Development of Gluten-Free Wrap Bread

A Thesis

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Dedication

To my mother and father, Xiangdong and Xiaofang.

For their love, support and kindness.

Abstract

Gluten, the storage protein in wheat, barley and rye is associated with coeliac disease, wheat allergy and non-coeliac gluten sensitivity. The clinical symptoms include diarrhoea, anaemia, nausea, mouth sore and psychological symptoms and in some cases a gluten free diet may reduce the severity of irritable bowel disease (IBD). Gluten-related disorders can be prevented by the omission of gluten from the diet. Currently, there is an increasing demand for gluten-free foods due to consumer awareness of gluten-related disorders as well as people seeking to reduce possible dietary risks. New Zealand's market for gluten-free foods is presently estimated at nearly four million US dollars.

The development and production of gluten-free bread presents major technological challenges due to the role of gluten in developing the characteristic structure of both the raw dough and subsequent loaf texture. The main ingredients of bread are water and cereal flours which provide the primary structure to the baked product. Wheat grain is a traditional and common cereal that is milled into bread flour. When wheat flour is hydrated with water, gluten, the protein component hydrates to become a continuous cohesive viscoelastic network entrapping starch granules. This highly elastic network retains CO_2 gas produced by yeast and sugar during leavening, thus forming the foam structure of bread. Gluten replacements that mimic the viscoelastic properties of gluten have been widely investigated for gluten free baked products including flatbread. Flatbread is popular for use in ready-to-eat convenient foods due to its large crust to crumb ratio. Wrap bread is a typical flatbread that can be rolled to hold various fillings. The manufacture of gluten-free wrap breads mainly suffers from poor rollability which is an essential property of the product. Thus, the present study investigated the development of gluten-free wrap bread (GFW) using xanthan gum, guar gum, carboxmethyl cellulose (CMC) as possible replacers for gluten, coconut oil was also added to improve flexibility of the bread. The formulations were investigated and optimised in four integrated phases.

In phase 1, guar and xanthan gums were studied as possible gluten replacers during the development of GFWs. GFW samples (n = 16) made from four formulations under four baking conditions (200°C/2 min, 200°C/4 min, 220°C/2 min, 220°C/4 min) were analysed for baking weight loss and rollability. Baking weight loss was determined as moisture loss during baking, while rollability was measured as the ability of the freshly cooked bread to conform to shape (1-5 scale) as it was rolled around a 3-cm diameter wooden dowel (rod). A mixture of guar and xanthan gums (1:1) produced GFWs with better rollability and less baking weight loss than either gum alone. GFW samples baked at the higher temperature for the longer time generally had higher rollability. The highest average rollability score (3) obtained for this phase was considered low for wrap breads developed in phase 2.

In phase 2, GFWs (n = 20) made from five formulations containing both xanthan and guar gums (1:1), CMC, and coconut oil were baked at 230°C for 2 or 4 min or at 240°C for 2 or 4 min. Freshly baked GFWs were analysed for baking weight loss, water activity, and colour. Rollability using 1 1-cm diameter dowel and visible mould growth of the GFWs were determined during storage for 28 days (4°C). Products produced in phase 2 had no visible mould growth during storage for 3 weeks (4°C). The inclusion of xanthan-guar gum, CMC and coconut oil into GFWs baked at 240°C/2 min may have contributed to high rollability and low baking weight loss. The effect of each test ingredient (xanthan-guar, CMC, and coconut oil) on the properties of GFWs was the subject of phase 3.

In phase 3, a basic formulation made with three levels (9 formulations) each of coconut oil, CMC and xanthan-gum gum were optimized using the Taguchi method to test the effect

of each ingredient in the basic formulation. GFWs made using the 9 formulations were analysed by physical and sensory tests over three weeks storage at 4°C during which mould growth was assessed visually. Products in phase 3 had no visible mould growth during storage for three weeks (4°C). GFWs with high level of coconut oil (12%) were characterised by high baking weight loss, high whiteness index and a shorter firmer texture (high rupture force and low rupture distance). CMC (0.3%) and xanthan-guar gum (1%) may have contributed to low water activity, high rollability, high rupture distance and high rupture force during storage. Results indicated that 0.3% CMC and 1% xanthan-guar gum were the optimum levels for these ingredients. As the optimized levels of coconut oil could not be confirmed in this phase, three promising formulations with different levels of coconut oil (8, 10, 12%) were evaluated in phase 4.

In phase 4, three products were produced using 3 optimised formulations from phase 3 and were analysed by physical tests and sensory evaluation during storage for two weeks (4°C). The 3 optimised formulations selected from phase 3 were: (1) base formulation plus 8% coconut oil, 0.3% CMC and 1% xanthan-guar gum; (2) base formulation plus 10% coconut oil, 0.3% CMC and 1% xanthan-guar gum; (3) base formulation plus 12% coconut oil, 0.3% CMC and 1% xanthan-guar gum. Among the three formulations, samples containing 12% coconut oil, 0.3% CMC and 1% xanthan-guar gum had the highest consumer sensory acceptability and were characterised by high rollability, and a more flexible texture (moderate rupture force and greater rupture distance) and low baking weight loss.

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List of Abbreviations

AACC	American Association for Clinical Chemistry
ANOVA	analysis of variance
AOAC	Association of Official Agricultural Chemists
B.C.	Before Christ
BWL	baking weight loss
ca	circa
CD	coeliac disease
CFU	colony forming units
CIE	International Commission on Illumination
cm	centimetre
CMC	carboxymethyl cellulose
DATEM	diacetyl tartaric acid ester of mono- and diglycerides
ERH	equilibrium relative humidity
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
GFW	gluten-free wrap bread
GLM	General linear model
HDP	hydroxypropyl distarch phosphate
Hi-Maize	high amylose maize resistant starch
HPMC	hydroxypropyl methycellose
IBD	Irritable bowel disease
L	litre
LDL	low density lipoprotein
M	molar, mole/litre
MCTs	medium chain triglycerides
min	minute
mL	millilitre
mm	millimetre
NaCl	sodium chloride
NCGS	non-coeliac gluten sensitivity
NMR	Nuclear Magnetic Resonance
No.	number
PA	Commonwealth of Pennsylvania
RS	resistant starch
SD	standard deviation of mean
SEM	standard error of mean
sec	second
SSL	sodium and calcium stearoyl lactylate
temp.	temperature
TPA	texture profile analysis
UK	United Kingdom of Great Britain and Northern Ireland
USA/US	United States of America
WHO	World Health Organization
xanthan-guar gum	mixture of xanthan gum and guar gum (1:1)

Chapter 1 Introduction

Bread is currently the most common staple food in the world and probably has been for at least 6,000 years (Kahlon & Chiu, 2014). It is a vital source of easily consumed and digested carbohydrate (Coulston et al., 1984). The Joint WHO/FAO recommendations state that carbohydrate should constitute 55-70% of total energy intake (WHO, 2003). Bread and baked cereals are the primary dietary sources of carbohydrate (Lambert et al., 2009).

Bread is not only a source of energy, but it delivers other aspects of nutrition and therefore contributes to health (Esteller, Amaral, & Lannes, 2004). Common foods are increasingly being considered as or modified to become functional foods (Siro, Kapolna, Kapolna, & Lugasi, 2008). Bread is an ideal matrix in which functional nutrients can be delivered to the consumer in an acceptable form. With increasing levels of gluten allergies being reported, particularly in western cultures, the demand for gluten-free bread is increasing (Sapone et al., 2012; Thompson, 2015).

Gluten-related disorders are caused by the ingestion of gluten by individuals with genetic and/or immunologic predispositions to these conditions (Sapone et al., 2012). This may be coeliac disease and wheat allergy which are the best known and non-coeliac gluten sensitivity (NCGS). The prevalence of coeliac disease is about 1% in US, Europe and New Zealand (Fasano et al., 2003; Mäki et al., 2003; West et al., 2003; Cook, Oxner, Chapman, Whitehead, & Burt, 2004). In a blood test to analyse the food hypersensitivity among nearly 2600 four-year-old children in Stockholm, the prevalence of wheat allergy was reported to be 5% (Östblom, Wickman, Van Hage, & Lilja, 2008). The prevalence of self-reported doctor-diagnosed wheat allergy was 0.4% among about 4500 US adults (Vierk, Koehler, Fein, & Street, 2007). The prevalence of self-reported NCGS in the USA is variable, ranging from 0.63 to 6% (Volta, Caio, Tovoli, & De Giorgio, 2013), but solid evidence-based clinical data on NCGS prevalence in the general population are not yet available (Di Sabatino & Corazza, 2012).

Apart from the medically-diagnosed gluten-related disorders, more people are choosing gluten-free diets for personal preferences. Typically, breads have a glycemic index of close to

100 and are comparable to ingesting glucose and consumption of large quantities of bread may increase the likelihood of type 2 diabetes (Jenkins, Wolever, & Jenkins, 1988; Willett, Manson, & Liu, 2002). Gluten-free bread is usually made by using composite flours which consist of beneficial ingredients such as legume flours, psyllium and guar gum which serve as gluten replacers (Abdul-Hamid & Luan, 2000; Dziki, Różyło, Gawlik-Dziki, & Świeca, 2014; Prabhasankar, 2014; Rebello, Greenway, & Finley, 2014).The content of proteins in cereals (wheat, 8-12%) was generally lower than in legumes (18-25%) (Tharanathan & Mahadevamma, 2003). Wheat protein (66% retention) is of lower nutritional quality compared to that of milk protein (74%), soy protein (71%), and legume proteins (70-74%) on the basis of postprandial utilisation in humans (Bos et al., 2005). Soluble dietary fibres, rich in legume flours, psyllium and gums, can slow down glucose absorption, reduce plasma cholesterol concentrations and are useful in the management of diabetes and heart diseases. Insoluble fibres in wheat bran contribute little to diabetes and other chronic diseases (Azizah & Zainon, 1997; Anderson et al., 2009; Bijkerk et al., 2009).

Besides being a basic source of nutrition, bread is a convenient food which is popular in today's busy life-style (Jabs & Devine, 2006). In the USA, tortilla, a typical flatbread has entered the main food stream and contributes a significant proportion of cereal products consumed (Kuk, 2006). Flatbreads were initially consumed in the Middle East, North Africa and Central Asia as ethic food. Due to their large crust-crumb ratios, flatbreads are being used as wrap breads which are popular all over the world (Qaroon, 1996). Wrap bread are commonly used to hold different types of fillings (Sommers & Boyd, 2005). Fillings may comprise of mixtures of foods, fresh or cooked (Cureton, 2007). Due to the rapid growth of the fast-food retail outlets, wrap breads offer potential for growth of convenience foods (Jekanowski, 1999). As part of this growth, there is need to develop gluten-free wrap bread.

