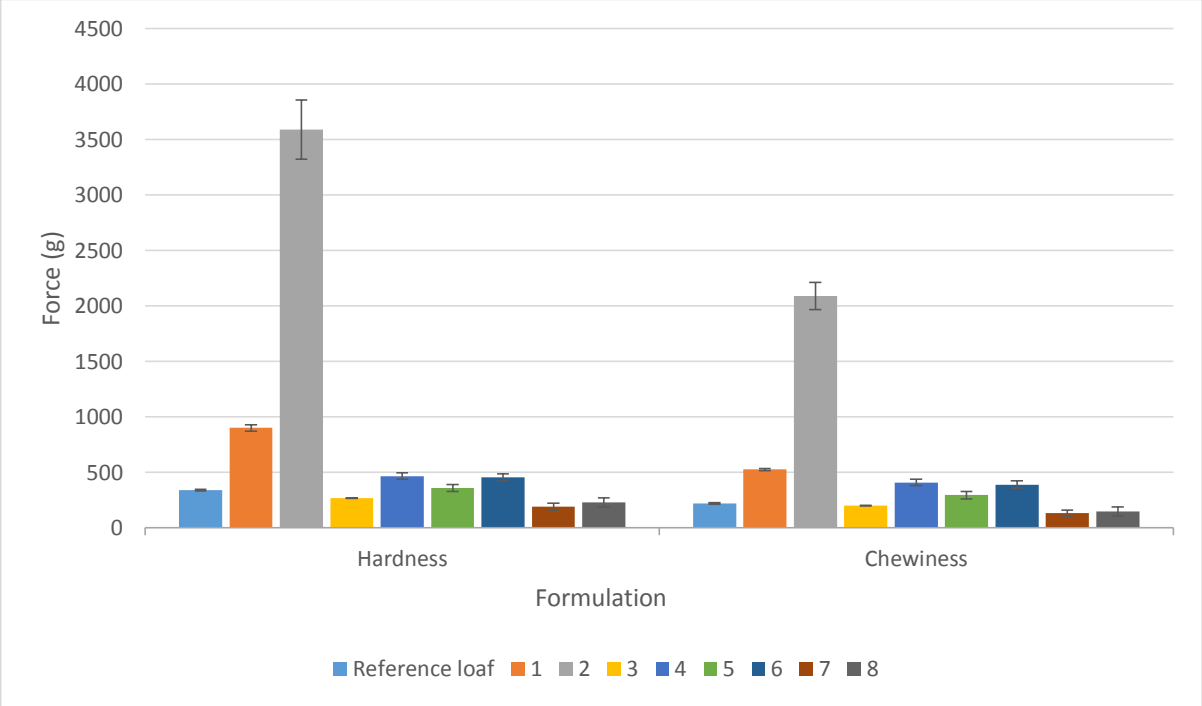


Figure 4.13 The mean values of the attributes hardness and chewiness for the different formulations with error bars indicating standard error of the means. Testing profile parameters: Full-scale force of 5 kg, compression speed: 100mm/min, to 25% compression (6.3mm for a 25mm slice), time interval between compressions: 5sec

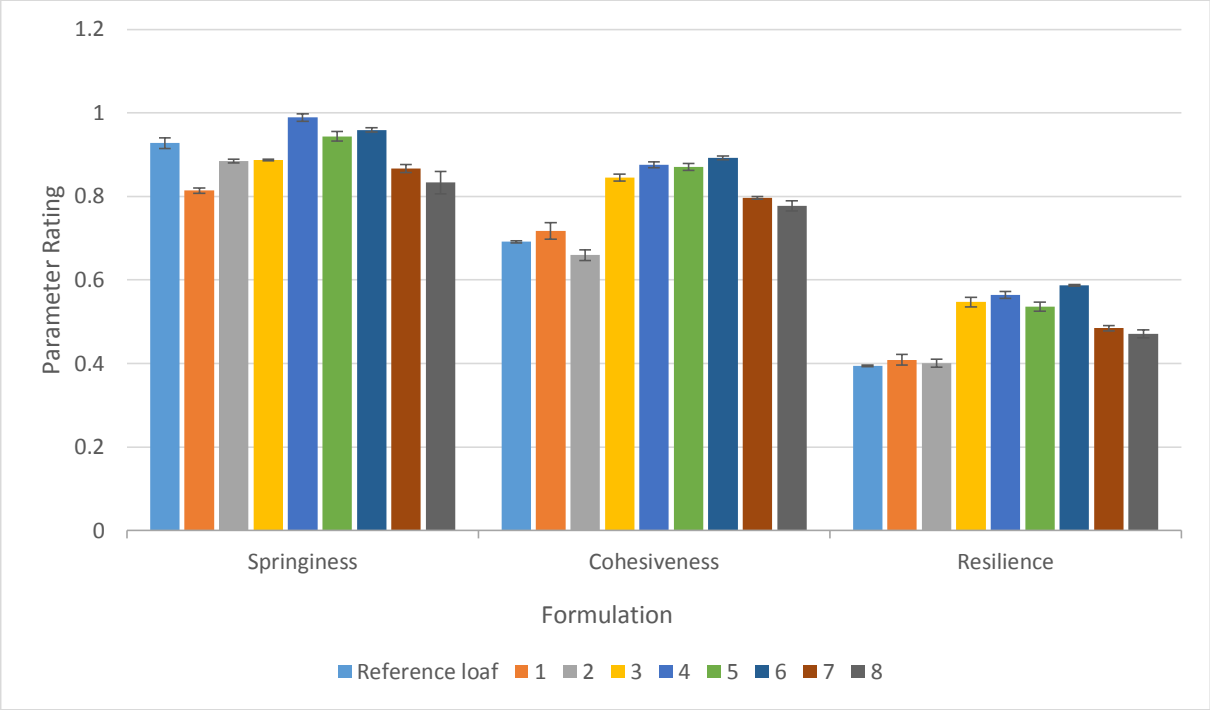


Figure 4.14 The mean values of the attributes springiness, cohesiveness, and resilience with the error bars indicating the standard error. Testing profile parameters: Full-scale force of 5 kg, compression speed: 100mm/min, to 25% compression (6.3mm for a 25mm slice), time interval between compressions: 5sec

Formulations 3-6

Formulations 3, 4, 5, and 6, contained the same ratio of tapioca starch to rice flour (70% tapioca starch to 30% rice flour) and varied in hydrocolloid type: 1% HPMC (formulation 3), 1% guar gum (formulation 4), 1% xanthan gum (formulation 5) and 1% carboxymethyl cellulose (CMC) (formulation 6). With regards to hardness, chewiness and cohesiveness no significant differences were observed between formulations 3-6 which may be due to the level of hydrocolloids used in each formulation (1%), as higher hydrocolloids concentrations above 1%, may result in differences in these parameters becoming more apparent (Lazaridou et al., 2007). Formulations 4 and 6 were shown to be significantly ($P<0.05$) higher in springiness when compared to formulation 3, while formulation 5 showed no significant differences when compared to the others. Resilience results showed formulation 6 rated significantly higher ($P<0.05$) than formulation 5. Differences in thickening and gelling potentials between each of the hydrocolloids may explain the variations in springiness and resilience as each hydrocolloid influences final crumb structure differently. CMC, guar gum and

xanthan gum are non-gelling agents (BeMiller, 2008; Saha & Bhattacharya, 2010) while HPMC forms a gel under baking conditions which may explain the reduced springiness in formulation 3 (Silva et al., 2008). Additionally, the inclusion of hydrocolloids has been shown to increase the water holding capacity of bread doughs to varying degrees which may increase softness and decrease chewiness (Kotoki & Deka, 2010).

Formulations 7 and 8

Formulations 7 and 8 contained a combination of hydrocolloids (HPMC, and guar gum), however formulation 8 contained a lowered starch to rice flour ratio in an attempt to reduce the visible presence of large gas cells. There were no significant differences in texture parameters between formulations 7 and 8 however they both showed the lowest values for chewiness when compared to all other formulations ($P<0.05$) and comparatively low ratings in hardness, which may have been due to the additive effects of the hydrocolloids (HPMC and guar gum). HPMC may have increased the softness and volume of the bread by increasing the water retention properties of the dough and increasing starch gelatinisation, thus aiding in the formation of gas cells (Crockett, Le, & Vodovotz, 2011). Simultaneously, guar gum may have increased elasticity in the dough via its thickening properties (BeMiller, 2008). Formulation 7 exhibited a very open crumb texture that contained large air bubbles which were most likely due to the higher ratio of starch which increased dough elasticity and allowed for the formation of excessively large gas bubbles (Crockett, Le, et al., 2011).

4.3.1.3 Colour measurements

Crust colour

Table 4.5 shows the results of the crust colour analysis of the eight GF formulations and the reference loaf. Colour parameters of the crust colour for each formulation varied significantly ($P<0.05$) with the L^* (lightness value) for formulation 7 being the highest, thus suggesting that the crust of this loaf was the lightest crust colour while formulation 6's L^* was the lowest indicating it possessed the darkest crust colour. The a^* value (red-green axis) values were all positive, showing that that the crust colour of all formulations reflected more red than green coloured light, formulation 6 had the highest redness and formulation 7 the lowest. All b^* values were positive thus representing that the crust colour was more yellow than blue, with formulation 1 possessing the highest values for b^* and formulation 6 the lowest. There is no clear explanation for the differences in crust colour and it is

possible differences in colours are in part due to varying baking conditions within the oven being used.

Table 4.5 Crust colour expressed in *L**, *a**, *b** and RGB values for the eight base formulations and reference loaf with different letters indicating significantly different groups (Tukey method, P<0.05)

Formulation	L*	a*	b*	RGB
Reference	53.87 ± 0.63 ^{ab}	11.98 ± 0.24 ^{bc}	26.22 ± 0.37 ^{abc}	
1	54.62 ± 3.69 ^{ab}	13.55 ± 0.83 ^{abc}	30.77 ± 2.21 ^{a}	
2	48.55 ± 1.27 ^{bcd}	14.66 ± 0.56 ^{a}	25.38 ± 3.04 ^{bc}	
3	46.21 ± 1.20 ^{de}	14.81 ± 0.40 ^{a}	26.53 ± 0.83 ^{abc}	
4	47.05 ± 0.39 ^{cd}	14.37 ± 0.19 ^{a}	24.71 ± 2.09 ^{cd}	
5	53.16 ± 4.40 ^{abc}	13.87 ± 1.51 ^{ab}	30.07 ± 0.81 ^{ab}	
6	40.70 ± 1.55 ^{e}	14.99 ± 0.33 ^{a}	20.01 ± 2.19 ^{d}	
7	58.07 ± 1.70 ^{a}	11.65 ± 0.85 ^{c}	30.59 ± 0.53 ^{a}	
8	54.53 ± 1.09 ^{ab}	14.61 ± 0.45 ^{a}	29.62 ± 0.17 ^{ab}	

Note: RGB = Red, Green, Blue colour profile, *L** = lightness axis, *a** = red-green axis, *b** = yellow-blue axis

Crumb colour

Significant differences in crumb colour were found between several formulations in each of the *L**, *a**, *b** values, as shown in Table 4.6. Formulation 8 had the highest *L** indicating it possessed the lightest crumb colour while formulation 4 obtained the lowest *L** values indicating the darkest crumb colour of all formulations. Meanwhile, the *a** values varied in positive and negative values with the most redness being measured in formulation 1, while the standard gluten-free bread possessed the highest greenness. The highest *b** values were determined for formulation 1 indicating the highest level in yellowness, the lowest values in yellowness were determined for formulation 4. Significant

differences in crumb colour may arise be due to differing starch to flour ratios and/or hydrocolloid type, however, there is no clear relationship present.

Table 4.6 Crumb colour expressed in *L**, *a**, *b** and RGB values for the eight base formulations and reference loaf with different letters indicating significantly different groups (Tukey method, P<0.05)

Formulation	L*	a*	b*	RGB
Reference	76.77 ± 2.22 ^{ab}	-0.75 ± 0.13 ^{f}	9.90 ± 0.47 ^{ab}	
1	79.62 ± 0.61 ^{a}	2.03 ± 0.08 ^{a}	10.33 ± 0.08 ^{a}	
2	71.29 ± 0.15 ^{c}	0.24 ± 0.12 ^{d}	8.72 ± 0.33 ^{bcd}	
3	70.51 ± 1.47 ^{c}	0.75 ± 0.19 ^{c}	7.71 ± 0.33 ^{de}	
4	69.53 ± 0.69 ^{c}	1.56 ± 0.12 ^{b}	7.19 ± 0.48 ^{e}	
5	74.41 ± 4.37 ^{abc}	-0.01 ± 0.27 ^{de}	8.96 ± 0.64 ^{bc}	
6	72.16 ± 0.45 ^{bc}	1.45 ± 0.07 ^{b}	8.48 ± 0.42 ^{cd}	
7	78.07 ± 1.30 ^{a}	-0.42 ± 0.06 ^{ef}	8.85 ± 0.50 ^{bcd}	
8	79.37 ± 0.68 ^{a}	0.89 ± 0.20 ^{c}	9.27 ± 0.24 ^{abc}	

Note: RGB = Red, Green, Blue colour profile, *L** = lightness axis, *a** = red-green axis, *b** = yellow-blue axis

4.3.1.4 Water activity

Results from the water activity measurements of the eight GF formulations (formulations 1-8) showed that the overall range for each was 0.978 - 0.986. These values were determined to be acceptable as they were similar to those determined in previous studies into GF breads (Lazaridou et al., 2007; Rosell & Rojas, 2001). Rosell and Rojas (2001) showed that gluten-free breads are typically slightly higher in water activity than standard wheat bread. This is in agreement with the results obtained in Section 4.2.2.5, which indicates the control wheat bread's water activity (0.965) was lower than those of the eight GF formulations tested in this study. This result may be due to the presence of hydroxyl

groups in the hydrocolloids of gluten-free bread which interact with water through hydrogen bonding, thus increasing the water-holding capacity of the loaf (Rosell & Rojas, 2001).

4.3.1.5 Selection of suitable gluten-free base formulation

Formulation 8 which contained 45% rice flour, 55% modified tapioca starch, 0.5% HPMC and 0.5% guar gum produced a loaf with the most preferred textural and visual characteristics and compared favourable against the reference loaf, therefore it was selected as the base formulation for further investigations.

4.3.2 Inclusion of mozuku powder

4.3.2.1 Loaf observations

Figure 4.15 shows the cross-sectional slices of the selected gluten free formulation (formulation 8) with mozuku powder concentrations ranging from 1% - 4% and NaCl adjustments based on mozuku powder added (Formulations 9 – 13). The most notable visual observations between loaves as mozuku concentrations increased were darkened crust and crumb colours, reduced loaf volumes and a denser crumb texture. Formulations 9 (1% mozuku) and 10 (2% mozuku) possessed an irregular crumb texture with large air bubbles. Formulation 9's crumb colour appeared much whiter than the other formulations, while formulation 11 (2.5% mozuku) and 12 (3% mozuku) possessed lower loaf heights, and a more regular crumb texture with fewer large gas bubbles. Formulation 13 had a visibly reduced loaf height, and volume as well as a denser crumb texture with a noticeably greenish crumb colour that was also darker in colour. Similarly to the wheat bread formulations mozuku powder particles were visibly present in all formulations containing mozuku powder.

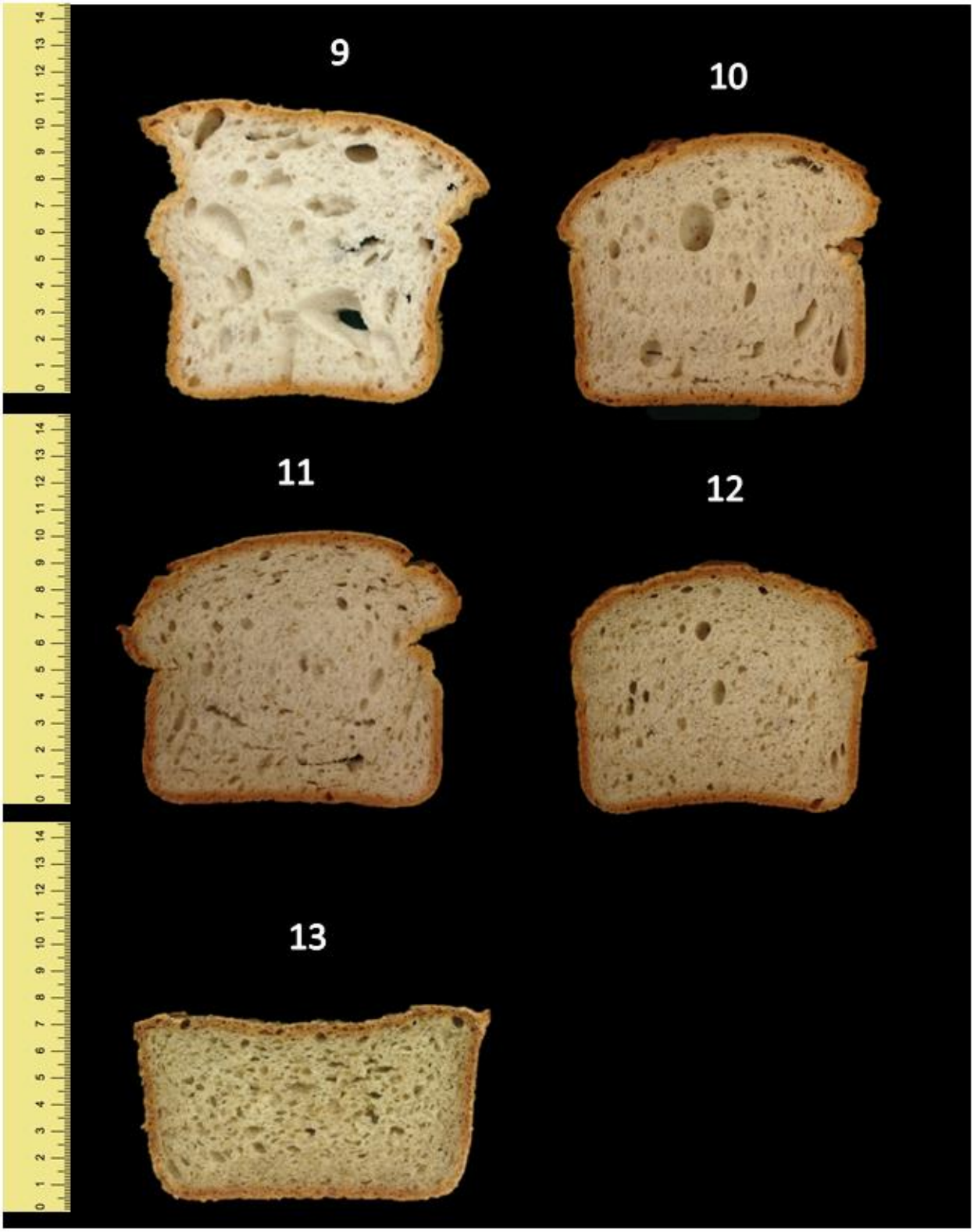


Figure 4.15 – Crumb texture and loaf height of the formulation 9 (1% mozuku), 10 (2% mozuku), 11 (2.5% mozuku), 12 (3% mozuku), and 13 (4% mozuku)

4.3.2.2 Texture analysis

The results of the texture analysis of five GF bread formulations and a reference GF loaf is shown in Figures 4.16 and 4.17. In general, hardness and chewiness ratings increased as mozuku quantities increased, although only formulation 13 was found to rank significantly ($P<0.05$) higher in these parameters. Formulation 13 (4% mozuku) showed the highest rating for hardness whereas formulation 9 (1% mozuku) had the lowest. The large increase in hardness and chewiness in formulation 13 when compared to the other formulations is most likely due to the higher solute concentration affecting yeast cell viability (Heggart et al., 1999). Environments with high solute concentrations impart osmotic pressure on all living cells and while yeast possesses a number of mechanisms to overcome such pressures their viability, growth and fermentative performance will still decline when exposed to high enough solute concentrations (D'Amore, Panchal, Russell, & Stewart, 1988). The osmotic pressure exerted on the yeast cells in formulation 13 due to the higher concentrations of mozuku powder may have inhibited their growth and fermentation thus reducing the amount of leavening taking place (D'Amore et al., 1988). A dough that is not adequately leavened results in a loaf with a dense, crumb texture and low volume as is the case in formulation 13. The gelatinisation of starches is also inhibited by high solute concentrations, this is because solutes decrease the availability of free water thus reducing starch hydration, a vital step in complete starch gelatinisation (D'Appolonia, 1977; Lim, Wu, & Reid, 2000). Incomplete starch gelatinisation results in reduced dough elasticity during oven spring which decreases the loaf's final volume (Arendt et al., 2003).

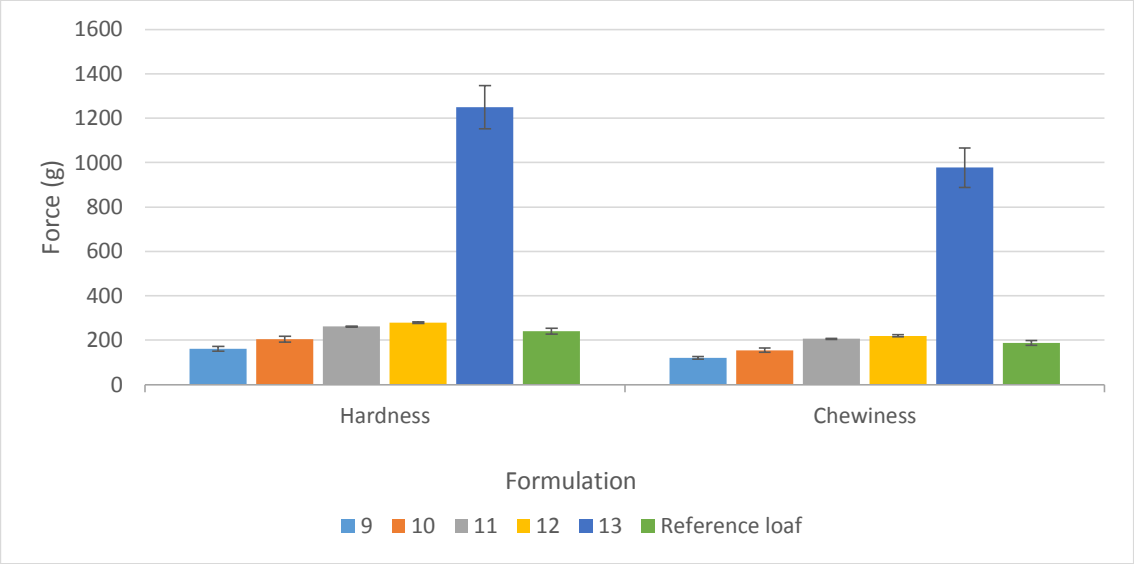


Figure 4.16 The mean values of the attributes hardness and chewiness. Error bars indicate standard error. Testing profile parameters: Full-scale force of 5 kg, compression speed: 100mm/min, to 25% compression (6.3mm for a 25mm slice), time interval between compressions: 5sec

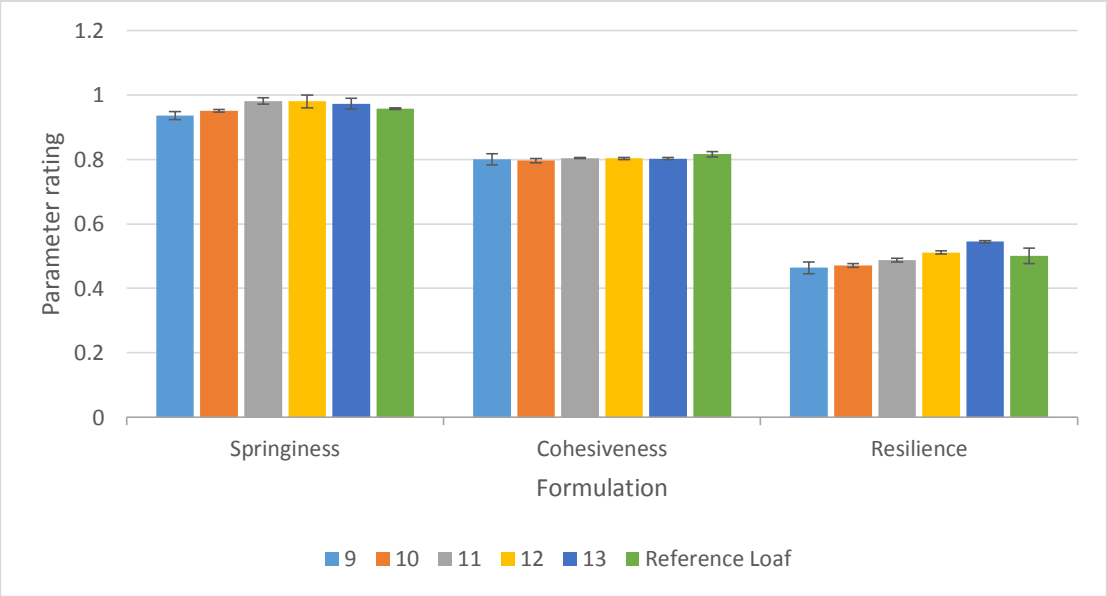


Figure 4.17 The mean values of the attributes springiness, cohesiveness, and resilience. Error bars indicate standard error. Testing profile parameters: Full-scale force of 5 kg, compression speed: 100mm/min, to 25% compression (6.3mm for a 25mm slice), time interval between compressions: 5sec

No significant differences were found in springiness and cohesiveness between samples, however formulation 11 (2.5% mozuku) exhibited the highest rating in springiness and the reference loaf exhibited the highest rating in cohesiveness. Resilience ratings increased with increasing mozuku powder concentrations, with formulation 13 rating significantly higher than formulations 9 and 10. These results are in contrast to those obtained from the wheat bread formulations, where resilience decreased as mozuku levels increased. The increase in resilience in GF loaves with added mozuku powder may be due to the hydrocolloids in the GF formulations interacting with components of the mozuku powder, as hydrocolloids have been shown to work synergistically (Saha & Bhattacharya, 2010). Additional research is required in order to further understand the interaction between mozuku powder and hydrocolloids in GF bread.

4.3.2.3 Specific loaf volume

Specific loaf volume of the different formulations was measured by the Rapeseed Displacement method. The results of the measurement of specific volume for the five GF formulations (formulations 9-13) and the reference GF loaf are shown in Figure 4.18. In general, specific volume decreased as mozuku concentrations increased. As discussed in previous sections the high mineral content present in the mozuku powder is the most likely reason for the reduction in bread quality and is likely also responsible for the resulting reduced specific volume. The increased solute concentrations of the dough, due to the mozuku powder, may have disrupted yeast fermentation and leavening capacity (Wei, Tanner, & Malaney, 1982). Another possible explanation for the reduced loaf volume at higher mozuku concentrations is the increased competition for water between components such as HPMC, minerals, guar gum, dietary fibres and starches thus leading to reduced hydrocolloid hydrations and starch gelatinisation (Suzuki, Ohsugi, Yoshie, & Hirano, 1996). It is likely that freeze dried mozuku powder attracts and retains a large amount of water due to it containing high concentrations of minerals, carbohydrates and dietary fibres, and therefore the seaweed powder may be in direct competition for water with the starches and hydrocolloids already present in the bread formulation (Rosell & Rojas, 2001). Therefore, the inclusion of mozuku powder may have resulted in insufficient amounts of water being available for complete starch gelatinisation and/or hydrocolloid hydration, this may explain the resulting volume decrease as mozuku concentrations increased.

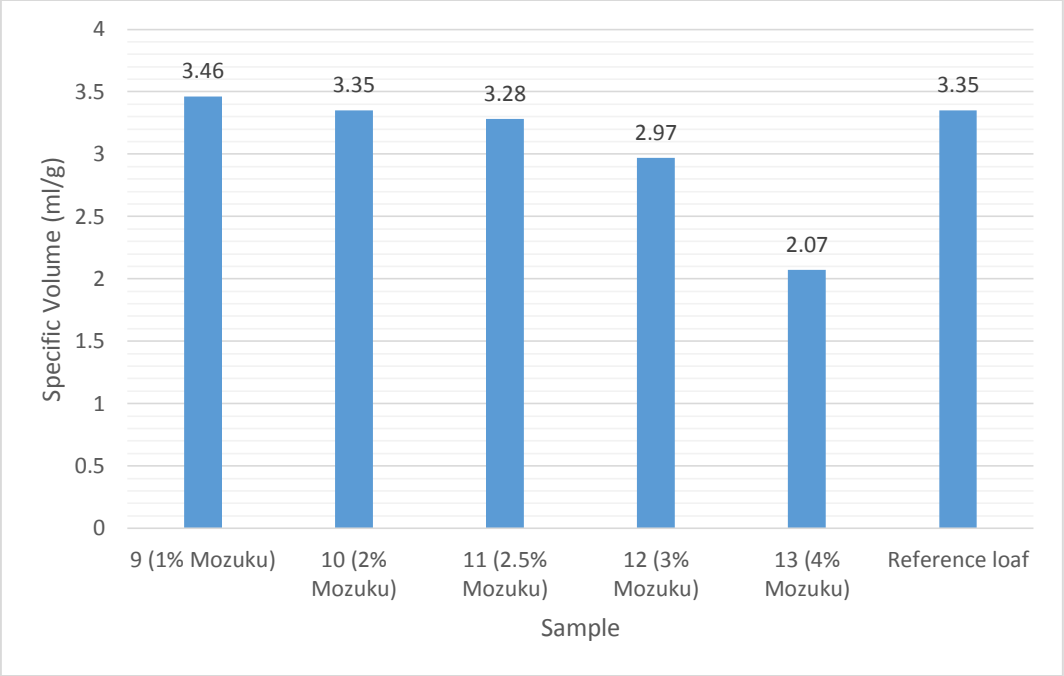


Figure 4.18 Outcomes of the specific loaf volume measurements. Specific volume was calculated by dividing volume by weight (ml/g)

4.3.2.4 Colour analysis

Tables 4.7 and 4.8 show the *L**, *a**, *b** values and RGB colours for crust and crumb colour respectively for the five GF formulations tested (formulations 9-13) and the reference GF loaf. Formulation 9 had the highest *L** value, while formulation 13 the lowest, thus, formulation 9 exhibited the lightest crust colour while formulation 13 exhibited the darkest. These results indicate that increasing mozuku concentrations lead to increased crust darkening, which is a similar result to that obtained in Section 4.2.2.4 for the wheat bread formulations. The *a** values for all samples were positive, indicating the crusts exhibited redness, with formulation 11 showing the highest *a** values and formulation 9 the lowest, however no clear relationship between mozuku concentration and redness was apparent. *b** values which represent yellowness in the crust were also positive for all the samples. The highest values for *b** were observed for formulation 10 and the lowest for formulation 13. The crust colour for formulation 9 was most similar to the reference loaf and overall.

Table 4.7 Crust colour expressed in *L**, *a**, *b** and RGB values for gluten-free formulations 9-13 including a standard reference bread loaf with different letters indicating significantly different groups (Tukey method, P<0.05)

Formulation	*L**		a*		b*		RGB
Reference	53.87	± 0.63^{ab}	11.98	± 0.20 ^{c}	26.22	± 0.37^{c}	
9	55.64	± 0.41^{a}	11.63	± 0.22 ^{c}	30.25	± 0.05^{b}	
10	51.56	± 0.52^{bc}	13.54	± 0.28 ^{bc}	33.02	± 0.26^{a}	
11	48.36	± 0.78^{cd}	16.18	± 0.17 ^{a}	31.32	± 0.82^{ab}	
12	45.46	± 0.77^{d}	14.41	± 1.22 ^{ab}	28.97	± 1.14^{b}	
13	40.57	± 3.35^{e}	13.25	± 1.14 ^{bc}	19.32	± 1.85^{d}	

Note: RGB = Red, Green, Blue colour profile, *L** = lightness axis, *a** = red-green axis, *b** = yellow-blue axis

Table 4.8 - Crumb colour expressed in *L**, *a**, *b** and RGB values for gluten-free formulations 9-13 including a standard reference bread loaf with different letters indicating significantly different groups (Tukey method, P<0.05)

Formulation	*L**		a*		b*		RGB
Reference	76.77	± 2.22^{a}	-0.76	± 0.13 ^{e}	9.90	± 0.47 ^{d}	
9	71.65	± 1.30^{b}	-0.20	± 0.12 ^{d}	13.39	± 0.25 ^{c}	
10	71.89	± 2.16^{b}	-0.01	± 0.14 ^{d}	14.12	± 0.36 ^{bc}	
11	71.91	± 0.74^{b}	1.98	± 0.03 ^{b}	15.08	± 0.68 ^{ab}	
12	66.64	± 0.68^{c}	0.98	± 0.34 ^{c}	15.33	± 0.47 ^{ab}	
13	52.32	± 1.85^{d}	2.62	± 0.18 ^{a}	16.05	± 0.82 ^{a}	

Note: RGB = Red, Green, Blue colour profile, *L** = lightness axis, *a** = red-green axis, *b** = yellow-blue axis

Significance differences ($P<0.05$) in crumb colour were found between formulations in all colour parameters with formulations 12 and 13 rating significantly ($P<0.05$) lower in the L^* value, thus indicating that the presence of mozuku powder darkens crumb colour. The a^* values varied between positive and negative but the highest values were found in formulation 13 indicating it possesses the most red colouration, while formulation 9 exhibited the highest green values. The highest b^* values were observed in formulation 13, while the lowest values were observed for formulation 9 however, b^* values were positive for all formulations indicating the crumb appeared more yellow than blue. All GF formulations showed significant differences ($P<0.05$) in crumb colour when compared to the reference loaf, specifically the reference loaf showed lighter colour (higher L^*), and more green/less red (lower a^*) and more blue/less yellow (higher b^*). These results indicate that mozuku power affected the crumb colour by increasing darkness, redness and yellowness.

4.2.2.4 Water activity

The water activities (a_w) of the five GF loaves and reference loaf are shown in Figure 4.19. Formulation 9 (1% mozuku, $a_w= 0.985$) and formulation 12 (3% mozuku, $a_w= 0.975$) were the only loaves found to be significantly different from each other, of which formulation 9 showed the highest water activity and formulation 12 the lowest. The lower water activity observed at the higher concentrations of mozuku powder may be due to minerals in the powder which are capable of decreasing water activity (Rosell & Rojas, 2001).

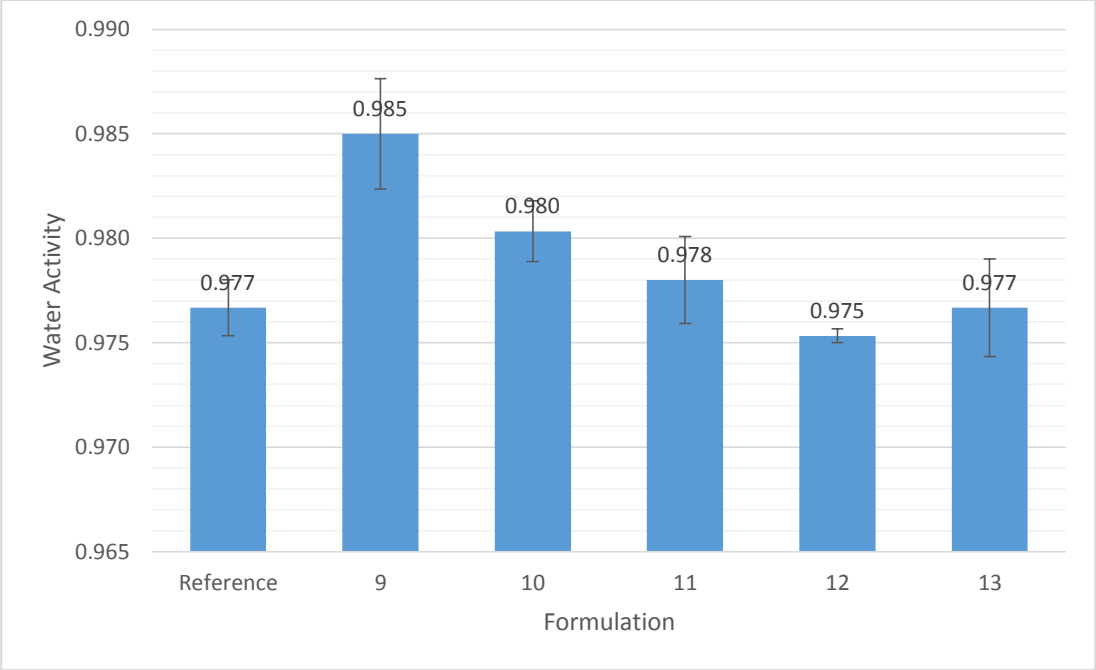


Figure 4.19 Mean values of water activity measurements for formulations 9-13 including a standard wheat bread loaf for reference, error bars indicate the standard error.