Developing gluten-free wrap bread with good textural quality faces challenges because the protein plays a vital role in the prime baking properties (Cauvain, 2015). When flour is hydrated to form dough, gluten is transformed into a continuous cohesive viscoelastic (gluten) protein network, entrapping the starch granules within it (Shewry, Halford, Belton, & Tatham, 2002; Mohamed & Rayas-Duarte, 2003). During baking, gluten proteins experience a combination of changes in protein surface hydrophobicity, sulfhydryl/disulphide interchanges and formation of new disulphide cross-links on heating (Schofield, Bottomley, Timms, & Booth, 1983; Lindsay & Skerritt, 1999). As a result of these changes with starch,

the typical foam structure of baked bread is formed. The gluten protein network assists with retaining gas which determines loaf volume and crumb structure of the bread (Dubreil et al., 1998).

Without gluten, breads will lack a protein network that can both hold water and form the network in which the starch is embedded. Substitutes of gluten should have water-binding capacity, ability to form a viscoelastic network to enhance baking performance of starches or starchy flours, providing structure to bread by entrapping gasses to form an open spongy cell crumb of gluten (Gallagher, 2009). To obtain the afore-mentioned properties of bread, different types of hydrocolloids (natural, synthetic and biotechnological) in bread formulations have been studied (Anton & Artfield, 2008). Xanthan gum, guar gum and carboxymethyl cellulose (CMC) have been widely used to make gluten-free bread due to their ability to control the rheology and texture of aqueous systems within starch (Gallagher, Gormley, & Arendt, 2004). Besides the role of gluten substitutes, hydrocolloids can replace part of fat to produce low calorie products (Mandala, Palogou, & Kostaropoulos, 2002). Addition of shortening to bread formulation can improve stabilisation of the gas bubble in bread dough to achieve better bread structure (Houben, Höchstötter, & Becker, 2012). Coconut oil is edible plant oil with good properties among shortening ingredients (Kappally, Shirwaikar, & Shirwaikar, 2015). Several commercial gluten-free breads with different formulation have been reported (Segura & Rosell, 2011; Houben et al., 2012). Currently, there is scanty published information on gluten-free wrap bread (GFW). Thus, there is a real opportunity for developing GFWs that meet consumer expectations (Berne, 2005).

Objectives

Main Objective

In this study, we aimed to develop gluten-free wrap bread (GFW) acceptable to consumers. The effects of baking conditions (baking temperature and baking time) were studied to achieve the desired properties of GFWs.

Specific objectives

The experiments were conducted into four integrated phases with the following specific objectives.

1. To select suitable ingredients and baking conditions (baking temperature and baking time) to produce gluten-free bread. In phases 1 and 2, nine formulations were tested to study effects of guar gum and xanthan gum, CMC and coconut oil on characteristics of GFWs. Each formulation was baked under four conditions to determine optimal baking conditions.
2. To optimise the levels of tested ingredients (xanthan gum, guar gum, CMC and coconut oil) in formulations. In phase 3, nine formulations containing three levels of test ingredients generated by a Taguchi design were analysed by physical properties during storage for 14 days at 4 °C.
3. To characterise physical properties of the GFW and determine the sensory acceptance of the products made by three optimised formulations. In phase 4, GFWs produced using three optimised formulations in phase 3 were investigated during storage (4 °C).

Chapter 2 Literature Review

2.1 Current trends

Bread is a basic dietary item used all over the world. The control of production and distribution of bread has been used as a means of exercising political influence over the populace for at least 2000 years (Coutton, 1925; Veyne, 1990). Typical bread is based on cereal flour and water (Table 1), prepared by mixing, dividing, proofing and baking to produce a complex solid matrix (Scanlon & Zghal, 2001). Trends in the innovation of bread are related to health, pleasure and convenience (Martínez-Monzó, García-Segovia, & Albors-Garrigos, 2013).

Convenience has been driven by changes in the social habits, increased working hours and changing household structures (Martínez-Monzó et al., 2013). The convenience food segment is rapidly increasing, with the global ready-made foods market expected to grow by over 3% from about $1 trillion in 2011 to nearly $1.5 trillion in 2016 (Rivera, Orias, & Azapagic, 2014). Consumers have less time to shop, cook or prepare their foods due to urbanization and industrialization resulting in rapid expansion of convenience foods (Scholliers, 2015). Several types of breads have been developed in the last few years, which include pre-packed sandwich wraps (Cauvain, 2015; Takagi & Shima, 2015). The increased sale of pre-packed sandwich wraps is now a large industry, comprising of large quantities of products.

Health trends are relevant drivers for innovation in the bread industry (Esteller et al., 2004). Reports by previous workers (McCullough et al. 2002; Roberts and Barnard, 2005), showed a direct association between unbalanced diets and rising incidences of chronic health-related issues, including cardiovascular disease, diabetes and obesity. The consumption of low-fat, high-fibre foods has been advocated by health and nutrition agencies (Martínez-Monzó et al., 2013). Consumers have an increasing interest in food that promotes and maintains energy, enhances satiety, or make consumers feel full after eating (Van Kleef, Van Trijp, Van Den Borne, & Zondervan, 2012). This demand gives the bread industry additional opportunities to develop products containing new functional ingredients compliant with these requirements. Recent studies suggest that breads with improved nutritional qualities, can be produced by the industry (Korus, Witczak, Ziobro, & Juszczak, 2009; Vierhile, 2012). Functional foods have

Table 1 Ingredients (%, w/w) of typical breads

Parameter	Flour[1]	Yeast	Salt	Water	Shortening	Emulsifier	Baking powder	Milk powder	Sugar	Yoghurt
UK white bread	100	2.1-2.3	1.7-2.1	60.0-62.0		1.0-1.5				
French baguette	100	3	1.7-2	64.0-66.0		1.0-2.0				
Irish soda bread	100	3.6	1.6	59-61.0	3.6	1.2-2.0	3.1	1.6		
Soft rolls	100	3.1	1.8	61.0-63.0	2.2	2.0		2.0-2.5	1.6	
Whole wheat bread	100[a]	2.7	1.8	32.5		1.0-2.0				
Mix-grain bread	100	2.1-2.3	1.8-2.1	58.0-62.0		1.0-2.0				
Rye bread	100[b]	3	1.6	60.7	0.71					
Pizza bases	100	6.5-7.0	2.0	56-58	0-0.8	1.0		0-2.5	0-1.5	
Chapattis	100		Optional	70	Optional					
Pita bread	100	0.5-1.0	0.75-1.5	50.0-60.0						
Naan	100	1	0.7-1.5	35					2.5	25
Tortilla (flour)	100		2	50	8.0-14.0		1.0-1.5			
Paratha	100		1.5-2.5	Variable	5.0-10.0					

Source: Qarooni,1996; Cauvain & Young, 2009.

Note: [1] Flour = wheat flour except for rye bread and whole meal bread; [a] = wholemeal wheat flour; [b] = rye flour

been developed in virtually all food categories. These foods may be defined “as foods and food components that provide a health benefit beyond basic nutrition (quantities necessary for normal growth and development) and include conventional foods, fortified, enriched or enhanced foods and dietary supplements” (Badaracco, 2015).

Alternative classification of functional products is firstly ‘‘to add good to your life’’, for example, improve regular stomach and colon functions (pre- and probiotics) or ‘‘improve children’s life’’ (Siro et al., 2008). A second group of functional food is designed to reduce an existing health risk problem such as high cholesterol or high blood pressure (Kraus, 2015). These two kinds of bread formulae are designed by including more whole grains, fibre, prebiotics and probiotics, or antioxidant ingredients (Korus et al., 2009; Thakur & Thakur, 2014). A third group comprises of lactose-free, gluten-free products (Mäkinen-Aakula, 2006; Jia, Kim, Huang, & Huang, 2008). As definitions differ, the percentage of population affected by food allergies is not accurately known. However, it is estimated that 2-10% of the population claim to be suffering from food allergies (Kulis, Wright, Jones, & Burks, 2015). The prevalence of food allergies is higher among children than adults (Nwaru et al., 2014; Sicherer & Sampson, 2014). Although any type of food can cause an allergic response, 90% of all reactions are caused by eight allergens comprising peanuts, tree nuts, eggs, milk, fish, shellfish, soy and wheat (Bock, Muñoz-Furlong, & Sampson, 2001; Lee & Burks, 2006; FSANZ, 2010).

Traditional baked products rely on many of these foods, especially wheat-based flour. Gluten is the protein portion of wheat flour, which triggers gluten-related disorders. The global market of gluten-free food sales has experienced rapid growth, with growth rates of 17% in 2014 and 12% in 2015 (Duan, 2016). However, gluten plays an important role in the structure of baked products (Toufeili et al., 1999; Cubadda, Carcea, Marconi, & Trivisonno, 2007). Gluten proteins have their specific function in bread making, as they contribute to the formation of cohesive, extensible and elastic dough, which can retain gas (Cauvain, 2015). Baking without gluten poses a big challenge for all bakers and researchers. Consumers want high quality gluten- and allergen-free bread products (Lee, Ng, Zivin, & Green, 2007). There is therefore need to use other cereals and flours to replace wheat while adding different components like hydrocolloids, dough treatment or by changing the method of baking to overcome the deficits of gluten-free bread (Durazzo, Turfani, Azzini, Maiani, & Carcea, 2013).

2.2 History of bread

Bread is one of the oldest foods (Randez-Gil, Sanz, & Prieto, 1999). Dating back to the Neolithic age (*ca* 10000 B.C.), grain flours became the mainstay of making bread as organized agriculture was spread (Mondal & Datta, 2008).

The origin of modern bread types can be linked to the cultivation of wheat and barley in an area described as the "Fertile Crescent" which extends from the Nile Valley of Egypt to Jordan, Syria, Lebanon, Tigris and the Euphrates Valley (Harlan & Zohary, 1966). Bread was the main staple food of that region. Wheat was first eaten as grain mixed with water. However, it tasted better when baked and the origin of bread was discovered (Stern et al., 2001). Early breads were unleavened and flat. The Egyptians are recognized as the pioneers of the art of bread-making through fermentation (Rubio-Tapia, Hill, Kelly, Calderwood, & Murray, 2013). The ancient technology of bread-making rapidly spread throughout the Mediterranean region, especially to Italy, where the fermentation process was improved considerably. The Romans later removed yeast from the surface of fermenting wine and used it to leaven bread (Tonutti & Bizzaro, 2014). This method of dough preparation, called bann, gained acceptance and soon spread to other countries including Britain (Farvili, Walker, & Qarooni, 1995).

Bread technology has improved throughout the years as our knowledge of fermentation and baking technology evolved. Through much of the history, white bread was consumed by upper classes of society while the poor had darker varieties (Smith, & Christian, 1984). A 500-g 100% whole grain loaf bread required 390 g flour while an 80% extraction loaf of white bread required about 488 g flour (Pearson, 1997). White bread used most of the grain resulting in high cost of milling to produce luxury foodstuff. In the middle ages, only the rich could afford white bread (Bobrow-Strain, 2012). Whereas, from the late 20th century people recognized that whole-grain bread had superior nutritional value compared to the white varieties (Montonen, Knekt, Järvinen, Aromaa, & Reunanen, 2003). Nowadays dark bread is commonly sold at a higher price than white bread (Kraus, 2015). Another outstanding progress in bread technology is the development of the Chorleywood Bread Process in 1961 (Chamberlain, Collins, Elton, & Cornford, 1962). This process, which combines high mechanical energy and chemical action to proceed from flour to bread in about four hours compared with over nine hours for bulk fermentation. Inferior grain can be used and it gives a

high yield of bread (Rawat & Indrani, 2015).

2.3 Classification of bread

Bread is divided into three main categories according to bread volume: (a) high specific volume bread such as pan bread; (b) medium specific volume bread such as rye bread; (c) low specific volume bread, flatbreads such as pita, chapatti, tortilla, roti, naan, paratha, poori, balady and barabri (El-Khoury, 1999).

2.3.1 Flatbread

Flatbread, characterised by a large surface area and a thin crumb, is the oldest well-known, and widely consumed bread type world-wide (Izydorczyk, Chornick, Paulley, Edwards, & Dexter, 2008; Quail, McMaster, & Wootton, 1991). Flatbread was initially a staple of the Middle East, North Africa and Central Asia. Due to its large crust-crumb ratio, flatbread is being used as wrap breads, which is popular for their versatility as it can be eaten with many different fillings as convenience food (Lind & Barham, 2004; Sommers & Boyd, 2006). Wrap breads consumed in fast retail outlet or home may continue to offer growth potential. As part of this growth, there is need to reformulate flatbread with new nutritional attributes and other functional properties. Thus, there is an opportunity to increase the number of flatbread on the market due to increasing consumer demand for functional, healthy and convenience foods (Berne, 2005).

Flatbread can be made from many types of cereals including wheat, corn, barley, oat, rye as well as the legumes. Among the cereals, wheat flour is the most common base that is used for baking flatbread (Al-Dmoor, 2012). Although wheat flour is popular for its superior baking properties, many types of flatbreads use composite flours to improve nutrition, flavour, colour and other properties (Ram, 2009).

Flatbread can be divided into two groups; single-layered and double-layered which may be referred as pocket-type. Widely consumed pocket-type flatbreads are pita and babali (Izydorczyk et al., 2008). Most flatbreads are single layered which also can be classified into

two categories according to leavening. Yeast leavened single-layered bread consists of pancake, pizza crust, lavash, naan and rye flatbread. Chapatti, paratha, arepa and tortilla constitute unleavened or chemically leavened single-layered flatbreads (Qarooni, 1996). The manufacturing process of flatbreads varies with the purpose of the end-product (Bråthen & Knutsen, 2005; Nordberg & Chandrasekhar, 2005).

Flatbread is also prepared from flour, water and other optional ingredients. After mixing of all ingredients, dough forms. Dough is divided into small pieces and moulded to a round shape. In single-layered flatbreads, dough pieces are baked after shaping while double-layered are proofed before baking. The proofing process allows the dough to relax, aerate and develop a thin skin. During baking, top and bottom crusts depart and two-layers develop because of steam from free water and CO_2 generated by yeast fermentation (El-Khoury, 1999).

2.3.2 Gluten-free bread

Coeliac disease is a chronic, immunologically determined form of enteropathy affecting the small intestine in genetically predisposed children and adults (Maleki, Hoseney, & Mattern, 1980; Fasno et al., 2003). It is precipitated by the ingestion of gluten-containing foods, such as wheat, barley and rye. The clinical symptoms of coeliac disease include diarrhea, anemia, and nausea, mouth sore and psychological symptoms such as headache, nervous depression and osteoporosis (Niewinski, 2008). The only treatment of coeliac disease is to maintain a life-long gluten-free diet (Mäki & Collin, 1997).

Gluten can be defined as a protein fraction from wheat, rye, barley, oats or their crossbred varieties (e.g. Triticale) and derivatives thereof, to which some individuals are intolerant and that is insoluble in water and 0.5M NaCl (Stern et al., 2001; Gallagher, Gormley, & Arendt, 2004). The major protein fractions of gluten, monomeric gliadin and polymeric glutenin are the storage proteins in wheat and increased gas retention of dough in breads, cakes and batters (Toufeili et al., 1999). In the formation of dough, the gliadins act as ‘plasticisers’ and associate with one another or the glutenins through hydrophobic interactions and hydrogen bonds (Veraverbeke & Delcour, 2002). Gliadins as polymorphic group of monomeric gluten proteins contribute elasticity and cohesiveness to the dough. Glutenins, as one of the largest protein polymers in nature are heterogeneous mixtures of multi-chain molecules formed from

polypeptides linked by disulphide bridges which provide extensibility to the dough system (Wieser, 2007). An appropriate balance between dough viscosity and extensibility/strength is vital for quality of bread which requires the gliadin/glutenin ratio of the gluten proteins. Up to a certain limit, higher dough strength increases loaf volume. Therefore, strong doughs have poor ability to rise during baking (Goesaert et al., 2005). Gluten plays an extreme important role in formation of soft crumb and crispy crust in bread products, and prevents crumbling (Primo-Martin et al., 2006). Bread crumb is a solid matrix of a continuous phase of gelatinized starch and a continuous gluten network that encloses the starch granules and fibre fragments (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001; Zannini, Jones, Renzetti, & Arendt, 2012). Dough prepared without gluten rarely has the character of cohesiveness and viscosity to stabilize air cells which leads to low quality of final bread because it usually lacks a protein network that can hold water and form the matrix to embed starch. Gluten-free doughs are much less cohesive and elastic than wheat dough, they are more sticky, less elastic and pasty; and as a result are difficult to handle (Bloksma, 1990; Moore, Schober, Dockery, & Arendt, 2004; Schober, Messerschmidt, Bean, Park, & Arendt, 2005).

There is a growing demand for gluten-free food, with global value sales doubling between 2008 and 2013 (Stadelman, 1999; Gallagher, 2009; Duan, 2016). The value of gluten-free market world-wide is estimated to reach about US $6 billion in 2018, for which 59% is in USA (Nijeboer, Bontkes, Mulder, & Bouma, 2013). According to Mandala and Kapsokefalou (2011), 15-25% parents in USA seek gluten-free products for their children. It is estimated that about 20% of USA consumers buy gluten-free products (Lee, Ng, Zivin, & Green, 2007; Siro et al., 2008; Nijeboer et al., 2013). Of these consumers, about 65% buy the products because they consider them healthy, 27% for weight loss, 11% for health reasons (inflammation, depression), and 20% are attributed to other reasons (Cauvain & Young, 2009). Some gluten-free products have their special functionality besides label of “gluten-free” to satisfy groups of people with nutritional needs (Rahaie, Gharibzahedi, Razavi, & Jafari, 2014).

Bread as staple food feed the majority of the world’s population (Rubio-Tapia et al., 2013). Consumption of bread places an important role in human nutrition (King, Mainous, & Lambourne, 2012). Among gluten-free products, 46% consist of bakery and confectionery products (Nijeboer et al., 2013). Development of new technologies and the use of gluten-free flours, starches, hydrocolloids and novel food ingredients will make it possible to find

alternative gluten-free products for the traditional bakery products. Various types of hydrocolloids (natural, synthetic and biotechnological) are commonly used to replace gluten in bread formulation, because of their high water-binding capacity (Matos & Rosell, 2014). Most of the gluten-free breads are based on flours and starches, especially in the case of starch-based formulations. With respect to nutritional composition, these products may contain lower levels of proteins, minerals and fibre. The nutritional composition of gluten-free bread formulation has been improved by using fibres, legume flour, addition of vitamins and minerals (Ćurić, Novotni, Tušak, Bauman, & Gabrić, 2007; Korus et al., 2012).

2.4 Functional properties of ingredients of breads

2.4.1 Introduction

Flour is the major ingredient in bread-making and the biggest single cost item. Water is used to hydrate flour proteins and is absorbed by the flour starch as it gelatinizes during cooking (Cauvain & Young, 2009). Salt is preferred for flavour development in the bread but will retard fermentation. Increases or decreases in salt level have to be matched by similar increases or decreases in yeast to maintain the same rate of fermentation (Lynch, Dal Bello, Sheehan, Cashman, & Arendt, 2009).

The optional ingredients of bread include acidulates, antimicrobial agents, fruits, herbs, meat, dairy product, emulsifiers, reducing and oxidizing agent, dietary fibre, shortening, and sugar. These ingredients may be used for adding new flavours, improving the quality, increasing the nutritive value of bread and as processing aids, which assist the bread-making process and retard staling. Small amounts of sugar or honey may be added to improve the taste, texture and flavour quality of bread (Aparna & Rajalakshmi, 1999). The fructose portion aids moisture retention for longer time to slow down staling and improves the texture of bread (Tong et al., 2010). With sugar addition the loaf weight of flatbread increased (Maleki, Vetter, & Hoover, 1981). The sweeteners also provide nutrients for the yeast and facilitate the browning of bread through caramelization and Maillard reaction (Ponte, 1990). Eggs can improve the quality of bread and are good sources of low-cost, high quality protein and foliate as well as vitamins B12 and B2 (Finney, Henry, & Jeffers, 1985; Stadelman, 1999). Milk is often added to impart sensory properties. Whole and skimmed milk powders improve

flavour and odour of bread (Kenny, Wehrle, Stanton, & Arendt, 2000). Sodium caseinate and hydrolysed casein contribute to lowering of proof time, increasing loaf volume and in making the bread loaf softer (Masoodi & Chauhan, 1998; Gallagher, Kunkel, Gormley, & Arendt, 2003). Dietary fibres have gained immense importance in bakery products because of their constructive role in reducing the digestion rate of carbohydrates, consequently reducing the severity of diabetes mellitus, blood glucose and cholesterol levels and functional physical properties for bread-making (Elleuch et al., 2011).

2.4.2 Flour and starch

Wheat

Wheat (*Triticum* spp.) is by far the most important and major cereal used in bread-making (Dewettinck et al., 2008). The ingredient influences the processing of most doughs and batters as well as determines the end-quality of wheat bread. The prominent role of wheat flour on bread is due to their unique proteins (gluten). Wheat flour is present in larger proportions than any other ingredients in the formulation of bread (Popov-Raljić, Mastilović, Laličić-Petronijević, & Popov, 2009; Demirkesen, Mert, Sumnu, & Sahin, 2010b).

Protein, starch, fibre and other important physico-chemical properties of wheat flour such as particle size and protein quality (wheat) commonly determines the effect of wheat flour on the characteristics of baked products. The key role of protein in wheat flour is the formation of gluten structures which is essential for bread-making. In general, an increase in protein content leads to an increase in gas-retention properties of dough, thereby increasing bread volume (Autio & Laurikainen, 1997; Mittag et al., 2004).