4.3.2.5 Selection of a suitable formulation for consumer evaluation

Formulation 11 was selected to undergo consumer sensory evaluations as it was determined to be a sufficient compromise between high levels of mozuku powder and satisfactory levels of bread quality. In comparison to the reference loaf, all formulations except formulation 13 (4% mozuku) exhibited similar textural properties and specific loaf volumes. Formulation 11 (2.5% mozuku) showed the greatest similarities for the textural attributes hardness, chewiness and cohesiveness and similar specific loaf volume and water activity results to the reference loaf. Based on the measured quality parameters formulation 12 (3% mozuku) may have also been suitable for consumer evaluation, however, discussions with bread manufacturers indicated the inclusion of 3% mozuku powder may be too high due to the associated production costs and therefore a lower percentage of 2.5% was selected (A. Smith, personal communication, June 15, 2016)

4.3.3 Consumer evaluation

Consumer evaluations of formulation 11, the gluten-free formulation containing 2.5% mozuku

powder, was carried out using a 9-point hedonic scale. The average overall acceptability of formulation 11 (2.5% mozuku) was ranked as 6.4 on a 9-point hedonic scale indicating a "moderate" to "very much" likeability (Figure 4.20). Mean values for appearance (6.7), texture (6.6), taste (6.1), and aroma (6.7), were also positive. Results indicated a great deal of diversity in consumer responses. Feedback from the additional comments section indicated some consumers perceived the crumb texture as soft and that the bread possessed a rich, pleasant aroma, however, the crust appeared to be less well received with comments describing it as bitter, tough, and powdery. One possible reason for the comments regarding bitterness in the crust may be due to the presence of certain minerals in the mozuku powder as indicated by its high ash content, these minerals may have become concentrated in the crust due the increased dehydration it goes through when compared to the crumb (Section 4.1.1). Previous studies into the inclusion of the minerals potassium and magnesium in breads has resulted in loaves with higher bitterness ratings (Salovaara, 1982). Additionally, some unknown compounds may have led to the increased production of bitter compounds during bread making, previous studies have shown that compounds found in bread such as 5-hydroxymethylfurfural and 2,3-dihydro-3,5-dihydroxy-6-methyl-4-pyran-4-one are highly correlated with bitterness and that these compounds are generated throughout Malliard reactions occurring in the crust (Bin, Jiang, Cho, & Peterson, 2012). .

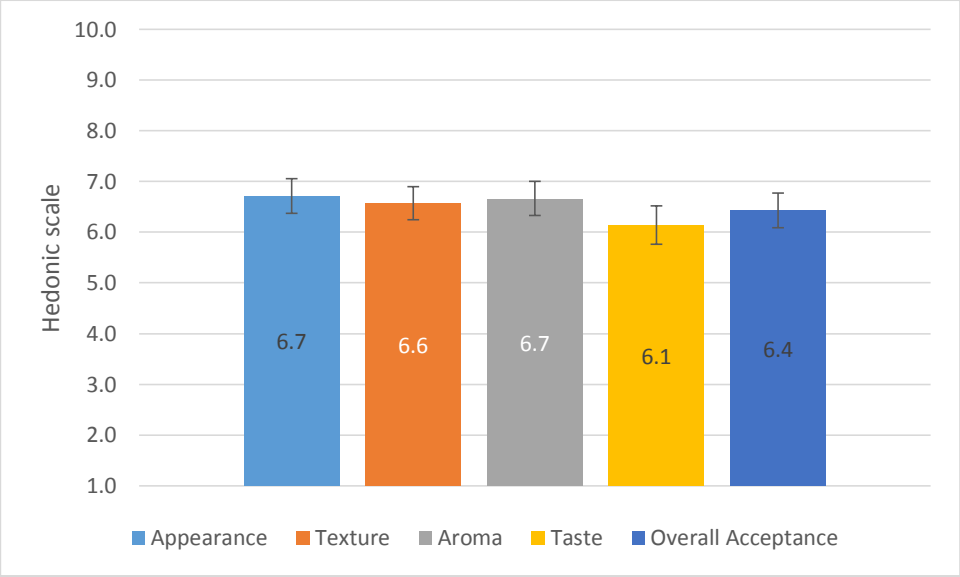


Figure 4.20 Average consumer (n=21) sensory evaluations results of formulation 11 on a hedonic scale with error bars indicating standard error.

In addition to scoring the different attributes, panellists were asked whether they would be willing to purchase the product if it were available. Figure 4.21 indicates that 24% of the consumers would purchase the product very often, 10% of the consumers liked it and would buy it now and then, 29% of the consumers would buy the product when available but would not go out of their way to do so, while 19% of the consumers did not like it and another 19% of the consumers would hardly ever buy the product. Overall, the 2.5% gluten-free formulation was deemed acceptable by the majority of consumers with it being ranked positively in all sensory parameters and the majority of the consumers indicating an intent to purchase the product on occasion. However, a number of participants commented negatively on the crust describing it as bitter and tough. Additional research is required in order to determine the cause of the crust's bitterness.

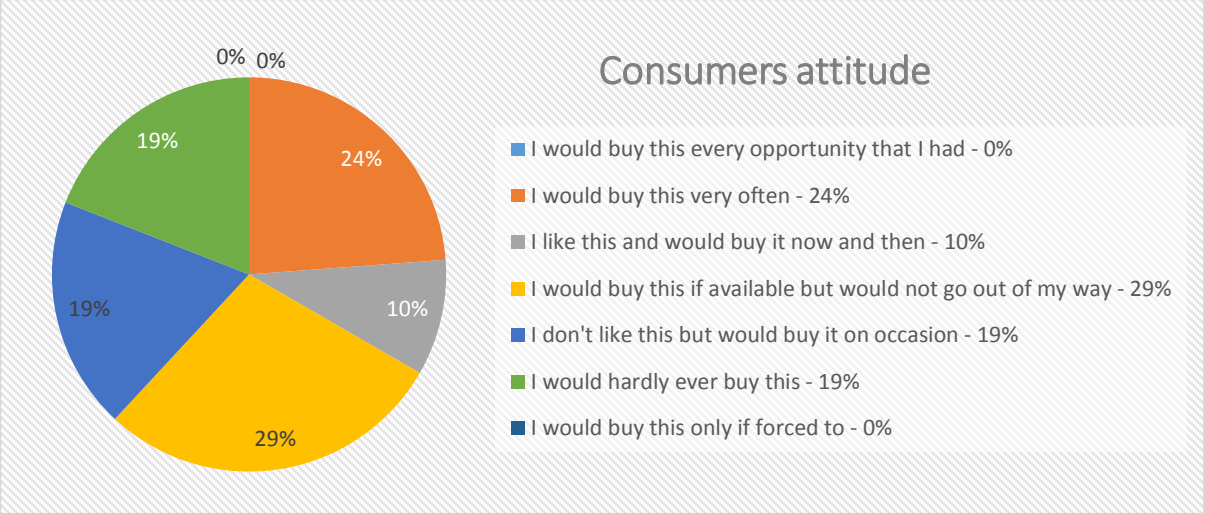


Figure 4.21 Outcomes of consumers' attitude regarding the final product. The percentages indicate the amount of consumers with a certain attitude regarding the final product

4.4 Limits of study and future research

This study shows that it is possible to develop wheat-based and gluten-free bread products that contain mozuku powder which retain satisfactory levels of bread quality. However, breadmaking consists of many complex biological, chemical and physical processes which are affected by interactions between functional bread components and external breadmaking processes. Therefore, while this study showed it is possible to produce bread products containing mozuku powder of acceptable quality, future research may desire to explore in detail interactions between mozuku powder and individual bread components, such as the interaction between mozuku powder and yeast fermentation. Understanding the mechanisms of the interactions between mozuku powder and specific bread components will allow bakery products to be efficiently optimised when incorporating mozuku powder into them.

Additionally, it was outside the scope of this thesis to determine whether fucoidan, the bioactive component of mozuku powder, was present in the finished loaves. Current fucoidan chemical analyses are unable to distinguish the fucoidan polysaccharide from other bread components and therefore additional techniques will need to be developed to determine its presence and bioactivity in the presence of other food compounds. Further research involving human clinical trials will also be required to determine any beneficial health effects of the mozuku bread formulations over standard bread recipes and, if present, whether fucoidan retained its bioactive functionalities following the breadmaking process.

5 Summary

Mozuku powder analysed in this study contained 46.9% ash, 30.4% dietary fibre, 19.0% sodium, 5.4% protein, 4.7% available carbohydrates, 1.0% fat, 0.13% sugar and an overall energy content of 209.1 kJ/100 g. From analysis the amino acids aspartic acid, methionine, histidine, glutamic were present in the highest concentrations while the fatty acids were mostly compromised of palmitic acid (68.8%), and oleic acid (12.5%). The high ash and sodium contents of the mozuku powder resulted in increased solute concentrations in the doughs and as a result bread formulations were adjusted by reducing added table salt by 0.5% per 1% mozuku powder added.

Addition of mozuku powder to both gluten-free and wheat bread formulations resulted in the visible presence of mozuku particles, decreased lightness and increased redness/yellowness, this effect being more pronounce as more mozuku powder was added. Specific loaf volumes were reduced as mozuku concentrations were increased for both gluten-free and wheat bread formulations regardless of adjustments in salt. The inclusion of mozuku powder also affected breadcrumb water activity, increasing it for wheat bread while decreasing it for gluten-free breads. Textural results indicated that in wheat bread mozuku powder increased hardness, while decreasing springiness, cohesiveness and resilience; however by adjusting the level of added salt many of the associated textural changes were reversed. For gluten-free bread, concentrations of mozuku powder (with adjustments in added salt) of up to 3% were not associated with any significant textural changes, although at 4% significant increases in hardness, chewiness and resilience were obtained.

Wheat bread containing mozuku powder at 1% and 2% (bakers' percentage) did not significantly affect consumer perceptions of each formulation when compared to a control. The wheat bread formulations containing 1% mozuku powder obtained an overall acceptance of 6.8 on a 9-point hedonic scale and was the most preferred sample. An acceptable gluten-free bread formulation was also able to be developed containing mozuku at an inclusion rate of 2.5% with an overall acceptance of 6.4 with 63% of panellists indicating willingness to purchase the product. The microbial stability of wheat breads stored at 20°C for 3 days were not significantly affected by the inclusion of mozuku powder (Control, 1%, 2%).

6 Conclusion

A review of the literature indicated mozuku powder and specifically fucoidan, exhibits a number of biological activities including anticancer, immunomodulatory, anti-inflammatory and antioxidative effects and therefore if successfully incorporated into bread formulations may confer those beneficial health effects to consumers. However, no studies were able to be found which showed how the biological activities of fucoidan may be affected by the breadmaking process and thus presents a future area of research. The incorporation of mozuku powder into wheat and gluten-free bread formulations yielded a range of effects on bread loaf quality, with the most notable effects being in increases in hardness and chewiness; and decreases in specific loaf volume in formulations without salt adjustments and at the highest levels of mozuku powder inclusion (4%). By adjusting levels of added salt wheat and gluten-free breads formulations were able to be developed which possessed similar quality levels results to a reference loaf, these formulations underwent consumer sensory evaluations which indicated they were in general well-received by participants. Mozuku powder was also not shown to affect the microbial stability of the developed wheat bread formulations. In conclusion, bread loaf quality was influenced by mozuku powder concentration, however, gluten-free and wheat-based bread loaves of acceptable quality were able to be developed by adjusting levels of added salt.

7 Recommendations

The mesh size analysis of the mozuku powder revealed the bimodality of the distribution and the relative abundance of particles in the 63-710 µm range, at these sizes mozuku particles were clearly visible in each bread formulation. If the visible presence of the mozuku powder particles was determined to be undesirable in the finished loaf, possibly by further consumer evaluations, additional milling of the mozuku powder so that 99% of particles are below 300 µm would be recommended. Below 300 µm mozuku particles would likely blend in with flour particles, thus becoming less noticeable.

Addition of 1% and 2% mozuku powder concentrations, with salt adjustments, to a standard wheat bread recipe resulted in bread loaves that were positively received by consumers. Therefore, when salt levels are adequately adjusted in wheat-based bakery products, the inclusion of up to 2% mozuku powder is unlikely to negatively affect consumer perception of the product. Reductions in added salt content of 0.5% per 1% mozuku powder may be recommended due to levels of ash and sodium found in mozuku powder which are likely to impact on yeast fermentation. Addition of mozuku powder into gluten-free breads at 2.5% was also well received by consumers and presents a real opportunity to improve on bread qualities such as nutritional composition, depth of colour and textural resilience of the loaf. However, a number of sensory participants commented on the presence of bitterness in the gluten-free crust, which may have been due to the high mineral content of the mozuku powder. The presence of minerals such as calcium and magnesium in mozuku powder may have had a negative effect on textural parameters such as loaf volume whilst also resulting in increased crust bitterness and therefore adjustments in formulations may be necessary. Thorough washing of raw mozuku prior to drying and milling may reduce levels of minerals in mozuku powder and any associated negative effects on bread quality.

These gluten-free and wheat bread formulations may be used in future clinical trials in order to determine whether the inclusion of mozuku powder at these levels into bread provides any beneficial health effects. Although, following the clinical trials these bread formulations will need to be optimised further prior to entering the market. Levels of added salt, yeast, hydrocolloids and water should be optimised to maximise loaf texture, volume and flavour.

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Appendices

Appendix A

White (wheat) bread formulation with added mozuku and reduced salt

	Control		1% M.		2% M.		1% RSM		2% RSM	
Ingredients	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)
Wheat flour	100	300	100	300	100	300	100	300	100	300
Water	66	198	66	198	66	198	66	198	66	198
Yeast	5	15	5	15	5	15	5	15	5	15
Sugar	5	15	5	15	5	15	5	15	5	15
Salt	1.5	4.5	1.5	4.5	1.5	4.5	1	3	0.5	1.5
Soy oil	3	9	3	9	3	9	3	9	3	9
Mozuku powder	0	0	1	3	2	6	1	3	2	6

White (wheat) bread formulation used for consumer and microbial stability evaluation

	Control		1% RSM		2% RSM	
Ingredients	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)
Wheat flour	100	300	100	300	100	300
Water	66	198	66	198	66	198
Yeast	5	15	5	15	5	15
Sugar	5	15	5	15	5	15
Salt	1.5	4.5	1	3	0.5	1.5
Soy oil	3	9	3	9	3	9
Mozuku powder	0	0	1	3	2	6

Gluten-free bread formulations – Base formulation

	Formulation							
	1		2		3		4	
Ingredients	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)
Rice flour	70	280	70	280	30	120	30	120
Tapioca starch	30	120	30	120	70	280	70	280
TOTAL	**70**	**400**	**70**	**400**	**100**	**400**	**100**	**400**
HPMC	1	4	0	0	1	4	0	0
Guar gum	0	0	1	4	0	0	1	4
Xanthan	0	0	0	0	0	0	0	0
CMC	0	0	0	0	0	0	0	0
Soy oil	12	48	12	48	12	48	12	48
Sugar	6	24	6	24	6	24	6	24
Salt	2	8	2	8	2	8	2	8
Yeast	2	8	2	8	2	8	2	8
Water	80	320	80	320	80	320	80	320

	Formulation							
	5		6		7		8	
Ingredients	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)
Rice flour	30	120	30	120	30	120	45	180
Tapioca starch	70	280	70	280	70	280	55	220
TOTAL	**100**	**400**	**100**	**400**	**100**	**400**	**100**	**400**
HPMC	0	0	0	0	0.5	2	0.5	2
Guar gum	0	0	0	0	0.5	2	0.5	2
Xanthan	1	4	0	0	0	0	0	0
CMC	0	0	1	4	0	0	0	0
Soy oil	12	48	12	48	12	48	12	48
Sugar	6	24	6	24	6	24	6	24
Salt	2	8	2	8	2	8	2	8
Yeast	2	8	2	8	2	8	2	8
Water	80	320	80	320	80	320	80	320

Gluten-free bread formulations – Mozuku introduction

	Formulation					
	9		10		11	
Ingredients	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)
Rice flour	45	180	45	180	45	180
Tapioca starch	55	220	55	220	55	220
TOTAL	**100**	**400**	**100**	**400**	**100**	**400**
Guar gum	0.5	2	0.5	2	0.5	2
HPMC	0.5	2	0.5	2	0.5	2
Soy oil	12	48	12	48	12	48
Sugar	6	24	6	24	6	24
Salt	**1**	**4**	**1**	**4**	**0.75**	**3**
Mozuku	**1**	**4**	**2**	**8**	**2.5**	**10**
Yeast	3.5	14	3.5	14	3.5	14
Water	80	320	80	320	80	320

	Formulation			
	12		13	
Ingredients	% Flour (w/w)	Weight (g)	% Flour (w/w)	Weight (g)
Rice flour	45	180	45	180
Tapioca starch	55	220	55	220
TOTAL	**100**	**400**	**100**	**400**
Guar gum	0.5	2	0.5	2
HPMC	0.5	2	0.5	2
Soy oil	12	48	12	48
Sugar	6	24	6	24
Salt	**0.5**	**2**	**0**	**0**
Mozuku	**3**	**12**	**4**	**16**
Yeast	3.5	14	3.5	14
Water	80	320	80	320

Gluten-free bread formulations – Sensory evaluation

	Formulation	
	11	
Ingredients	**%**	**gram**
Rice flour	45	180
Tapioca starch	55	220
TOTAL	**100**	**400**
Guar gum	0.5	2
HPMC	0.5	2
Soy oil	12	48
Sugar	6	24
Salt	**0.75**	**3**
Mozuku	**2.5**	**10**
Yeast	3.5	14
Water	80	320

Appendix B

SENSORY EVALUATION OF GLUTEN-FREE MOZUKU BREAD

Please taste the sample and indicate how much you like/dislike it by encircle the appropriate box. You may taste the sample more than once. Use the water provided to cleanse your palate before tasting.

1. How would you rate the **APPERARANCE** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

2. How would you rate the **TEXTURE** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

3. How would you rate the **AROMA** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

4. How would you rate the **TASTE** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

5. How would you rate the **OVERALL ACCEPTABILITY** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

6. How do you feel about the final product? Thick the box that describes best.

	1. I would buy this every opportunity that I had
	2. I would buy this very often
	3. I like this and would buy it now and then
	4. I would buy this if available but would not go out of my way
	5. I don't like this but would buy it on occasion
	6. I would hardly ever buy this
	7. I would buy this only if forced to

Additional comments:

_____

_____

_____

_____

Thank you

SENSORY EVALUATION OF MOZUKU ENRICHED WHITE WHEAT BREAD

You will be given three samples of bread, each coded with a 3 digit number.

Please taste each sample and indicate how much you like/dislike it by ticking ☑the appropriate box. You may taste the sample more than once. Use the water provided to cleanse your palate before and after tasting.

Sample code: **Date:**

1. How would you rate the **APPERARANCE** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

2. How would you rate the **TEXTURE** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

3. How would you rate the **AROMA** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

4. How would you rate the **TASTE** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

5. How would you rate the **OVERALL ACCEPTABILITY** of this sample?

Dislike extremely	Dislike very much	Dislike Moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

6. Which sample would you prefer to purchase in a store? (Please write the code corresponding to the sample)

Preferred Sample Code:_____

Additional comments:

_____

_____

_____

Thank you

INFORMATION SHEET MOZUKU ENRICHED GLUTEN-FREE BREAD

Introduction

Hi, my name's Stephen Grubb, a Master of Food Technology student in the School of Food and Nutrition, Albany campus, Massey University. This study is part of my research project and may contribute to the development of gluten-free bread containing mozuku, an approved food ingredient. You are invited to take part in a study that assesses the sensory characteristics of the developed gluten-free bread. The aim of the sensory evaluation is to evaluate the level of acceptance of the gluten-free product by potential consumers.

Participant involvement

The trial involves tasting and evaluating gluten-free bread. Your participation will take 3 to 5 minutes. The breads you will be tasting may contain ingredients which may be harmful or cause allergic reactions with certain groups of people. You should not take part if you are allergic or may be negatively affected by the any following ingredients: Tapioca starch, rice flour, soybean oil, dried mozuku, yeast, guar gum, hydroxypropyl methyl cellulose (HPMC). In the unlikely event of any adverse reaction, medical assistance will be provided. You may advise one of the researchers of any potentially relevant cultural, religious or ethical beliefs which may prevent you from consuming the food under consideration.

The information collected in this study will not be linked to any individual's identity and will be used to complete an assignment in partial fulfilment of the Master of Food Technology.

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular question;
- Withdraw from the study (at any time);
- Ask any questions about the study at any time during participation;
- Provide information on the understanding that your name will not be used unless you give permission to the researcher;

Project Contacts

- Stephen Grubb (Masters student)- sgru016@aucklanduni.ac.nz
- Dr Tony Mutukumira (Supervisor) - a.n.mutukumira@massey.ac.nz

"This project has been evaluated by peer review and judged to be low risk. Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named above are responsible for the ethical conduct of this research.

If you have any concerns about the conduct of this research that you wish to raise with someone other than the researcher(s), please contact Professor John O'Neill, Director, Research Ethics, telephone 06 350 5249, email humanethics@massey.ac.nz".

INFORMATION SHEET MOZUKU ENRICHED WHITE BREAD

Introduction

Hi, my name's Stephen Grubb, a Master of Food Technology student in the School of Food and Nutrition, Albany campus, Massey University. This study is part of my research project and may contribute to the development of wheat bread containing a Japanese seaweed variety (Mozuku). You are invited to take part in a study that assesses the sensory characteristics of the developed gluten-free bread. The aim of this sensory testing is to evaluate the level of acceptance of the bread product by potential consumers.

Participant involvement

The trial involves tasting and evaluating wheat bread, which may or may not contain varying levels of Mozuku. Your participation will take 5 to 10 minutes. The breads you will be tasting may contain ingredients which may be harmful or cause allergic reactions with certain groups of people. You should not take part if you are allergic or may be negatively affected by the any following ingredients: White wheat flour, gluten, yeast, soybean oil and dried mozuku. In the unlikely event of any adverse reaction, medical assistance will be provided. You may advise one of the researchers of any potentially relevant cultural, religious or ethical beliefs which may prevent you from consuming the food under consideration.

The information collected in this study will not be linked to any individual's identity and will be used to complete an assignment in partial fulfilment of the Master of Food Technology degree.

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular question;
- Withdraw from the study (at any time);
- Ask any questions about the study at any time during participation;
- Provide information on the understanding that your name will not be used unless you give permission to the researcher;

Project Contacts

- Stephen Grubb (Masters student)- sgru016@aucklanduni.ac.nz
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If you have any concerns about the conduct of this research that you wish to raise with someone other than the researcher(s), please contact Professor John O'Neill, Director, Research Ethics, telephone 06 350 5249, email humanethics@massey.ac.nz".

PARTICIPANT CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction.

I understand that I have the right to withdraw from the study at any time and decline my answers.

I understand the allergen risks and agree to voluntarily participate in this study under the conditions set out in the Information Sheet.

Signature:…… Date: ……..…..

Full Name(s) (Printed):……..……

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Date: 20 May 2016

Dear Stephen Grubb

Re: Ethics Notification **- 4000016152 - Development of wheat- and gluten-free bread enriched with mozuku**

Thank you for your notification which you have assessed as Low Risk.

Your project has been recorded in our system which is reported in the Annual Report of the Massey University Human Ethics Committee.

The low risk notification for this project is valid for a maximum of three years.

If situations subsequently occur which cause you to reconsider your ethical analysis, please go to http://rims.massey.ac.nz and register the changes in order that they be assessed as safe to proceed.

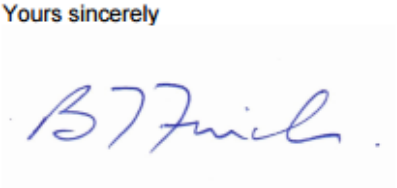
Please note that travel undertaken by students must be approved by the supervisor and the relevant Pro Vice-Chancellor and be in accordance with the Policy and Procedures for Course-Related Student Travel Overseas. In addition, the supervisor must advise the University's Insurance Officer.

A reminder to include the following statement on all public documents:
"This project has been evaluated by peer review and judged to be low risk. Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named in this document are responsible for the ethical conduct of this research.

If you have any concerns about the conduct of this research that you want to raise with someone other than the researcher(s), please contact Dr Brian Finch, Director - Ethics, telephone 06 3569099 ext 86015, email humanethics@massey.ac.nz. "

Please note, if a sponsoring organisation, funding authority or a journal in which you wish to publish requires evidence of committee approval (with an approval number), you will have to complete the application form again, answering "yes" to the publication question to provide more information for one of the University's Human Ethics Committees. You should also note that such an approval can only be provided prior to the commencement of the research.

Yours sincerely



Research Ethics Office, Research and Enterprise
Massey University, Private Bag 11 222, Palmerston North, 4442, New Zealand **T** 06 350 5573; 06 350 5575 **F** 06 355 7973
E humanethics@massey.ac.nz **W** http://humanethics.massey.ac.nz

Mozuku wheat bread sensory evaluation poster

Sensory Evaluation

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Mozuku Bread Tasting

- **When ?:** Between 9am and 2pm, Wednesday 20th of July
- **Where?:** Building 26 Sensory Lab, Oteha Rohe precinct, Albany campus, Massey University
- **What?:** Tasting sessions will include three samples of white bread, some of which have been enriched with a Japanese seaweed variety (Mozuku). You will be asked to complete a short questionnaire which should take between 5-10mins.
- **Who?:** Open to general public, minimum age of 18

Mozuku gluten-free bread sensory evaluation poster

Sensory Evaluation

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Mozuku Gluten-Free Bread Tasting

- **When ?:** Between 9am and 2pm, Wednesday 20th of August
- **Where?:** Building 26 Sensory Lab, Oteha Rohe precinct, Albany campus, Massey University
- **What?:** Tasting sessions will include one sample of gluten-free bread which has been enriched with a Japanese seaweed variety (Mozuku). You will be asked to complete a short questionnaire which should take between 5-10mins.
- **Who?:** Open to general public, minimum age of 18

Appendix C

Nutritional analysis of mozuku

NutLab ID	Sample Name	Moisture %	Ash %	Protein %	Fat %	Carb %	TDF %	Energy KJ/100g
TN15-617-01	Mozuku Powder	11.5	46.9	5.4	1.0	4.7	30.4	209.1

NutLab ID	Sugars g/100g	Sodium g/100g
Mozuku	0.130	13.4

AMINO ACIDS	Mozuku powder
Aspartic Acid	0.59
Threonine	0.28
Serine	0.24
Glutamic Acid	0.55
Proline	0.22
Glycine	0.30
Alanine	0.37
Valine	0.33
Methionine	0.07
Isoleucine	0.24
Leucine	0.42
Tyrosine	0.16
Phenylalanine	0.25
Histidine	0.07
Lysine	0.21
Arginine	0.27
Units	mg/100mg

Fatty acids	g/100g	C18:2n6c Linoleic	0.02
C6:0 Caproic	<0.01	C20:0 Arachidic	0.01
C8:0 Caprylic	<0.01	C18:3n6 - cis-6,9,12-Gamma linolenic	ND
C10:0 Capric	<0.01	C20:1n9 - cis-11-Eicosenoic	ND
C11:0 Undecanoic	<0.01	C18:3n3 - cis-9,12,15-Alpha linolenic	0.01
C12:0 Lauric	<0.01	C21:0 Heneicosanoic	ND
C13:0 Tridecanoic	<0.01	C20:2n6 - cis-11,14-Eicosadienoic	ND
C14:0 Myristic	<0.01	C22:0 Behenic	0.02
C14:1n5 - cis-9-Myristoleic	ND	C20:3n6 - cis-8,11,14-Eicosatrienoic	ND
C15:1n5 - cis-10-Pentadecenoic	ND	C22:1n9 - cis-13-Erucic	<0.01
C16:0 Palmitic	0.33	C20:3n3 - cis-11,14,17-Eicosatrienoic	ND
C16:1n7 - cis-9-Palmitoleic	0.01	C23:0 Tricosanoic	ND
C17:0 Margaric	<0.01	C20:4n6 - cis-5,8,11,14-Arachidonic	0.01
C17:1n7 - cis-10-Heptadecenoic	ND	C22:2n6 - cis-13,16-Docosadienoic	ND
C18:0 Stearic	0.02	C24:0 Lignoceric	ND
C18:1n9t Elaidic	ND	C20:5n3 - cis-5,8,11,14,17-Epa	<0.01
C18:1n7t Vaccenic	ND	C24:1n9 - cis-15- Nervonic	ND
C18:1n9c Oleic	0.06	C22:5n3 - cis-7,10,13,16,19-DPA	ND
C18:1n7c Vaccenic	0.01	C22:6n3 - cis-4,7,10,13,16,19-DHA	ND
C18:2n6t Linolelaidic	ND		

Amino acids: HCl hydrolysis followed by Ninhydrin method (sub-contracted the HPLC separation)

Fat : Mojonnier, AOAC 954.02

****Energy : By calculation***

Moisture + Ash: AOAC 930.15/925.10/942.05

Total Dietary fibre : Megazyme , AOAC 991.43

****Avail Carbohydrate (Carb): By difference***

Sugars : Phenol sulphuric, sub-contracted

Crude protein: Leco, AOAC 968.06 (Dumas method). N-P = 6.25

Sodium: ICP-OES, sub-contracted

****Tests marked with an asterix are currently outside the scope of the Nutrition Laboratory's accreditation***

MOLAB Ltd.

Consulting Analytical Chemist & Food Technologist.

PO Box 18396, Glen Innes, Auckland 1743, New Zealand.

eMail molab@ww.co.nz Web Site www.molab.co.nz

Lab Ph/Fax (09) 5706040, Home (09) 5217292, Mobile (027) 4523984.

CLIENT

Glenorie

DATE & JOB No.

2/9/15 51872

JOB

Dried Limu

REPORT

Analysis

Limu as received

Moisture % w/w	11.0
Soluble Gums %w/w	38.5

Soluble Gums

Sulphate % w/w	18.15

Sulphate in soluble gums	7.37

as a percent of Limu % w/w

Fucoidan ex sulphate % w/w	22.7

At eq wt of 296

Appendix D

Edmonds Flour High Grade

Ingredients: Wheat Flour

Nutrient	Per 100g
Energy	1450kJ
Protein	11g
Fat - Total	1.4g
Saturated	<1g
Carbohydrate	69.6g
Sugars	<1g
Dietary Fibre	3.5g
Sodium	5mg

Edmonds Active Dried Yeast

Ingredients: Active Dry Yeast (Saccharomyces Cerevisiae), Salt

Nutrient	Per 100g
Energy	1250kJ
Protein	40g
Fat - Total	2.2g
Saturated	0.9g
Carbohydrate	18.5g
Sugars	0g
Sodium	210mg

Chelsea White Sugar

Ingredients: Cane Sugar

Nutrient	Per 100g
Energy	1700kJ
Protein	0g
Fat - Total	0g
Saturated	0g
Carbohydrate	100g
Sugars	100g
Sodium	<2.5mg

Cerebos Salt – Iodised

Ingredients: Salt, Anticaking Agent (551), Potassium Iodate

Nutrient	Per 100g
Energy	0kJ
Protein	0g
Fat - Total	0g
Saturated	0g
Carbohydrate	0g
Sugars	0g
Sodium	39000mg
Potassium	11g

Simply Soya Oil

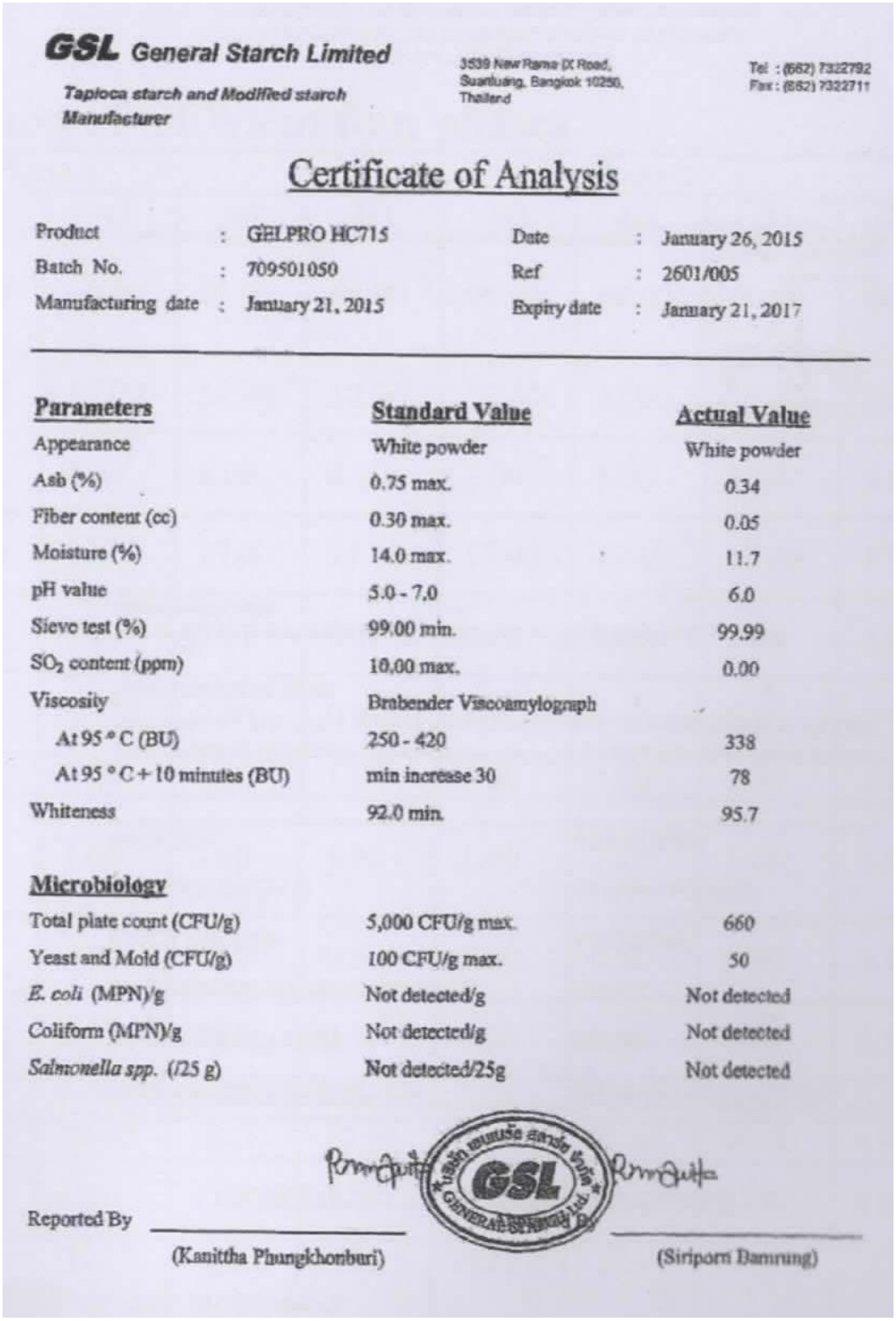
Ingredients: Soyabean Oil

Nutrient	Per 100g
Energy	3700kJ
Protein	0g
Fat - Total	100g
Saturated	20g
Trans	1g
Polyunsaturated	55g
Monounsaturated	15g
Cholesterol	0mg
Carbohydrate	0g
Sugars	0g
Sodium	0mg

Freshlife Rice Flour Gluten Free

Nutrient	Per 100g
Energy	1500kJ
Protein	6.4g
Fat - Total	1g
Saturated	0.5g
Carbohydrate	79.3g
Sugars	0.5g
Dietary Fibre	0.6g
Sodium	5mg
Gluten	0mg

Modified Tapioca Starch



GSL General Starch Limited

Tapioca starch and Modified starch Manufacturer

3539 New Rama IX Road, Suanluang, Bangkok 10250, Thailand

Tel : (662) 7322792
Fax : (662) 7322711

Certificate of Analysis

Product	:	GELPRO HC715	Date	:	January 26, 2015
Batch No.	:	709501050	Ref	:	2601/005
Manufacturing date	:	January 21, 2015	Expiry date	:	January 21, 2017