Rye

Rye (*Secale cereal*) is placed second to wheat in the production of bread (Dhingra, Michael, Rajput, & Patil, 2012). Rye is a grain of cold climates, which grows well in Northern and Eastern Europe. Similar to wheat, rye has potential to make bread (Esteller & Lannes, 2008). Rye is often consumed as a whole grain product (Karppinen, Myllymäki, Forssell, &

Poutanen, 2003; Bejosano, Joseph, Lopez, Kelekci, & Waniska, 2005).

Bread based on rye is commonly found in the USA, and northern, central and eastern Europe. Rye bread is effective in reducing serum and low density lipoprotein (LDL) cholesterol concentrations in people with elevated serum cholesterol (Leinonen, Poutanen, & Mykkänen, 2000; Collado-Fernández, 2003a). Whole-meal rye bread significantly improves bowel function in healthy adults and may decrease the concentration of some compounds that are putative colon cancer risk markers (McKeown, Meigs, Liu, Wilson, & Jacques, 2002; Collado-Fernández, 2003b). However, utilization of rye in bread is still limited mainly because of the problems arising from its flavour; many consumers are not familiar with the somewhat mild, rye-like flavour, perceived as bitter and intense (Heiniö, Liukkonen, Katina, Myllymäki, & Poutanen, 2003).

Barley

Barley (*Hordeum vulgare* L.) is one of the oldest domesticated grains, which grows in a wide range of climates and altitudes, including the arid conditions of the Sahara and high altitudes of Tibet. Barley is not suitable as a main flour ingredient for bread because it lacks gluten, which cannot form cohesive dough (Andersson et al., 2004). Barley bread has poor gas retaining ability, texture sensory qualities and low volume loaf compared to wheat bread (Newman & Newman, 2006; Kim & Yokoyama, 2010; Rieder, Holtekjølen, Sahlstrøm, & Moldestad, 2012). The drought-tolerant crop is however used as malt flour in baking. Although malt flour is not suitable for bread-making, it is added to wheat flour in small quantities to support the growth of yeast, develop texture and improve flavour. Minor replacement with barley flour in wheat bread has potential applications in hypoglycaemic, antioxidant and anti-diabetic products (Alu'datt et al., 2012).

Gluten-free flour and starches

Non-gluten flours (maize, rice, sorghum, millets, pseudocereals, legumes, chestnut and coconut) and starches are used in producing gluten-free bakery products (Cauvain & Young, 2009; Krupa-Kozak, Troszyńska, Bączek, & Soral-Śmietana, 2011; Demirkesen, Campanella,

Sumnu, Sahin, & Hamaker, 2014). Cereals such as sorghum, millet, teff and pseudocereals like quinoa and buckwheat are usually used in their milled form. However, maize and rice are commonly used as isolated starch besides milled into flour. Flours from roots, tubers, legumes and other sources can also be used as components of composite mixes (Giuberti, Gallo, Cerioli, Fortunati, & Masoero, 2015).

Sorghum

Sorghum (*Sorghum bicolour*), is widely grown all over the world for human food and animal feed. It is a key staple in many parts of the developing world, especially in the drier and more marginal areas of the semi-tropics (Shambat, 2011). In the southern and southwestern regions of Saudi Arabia and Yemen, sorghum is a staple food in the form of thick flatbread, usually baked at home (Khalil, Sawaya, Safi, & Al-Mohammad, 1984).

Various processing methods are used for preparation of foods from sorghum. There has been some interest in using sorghum for making bread-like products as an alternative to wheat (Taylor, Schober, & Bean, 2006). Sorghum gluten-free bread has been successfully produced in pilot studies (Taylor et al., 2006; Velázquez, Sánchez, Osella, & Santiago, 2012). However, sorghum baked bread is characterized by low loaf volume, poor flavour, and becomes firm and brittle during storage (Hart, Graham, Gee, & Morgan, 1970; Schober et al., 2005). Torres, Ramirez-Wong, Serna-Saldivar, and Rooney (1993) reported that tortillas containing sorghum flour had black spots throughout the loaf which detracted appearance; sorghum tortillas were prone to stale much faster during storage compared to wheat tortillas.

Maize

Maize (*Zea mays* L.), a native plant of the Americas, originated from Peru and Ecuador is commonly known as corn. Maize forms a substantial staple food in many parts of the world, particularly in the developing countries (Cauvain, 2015). Maize has potential to make bread and it has been used to produce specific type of bread in some places. In Portugal, maize is used to make “Broa” with rye, which is fermented bread with yeast (Patto, Moreira, Carvalho, & Pego, 2007). In central and south America, maize is generally consumed in the

form of tortillas, flat thin disks of baked masa (maize dough). Maize and maize-based products have been proposed as sources of beneficial carbohydrates, such as resistant starch and β-glucans (Niba, 2003). Maize-breads (Arepas) made from regular dent corn with 25% amylose content have been shown to have high resistant starch and high fermentability for colonic microorganisms in the hindgut of rats (Granfeldt, Drews, & Björck, 1993). A study on application of maize into gluten-free bread reported that the coarser maize flours increased volume and decreased firmness of bread compared to finer flours due to their higher capacity to retain gas during fermentation (de la Hera, Talegón, Caballero, & Gómez, 2013). Maize-breads (Broa) made from composite maize-rye-wheat flour usually have compact crumb texture and low specific volume (Brites, Trigo, Santos, Collar, & Rosell, 2010).

Rice

Rice (*Oryza sativa*) is an important grain that is highly consumed in Asia as staple food. It has many unique attributes, such as ease of digestion, bland taste, and hypoallergenic properties. Rice flour can serve as a good substitute for wheat flour in gluten-free breads (Mandala & Kapsokefalou, 2011). However, the rice proteins have little elasticity and cannot retain gas produced during the fermentation process (Gujral & Rosell, 2004). Rice breads have lower specific volume, harder texture, and are more prone to retrograde during storage than wheat bread (Kadan, Robinson, Thibodeaux, & Pepperman, 2001). To substitute the technological effect of gluten, hydroxypropyl methycellose (HPMC), locust bean gum, guar gum, carrageenan, xanthan gum and agar were used to improve rice bread (Anton & Artfield, 2008). Due to its functional characteristics of its fibre, rice bran has been used to develop fortified breads (Sairam, Krishna, & Urooj, 2011). β-type hemicellulose of rice bran, commercially called 'Fibrex®', contributes to soft-texture of bread by binding fat and water (Hu, Huang, Cao, & Ma, 2009; Rahaie et al., 2014).

Pseudocereals

Pseudocereals are defined as those seeds, which resemble true cereals in function and composition. The most common pseudocereals, amaranth, quinoa and buckwheat are not true cereals in botanical terms, but are dicotyledonous plants in contrast to most cereals which are monocotyledonous (wheat, rice, barley).

An increasing trend in research is focusing on the use of pseudocereals in the formulation of high quality, healthy gluten-free bread (Alvarez-Jubete, Arendt, & Gallagher, 2010). Acceptable breads with amaranth and quinoa flour have been produced to improve nutritional quality with higher levels of protein, fibre and minerals (Alencar, Steel, Alvim, de Morais, & Bolini, 2015). The volume of gluten-free bread made from quinoa white flour increased by 33% compared to rice/maize flour and the foam properties also improved (Elgeti et al., 2014). Gluten-free bread with low calories and high antioxidant activity was produced using chia seed and buckwheat (Costantini et al., 2014). Pseudocereal breads are characterised by a softer crumb texture with specific emulsifier that obtained the same sensory acceptability in comparison with the rice and potato starch-based gluten-free bread (Alvarez-Jubete, Auty, Arendt, & Gallagher, 2010). However, acceptability of pseudocereal breads by customers is still low (Del Castillo, Lescano, & Armada, 2009).

Chestnut

Chestnut (*Castanea* spp.) flour contains high quality proteins with essential amino acids (4-7%), adequate amount of sugar (13.9-32.6%), starch (50-60%), dietary fibre (4-10%), low amount of fat (2-4%), vitamins E and B group and essential elements such as potassium, phosphorous and magnesium (Demirkesen et al., 2010b). The flour can be used in gluten-free breads with good nutritional quality and health benefits since most of the gluten-free products do not contain sufficient amounts of vitamin B, iron, folate, and dietary fibre (Moroni, Bello, & Arendt, 2009; Durazz et al., 2013). However, the flour suffers from low volume and unacceptable dark colour when used in bread-making. To alleviate these defects, small portions of chestnut flour may be mixed with other types of flours (Rahaie et al., 2014). Addition of chestnut flour into gluten-free rice bread baked in conventional or infra-red-microwave combination ovens delayed the staling process of bread (Demirkesen et al., 2014).

Tapioca

Tapioca *(Manihot esculenta*) is also called yucca, mandioca, manioc and cassava has not been fully exploited for making bakery products, mainly due to high levels of carbohydrates and low protein content which contribute to poor dough characteristics (Shittu, Dixon, Awonorin, Sanni, & Maziya-Dixon, 2008). Induced malting using amylolytic enzymes and pregelatinization through hydrothermal cooking have been used to modify the textural and functional attributes of tapioca flour, which is then blended with various cereal and legume ingredients as well as rice bran and used for making baked products. Tapioca flour can replace wheat flour and is used by people with wheat allergies or coeliac disease (Eggleston, Omoaka, & Ihedioha, 1992).

Tapioca starch is extracted from the roots of the tapioca tuber. In some South American countries, modified tapioca starch is used for production of special kinds of popular gluten-free breads and biscuits (Mestres & Rouau, 1997). Bread made with modified tapioca starch stales at a lower rate than native starch (Miyazaki, Maeda, & Morita, 2005). Modified (UV irradiation) tapioca starch contributed to the volume of baked bread forming stable network structures (Vatanasuchart, Naivikul, Charoenrein, & Sriroth, 2005). Pregelatinized tapioca starch increased volume and softened the crumb texture of gluten-free bread with the addition of rice flours (Pongjaruvat, Methacanon, Seetapan, Fuongfuchat, & Gamonpilas, 2014).

Coconut

In many developing countries, such as Sri Lanka and The Philippines, coconut (*Cocos nucifera*) milk is used for culinary purposes while the residue, ‘copra’, is discarded. Copra can be defatted or directly processed into coconut flour (Taheri et al., 2010). Coconut flour contains about 60% carbohydrates, 22% protein, 11% fibre and 8% fat (Gunathilake, Yalegama, & Kumara, 2009). Pareyt, Finnie, Putseys, and Delcour (2011) reported the constituents of coconut fibre to be neutral detergent fibre (38%), acid detergent fibre (24%), hemicelluloses (14%), celluloses (10%) and dietary fibre (38%). Experiments on animals showed that high-fibre coconut flour in bakery foods increased faecal bulk and lowered the serum cholesterol of the animals and the glycemic index of foods (Trinidad et al., 2003). The presence of high lauric acid content in coconut flour was effective against mouth sores and

some oral infection due to its antimicrobial properties (Hornung, Amtmann, & Sauer, 1994; Taheri et al., 2010; Hristov et al., 2011).