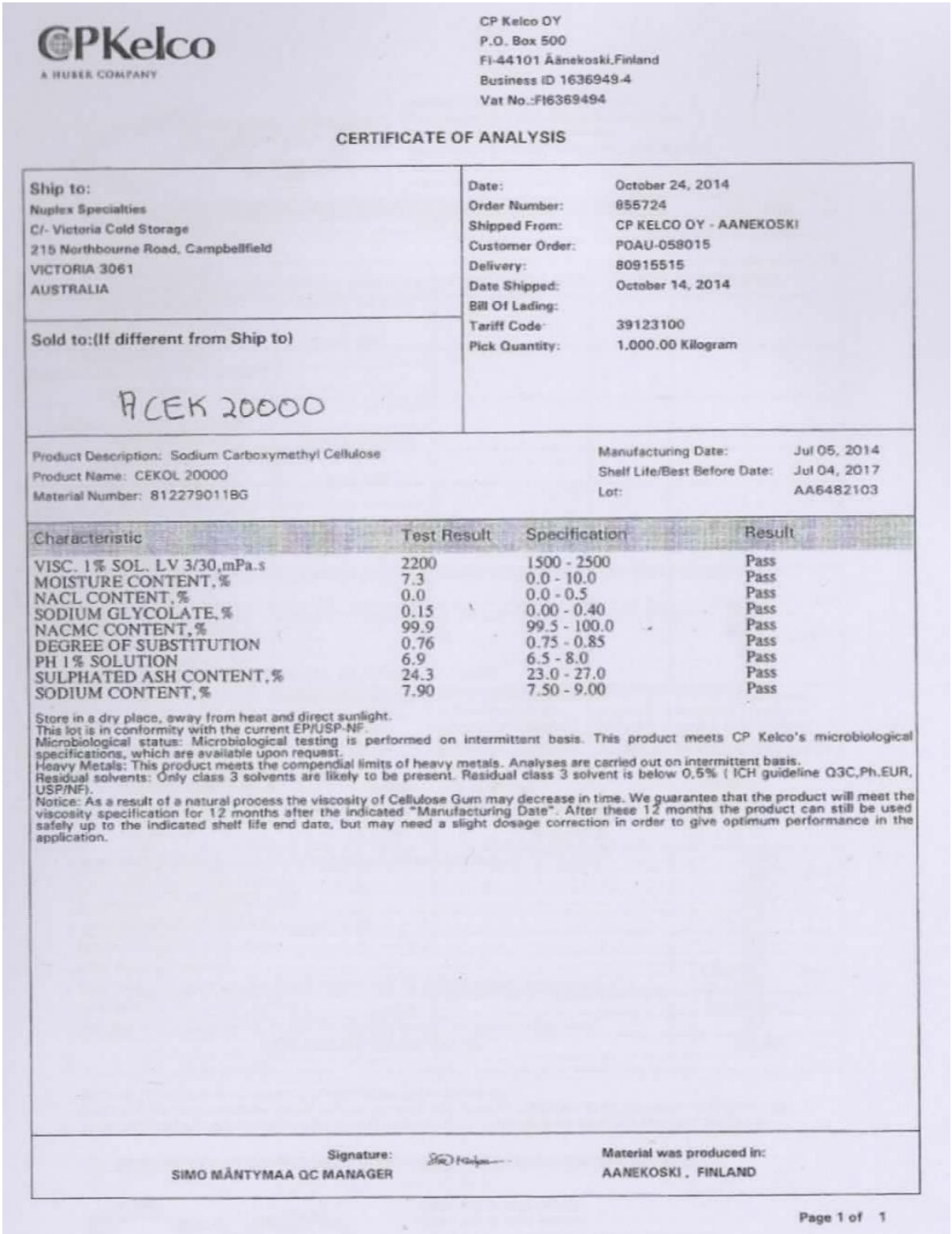
Parameters	Standard Value	Actual Value
Appearance	White powder	White powder
Ash (%)	0.75 max.	0.34
Fiber content (cc)	0.30 max.	0.05
Moisture (%)	14.0 max.	11.7
pH value	5.0 - 7.0	6.0
Sieve test (%)	99.00 min.	99.99
SO_2 content (ppm)	10.00 max.	0.00
Viscosity	Brabender Viscoamylograph	
At 95 ° C (BU)	250 - 420	338
At 95 ° C + 10 minutes (BU)	min increase 30	78
Whiteness	92.0 min.	95.7
Microbiology		
Total plate count (CFU/g)	5,000 CFU/g max.	660
Yeast and Mold (CFU/g)	100 CFU/g max.	50
E. coli (MPN)/g	Not detected/g	Not detected
Coliform (MPN)/g	Not detected/g	Not detected
Salmonella spp. (/25 g)	Not detected/25g	Not detected

Reported By

(Kanittha Phungkhonburi)

(Siriporn Damrung)

Carboxymethyl Cellulose



CPKelco
A HUBER COMPANY

CP Kelco OY
P.O. Box 500
FI-44101 Äänekoski,Finland
Business ID 1636949-4
Vat No.:FI6369494

CERTIFICATE OF ANALYSIS

Ship to:		Date:	October 24, 2014
Nuplex Specialties		Order Number:	855724
C/- Victoria Cold Storage		Shipped From:	CP KELCO OY - AANEKOSKI
215 Northbourne Road, Campbellfield		Customer Order:	POAU-058015
VICTORIA 3061		Delivery:	80915515
AUSTRALIA		Date Shipped:	October 14, 2014
		Bill Of Lading:	
Sold to:(If different from Ship to)		Tariff Code:	39123100
		Pick Quantity:	1,000.00 Kilogram

ACEK 20000

Product Description: Sodium Carboxymethyl Cellulose
Product Name: CEKOL 20000
Material Number: 812279011BG

Manufacturing Date: Jul 05, 2014
Shelf Life/Best Before Date: Jul 04, 2017
Lot: AA6482103

Characteristic	Test Result	Specification	Result
VISC. 1% SOL. LV 3/30,mPa.s	2200	1500 - 2500	Pass
MOISTURE CONTENT,%	7.3	0.0 - 10.0	Pass
NACL CONTENT,%	0.0	0.0 - 0.5	Pass
SODIUM GLYCOLATE,%	0.15	0.00 - 0.40	Pass
NACMC CONTENT,%	99.9	99.5 - 100.0	Pass
DEGREE OF SUBSTITUTION	0.76	0.75 - 0.85	Pass
PH 1% SOLUTION	6.9	6.5 - 8.0	Pass
SULPHATED ASH CONTENT,%	24.3	23.0 - 27.0	Pass
SODIUM CONTENT,%	7.90	7.50 - 9.00	Pass

Store in a dry place, away from heat and direct sunlight.
This lot is in conformity with the current EP/USP-NF.
Microbiological status: Microbiological testing is performed on intermittent basis. This product meets CP Kelco's microbiological specifications, which are available upon request.
Heavy Metals: This product meets the compendial limits of heavy metals. Analyses are carried out on intermittent basis.
Residual solvents: Only class 3 solvents are likely to be present. Residual class 3 solvent is below 0,5% (ICH guideline Q3C,Ph.EUR, USP/NF).
Notice: As a result of a natural process the viscosity of Cellulose Gum may decrease in time. We guarantee that the product will meet the viscosity specification for 12 months after the indicated "Manufacturing Date". After these 12 months the product can still be used safely up to the indicated shelf life end date, but may need a slight dosage correction in order to give optimum performance in the application.

Signature:
SIMO MÄNTYMAA QC MANAGER

Material was produced in:
AANEKOSKI , FINLAND

Page 1 of 1

Guar Gum

CERES ENTERPRISES LTD.
121 Carbine Rd, Mt. Wellington,
Auckland, New Zealand.
P.O. Box 11-336, Ellerslie, Auckland.
Tel: 64 9 574 0373
Fax: 64 9 527 4513

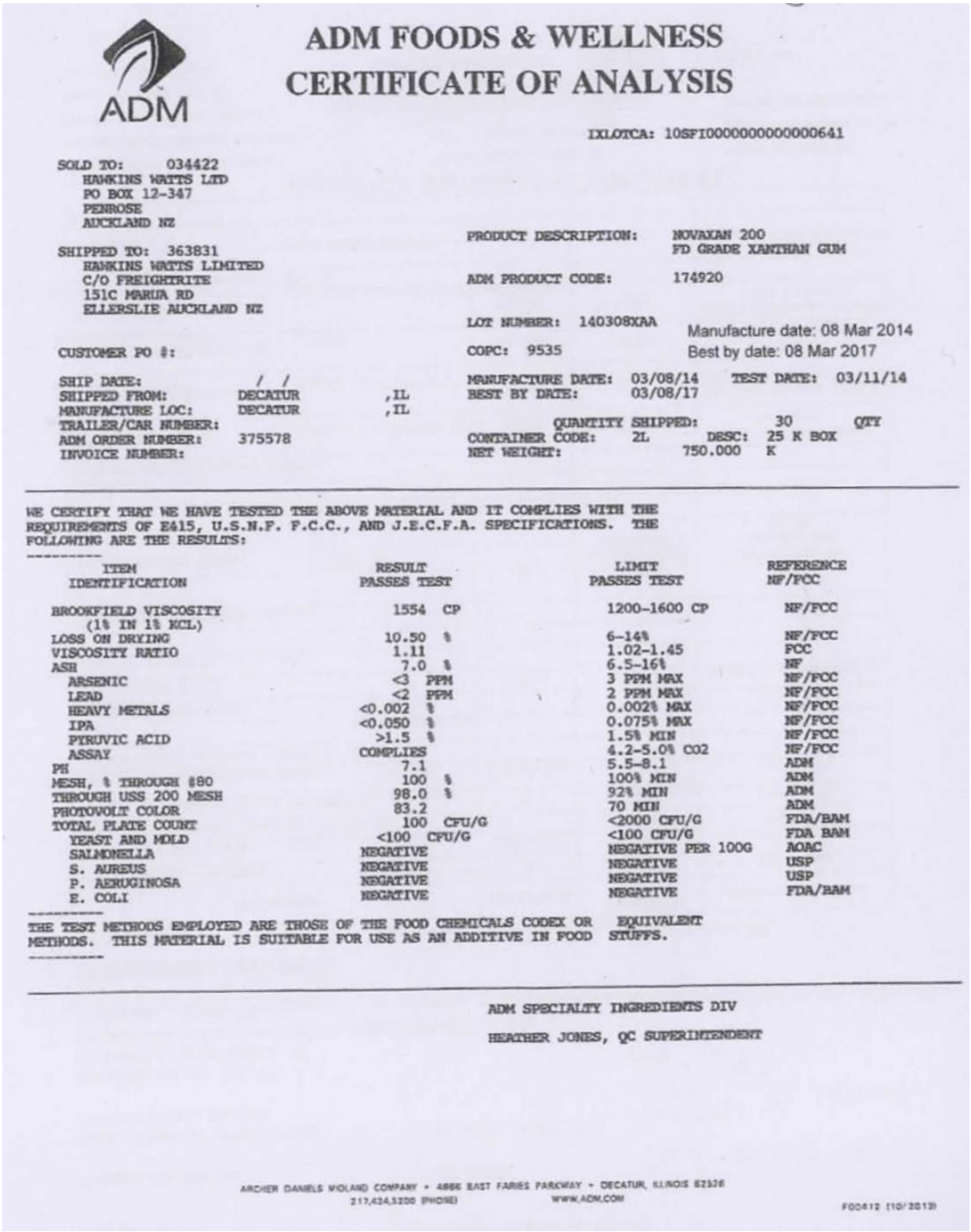
Product Specifications

Product Name/description	**ORGANIC GUAR GUM**
Code	40622
Country of Origin	India
Shelf life	18 months from manuf. date
Packaging	25 kg Bags
Ingredients (incl.additives)	Guar Gum

Physical, Chemical and Microbiological Analysis	
Moisture	12% Max
Ashes	4% max
Gum Content	80% min
Acid insoluble residue	10% max
pH (1% solution at 27 ° C)	5-8
Viscosity	3500 cps min
Starch	Absent
Total Plate Count (APC/TVC)	<100 000 cfu/gm
Yeast and mould	<5000 cfu/gm
Salmonella	Absent
E coli	< 10 cfu/gm
Quality Parameters	
Appearance	Fine powder
colour	Creamy white
Odour/Flavour	Product specific. Not off
Sieving 100 Mesh 200 Mesh	100% material passing 95% material passing

Nutrition Information	
Nutrition	**Amount per100g**
Protein (g)	5% min
Gum Content	80% min
Fat, Total (g)	1 max
Certification	
Certified Organic	BioGro
Genetic Modification	No
Kosher-certified	Yes

Xanthan Gum



ADM FOODS & WELLNESS
CERTIFICATE OF ANALYSIS

IXLOTCA: 10SFI0000000000000641

SOLD TO: 034422
HAWKINS WATTS LTD
PO BOX 12-347
PENROSE
AUCKLAND NZ

SHIPPED TO: 363831
HAWKINS WATTS LIMITED
C/O FREIGHTRITE
151C MARUA RD
ELLERSLIE AUCKLAND NZ

CUSTOMER PO #:

SHIP DATE: / /
SHIPPED FROM: DECATUR ,IL
MANUFACTURE LOC: DECATUR ,IL
TRAILER/CAR NUMBER:
ADM ORDER NUMBER: 375578
INVOICE NUMBER:

PRODUCT DESCRIPTION: NOVAXAN 200
FD GRADE XANTHAN GUM

ADM PRODUCT CODE: 174920

LOT NUMBER: 140308XAA
Manufacture date: 08 Mar 2014
COPC: 9535
Best by date: 08 Mar 2017

MANUFACTURE DATE: 03/08/14 TEST DATE: 03/11/14
BEST BY DATE: 03/08/17

QUANTITY SHIPPED: 30 QTY
CONTAINER CODE: 2L DESC: 25 K BOX
NET WEIGHT: 750.000 K

WE CERTIFY THAT WE HAVE TESTED THE ABOVE MATERIAL AND IT COMPLIES WITH THE REQUIREMENTS OF E415, U.S.N.F. F.C.C., AND J.E.C.F.A. SPECIFICATIONS. THE FOLLOWING ARE THE RESULTS:

ITEM IDENTIFICATION	RESULT PASSES TEST	LIMIT PASSES TEST	REFERENCE NF/FCC
BROOKFIELD VISCOSITY (1% IN 1% KCL)	1554 CP	1200-1600 CP	NF/FCC
LOSS ON DRYING	10.50 %	6-14%	NF/FCC
VISCOSITY RATIO	1.11	1.02-1.45	FCC
ASH	7.0 %	6.5-16%	NF
ARSENIC	<3 PPM	3 PPM MAX	NF/FCC
LEAD	<2 PPM	2 PPM MAX	NF/FCC
HEAVY METALS	<0.002 %	0.002% MAX	NF/FCC
IPA	<0.050 %	0.075% MAX	NF/FCC
PIRUVIC ACID	>1.5 %	1.5% MIN	NF/FCC
ASSAY	COMPLIES	4.2-5.0% CO2	NF/FCC
PH	7.1	5.5-8.1	ADM
MESH, % THROUGH #80	100 %	100% MIN	ADM
THROUGH USS 200 MESH	98.0 %	92% MIN	ADM
PHOTOVOLT COLOR	83.2	70 MIN	ADM
TOTAL PLATE COUNT	100 CFU/G	<2000 CFU/G	FDA/BAM
YEAST AND MOLD	<100 CFU/G	<100 CFU/G	FDA BAM
SALMONELLA	NEGATIVE	NEGATIVE PER 100G	AOAC
S. AUREUS	NEGATIVE	NEGATIVE	USP
P. AERUGINOSA	NEGATIVE	NEGATIVE	USP
E. COLI	NEGATIVE	NEGATIVE	FDA/BAM

THE TEST METHODS EMPLOYED ARE THOSE OF THE FOOD CHEMICALS CODEX OR EQUIVALENT METHODS. THIS MATERIAL IS SUITABLE FOR USE AS AN ADDITIVE IN FOOD STUFFS.