Chastain, Sheen, Cooper, and Strength (1975) reported an organoleptically acceptable coconut bread product with high protein content. The glycemic index of bakery foods decreased with increasing level of coconut flour content (Gunathilake & Abeyrathne, 2008). Cupcakes, brownies, and maroons containing coconut flour had lower glycemic indices than the multigrain products (Trinidad et al., 2006). Replacement of wheat flour with coconut flour decreased volume and increased hardness of bread, thus, small portions of the flour (coconut) can be used in gluten-free bread (Tangkanakul, Tungtrakul, Vatanasuchart, Auttaviboonkul, & Niyomvit, 1995).

Legume flour

Food legumes are crops of the family *Leguminosae* also called *Fabacae*. They are mainly grown for their edible seeds, and thus are also named grain legumes. Among legumes, soybean, chickpea, pea, mung bean, small red bean, cowpea, kidney bean, and pigeon bean are the common types of food (Du, Jiang, Yu, & Jane, 2014). Legumes are good sources of slow release carbohydrates (dietary fibre) and are rich in proteins (18-25%) compared to wheat (Tharanathan & Mahadevamma, 2003). Combinations of legumes with cereal-based foods are of interest due to the presence of the amino acid lysine which tends to be deficient in dietary terms in the cereals (wheat) primarily used for breads (Angioloni & Collar, 2012).

Legume consumption has many beneficial physiological effects in preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer (Azevedo et al., 2003). The benefits may not be entirely associated to dietary fibre, but to phenolics and other non-nutritive compounds (Oomah, Tiger, Olson, & Balasubramanian, 2006). Polyphenols from legumes can act as antioxidants, hindering the formation of free radicals (Oomah, Cardador-Martínez, & Loarca-Piña, 2005; Fratianni et al., 2014). The general consensus on healthy eating habits favours an increase in the proportion of legume-based polymeric plant carbohydrates including starch. Besides that, legumes are low-energy density and are nutrient dense food, making them valuable sources of nutrients in undernourished or under-served people in developing countries (Rebello et al. , 2014).

Breads baked partly with legume flours have shown good physicochemical characteristics and adequate sensory profile (Geil & Anderson, 1994). Since the 1930s, soybean flour has been used in bread. Soybean flour contains a lipoxygenase system that contributes to dough development by the retention of gas (Wolf, 1970). Partially substituting wheat flour with soy protein isolate, oat bran and chickpea flour can be used to make bread with high protein, high fibre and low carbohydrate content (Dhinda, Prakash, & Dasappa, 2012). Utilisation of 5% and 10% lupin and soybean flour in replacement of wheat flour decreased bread loaf volume as the high protein component of legume flour diluted the gluten structure (Doxastakis, Zafiriadis, Irakli, Marlani, & Tananaki, 2002). White wheat bread fortified with chickpea and lentil flour had lower starch hydrolysis indices and good acceptability (Rizzello, Calasso, Campanella, Angelis, & Gobbetti, 2014). In wheat-chickpea bread, addition of chickpea flour increased the water absorption and dough development time, thus, the extensibility of dough and the resistance to deformation were reduced. The dough surface of the blend with 10% chickpea was classified as “normal” while “sticky” dough surface appeared with 20% and 30% chickpea flour (Mohammed, Ahmed, & Senge, 2012).

Coarse legume flours of navy beans, green lentils and pinto beans were more suitable in flatbread; the composite flours containing 25% legume flour produced products with better sensory profile (Borsuk, Arntfield, Lukow, Swallow, & Malcolmson, 2012). Tortilla made from composite flours of small red, black, pinto, or navy beans and wheat produced acceptable textural properties and improved nutritional value compared to wheat flour tortilla (Anton, Ross, Lukow, Fulcher, & Arntfield, 2008). In another study, the formulation of flour tortillas with 25% pinto bean flour was acceptable to customers (Anton, Lukow, Fulcher, & Arntfield, 2009).

Large amounts of legumes incorporated into baked products are cost-effective and nutritionally advantageous, without any structuring agent, it is technologically very difficult to achieve. In substitution of wheat flour of bread development applications, the lack of gluten to achieve desirable viscoelastic properties in the dough restricts the incorporation of high levels of legume flour into wheat dough systems. Composites of legume-wheat-structuring agents have successfully developed highly nutritious breads with increasing dietary fibre fractions, lower and slower starch hydrolysis and reduced glycemic index. Moreover, viscoelastic properties of dough which gains gas retention and sensory acceptance of bread are achieved (Angioloni & Collar, 2012).

Gluten-free bread produced with extruded blend of 75% corn meal and 25% defatted soybean flour with addition of guar gum had the greatest volume, the best crumb, elasticity, softness and porosity (Ćurić et al., 2007). Gluten-free bread made with rice and tapioca flour can be improved by partially replacing with soybean flour; the protein solubility in soybean flour benefit air-retention and stabilisation in the batter (Ribotta et al., 2004). In addition, gluten-free bread with pea, lupin and soybean proteins decreased hardness and chewiness of the crumb. Soybean is the most widely used legume in food yet it is an allergic food (Mittag et al., 2004). In gluten-free bread, pea isolate and chickpea flour with corn starch produced good bread crumb and loaf volume which could be promising alternative to soybean (Miñarro, Albanell, Aguilar, Guamis, & Capellas, 2012). Among chickpea flour, pea isolate, carob germ flour or soybean flour, gluten-free bread made by chickpea produced the softest crumb, indicating that it could be a promising alternative to soybean (Miñarro et al., 2012).

Starch

Starch, one of the most important polysaccharides, is widely used in food industry (Zobel, 1988; Taggart, 2004). It is the basic carbohydrate in the human diet. Starch is made by two forms of molecules, amylose and amylopectin. Amylose has a lower molecular weight than amylopectin but forms a linear chain while amylopectin has branched chains. Both of them are based on α- (1→4)-D-glucose units while amylopectin is branched at the α- (1→6)-D-glucose units. Amylose contributes to gelling properties and is prone to crystallization called retrogradation while amylopectin could disperse in water and retrograde slower thus leading high viscosity of paste (Mua & Jackson, 1997; Blazek & Copeland, 2008). The ratio of two types depends on the origins of starch (MacMasters, 1964).

As the major component of flour, starch has a direct and important impact on flour properties (Cauvain & Young, 2009). Native starch is accumulated in granules as energy store in the endosperm of plants (Svihus, Uhlen, & Harstad, 2005). Size and shape of granules differ due to their plant origins as shown in Table 2. Starch granule size affects quality of bread (Mais, 2008). Flour within larger starch granules produces bread with a more open grained crumb with larger gas cells (Hayman, Sipes, Hoseney, & Faubion, 1998).

Table 2 Characteristics of starch granules from typical plants

Starch	Gelatinization temperature range (°C)	Granule shape	Granule size (μm)
Wheat	58-64	Round or lenticular	20-35
Maize	62-72	Round or polyhedral	15
Sorghum	68-78	Round	25
Rice	68-78	Polygonal	3-8
Tapioca	59-65	Round or polyhedral	20
Potato	57-65	Oval	100

Source: Hines, 2007.

In baked goods, gelatinization of starch is vital to build structure and texture. When temperature rises, the primary function of starch is absorbing water and swelling, especially during baking. The process of water absorption by starch, and the input of heat results in gelatinization. Gelatinization during baking plays an important role in the formation of product structure, and together with the denaturing of protein forms an extensible matrix, which contributes to carbon dioxide retention, assists with expansion of the growing bubble, prevents coalescence with neighbouring bubbles during growth and stabilises final structure on cooling (Houben et al., 2012). During storage, reorganization of the gelatinized starch structure occurs, firming the bread crumb. This process, typically accepted as retrogradation or staling, can take place irrespective of moisture loss from the bread. Staling caused by retrogradation remains a challenge to the baking industry (Gray & Bemiller, 2003).

For gluten-free bread, starch plays an especially vital role in the structure and texture due to elimination of gluten. The most important starches used in gluten-free bakery products are extracted from potatoes, wheat, maize, rice and tapioca (Taggart, 2004). The properties of starch used in formulation largely influence the characteristics of final and intermediate products. Starch influences microstructure, rheology of the dough, water retention and final structure and quality of the products (Abebe, Collar, & Ronda, 2015). The comparable properties of starch are referred as water swelling and solubility, granule size, pasting and gelling, rheological properties of starch solution, ability of amylose to form a composite mixture with fats and emulsifiers (Witczak, Juszczak, Ziobro, & Korus, 2012; de la Hera, Rosell, & Gomez, 2014). Gluten-free bread prepared from starch-based formulation (17.2% rice flour, 74.2% maize starch, and 8.6% tapioca starch) had good acceptable sensory scores and crumb-grain score (Sanchez, Osella, & Torre, 2002). High levels of starch content from tapioca, maize, potato or rice starch used in gluten-free sorghum bread decreased crumb firmness and chewiness (Onyango, Mutungi, Unbehend, & Lindhauer, 2011). Among the four

types of starch, 50% tapioca starch in gluten-free sorghum bread contributed to the best overall texture.

Modified starch

Apart from native starch, modified starch can also be used to improve the structure of gluten-free bread (Chiu & Solarek, 2009). It is a key starch in industry which is modified natural starch used for specific application (Luallen, 2004). Modified starch is chemically, physically or enzymatically modified to improve its functionality during normal processing conditions such as high heat treatment, storage, cooling and freezing (Jobling, 2004; Singh, Kaur, & McCarthy 2007).

The utilization of modified starches encourages faster food preparation, better control of viscosity and increases stability of crumb structure and retards retrogradation, thus slowing staling of bread (Witczak et al., 2012). Common modified starches used for making gluten-free bread are modified rice, maize and tapioca starch based on starch origin. According to modified method, pre-gelatinized, hydroxypropyl modified starch has been usually used in gluten-free factory due to its ability to form highly viscous slurries and pastes (Abdel-Aal, 2009).

Modified rice starches produced with low to high levels of starch hydrolysis have been applied on a replacement basis for wheat starch in gluten-free bread formulations (Gallagher, Polenghi, & Gormley, 2002, as cited in Gallagher et al., 2004). Pre-gelatinized starches are able to form a matrix in which gas and air bubbles are entrapped, which are a major structural component in bread (Purhagen, Sjöö, & Eliasson, 2012). Hydroxypropyl modified starch has the best properties to retard bread staling, which is associated with slow retrogradation of amylopectin. Utilization of hydroxypropyl distarch phosphate (HDP) deviated from high amylose maize starch in gluten-free bread contributed to the increased bread volume, caused by changes in structure while the properties of textural parameters were similar to those of bread with high amylose starch (Witczak et al., 2012).

Resistant starch

Resistant starch has been defined as the sum of starch degradation not absorbed in the small intestine of healthy individuals (Champ, 2004). It is considered the third type of dietary fibre. Resistant starch has a positive effect on the functioning of the digestive tract, microbial flora, blood cholesterol level, glycemic index and helps to control diabetes (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). It also has improved textural properties of bread. Bread containing 40% resistant starch (high amylose maize starch) had greater loaf and better cell structure compared with traditional fibres (Baghurst, Baghurst, & Record, 1996).