ADM SPECIALTY INGREDIENTS DIV

HEATHER JONES, QC SUPERINTENDENT

ARCHER DANIELS MIDLAND COMPANY • 4666 EAST FARIES PARKWAY • DECATUR, ILLINOIS 62526
217.424.5200 (PHONE) WWW.ADM.COM

F00412 (10/2013)

Formulation 11 – Nutritional Information Panel

Nutrient	Per 100g
Energy (kJ)	1075.6
Protein (g)	2.3
Fat - Total(g)	6.5
- Saturated (g)	1.4
- Trans (g)	0.1
- Polyunsaturated (g)	3.4
- Monounsaturated (g)	0.9
Carbohydrate (g)	22.9
Sugars (g)	3.2
Sodium (mg)	401.0

Ingredients: Water, Modified Tapioca Starch, Rice Flour, Soya Oil, Sugar, Yeast, Mozuku Powder, Salt, HPMC, Guar Gum

Appendix E – Descriptive statistics of mesh size analysis

One-Sample T: Test Collec, 45, 63, 90, 150, 250, 355, 500, 710

Variable	N	Mean	StDev	SE Mean	95% CI
Collec	3	2.9900	0.0520	0.0300	(2.8609, 3.1191)
45	3	23.820	0.226	0.131	(23.258, 24.382)
63	3	49.917	0.550	0.318	(48.551, 51.283)
90	3	39.337	0.523	0.302	(38.039, 40.635)
150	3	28.400	1.216	0.702	(25.378, 31.422)
250	3	22.0533	0.0723	0.0418	(21.8736, 22.2330)
355	3	24.280	0.670	0.387	(22.616, 25.944)
500	3	7.7700	0.0529	0.0306	(7.6386, 7.9014)
710	3	0.07667	0.00577	0.00333	(0.06232, 0.09101)

Appendix F – Wheat bread microbial data and analysis

Day	Recipe	Dilution Factor	Sample	CFU Count	Recipe	Dilution Factor	Sample	CFU Count	Recipe	Dilution Factor	Sample	CFU Count
1	Formulation 2	1	a	TFTC	Formulation 5	1	a	TFTC	Formulation 6	1	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	2	a	TFTC	Formulation 5	2	a	TFTC	Formulation 6	2	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	3	a	TFTC	Formulation 5	3	a	TFTC	Formulation 6	3	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	4	a	TFTC	Formulation 5	4	a	TFTC	Formulation 6	4	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	5	a	TFTC	Formulation 5	5	a	TFTC	Formulation 6	5	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	6	a	TFTC	Formulation 5	6	a	TFTC	Formulation 6	6	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
2	Formulation 2	1	a	TMTC	Formulation 5	1	a	TMTC	Formulation 6	1	a	TMTC
	Formulation 2		b	TMTC	Formulation 5		b	TMTC	Formulation 6		b	TMTC
	Formulation 2	2	a	110	Formulation 5	2	a	106	Formulation 6	2	a	95
	Formulation 2		b	102	Formulation 5		b	97	Formulation 6		b	107
	Formulation 2	3	a	TFTC	Formulation 5	3	a	TFTC	Formulation 6	3	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	4	a	TFTC	Formulation 5	4	a	TFTC	Formulation 6	4	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	5	a	TFTC	Formulation 5	5	a	TFTC	Formulation 6	5	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	6	a	TFTC	Formulation 5	6	a	TFTC	Formulation 6	6	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
3	Formulation 2	1	a	TFTC	Formulation 5	1	a	TMTC	Formulation 6	1	a	TMTC
	Formulation 2		b	TFTC	Formulation 5		b	TMTC	Formulation 6		b	TMTC
	Formulation 2	2	a	TFTC	Formulation 5	2	a	TMTC	Formulation 6	2	a	TMTC
	Formulation 2		b	TFTC	Formulation 5		b	TMTC	Formulation 6		b	TMTC
	Formulation 2	3	a	118	Formulation 5	3	a	123	Formulation 6	3	a	128
	Formulation 2		b	125	Formulation 5		b	115	Formulation 6		b	122
	Formulation 2	4	a	TFTC	Formulation 5	4	a	TFTC	Formulation 6	4	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	5	a	TFTC	Formulation 5	5	a	TFTC	Formulation 6	5	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC
	Formulation 2	6	a	TFTC	Formulation 5	6	a	TFTC	Formulation 6	6	a	TFTC
	Formulation 2		b	TFTC	Formulation 5		b	TFTC	Formulation 6		b	TFTC

One-way ANOVA Day 2: Control, Formulation 5, Formulation 6

Method

Null hypothesis: All means are equal
Alternative hypothesis: At least one mean is different
Significance level: α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	Control, Formulation 5, Formulation 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	303333	151667	0.31	0.751
Error	3	1445000	481667		
Total	5	1748333			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
694.022	17.35%	0.00%	0.00%

Means

Factor	N	Mean	StDev	95% CI
Control	2	10600	566	(9038, 12162)
Formulation 5	2	10150	636	(8588, 11712)
Formulation 6	2	10100	849	(8538, 11662)

Pooled StDev = 694.022

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
Control	2	10600	A
Formulation 5	2	10150	A
Formulation 6	2	10100	A

Means that do not share a letter are significantly different.

One-way ANOVA Day 3: Control, Formulation 5, Formulation 6

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  Control, Formulation 5, Formulation 6


Analysis of Variance

Source  DF     Adj SS    Adj MS  F-Value  P-Value
Factor   2   36333333  18166667     0.73    0.551
Error    3   74500000  24833333
Total    5  110833333


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
4983.31  32.78%      0.00%       0.00%


Means

Factor         N    Mean  StDev       95% CI
Control        2  121500   4950  (110286, 132714)
Formulation 5  2  119000   5657  (107786, 130214)
Formulation 6  2  125000   4243  (113786, 136214)

Pooled StDev = 4983.31
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor         N    Mean  Grouping
Formulation 6  2  125000  A
Control        2  121500  A
Formulation 5  2  119000  A

Means that do not share a letter are significantly different.
```

Appendix G-I – Statistical analysis of wheat bread

Crust Colour

One-way ANOVA: L versus Sample

Method

```
Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       5  1% M. Red. Salt, 1% Mozuku, 2% M. Red. Salt, 2% Mozuku, Control


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Sample   4   26.78   6.695     1.36    0.260
Error   55  271.06   4.928
Total   59  297.83


Model Summary

      S   R-sq  R-sq(adj)  R-sq(pred)
2.21997  8.99%      2.37%       0.00%


Means

Sample            N    Mean  StDev       95% CI
1% M. Red. Salt  12  57.834  2.508  (56.550, 59.118)
1% Mozuku        12  57.264  2.366  (55.980, 58.548)
2% M. Red. Salt  12  57.991  0.677  (56.707, 59.275)
2% Mozuku        12  57.073  1.935  (55.789, 58.358)
Control          12  58.976  2.925  (57.692, 60.260)

Pooled StDev = 2.21997
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample            N    Mean  Grouping
Control          12  58.976  A
2% M. Red. Salt  12  57.991  A
1% M. Red. Salt  12  57.834  A
1% Mozuku        12  57.264  A
2% Mozuku        12  57.073  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: *a** versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       5  1% M. Red. Salt, 1% Mozuku, 2% M. Red. Salt, 2% Mozuku, Control


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Sample   4   15.01   3.754     1.21    0.318
Error   55  170.81   3.106
Total   59  185.83


Model Summary

      S   R-sq  R-sq(adj)  R-sq(pred)
1.76229  8.08%      1.39%       0.00%


Means

Sample            N    Mean  StDev       95% CI
1% M. Red. Salt  12  12.645  1.626  (11.626, 13.665)
1% Mozuku        12  11.704  2.216  (10.684, 12.723)
2% M. Red. Salt  12  12.234  1.565  (11.215, 13.254)
2% Mozuku        12  11.636  1.529  (10.616, 12.655)
Control          12  12.900  1.784  (11.880, 13.920)

Pooled StDev = 1.76229
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample            N    Mean  Grouping
Control          12  12.900  A
1% M. Red. Salt  12  12.645  A
2% M. Red. Salt  12  12.234  A
1% Mozuku        12  11.704  A
2% Mozuku        12  11.636  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: *b** versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       5  1% M. Red. Salt, 1% Mozuku, 2% M. Red. Salt, 2% Mozuku, Control


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Sample   4   43.69  10.923    10.16    0.000
Error   55   59.14   1.075
Total   59  102.83


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
1.03692  42.49%     38.31%      31.56%


Means

Sample            N    Mean  StDev       95% CI
1% M. Red. Salt  12  34.713  0.744  (34.113, 35.312)
1% Mozuku        12  34.465  1.294  (33.865, 35.065)
2% M. Red. Salt  12  33.201  0.926  (32.601, 33.801)
2% Mozuku        12  33.252  1.279  (32.653, 33.852)
Control          12  35.388  0.810  (34.788, 35.988)

Pooled StDev = 1.03692
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample            N    Mean  Grouping
Control          12  35.388  A
1% M. Red. Salt  12  34.713  A
1% Mozuku        12  34.465  A
2% Mozuku        12  33.252    B
2% M. Red. Salt  12  33.201    B

Means that do not share a letter are significantly different.
```

Crumb

One-way ANOVA: L versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       5  1% M. Red. Salt, 1% Mozuku, 2% M. Red. Salt, 2% Mozuku, Control


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Sample   4  306.03  76.508    31.98    0.000
Error   35   83.74   2.392
Total   39  389.77


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
1.54675  78.52%     76.06%      71.94%


Means

Sample           N    Mean  StDev       95% CI
1% M. Red. Salt  8  69.912  1.179  (68.802, 71.023)
1% Mozuku        8  69.590  1.202  (68.480, 70.700)
2% M. Red. Salt  8  66.013  1.433  (64.902, 67.123)
2% Mozuku        8  64.983  2.095  (63.872, 66.093)
Control          8  72.571  1.639  (71.461, 73.681)

Pooled StDev = 1.54675
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample           N    Mean  Grouping
Control          8  72.571  A
1% M. Red. Salt  8  69.912    B
1% Mozuku        8  69.590    B
2% M. Red. Salt  8  66.013      C
2% Mozuku        8  64.983      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: *a** versus Sample

Method

Null hypothesis: All means are equal
Alternative hypothesis: At least one mean is different
Significance level: α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	5	1% M. Red. Salt, 1% Mozuku, 2% M. Red. Salt, 2% Mozuku, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	4.2115	1.05289	52.81	0.000
Error	35	0.6977	0.01994		
Total	39	4.9093			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.141193	85.79%	84.16%	81.44%

Means

Sample	N	Mean	StDev	95% CI
1% M. Red. Salt	8	-1.9825	0.1162	(-2.0838, -1.8812)
1% Mozuku	8	-2.0275	0.1491	(-2.1288, -1.9262)
2% M. Red. Salt	8	-1.8200	0.1231	(-1.9213, -1.7187)
2% Mozuku	8	-1.7175	0.2123	(-1.8188, -1.6162)
Control	8	-2.6487	0.0610	(-2.7501, -2.5474)

Pooled StDev = 0.141193

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
2% Mozuku	8	-1.7175	A
2% M. Red. Salt	8	-1.8200	A B
1% M. Red. Salt	8	-1.9825	B C
1% Mozuku	8	-2.0275	C
Control	8	-2.6487	D

Means that do not share a letter are significantly different.

One-way ANOVA: *b** versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       5  1% M. Red. Salt, 1% Mozuku, 2% M. Red. Salt, 2% Mozuku, Control


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Sample   4  28.599  7.1498    27.82    0.000
Error   35   8.994  0.2570
Total   39  37.593


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.506927  76.08%     73.34%      68.75%


Means

Sample           N    Mean  StDev       95% CI
1% M. Red. Salt  8  18.771  0.592  (18.407, 19.135)
1% Mozuku        8  18.430  0.390  (18.066, 18.794)
2% M. Red. Salt  8  19.630  0.362  (19.266, 19.994)
2% Mozuku        8  19.627  0.526  (19.264, 19.991)
Control          8  17.361  0.612  (16.997, 17.725)

Pooled StDev = 0.506927
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample           N    Mean  Grouping
2% M. Red. Salt  8  19.630  A
2% Mozuku        8  19.627  A
1% M. Red. Salt  8  18.771    B
1% Mozuku        8  18.430    B
Control          8  17.361      C

Means that do not share a letter are significantly different.
```

Specific volume

One-way ANOVA: Specific Volume versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample         5  1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku,
Control


Analysis of Variance

Source  DF   Adj SS    Adj MS  F-Value  P-Value
Sample   4  0.35383  0.088458    38.81    0.000
Error   15  0.03419  0.002279
Total   19  0.38802


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0.0477434  91.19%     88.84%      84.33%


Means

Sample              N     Mean    StDev        95% CI
1% M. Reduced salt  4   2.3662   0.0335  ( 2.3153,  2.4171)
1% Mozuku           4   2.2717   0.0238  ( 2.2208,  2.3225)
2% M. Reduced salt  4  2.19041  0.01568  (2.13953, 2.24130)
2% Mozuku           4   2.0931   0.0219  ( 2.0422,  2.1439)
Control             4   2.4749   0.0948  ( 2.4240,  2.5258)

Pooled StDev = 0.0477434
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample              N     Mean  Grouping
Control             4   2.4749  A
1% M. Reduced salt  4   2.3662    B
1% Mozuku           4   2.2717    B C
2% M. Reduced salt  4  2.19041      C D
2% Mozuku           4   2.0931        D

Means that do not share a letter are significantly different.
```

Water activity

One-way ANOVA: Water activity versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample         5  1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku,
Control


Analysis of Variance

Source  DF    Adj SS    Adj MS  F-Value  P-Value
Sample   4  0.000086  0.000021     1.29    0.318
Error   15  0.000249  0.000017
Total   19  0.000335


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0.0040764  25.59%      5.74%       0.00%


Means

Sample              N     Mean    StDev        95% CI
1% M. Reduced salt  4  0.96800  0.00216  (0.96366, 0.97234)
1% Mozuku           4  0.96925  0.00532  (0.96491, 0.97359)
2% M. Reduced salt  4  0.96775  0.00206  (0.96341, 0.97209)
2% Mozuku           4  0.97075  0.00556  (0.96641, 0.97509)
Control             4  0.96450  0.00387  (0.96016, 0.96884)

Pooled StDev = 0.00407635
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample              N     Mean  Grouping
2% Mozuku           4  0.97075  A
1% Mozuku           4  0.96925  A
1% M. Reduced salt  4  0.96800  A
2% M. Reduced salt  4  0.96775  A
Control             4  0.96450  A

Means that do not share a letter are significantly different.
```

Texture

One-way ANOVA: Hardness versus Sample

Method

Null hypothesis: All means are equal
Alternative hypothesis: At least one mean is different
Significance level: α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	5	1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	516075	129019	58.16	0.000
Error	55	122011	2218		
Total	59	638086			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
47.0997	80.88%	79.49%	77.24%

Means

Sample	N	Mean	StDev	95% CI
1% M. Reduced salt	12	860.6	38.2	(833.3, 887.8)
1% Mozuku	12	971.52	29.11	(944.27, 998.77)
2% M. Reduced salt	12	881.0	38.3	(853.7, 908.2)
2% Mozuku	12	1095.7	70.3	(1068.4, 1122.9)
Control	12	848.6	48.8	(821.4, 875.9)

Pooled StDev = 47.0997

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
2% Mozuku	12	1095.7	A
1% Mozuku	12	971.52	B
2% M. Reduced salt	12	881.0	C
1% M. Reduced salt	12	860.6	C
Control	12	848.6	C

Means that do not share a letter are significantly different.

One-way ANOVA: Adhesiveness versus Sample

Method

Null hypothesis All means are equal
Alternative hypothesis At least one mean is different
Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	5	1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	2.999	0.7497	1.03	0.401
Error	55	40.098	0.7291		
Total	59	43.097			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.853846	6.96%	0.19%	0.00%

Means

Sample	N	Mean	StDev	95% CI
1% M. Reduced salt	12	-0.5634	0.3270	(-1.0573, -0.0694)
1% Mozuku	12	-0.927	0.573	(-1.421, -0.433)
2% M. Reduced salt	12	-0.8503	0.3019	(-1.3442, -0.3563)
2% Mozuku	12	-1.160	1.719	(-1.654, -0.666)
Control	12	-0.583	0.406	(-1.077, -0.089)

Pooled StDev = 0.853846

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
1% M. Reduced salt	12	-0.5634	A
Control	12	-0.583	A
2% M. Reduced salt	12	-0.8503	A
1% Mozuku	12	-0.927	A
2% Mozuku	12	-1.160	A

Means that do not share a letter are significantly different.

One-way ANOVA: Springiness versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample         5  1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku,
Control


Analysis of Variance

Source  DF   Adj SS    Adj MS  F-Value  P-Value
Sample   4  0.02607  0.006518     8.26    0.000
Error   55  0.04341  0.000789
Total   59  0.06948


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0.0280933  37.53%     32.98%      25.65%


Means

Sample               N     Mean    StDev        95% CI
1% M. Reduced salt  12  0.94106  0.02457  (0.92481, 0.95731)
1% Mozuku           12  0.92427  0.02123  (0.90802, 0.94052)
2% M. Reduced salt  12  0.93512  0.02481  (0.91887, 0.95137)
2% Mozuku           12   0.8911   0.0401  ( 0.8748,  0.9073)
Control             12  0.95200  0.02582  (0.93575, 0.96826)

Pooled StDev = 0.0280933
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample               N     Mean  Grouping
Control             12  0.95200  A
1% M. Reduced salt  12  0.94106  A
2% M. Reduced salt  12  0.93512  A
1% Mozuku           12  0.92427  A
2% Mozuku           12   0.8911    B

Means that do not share a letter are significantly different.
```

One-way ANOVA: Cohesiveness versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample         5  1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku,
Control


Analysis of Variance

Source  DF   Adj SS    Adj MS  F-Value  P-Value
Sample   4  0.05040  0.012600    12.96    0.000
Error   55  0.05347  0.000972
Total   59  0.10387


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0.0311806  48.52%     44.78%      38.74%


Means

Sample               N     Mean    StDev        95% CI
1% M. Reduced salt  12  0.75554  0.01228  (0.73750, 0.77358)
1% Mozuku           12   0.7473   0.0360  ( 0.7292,  0.7653)
2% M. Reduced salt  12  0.74840  0.01167  (0.73036, 0.76643)
2% Mozuku           12   0.6990   0.0551  ( 0.6810,  0.7170)
Control             12  0.78973  0.01551  (0.77170, 0.80777)

Pooled StDev = 0.0311806
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample               N     Mean  Grouping
Control             12  0.78973  A
1% M. Reduced salt  12  0.75554  A B
2% M. Reduced salt  12  0.74840    B
1% Mozuku           12   0.7473    B
2% Mozuku           12   0.6990      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: Gumminess versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample         5  1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku,
Control


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Sample   4   28698    7175     1.58    0.194
Error   55  250305    4551
Total   59  279004


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
67.4611  10.29%      3.76%       0.00%


Means

Sample               N   Mean  StDev      95% CI
1% M. Reduced salt  12  736.3   55.2  (697.3, 775.3)
1% Mozuku           12  740.3   86.7  (701.3, 779.4)
2% M. Reduced salt  12  733.8   40.9  (694.7, 772.8)
2% Mozuku           12  766.3   83.1  (727.2, 805.3)
Control             12  697.8   60.1  (658.8, 736.8)

Pooled StDev = 67.4611
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample               N   Mean  Grouping
2% Mozuku           12  766.3  A
1% Mozuku           12  740.3  A
1% M. Reduced salt  12  736.3  A
2% M. Reduced salt  12  733.8  A
Control             12  697.8  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: Chewiness versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample         5  1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku,
Control


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
Sample   4    5468    1367     0.45    0.769
Error   55  165641    3012
Total   59  171109


Model Summary

      S   R-sq  R-sq(adj)  R-sq(pred)
54.8785  3.20%      0.00%       0.00%


Means

Sample               N    Mean  StDev       95% CI
1% M. Reduced salt  12  664.20  27.12  (632.45, 695.95)
1% Mozuku           12   678.3   53.3  ( 646.6,  710.1)
2% M. Reduced salt  12  686.15  29.42  (654.40, 717.90)
2% Mozuku           12   683.9   87.8  ( 652.2,  715.7)
Control             12   663.8   53.8  ( 632.1,  695.6)

Pooled StDev = 54.8785
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample               N    Mean  Grouping
2% M. Reduced salt  12  686.15  A
2% Mozuku           12   683.9  A
1% Mozuku           12   678.3  A
1% M. Reduced salt  12  664.20  A
Control             12   663.8  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: Resilience versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample         5  1% M. Reduced salt, 1% Mozuku, 2% M. Reduced salt, 2% Mozuku,
Control


Analysis of Variance

Source  DF   Adj SS    Adj MS  F-Value  P-Value
Sample   4  0.08723  0.021807    19.93    0.000
Error   55  0.06017  0.001094
Total   59  0.14740


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0.0330763  59.18%     56.21%      51.42%


Means

Sample               N     Mean    StDev        95% CI
1% M. Reduced salt  12  0.46412  0.01664  (0.44499, 0.48326)
1% Mozuku           12  0.43572  0.01981  (0.41658, 0.45486)
2% M. Reduced salt  12  0.45415  0.01726  (0.43502, 0.47329)
2% Mozuku           12   0.3711   0.0633  ( 0.3520,  0.3903)
Control             12  0.48166  0.02235  (0.46252, 0.50079)

Pooled StDev = 0.0330763
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample               N     Mean  Grouping
Control             12  0.48166  A
1% M. Reduced salt  12  0.46412  A B
2% M. Reduced salt  12  0.45415  A B
1% Mozuku           12  0.43572    B
2% Mozuku           12   0.3711      C

Means that do not share a letter are significantly different.
```