Resistant starch (RS) has been categorized into for four types comprising RS1 to RS4. RS1 is found in starchy foods, which are not fractionated and refined, and mostly found in pulses and cereals. It is not physically accessible by enzymes. RS2 types are native resistant starch granules, generally starches, such as unripe banana, potatoes and high amylose starches (mostly high amylose maize starch, Hi-Maize) which cannot be digested by enzymes. RS3 types are retrograded starches formed during storage of starch gels and are more or less resistant to enzyme hydrolysis (Haralampu, 2000). RS4 types are chemically modified starches, typically those which have been etherized, esterified or cross-bonded with chemicals to decrease their digestibility in the small intestine.

Hi-Maize resistant starch is a natural, unmodified high amylose maize starch (Englyst, Wiggins, & Cummings, 1982; Homayouni et al., 2014). When Hi-Maize starch is added in bread doughs, it exhibited increased gelatinization temperatures, which stabilised the structure of bread (Houben et al., 2012; Tsatsaragkou, Gounaropoulos, & Mandala, 2014). Hi-Maize and tapioca resistant starch have been used to partially replace starch in gluten-free bread formulations, resulting in increased shelf-life of bread and reduction in crumb hardness of loaf bread (Korus et al., 2009). Incorporation of Hi-Maize in gluten-free rice bread increased the elasticity of bread crumb (Tsatsaragkou et al., 2014).

2.4.3 Hydrocolloids

Hydrocolloids (or gums), are a group of high molecular weight polymers, widely used in food technology (Burey, Bhandari, Howes, & Gidley, 2008). In the food industry, hydrocolloids are multifunctional additives that add flexibility, functioning as fat replacers, water binders, texturizers and adhesives to modify rheology (in the form of thickening and gelling) and water-binding as well as emulsion stabilisation, prevention of ice recrystallization and enhancement of organoleptic properties (Dickinson, 2009; Saha & Bhattacharya, 2010).

In bakery products, hydrocolloids assist with improvement of food texture and moisture retention, reducing starch retrogradation, and thus increase the overall quality of the end-products during storage (Kohajdová & Karovičová, 2009). Recently, hydrocolloids have been focused on application as fat replacers to produce low calorie bakery products and also replace gluten to fortify gluten-free breads due to their polymeric structure (Anton & Artfield, 2008). Most gluten-free breads formulation or recipes contain hydrocolloids as well as non-wheat or non-gluten containing flours and starches that may form dough exhibiting poor viscoelastic and gas retaining properties (Velázquez et al., 2012). Hydrocolloids as gluten replacer aid the formation of elastic dough and stabilize air cells in bread-making to enhance baking performance of starches or starchy flours. The interactions between gums and starches may improve rheological and textural properties contributing to enhanced product acceptability and stability (Gallagher, 2009).

Cellulose derivatives

Cellulose is a polysaccharide consisting of a linear chain of several hundred to many thousands of β-(1→4) linked D-glucose units (Blackwell, Vasko, & Koenig, 1970). It origins from most land plants and is utilized in various ways. It is modified in different ways to utilize its derivatives for diverse applications (Ioelovich, 2008). Commonly used derivatives comprise carboxymethyl cellulose (CMC) and hydroxypropyl methylcellulose (HPMC) which are obtained by chemical modification of cellulose. HPMC is generated by addition of methyl and hydroxypropyl groups to the cellulose linear chain. CMC is a cellulose derivative with carboxymethyl groups (-CH_2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone.

HPMC has high surface activity, forms thermo-reversible gel networks on heating and exhibits lower variability regarding its hydration–dehydration properties during variable temperatures (Bárcenas & Rosell, 2005). In bread-making, HPMC acts as an emulsifier and strengthens the crumb (Guarda, Rosell, Benedito, & Galotto, 2004). HPMC improves bread quality including increasing loaf volume, moisture content, improving texture of crumb, and sensory properties (Kohajdová & Karovičová, 2009). In addition, HPMC acts as a good anti-staling agent, retarding staling of the crumb and retrogradation of amylopectin (Guarda et al., 2004). In gluten-free bread, HPMC can be used as a binding agent and gluten substitutes; it is suitable in rice bread-making (Kang, Choi, & Choi, 1997). The positive effects of HPMC on rheological properties of rice dough and rice bread have shown potential prospects in gluten-free bread market (Sivaramakrishnan, Senge, & Chattopadhyay, 2004).

CMC is used to maintain moisture, improve mouth-feel, rheological properties of dough and structural consistency of bakery goods. Addition of CMC (1%) increased volumes, crumb porosity and elasticity in gluten-free breads, without changing crumb firmness (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). CMC is also used as a combination agent with other stabilizers and hydrocolloids because of its high water-absorbing ability, such as pectin or locust bean gum (Gimeno, Moraru, & Kokini, 2004; Kohajdová & Karovičová, 2009). Gluten-free bread with better quality was produced when CMC (0.8%) was combined with HPMC (3.3%) added to rice flours (Cato, Gan, & Small, 2002, as cited in Gallagher et al., 2004).

Guar gum

Guar gum is a galactomannan derived from the seed of a bean plant *Cyamopsis tetragonolobus* (Chudzikowski, 1971). It has been widely used as a food additive due to its high viscosity of its aqueous solutions even at low concentrations (Miyazawa & Funazukuri, 2006). In baked products, guar gum is used to improve mouth feel, change their rheological properties and enhance the shelf-life through moisture retention (Keskin, Sumnu, & Sahin, 2007; Kohajdová & Karovicova, 2008; Ghodke, 2009). In addition, results of some human studies indicated that guar-containing bread was more effective in improving glycemic control (Ellis, Apling, Leeds, & Bolster, 1981).

Utilization of guar gum in pinto bean-wheat flour tortilla had a positive influence on water holding capacity and texture profiles under storage (Anton et al., 2008). Compared to HPMC, CMC and carrageenan, fresh and stored wheat chapatti with guar gum had higher extensibility and sensory acceptability (Shalini & Laxmi, 2007).

Production of gluten-free bread based on rice combination with guar gum increased volume with lower crumb hardness (Galle et al., 2012). Gluten-free French style bread based on buckwheat and rice flour with guar gum addition has been successfully developed which had the most heterogeneous cell size distribution compared to CMC, HPMC or xanthan gum (Mezaize, Chevallier, Le Bail, & De Lamballerie, 2009). Meanwhile, Gluten-free loaf bread with guar gum had better quality compared to addition of pectin; bread with guar gum manifested in higher loaf volume, lower baking weight loss and better water retention (Gambuś, Nowotna, Ziobro, Gumul, & Sikora, 2001).

Xanthan gum

Xanthan gum produced by *Xanthomonas campestris,* is an anionic natural polysaccharide, with a primary structure consisting of repeated pentasaccharide units formed by two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.8:2.0:2.0 (García-Ochoa, Santos, Casas, & Gomez, 2000). Xanthan gum has its specific property on the interactions with plant galactomannans such as locust bean gum or guar gum. Addition of galactomannans to a solution of xanthan gum leads a synergistic increase in viscosity (Casas & García-Ochoa, 1999; Casas, Mohedano, & García-Ochoa, 2000). It is a major commercial microbial polysaccharide, with over 20000 tons xanthan gum produced every year (Khan, Park, & Kwon, 2007).

Xanthan gum in bakery products improves the cohesion of starch granules, assists with the structure and retention of CO_2, increases volume and retards staling during storage by retaining moisture (Katzbauer, 1998). Gluten-free bread with xanthan gum made from rice, corn and soybean flours had larger bread volume, lower crumb firmness and staling rate compared to the use of carrageenan, alginate, xanthan gum, CMC or gelatine (Sciarini, Ribotta, León, & Pérez, 2010). The formulation of gluten-free breads based on potato starch, tomato starch, and maize flour revealed that bread with xanthan gum had higher volume in

comparison to bread with pectin-guar mixture; besides when the amount of xanthan gum was increased, crumb hardness decreased during baking and storage (Gambuś, Sikora, & Ziobro, 2007). Gluten-free tortilla made from sorghum flour with xanthan gum had acceptable sensory attributes (Winger, Khouryieh, Aramouni, & Herald, 2014). Xanthan gum with composite flours made from 45% rice flour, 35% corn starch and 20% tapioca starch were used to make gluten-free bread; the gluten-free bread had acceptable flavour and bread crumb which was well-distributed with cells (López, Pereira, & Junqueira, 2004).

Carrageenan

Carrageenan is a linear sulphated water-soluble galactans extracted from red seaweeds (De Ruiter & Rudolph, 1997). Due to its gelling, thickening, and stabilising properties, carrageenan is commonly used in the food industry. Carrageenan as bakery additive can improve the specific volume of bread, and increase moisture content of end-product while reducing water activity (León et al., 2000). In a gluten-free bread study by Shambat (2011), carrageenan had more significant positive effects on specific volume of gluten-free bread made by rice flour, tapioca starch and soybean flour compared to xanthan gum, CMC or alginate.

Alginate

Alginate (alginic acid) is a linear polymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate and its C-5 epimer α-L-guluronate residues, respectively, covalently linked together in different sequences or blocks (Augst, Kong, & Mooney, 2006). Currently, commercial sources of alginate are brown seaweeds such as *Laminaria digitata*, *L. hyperborea*, *Ascophyllium nodosum* and *Fucus serratus* (Mabeau & Fleurence, 1993). Alginates exhibit different properties to other seaweeds. The ability of alginate to entrap water, form gels, and to form and stabilize emulsions has led to many food and industrial applications (Kohajdová & Karovičová, 2009). Alginate shows a positive impact on shelf life and moisture retention in bread-making (Brownlee et al., 2005). Alginate delays staling, inhibits crumb hardening and reduces loss of moisture content of bread during storage (Guarda et al., 2004). Fortification of alginate in gluten-free bread has not been however

widely reported.

Locust bean gum

Locust bean gum, also called carob gum, is a galactomannan polysaccharide extracted from the seeds of carob tree *(Ceratonia siliqua* L.) after the removal of testa (seed coat) (Doublier & Launay, 1981). The hydrocolloid adds viscosity to the dough, eventually improving final product texture and yields (Kohajdová & Karovičová, 2009). In addition, it is effective in decreasing casein digestibility, which may be applied in dietary treatment of diabetics (Lamghari El Kossori et al., 2000; Mandala, Karabela, & Kostaropoulos, 2007). With addition of locust bean gum into bread, bread loaves were increased which encouraged the development of gluten-free bread (Schwarzlaff, Johnson, Barbeau, & Duncan, 1996). Locust bean gum has been used to make acceptable gluten-free rice bread (Kang et al., 1997).

Effects of mixed hydrocolloids on bread

Combinations of different hydrocolloids in formulation of bakery products may act synergically to increase functionality such as viscosity which contributes to the stabilisation by preventing settling, phase separation, foam collapse and crystallization (Marcotte, Hoshahili, & Ramaswamy, 2001). Mixtures of HPMC and CMC , as gluten replacers, produced better bread characteristics than guar gum in wheat-rice flour formulations (Gan, Rafael, Cato, & Small, 2001, as cited in Gallagher et al., 2004). Mixed addition of xanthan gum and guar gum can delay staling in gluten-free rice cake (Sumnu, Koksel, Sahin, Basman, & Meda, 2010). Mixed hydrocolloids have been applied as gluten replacements to enhance quality of gluten-free bread. Incorporation of guar gum in bread formulation with pectin behaved better than using a single hydrocolloid. Bread with guar gum and pectin improved textural features, reduced gumminess, chewiness and crispness (Gambuś et.al, 2001). A blend of xanthan gum and guar gum improved the structure and texture of gluten-free rice bread better than using each single hydrocolloid (Demirkesen, Mert, Sumnu, & Sahin, 2010a).

2.4.4 Other Ingredients

Shortening

In bakery products, added oils and fats are generally described as shortening as they tenderize the texture of the breads or cakes (Smith & Johansson, 2004). Shortenings contribute to lubrication, incorporation of air, and transformation of heat. Fatty acid chain lengths, degree of unsaturation, dominant polymorphic form, source and these fatty acid species be can classified shortenings. Shortening is the first ingredient affected by oven heat and the solid components of shortening turns into liquid due to their low melting temperatures (Mondal & Datta, 2008). As shortening melts, it coats proteins and the starch granules, thus preventing the structure becoming rigid and therefore contributes to the tenderness of the bread. Addition of shortening also stabilises of gas bubbles in bread dough. During kneading, the shortening crystals absorb at the interface of the gas bubbles inside the dough, and during baking, they melt and expand without destruction. Solid fats come from both animal and plant sources and are usually solid at room temperature. Oils are produced mainly from plants and are liquid at room temperature. The most common oils are extracted from seeds (safflower, sunflower, sesame, canola, flaxseed), beans (soybean), grains (corn, wheat germ), fruits (avocado, olive), and nuts (almond, coconut, walnut, palm kernel) (de la Hera et al., 2014). The fatty acids in the solid fraction are generally more saturated than those in the liquid fraction. Polyunsaturated fatty acids contribute to the development of oxidative rancidity which should be avoided in shortenings that are especially exposed to high temperatures and air, and for products that need a long shelf life (Not et al., 2001).

Butter, the most common solid shortening in bakery is made from milk fat and remains the industrial favourite despite the general move towards the use of vegetable-based fats (Chisholm et al., 1996). Despite its popularity with consumers, it is technically one of hardest fats for the baker to use. Firstly, butter is a natural material that suffers from natural variability. Secondly, the crystal form and solid-fat content profile of butter is not entirely compatible with the functional role of fat which is required in the manufacture of baked products. As butter is a solid dairy product made by churning fresh or fermented cream or milk, people who are allergenic to dairy food cannot consume it. Besides these factors, the low melting point drives bakers to look for alternative oils to replace butter (Deffense, 1993; Lipp & Anklam, 1998).

Coconut oil has excellent frying stability when isolated from other oils because of its high level (90%) of saturated fatty acids, and it is a popular frying medium for Mexican foods (Edwards, 2007). Epidemiological studies suggest that the consumption of high amounts of saturated fat and cholesterol can lead to high blood cholesterol (Vogel, Corretti, & Plotnick, 1997; Clark & Slavin, 2013). Therefore, coconut oil has received bad reputation. However, clinical studies conducted on coconut oil and virgin coconut oil has shown positive outcomes, which contradict these arguments (Nevin & Rajamohan, 2004; Nevin & Rajamohan, 2006). Virgin coconut oil is the emerging product with high demand. Various types of cold presses are used for the extraction of virgin coconut oil from the coconut kernel at low temperature (Gunathilake & Abeyrathne, 2008).

Coconut oil is rich in lauric acid, a fatty acid with strong antimicrobial property, which probably inhibits various pathogenic bacteria including *Listeria monocytogenes* (Houben et al., 2012). Meanwhile, coconut oil is nature's richest source of medium chain triglycerides (MCTs), which are resistant to oxidation (Rahilly-Tierney, Spiro, Vokonas, & Gaziano, 2011). The MCTs in coconut oil destroy microorganisms by disrupting their membranes, thus interfering with the assembly of genetic materials (Wang & Johnson, 1992). A study involving patients with Alzheimer's disease indicated that MCTs in coconut oil may be associated with the formation and functioning of synapses in the brain (Reger et al., 2004). Another study with supplementation of coconut oil showed beneficial effects on the biochemical and anthropometric profiles of women with abdominal obesity. The intake of dietary supplement with virgin coconut oil instead of other fats decreased the amount of abdominal fat (Assunçao, Ferreira, dos Santos, Cabral Jr, & Florêncio, 2009).

Salt

In the manufacture of bread, common table salt (sodium chloride) is used for a variety of purposes. Firstly, it is major contributor to product flavour (Homayouni et al., 2014). Besides, gluten protein hydration can be delayed by salt at the dough-forming stage, which results in the formation of more fibril gluten network (Husby et al., 2012; Sapone et al., 2012). Also, salt can increase the strength of dough prepared from low protein flour compared to those from high protein flour (Niewinski, 2008; Nijeboer et al., 2013).

Sugar

Sugar plays a significant role in structure formation by affecting the gelatinization temperature of starch (DiGiacomo, Tennyson, Green, & Demmer, 2013). Addition of sugar can increase the volume of bread (Singh & MacRitchie, 2001). Sucrose in different forms is widely used in baked products. In fermented bread, the addition of low sucrose increases gas-producing ability of baker's yeast (Thomas, 2013). Dextrose and glucose syrups play similar roles to that of sucrose in imparting sweetness and colour to baked products, but less sweet than sucrose. Glucose and other non-sucrose syrups often lead to excessive browning of products and, therefore their levels in many baked products are much lower than those commonly used with sucrose (Schober et al., 2005).

Emulsifier

Emulsions are two-phased systems in baking, usually hydrophilic and hydrophobic in which one phase (dispersed) is suspended as small droplets in the second phase (continuous) (Edwards, 2007). Ingredients that enhance stability in emulsions are known as emulsifiers, which work by providing a bridge between two phases. Doughs are complex emulsions and various emulsifiers are used successfully to help oil, and more importantly air dispersions and their stability during all stages of baking process. In addition to potential interactions with liquids, gases and oils, emulsifiers still play a role in starch-complexing (anti-staling) and interact with proteins. Egg yolk, mustard, soy and sunflower lecithin, sodium phosphates, sodium stearoyl lactylate (SSL), diacetyl tartaric acid ester of mono- and diglycerides (DATEM) are widely used food emulsifiers (Rousseau, 2000). Addition of emulsifiers is particularly important for large scale, industrial baking of bread as they impart greater dough strength to withstand machine handling, improve rate of hydration, crumb structure, slicing characteristic, gas holding capacity, reduce stalling and extend shelf-life (Stampfli & Nersten, 1995).

In yeast raised, chemically leavened and non-leavened baked goods, emulsifiers, which can be divided into synthetic, and natural has been increasingly used to improve the quality of bakery products. Chemical emulsifiers have the advantage of being tailor-made to meet specific functional needs. However, natural food is preferred by health-conscious consumers

(Siro et al., 2008). Lecithin is the most widely used natural emulsifier (DiGiacomo et al., 2013). It has well-known nutritional properties, both therapeutic as well as medicinal. According to Shalini and Laxmi (2007), the digestion of lecithin may have a positive effect on treating Alzheimer's disease and dementia. Lecithin comprises of a group of complex phospholipids found naturally in a wide range of animals and plants (Van Nieuwenhuyzen, 1981). Lecithin was and still is the only legal source of lipid permitted in traditional French baguette.

Water

Due to its unique properties, water plays many significant roles in baking, final product quality and product shelf-life (Korus et al., 2012). Water has key roles associated with solubilising and dispersion of ingredients during the mixing process and in the formation of complexes such as gluten in bread. During baking, the evaporation of water causes changes of other components in whole recipe matrices. In the final product, the water (moisture) content makes major contributions to eating quality and shelf-life (Smith & Johansson, 2004).

Following baking, the moisture content of crusts is lower than the crumb of bread. However, the moisture gradually migrates from higher area (crumb) to lower moisture content area (crust) causing the crust to lose its crispness and crumb softness resulting in staling. Water migration leads to inferior quality of bread and reduces the shelf life of bread (Rousseau, 2000). That leads to a firmer texture and a drier, harder characteristic of bread, which gives a chewy impression to consumers (Van Nieuwenhuyzen, 1981; Rousseau, 2000). The level of water used in baking needs to be optimised to achieve the required handling properties of the dough and final product characteristic.

Baking powder

Baking powder comprising sodium bicarbonate and a food-grade acid is used only in chemically leavened breads to provide a source of carbon dioxide gas (Stauffer, 2005). The level of baking acid is usually balanced to make a complete reaction with the sodium bicarbonate. This is commonly referred to as the neutralization value of the acid: the quality

of the baking acid required to release all of the available carbon dioxide from the sodium bicarbonate. Several different kinds of acidulants (organic acids) may be used in the manufacture of bread, ranging from reacting with sodium bicarbonate to yield carbon dioxide and aiding structure formation, to lowering pH, which assists against mould growth (Smith & Johansson, 2004; Chen & Opara, 2013).

Dietary fibre

Carbohydrates have usually comprised 50–80% on a dry weight basis of cereals as major ingredients of bread (Shelton & Lee, 2000). Carbohydrates can be classified into two broad categories: available and unavailable. Available carbohydrates are those digested and absorbed by humans, which include starch (non-resistant) and soluble sugars. In contrast, unavailable carbohydrates (dietary fibre) are not digested by the endogenous secretion of the human digestive tract (Southgate, 1991).

According to AACC (2011), dietary fibre is the edible part of plants or analogous carbohydrates that is resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine (Dhingra et al., 2012). Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances (Bonn, 2005). Oats, rice, soya, apple, tomato, legumes and psyllium are good sources of dietary fibre.

Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, blood glucose attenuation and increase satiety (Clark & Slavin, 2013). It has been recommended to consume dietary fibre as daily by the American Heart Association, Institute of Medicine and United States Department of Agriculture (Yon, & Johnson, 2005; McGuire, 2011; King et al., 2012; Eckel et al., 2014). Cereals, such as wheat, are rich in insoluble dietary fibre, which increases faecal weight, bulk and softness, frequency of defecation and reduces intestinal transit times (Muralikrishna & Subba Rao, 2007). These effects probably play a role in preventing colon cancer and other bowel disorders. Soluble fibres of cereals such as oats (3–4%) and barley (4–5%) slow down glucose absorption, reduce plasma cholesterol concentrations and are useful in the management of diabetes as well as heart diseases (Plaami, 1997).