Wheat bread consumer survey

One-way ANOVA: Appearance versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       3  1% Mozuku, 2% Mozuku, Control


Analysis of Variance

Source   DF   Adj SS  Adj MS  F-Value  P-Value
Sample    2    2.562   1.281     0.88    0.416
Error   117  169.787   1.451
Total   119  172.349


Model Summary

      S   R-sq  R-sq(adj)  R-sq(pred)
1.20465  1.49%      0.00%       0.00%


Means

Sample      N   Mean  StDev      95% CI
1% Mozuku  40  6.303  1.047  (5.925, 6.680)
2% Mozuku  40  6.018  1.304  (5.640, 6.395)
Control    40  6.348  1.248  (5.970, 6.725)

Pooled StDev = 1.20465
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample      N   Mean  Grouping
Control    40  6.348  A
1% Mozuku  40  6.303  A
2% Mozuku  40  6.018  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: Texture versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       3  1% Mozuku, 2% Mozuku, Control


Analysis of Variance

Source   DF  Adj SS  Adj MS  F-Value  P-Value
Sample    2   35.56  17.778     9.66    0.000
Error   117  215.35   1.841
Total   119  250.90


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
1.35668  14.17%     12.70%       9.71%


Means

Sample      N   Mean  StDev      95% CI
1% Mozuku  40  6.228  1.369  (5.803, 6.652)
2% Mozuku  40  6.465  1.034  (6.040, 6.890)
Control    40  5.210  1.605  (4.785, 5.635)

Pooled StDev = 1.35668
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample      N   Mean  Grouping
2% Mozuku  40  6.465  A
1% Mozuku  40  6.228  A
Control    40  5.210    B

Means that do not share a letter are significantly different.
```

One-way ANOVA: Aroma versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       3  1% Mozuku, 2% Mozuku, Control


Analysis of Variance

Source   DF   Adj SS  Adj MS  F-Value  P-Value
Sample    2    7.590   3.795     2.82    0.064
Error   117  157.728   1.348
Total   119  165.318


Model Summary

      S   R-sq  R-sq(adj)  R-sq(pred)
1.16108  4.59%      2.96%       0.00%


Means

Sample      N   Mean  StDev      95% CI
1% Mozuku  40  6.310  1.230  (5.946, 6.674)
2% Mozuku  40  6.120  1.250  (5.756, 6.484)
Control    40  5.708  0.984  (5.344, 6.071)

Pooled StDev = 1.16108
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample      N   Mean  Grouping
1% Mozuku  40  6.310  A
2% Mozuku  40  6.120  A
Control    40  5.708  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: Taste versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       3  1% Mozuku, 2% Mozuku, Control


Analysis of Variance

Source   DF  Adj SS  Adj MS  F-Value  P-Value
Sample    2   16.41   8.204     4.60    0.012
Error   117  208.52   1.782
Total   119  224.93


Model Summary

      S   R-sq  R-sq(adj)  R-sq(pred)
1.33499  7.29%      5.71%       2.48%


Means

Sample      N   Mean  StDev      95% CI
1% Mozuku  40  7.085  1.275  (6.667, 7.503)
2% Mozuku  40  6.248  1.394  (5.829, 6.666)
Control    40  6.368  1.333  (5.949, 6.786)

Pooled StDev = 1.33499
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample      N   Mean  Grouping
1% Mozuku  40  7.085  A
Control    40  6.368    B
2% Mozuku  40  6.248    B

Means that do not share a letter are significantly different.
```

One-way ANOVA: Overall Acceptability versus Sample

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Sample       3  1% Mozuku, 2% Mozuku, Control


Analysis of Variance

Source   DF   Adj SS  Adj MS  F-Value  P-Value
Sample    2    7.584   3.792     2.60    0.078
Error   117  170.544   1.458
Total   119  178.128


Model Summary

      S   R-sq  R-sq(adj)  R-sq(pred)
1.20733  4.26%      2.62%       0.00%


Means

Sample      N   Mean  StDev      95% CI
1% Mozuku  40  6.830  1.241  (6.452, 7.208)
2% Mozuku  40  6.410  1.301  (6.032, 6.788)
Control    40  6.230  1.067  (5.852, 6.608)

Pooled StDev = 1.20733
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Sample      N   Mean  Grouping
1% Mozuku  40  6.830  A
2% Mozuku  40  6.410  A
Control    40  6.230  A

Means that do not share a letter are significantly different.
```

Chi-squared test of most preferred sample

Sample	N	Obtained	Expected
1% Mozuku	40	18	13.3
2% Mozuku	40	11	13.3
Control	40	11	13.3

P = 0.29282

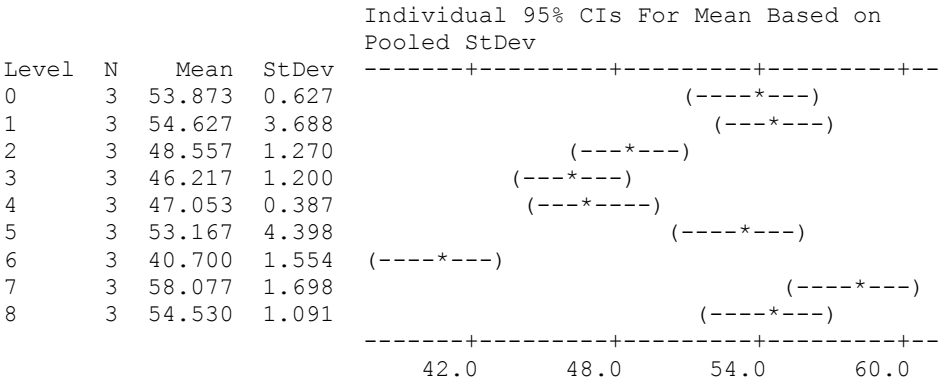
H_0: 1% Mozuku = 2% Mozuku = Control
Testing at 95% significance level reject null if P<0.05

Appendix G-2 – Phase I statistical analysis of gluten-free bread

Crust Colour

One-way ANOVA: Crust - L versus Sample

```
Source  DF      SS     MS      F      P
Sample   8  715.87  89.48  18.72  0.000
Error   18   86.06   4.78
Total   26  801.93

S = 2.187   R-Sq = 89.27%   R-Sq(adj) = 84.50%
```



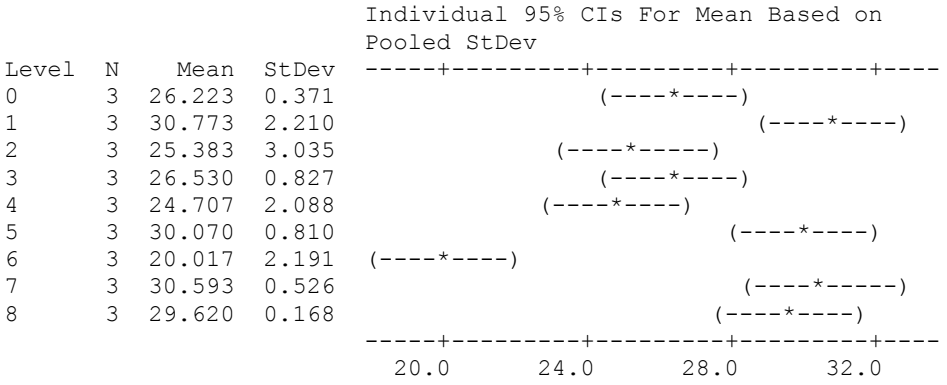

```
Pooled StDev = 2.187

Grouping Information Using Tukey Method

Sample  N    Mean  Grouping
0       3  53.873  A B
1       3  54.627  A B
2       3  48.557    B C D
3       3  46.217        D E
4       3  47.053      C D
5       3  53.167  A B C
6       3  40.700          E
7       3  58.077  A
8       3  54.530  A B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
```

One-way ANOVA: Crust - a versus Sample

```
Source  DF      SS     MS     F      P
Sample   8  36.528  4.566  8.98  0.000
Error   18   9.151  0.508
Total   26  45.680

S = 0.7130   R-Sq = 79.97%   R-Sq(adj) = 71.06%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
0      3  11.980  0.242    (-----*-----)
1      3  13.557  0.827               (----*-----)
2      3  14.667  0.562                      (-----*-----)
3      3  14.810  0.397                       (-----*----)
4      3  14.373  0.191                    (-----*-----)
5      3  13.870  1.514                  (----*-----)
6      3  14.997  0.333                         (-----*-----)
7      3  11.650  0.846  (-----*----)
8      3  14.610  0.451                      (----*-----)
                          --------+---------+---------+---------+-
                               12.0      13.5      15.0      16.5

Pooled StDev = 0.713


Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
0       3  11.9800    B C
1       3  13.5567  A B C
2       3  14.6667  A
3       3  14.8100  A
4       3  14.3733  A
5       3  13.8700  A B
6       3  14.9967  A
7       3  11.6500      C
8       3  14.6100  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: Crust - b versus Sample

```
Source  DF      SS     MS      F      P
Sample   8  302.43  37.80  13.59  0.000
Error   18   50.08   2.78
Total   26  352.51

S = 1.668   R-Sq = 85.79%   R-Sq(adj) = 79.48%
```




```
Pooled StDev = 1.668
```

Grouping Information Using Tukey Method

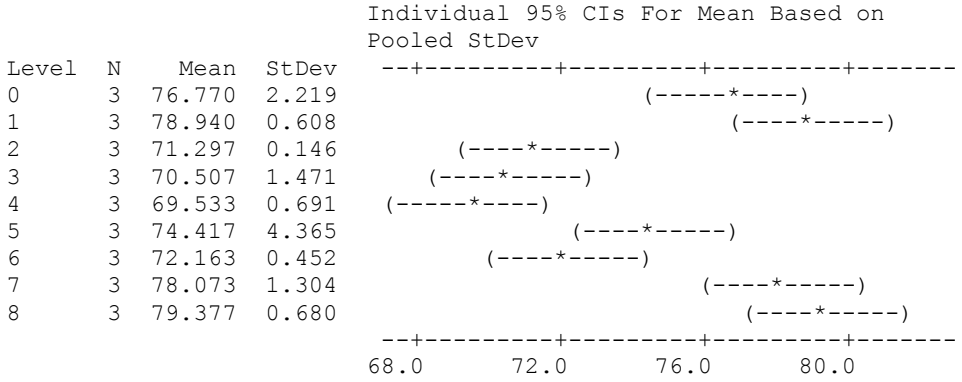
Sample	N	Mean	Grouping
0	3	26.223	A B C
1	3	30.773	A
2	3	25.383	B C
3	3	26.530	A B C
4	3	24.707	C D
5	3	30.070	A B
6	3	20.017	D
7	3	30.593	A
8	3	29.620	A B

Means that do not share a letter are significantly different.

Crumb

One-way ANOVA: Crumb - L versus Sample

```
Source  DF      SS     MS      F      P
Sample   8  353.17  44.15  13.52  0.000
Error   18   58.76   3.26
Total   26  411.93

S = 1.807   R-Sq = 85.73%   R-Sq(adj) = 79.39%
```




```
Pooled StDev = 1.807
```

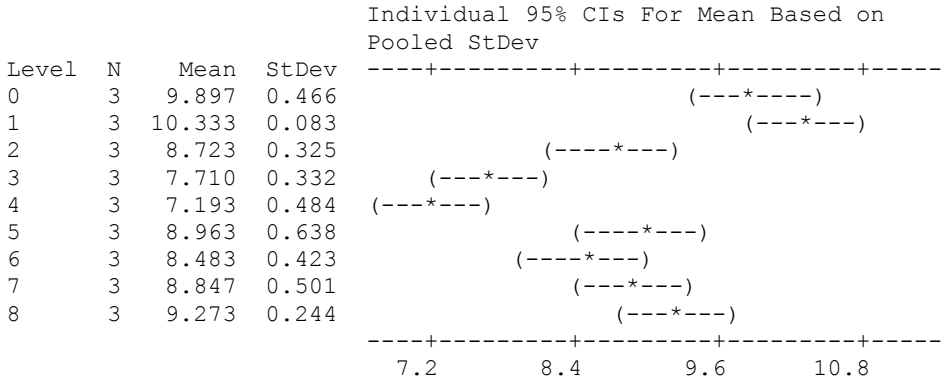
```
Grouping Information Using Tukey Method

Sample  N    Mean  Grouping
0       3  76.770  A B
1       3  78.940  A
2       3  71.297      C
3       3  70.507      C
4       3  69.533      C
5       3  74.417  A B C
6       3  72.163    B C
7       3  78.073  A
8       3  79.377  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: Crumb - a versus Sample

```
Source  DF       SS      MS       F      P
Sample   8  21.5435  2.6929  115.96  0.000
Error   18   0.4180  0.0232
Total   26  21.9615

S = 0.1524   R-Sq = 98.10%   R-Sq(adj) = 97.25%


                          Individual 95% CIs For Mean Based on Pooled StDev
Level  N     Mean   StDev   --+---------+---------+---------+-------
0      3  -0.7567  0.1332   (--*-)
1      3   2.0367  0.0757                                        (-*--)
2      3   0.2400  0.1212               (-*-)
3      3   0.7500  0.1868                     (-*--)
4      3   1.5567  0.1201                               (-*--)
5      3  -0.0067  0.2706             (-*-)
6      3   1.4533  0.0651                            (-*-)
7      3  -0.4200  0.0624       (--*-)
8      3   0.8933  0.2003                      (-*-)
                          --+---------+---------+---------+-------
                         -0.80      0.00      0.80      1.60

Pooled StDev = 0.1524


Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
0       3  -0.7567           F
1       3   2.0367  A
2       3   0.2400        D
3       3   0.7500      C
4       3   1.5567    B
5       3  -0.0067        D E
6       3   1.4533    B
7       3  -0.4200          E F
8       3   0.8933      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: Crumb - b versus Sample

```
Source  DF      SS     MS      F      P
Sample   8  23.030  2.879  16.50  0.000
Error   18   3.140  0.174
Total   26  26.170

S = 0.4176   R-Sq = 88.00%   R-Sq(adj) = 82.67%
```




```
Pooled StDev = 0.418
```

```
Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
0       3   9.8967  A B
1       3  10.3333  A
2       3   8.7233    B C D
3       3   7.7100        D E
4       3   7.1933          E
5       3   8.9633    B C
6       3   8.4833      C D
7       3   8.8467    B C D
8       3   9.2733  A B C
```

Texture

One-way ANOVA: Hardness versus Sample

```
Source  DF        SS       MS       F      P
Sample   8  28163080  3520385  133.58  0.000
Error   18    474380    26354
Total   26  28637461

S = 162.3   R-Sq = 98.34%   R-Sq(adj) = 97.61%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  +---------+---------+---------+---------
0      3   339.3   15.5   (-*-)
1      3   901.0   50.0         (-*-)
2      3  3589.0  463.9                                    (-*-)
3      3   267.8   45.0   (-*-)
4      3   465.8   68.9     (-*-)
5      3   358.9   62.7    (-*-)
6      3   455.6   89.0     (-*-)
7      3   190.5   24.8  (-*-)
8      3   229.7    5.0  (-*-)
                         +---------+---------+---------+---------
                         0      1000      2000      3000

Pooled StDev = 162.3


Grouping Information Using Tukey Method

Sample  N    Mean  Grouping
2       3  3589.0  A
1       3   901.0    B
4       3   465.8    B C
6       3   455.6    B C
5       3   358.9      C
0       3   339.3      C
3       3   267.8      C
8       3   229.7      C
7       3   190.5      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: Chewiness versus Sample

```
Source  DF       SS       MS       F      P
Sample   8  9051268  1131408  179.34  0.000
Error   18   113560     6309
Total   26  9164827

S = 79.43   R-Sq = 98.76%   R-Sq(adj) = 98.21%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
0      3   217.9   13.1   (-*)
1      3   525.0   15.8        (-*)
2      3  2089.3  209.0                                    (-*)
3      3   200.7   33.7   (*-)
4      3   407.5   57.7      (-*)
5      3   294.0   44.1    (-*-)
6      3   390.0   76.3      (*-)
7      3   131.4   17.8  (*-)
8      3   148.8   11.7  (*-)
                          ---------+---------+---------+---------+
                                 600      1200      1800      2400

Pooled StDev = 79.4


Grouping Information Using Tukey Method

Sample  N    Mean  Grouping
2       3  2089.3  A
1       3   525.0    B
4       3   407.5    B C
6       3   390.0    B C
5       3   294.0      C D
0       3   217.9      C D
3       3   200.7      C D
8       3   148.8        D
7       3   131.4        D

Means that do not share a letter are significantly different.
```

One-way ANOVA: Springiness versus Sample

```
Source  DF        SS        MS      F      P
Sample   8  0.082571  0.010321  23.93  0.000
Error   18  0.007764  0.000431
Total   26  0.090335

S = 0.02077   R-Sq = 91.41%   R-Sq(adj) = 87.59%


                           Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean   StDev  ---------+---------+---------+---------+
0      3  0.9277  0.0225                    (----*---)
1      3  0.8140  0.0111  (----*---)
2      3  0.8847  0.0074              (---*----)
3      3  0.8870  0.0036               (---*---)
4      3  0.9890  0.0156                                (---*---)
5      3  0.9440  0.0209                          (---*----)
6      3  0.9590  0.0089                             (---*---)
7      3  0.8663  0.0168           (---*----)
8      3  0.8330  0.0463      (---*---)
                           ---------+---------+---------+---------+
                                  0.840     0.900     0.960     1.020

Pooled StDev = 0.0208
```

Grouping Information Using Tukey Method

Sample	N	Mean	Grouping
4	3	0.98900	A
6	3	0.95900	A B
5	3	0.94400	A B C
0	3	0.92767	B C
3	3	0.88700	C D
2	3	0.88467	C D
7	3	0.86633	D E
8	3	0.83300	D E
1	3	0.81400	E

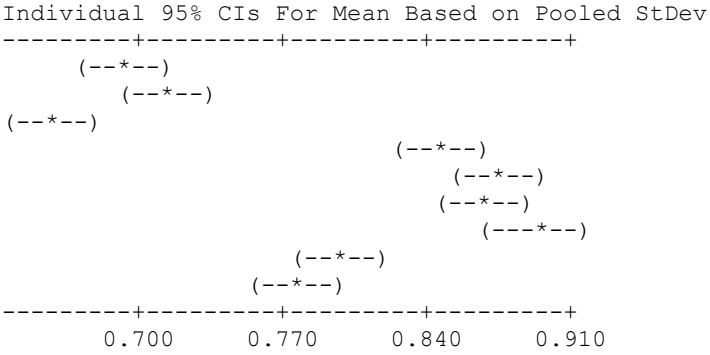
Means that do not share a letter are significantly different.

One-way ANOVA: Cohesiveness versus Sample

Source	DF	SS	MS	F	P
Sample	8	0.178759	0.022345	72.46	0.000
Error	18	0.005551	0.000308		
Total	26	0.184309			

S = 0.01756   R-Sq = 96.99%   R-Sq(adj) = 95.65%

Level	N	Mean	StDev
0	3	0.69133	0.00462
1	3	0.71700	0.03470
2	3	0.65967	0.02196
3	3	0.84500	0.01473
4	3	0.87567	0.01137
5	3	0.87033	0.01387
6	3	0.89267	0.00737
7	3	0.79667	0.00493
8	3	0.77733	0.02122



Pooled StDev = 0.01756

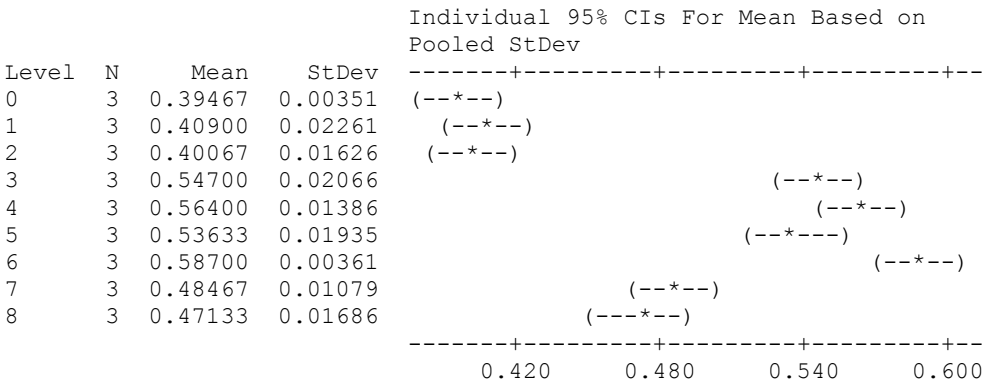
Grouping Information Using Tukey Method

Sample	N	Mean	Grouping
6	3	0.89267	A
4	3	0.87567	A
5	3	0.87033	A
3	3	0.84500	A B
7	3	0.79667	B C
8	3	0.77733	C
1	3	0.71700	D
0	3	0.69133	D E
2	3	0.65967	E

Means that do not share a letter are significantly different.

One-way ANOVA: Resilience versus Sample

```
Source  DF        SS        MS      F      P
Sample   8  0.132784  0.016598  68.07  0.000
Error   18  0.004389  0.000244
Total   26  0.137174

S = 0.01562   R-Sq = 96.80%   R-Sq(adj) = 95.38%
```




```
Pooled StDev = 0.01562
```

```
Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
6       3  0.58700  A
4       3  0.56400  A B
3       3  0.54700  A B
5       3  0.53633    B
7       3  0.48467      C
8       3  0.47133      C
1       3  0.40900        D
2       3  0.40067        D
0       3  0.39467        D

Means that do not share a letter are significantly different.
```

Appendix G-3 – Phase II statistical analysis of gluten-free bread

Crust Colour

One-way ANOVA: Crust - L versus Sample

```
Source  DF      SS     MS      F      P
Sample   5  474.19  94.84  43.02  0.000
Error   12   26.46   2.20
Total   17  500.65

S = 1.485   R-Sq = 94.72%   R-Sq(adj) = 92.51%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 R     3  53.873  0.627                              (---*--)
 9     3  55.637  0.410                                  (--*---)
10     3  51.560  0.521                         (---*---)
11     3  48.357  0.779                    (---*--)
12     3  45.460  0.756              (---*---)
13     3  40.567  3.349  (---*---)
                         ---+---------+---------+---------+------
                         40.0      45.0      50.0      55.0

Pooled StDev = 1.485


Grouping Information Using Tukey Method

Sample  N    Mean  Grouping
 9      3  55.637  A
 R      3  53.873  A B
10      3  51.560    B C
11      3  48.357      C D
12      3  45.460        D
13      3  40.567          E

Means that do not share a letter are significantly different.
```

One-way ANOVA: Crust - a versus Sample

```
Source  DF      SS     MS      F      P
Sample   5  41.578  8.316  16.63  0.000
Error   12   6.002  0.500
Total   17  47.580

S = 0.7072   R-Sq = 87.39%   R-Sq(adj) = 82.13%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 R     3  11.980  0.242    (-----*----)
 9     3  11.633  0.215  (-----*----)
10     3  13.540  0.277              (-----*----)
11     3  16.177  0.169                                (----*-----)
12     3  14.413  1.220                       (----*-----)
13     3  13.247  1.141            (-----*----)
                          ---+---------+---------+---------+------
                          11.2      12.8      14.4      16.0

Pooled StDev = 0.707


Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
11      3  16.1767  A
12      3  14.4133  A B
10      3  13.5400    B C
13      3  13.2467    B C
 R      3  11.9800      C
 9      3  11.6333      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: Crust - b versus Sample

```
Source  DF       SS      MS      F      P
Sample   5  361.471  72.294  77.48  0.000
Error   12   11.197   0.933
Total   17  372.668

S = 0.9660   R-Sq = 97.00%   R-Sq(adj) = 95.74%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 R     3  26.223  0.371                (-*--)
 9     3  30.250  0.046                       (--*-)
10     3  33.023  0.264                             (-*-)
11     3  31.320  0.816                          (--*-)
12     3  28.967  1.135                    (-*-)
13     3  19.323  1.854  (--*-)
                          ----+---------+---------+---------+-----
                           20.0      25.0      30.0      35.0

Pooled StDev = 0.966

Grouping Information Using Tukey Method

Sample  N    Mean  Grouping
10      3  33.023  A
11      3  31.320  A B
 9      3  30.250    B
12      3  28.967    B
 R      3  26.223      C
13      3  19.323        D

Means that do not share a letter are significantly different.
```

Crumb

One-way ANOVA: Crumb - L versus Sample

```
Source  DF       SS      MS      F      P
Sample   5  1100.50  220.10  84.14  0.000
Error   12    31.39    2.62
Total   17  1131.89

S = 1.617   R-Sq = 97.23%   R-Sq(adj) = 96.07%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 R     3  76.770  2.219                                (--*--)
 9     3  71.653  1.295                           (--*-)
10     3  71.893  2.161                           (--*-)
11     3  71.910  0.735                           (--*-)
12     3  66.640  0.676                     (-*--)
13     3  52.317  1.851  (-*--)
                          -------+---------+---------+---------+--
                              56.0      64.0      72.0      80.0

Pooled StDev = 1.617


Grouping Information Using Tukey Method

Sample  N    Mean  Grouping
 R      3  76.770  A
11      3  71.910    B
10      3  71.893    B
 9      3  71.653    B
12      3  66.640      C
13      3  52.317        D

Means that do not share a letter are significantly different
```

One-way ANOVA: Crumb - a versus Sample

```
Source  DF       SS      MS       F      P
Sample   5  26.3844  5.2769  160.69  0.000
Error   12   0.3941  0.0328
Total   17  26.7784

S = 0.1812   R-Sq = 98.53%   R-Sq(adj) = 97.92%


                            Individual 95% CIs For Mean Based on Pooled StDev
Level  N     Mean   StDev     +---------+---------+---------+---------
 R     3  -0.7567  0.1332     (-*--)
 9     3  -0.1967  0.1234           (-*-)
10     3  -0.0100  0.1411             (-*-)
11     3   1.9767  0.0252                                (--*-)
12     3   0.9767  0.3350                         (--*-)
13     3   2.6200  0.1769                                     (-*-)
                              +---------+---------+---------+---------
                            -1.0       0.0       1.0       2.0

Pooled StDev = 0.1812
```

```
Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
13      3   2.6200  A
11      3   1.9767    B
12      3   0.9767      C
10      3  -0.0100        D
 9      3  -0.1967        D
 R      3  -0.7567          E

Means that do not share a letter are significantly different.
```

One-way ANOVA: Crumb - b versus Sample

```
Source  DF      SS      MS      F      P
Sample   5  72.974  14.595  49.65  0.000
Error   12   3.528   0.294
Total   17  76.502

S = 0.5422   R-Sq = 95.39%   R-Sq(adj) = 93.47%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 R     3   9.897  0.466  (--*---)
 9     3  13.393  0.251                   (--*--)
10     3  14.120  0.360                      (---*--)
11     3  15.077  0.681                           (--*---)
12     3  15.327  0.469                            (---*--)
13     3  16.047  0.819                                (--*---)
                        ----+---------+---------+---------+-----
                         10.0      12.0      14.0      16.0

Pooled StDev = 0.542


Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
13      3  16.0467  A
12      3  15.3267  A B
11      3  15.0767  A B
10      3  14.1200    B C
 9      3  13.3933      C
 R      3   9.8967        D

Means that do not share a letter are significantly different.
```

Texture

One-way ANOVA: Hardness versus Sample

```
Source  DF       SS      MS       F      P
Sample   5  2628027  525605  107.25  0.000
Error   12    58807    4901
Total   17  2686834

S = 70.00   R-Sq = 97.81%   R-Sq(adj) = 96.90%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 R     3   240.1   21.5    (--*-)
 9     3   161.3   20.4  (--*-)
10     3   205.1   22.8   (--*-)
11     3   261.0    3.4     (-*--)
12     3   278.8    6.3     (--*-)
13     3  1249.4  167.2                              (--*-)
                         --------+---------+---------+---------+-
                               350       700      1050      1400

Pooled StDev = 70.0


Grouping Information Using Tukey Method

Sample  N    Mean  Grouping
13      3  1249.4  A
12      3   278.8    B
11      3   261.0    B
 R      3   240.1    B
10      3   205.1    B
 9      3   161.3    B

Means that do not share a letter are significantly different.
```

One-way ANOVA: Chewiness versus Sample

```
Source  DF       SS      MS      F      P
Sample   5  1616009  323202  78.90  0.000
Error   12    49156    4096
Total   17  1665165

S = 64.00   R-Sq = 97.05%   R-Sq(adj) = 95.82%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N   Mean  StDev  ---------+---------+---------+---------+
 R     3  187.7   17.4     (-*--)
 9     3  120.1    8.8  (--*--)
10     3  155.1   15.8   (--*--)
11     3  205.8    5.1     (--*--)
12     3  219.2    8.0      (-*--)
13     3  976.7  154.5                             (--*-)
                         ---------+---------+---------+---------+
                                300       600       900      1200
```

```
Pooled StDev = 64.0


Grouping Information Using Tukey Method

Sample  N   Mean  Grouping
13      3  976.7  A
12      3  219.2    B
11      3  205.8    B
 R      3  187.7    B
10      3  155.1    B
 9      3  120.1    B

Means that do not share a letter are significantly different.
```

One-way ANOVA: Springiness versus Sample

```
Source  DF        SS        MS     F      P
Sample   5  0.004969  0.000994  2.10  0.136
Error   12  0.005677  0.000473
Total   17  0.010646

S = 0.02175   R-Sq = 46.68%   R-Sq(adj) = 24.46%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  -------+---------+---------+---------+--
 R     3  0.9570  0.0053         (--------*--------)
 9     3  0.9353  0.0223  (--------*--------)
10     3  0.9503  0.0075       (--------*--------)
11     3  0.9810  0.0161                 (--------*--------)
12     3  0.9797  0.0342                (---------*--------)
13     3  0.9727  0.0287              (--------*--------)
                          -------+---------+---------+---------+--
                               0.930     0.960     0.990     1.020

Pooled StDev = 0.0217


Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
11      3  0.98100  A
12      3  0.97967  A
13      3  0.97267  A
 R      3  0.95700  A
10      3  0.95033  A
 9      3  0.93533  A

Means that do not share a letter are significantly different.
```

One-way ANOVA: Cohesiveness versus Sample

```
Source  DF        SS        MS     F      P
Sample   5  0.000698  0.000140  0.65  0.664
Error   12  0.002562  0.000213
Total   17  0.003260

S = 0.01461   R-Sq = 21.42%   R-Sq(adj) = 0.00%
```

```
                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev   -+---------+---------+---------+--------
 R     3  0.81633  0.01457               (-----------*-----------)
 9     3  0.79967  0.02970     (-----------*-----------)
10     3  0.79633  0.01097   (-----------*-----------)
11     3  0.80367  0.00252        (-----------*-----------)
12     3  0.80267  0.00586      (-----------*-----------)
13     3  0.80233  0.00503      (-----------*-----------)
                            -+---------+---------+---------+--------
                           0.780     0.795     0.810     0.825

Pooled StDev = 0.01461


Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
 R      3  0.81633  A
11      3  0.80367  A
12      3  0.80267  A
13      3  0.80233  A
 9      3  0.79967  A
10      3  0.79633  A
```

One-way ANOVA: Resilience versus Sample

```
Source  DF        SS        MS     F      P
Sample   5  0.013017  0.002603  5.32  0.008
Error   12  0.005875  0.000490
Total   17  0.018892

S = 0.02213   R-Sq = 68.90%   R-Sq(adj) = 55.94%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  -----+---------+---------+---------+----
 R     3  0.50033  0.04105           (-------*-------)
 9     3  0.46400  0.03118  (-------*-------)
10     3  0.47133  0.00971    (-------*-------)
11     3  0.48800  0.00954        (-------*-------)
12     3  0.51100  0.00819                (-------*-------)
13     3  0.54500  0.00529                         (-------*-------)
                           -----+---------+---------+---------+----
                              0.455     0.490     0.525     0.560

Pooled StDev = 0.02213


Grouping Information Using Tukey Method

Sample  N     Mean  Grouping
13      3  0.54500  A
12      3  0.51100  A B
 R      3  0.50033  A B
11      3  0.48800  A B
10      3  0.47133    B
 9      3  0.46400    B

Means that do not share a letter are significantly different.
```

Water activity

One-way ANOVA: Aw versus Sample

```
Source  DF         SS         MS     F      P
Sample   5  0.0001873  0.0000375  3.61  0.032
Error   12  0.0001247  0.0000104
Total   17  0.0003120

S = 0.003223   R-Sq = 60.04%   R-Sq(adj) = 43.39%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  -------+---------+---------+---------+--
 R     3  0.97667  0.00231    (-------*-------)
 9     3  0.98500  0.00458                    (-------*-------)
10     3  0.98033  0.00252            (-------*-------)
11     3  0.97800  0.00361       (-------*-------)
12     3  0.97533  0.00058  (-------*-------)
13     3  0.97667  0.00404    (-------*-------)
                           -------+---------+---------+---------+--
                                0.9750    0.9800    0.9850    0.9900

Pooled StDev = 0.00322


Grouping Information Using Tukey Method

Sample  N      Mean  Grouping
 9      3  0.985000  A
10      3  0.980333  A B
11      3  0.978000  A B
13      3  0.976667  A B
 R      3  0.976667  A B
12      3  0.975333    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample

Individual confidence level = 99.43%
```

Appendix G-4 – Phase III Consumer evaluation of gluten-free bread

Raw Data

	Tester	Apperance	Texture	Aroma	Taste	all Acceptab	attitude	Comment
	1	7	4	5	6	6	5	
	2	8	7	6	6	7	4	
	3	8	7	7	8	7	2	Quite chewy, looks like it would be good toasted
	4	5	7	4	6	6	5	
	5	5	3	5	3	4	5	
	6	7	6	7	6	7	4	Has a strange aftertaste, a bit bitter maybe?
	7	9	7	8	8	7	4	Crust is too tough
	8	5	6	4	5	4	4	The crust is a little bit hard, the aroma smell like artifical additives
	9	5	7	5	3	5	6	Not my taste but people might like it
	10	7	4	6	7	6	6	The crust was too hard which I did not like, the taste of the bread is new for me but I like it slightly
	11	4	8	7	5	6	4	The crust is too bitter
	12	9	8	8	9	9	2	The crust hass a funny flavour/texture (powdery) but overall its relly good. Better than other gluten free bread by miles.
	13	8	8	9	9	9	2	Crust looks like t might dry out quickly. Really yum though. Especially for GF bread.
	14	7	5	7	3	4	6	For the texture the rust is quite hard to chew, but the bread is soft. For the taste its bitter after taste for the crust
	15	7	8	7	6	8	4	The crust is very bitter
	16	8	8	8	7	8	3	
	17	7	7	5	6	6	5	The crust was a bit tough and bitter
	18	8	8	9	7	8	2	
	19	8	8	8	7	7	2	
	20	4	6	7	5	4	6	Dislike is mainly the crust. The middle part is good. Rubbery texture to the crust and a slightly burnt taste is left on my mouth
	21	5	6	8	7	7	3	The bread crust had a slight bitter aftertaste that was unpleasant otherwise it was all good
TOTAL	21	141	138	140	129	135		
AVERAGE		6,7	6,6	6,7	6,1	6,4		
PERCENTAGE		**75**	**73**	**74**	**68**	**71**		
STD DEV		1,59	1,50	1,53	1,74	1,57		