Psyllium (seed of plant), besides being an excellent source of natural soluble fibre, has been widely recognized for its cholesterol-lowering effect and ability to improve insulin sensitivity (Yu, Perret, Parker, & Allen, 2003). In addition to its beneficial health properties, dietary fibre has pronounced effects on dough properties of bread. It assists with water absorption, mixing tolerance, tenacity of dough (Gómez, Ronda, Blanco, Caballero, & Apesteguía, 2003). With dietary fibre, the viscous and elastic moduli of dough increases, and the dough becomes stiffer in some cases. Dietary fibre can also affect loaf volume, increase hardness of crumb, change colour, surface properties and the density of bread (Elleuch et al., 2011).

As coeliac patients generally have lower intake of fibre attributed to their gluten-free diet, gluten-free bread with enrichment of dietary fibre is highly demanded (Shepherd & Gibson, 2013). A muffin with 10% psyllium husk received more sensory acceptance (Bhise, 2015). When psyllium was added to gluten-free bread, it leaded to a softer crumb during storage and it could replace gluten in some products (Mariotti, Lucisano, Ambrogina Pagani, & Ng, 2009; Zandonadi, Botelho, & Araújo, 2009). In addition, the effect of psyllium and sugar beet fibre on gluten-free dough and bread has been studied. Psyllium and sugar beet fibres can both improve the dough working ability. Meanwhile, psyllium contributes to bread making due to its anti-staling effect on bread because of its water binding ability (Cappa, Lucisano, & Mariotti, 2013).

2.5 Technology and science of making flatbread

The present study focuses on the development of a gluten-free wrap bread. In essence, gluten-free wrap bread is flatbread but it is not identical to any existing categories of bread. Review of literature reveals that there is no specific published data for making gluten-free wrap bread. Therefore, general knowledge of making bread and flatbread are instead reviewed (Qaroon, 1996; Mandala & Kapsokefalou, 2011; Al-Dmoor, 2012).

The bakery industry has undergone a revolution over the past 150 years. The small artisan bakeries, which were present in every village, have made way for a high technology bakery industry. Industrial mono-production took over from the small bakeries as bread could be produced more efficiently and cheaper. Different baking technologies have been developed to respond better to new market demands (Decock & Cappelle, 2005).

2.5.1 Mixing

Mixing, as the first significant step in the manufacture of any bread, it blends the ingredients into a quasi-homogeneous mixture to develop a three-dimensional matrix in dough, thereby entrapping air (Autio & Laurikainen, 1997). Mixing also acts as a dough development process. During mixing, water, flour and other ingredients are transformed into a viscoelastic dough. Several changes occur in the dough, beginning with solubilisation, hydration and redistribution of ingredients and their components. Starch and proteins are unevenly distributed if dough is not well-mixed, and compact protein masses are stretched out into sheets (Autio & Laurikainen, 1997). Over-mixed dough can become sticky. A sticky dough usually forms when mechanical forces applied to the dough decrease the molecular weight of the protein resulting in the reduction of extensibility (Autio & Laurikainen, 1997). In a multigrain mixed dough (containing wheat), dough development time is increased. The presence of grains or flours other than wheat delays the hydration and development of gluten (Indrani, Soumya, Rajiv, & Venkateswara Rao, 2010).

Three mixing methods are commonly used in bakery products. The simplest method is single-stage process called ‘straight-dough method’ where mixing of ingredients is performed in one step. A minimum of one hour resting periods in the ‘straight-dough method’ is required after mixing and before dividing. It is the most traditional and natural method (Corke, De Leyn, Nip, Cross, & Hui, 2008). The second method, the ‘sponge and dough method’, involves mixing ingredients in two steps. Yeast, water and flour are mixed during the first step. The mixture is left for several hours and then the rest of the ingredients are added. The last method is known as the Chorleywood Process, and is widely used in the United Kingdom and Australia. In the Chorleywood Process, all the ingredients are blended in a high speed mixer for 2-5 min to form dough, which is removed and directly placed into a divider (Giannou, Kessoglou, & Tzia, 2003). The Chorleywood Process is advantageous as it can be used with lower quality flour and produces higher yields of bread (Buchanan, & Nicholas, 1980). However, the Chorleywood Process is associated with flavour reduction of bread.

2.5.2 Dividing and moulding

After mixing, the dough is divided into smaller pieces of certain weight as a unit and the pieces are moulded into the final expected shapes for further processing. Dividing helps with weight control of end product to meet specified national standard. For some breads, dividing and moulding also modifies the structure of gas cells as they induce coalesce of small cells into larger ones and contribute to the final development of the matrix network of the dough (Chakrabarti-Bell, Bergström, Lindskog, & Sridhar, 2010). For flatbread, the shaping process is commonly called sheeting, which is the most important step as it affects product quality. The dough of flatbread is shaped to flat, round or oval pieces. The modified shape of the sheeted dough pieces aims to achieve the desired configuration, further expansion of dough units and fixing the final bread structure. For double-layer flatbreads, the thickness of sheeted dough units determines the separation and evenness of layers (Rubenthaler & Faridi, 1982).

2.5.3 Proofing and retarding

Proofing is the final step for dough expansion before baking which refers to a specific rest period. When the recipe of bakery products consists of yeast, this process is commonly known as fermentation. In the fermentation step, yeast leavens the dough and makes the dough rise. The rest period provides proper temperature and humidity environment for yeast to grow and generate CO_2 (Cauvain, 2015). To allow the dough to maximise relaxing, the relative humidity and temperature must be controlled. In general, humidity at 65-80% is considered sufficient to prevent either drying of skin formation or water condensation on the surface of dough units. The temperature of proofing varies according to the type of product, ranging from 25-45°C. Longer proofing time is required when the proofing temperature is low (Qarooni, 1996).

Retarding involves holding the dough at relatively low temperatures, typically around refrigeration temperature (4-5°C), to decelerate the fermentation process. The activity of the yeast during this period is reduced while the enzymatic activity of flour amylase is further diminished. Retarding can inhibit dough to become too sticky to handle by reducing the action of amylase on starch breakdown (Edwards, 2007).

2.5.4 Baking

Baking is the last stage of the bread-making process. A complex series of physical, chemical and biological changes including evaporation of water, formation of porous structure, volume expansion, protein denaturation, starch gelatinization and crust formation take place. These changes are the result of the action of heat, either by convection, conduction or radiation, or a combination of these factors (Figoni, 2008; Mondal & Datta, 2008). The baking step converts the dough into an edible baked product with excellent organoleptic and nutritive properties called bread (Therdthai & Zhou, 2002).

At this stage of baking, it is important to control the baking temperature and baking time, which depends on the type of oven used, the size of dough pieces and the kind of bread desired (formulation used). The dough is transformed into the final baked product by firming, stabilisation of the structure and generation of typical aroma substances and colour. For flatbread, the baking time is short, only a few minutes at high temperature.

Depending on the temperature, three phases are classified in the baking process: 1. oven spring (enzyme active zone) (30 to 60/70°C); 2. gelatinization of starch (55 – 60°C) to no higher than 90°C; 3. browning and aroma formation above 100°C (Quail, McMaster, Tomlinson, & Wootton, 1990). Water is lost during the three stages of bread-baking.

In yeast-leavening and chemical-leavening bread, the last expansion occurs at the beginning of baking, called oven-spring. A sudden increase in the volume of dough during the first several minutes of baking occurs due to increased rate of fermentation and expansion of gases in the bread. The final expansion comes from expansion of the carbon dioxide and expansion of air and water converting to water vapour (Figoni, 2008). The expansion of dough is the same in chemically leavened bread. Chemical leavening agent determines the temperature and time of generation of carbon dioxide. Once the final expansion has taken place, and the temperature continues to rise, the proteins start to denature and the starch gelatinizes. Proteins at the surface of the bread (crust) undergo Maillard reactions and the typical fresh baked flavour develops. The temperature of the crumb does not exceed the boiling point of water (100°C) while the temperature of the crust can reach approximately 205°C when the oven remains at a constant temperature zone of 220 – 240°C (Lai & Lin, 2006). The compounds produced by Maillard reaction benefits bakery products by improving their flavour, colour

and texture (García-Baños, Villamiel, Olano, & Rada-Mendoza, 2004; Mottram, 2007).

2.5.5 Cooling and packaging

After baking, bread is cooled to stop the cooking process before packaging. Cooling helps to prevent condensation occurring with wrapping material and possible growth of mould spoilage (Edwards, 2007). Also cooling ensures that the correct moisture content is retained in the product, not only to maintain eating quality and also to minimise weight loss if excessive moisture is lost. Cooling also helps to keep the finished bread in a stable condition for further processing (He & Hoseney, 1990).

Packaging is vital in extending the shelf-life of foods and reduces the risk of microbial growth. Any packaging material must minimize the loss of moisture by providing an effective and functional barrier (Giannou et al., 2003). The package must be stable during transportation and storage. Diverse types of materials and technology are used to store bread for longer shelf life such as modern antimicrobial materials and modified atmosphere packaging (Appendini & Hotchkiss, 2002; Kotsianis, Giannou, & Tzia, 2002).

2.6 Characteristics and analysis of bread

2.6.1 Moisture content

Moisture content plays an important role in bread quality, particularly to eating characteristics (Maleki et al., 1980). Generally, higher moisture content maintains the softness in bread (He & Hoseney, 1990). Depending on product type, bread has specific softness, springiness and chewiness. Higher moisture content can lead to a decrease in firmness while increasing chewiness and springiness. The techniques used to evaluate moisture content of bakery products comprise of oven-drying and electrical methods. Oven-drying is based on removing water with heat, which is the most widely used method due to its simplicity and veracity. Moisture content can be determined by the method of oven air-drying or vacuum-drying. Nuclear magnetic resonance (NMR), near infrared (IR) and IR-direct heating are also good alternative electrical methods for determining moisture content (Cauvain & Young, 2009).

2.6.2 Colour

Colour is one of the three main features that consumers consider to make their assessment of bread quality; the other two are texture and flavour. Esteller and Lannes (2008) reported that colour of bread is depended on physicochemical properties of dough (water content, pH, reducing sugars and amino acid content). Colour of bread is usually determined by standard charts or colorimeters. The theory for colorimeters uses three parameters: black to white, red to green, and yellow to blue. Measurement of these data helps to provide valuable information on how consumers view the attribute (colour). The existence of the relationship between colour of bread and moisture loss has been reported (Purlis & Salvadori, 2007).

2.6.3 Baking weight loss

Baking weight loss is moisture lost when dough forms into baked bread. The presence of water in baked bread impacts on the sensory properties (Smith & Johansson, 2004). High moisture loss during baking has negative effects on final product, resulting hard bread crumb and low product yield (Kotoki & Deka, 2010). Baking conditions such as baking temperature and baking time have significant effects on the loss (Mariotti, Pagani, & Lucisano, 2013).

2.6.4 Water activity

Water activity (a_w), or its equilibrium relative humidity (ERH) is a key characteristic for the shelf-life of bread (Troller & Christian, 1978). The level of water activity can be used as an indicator for potential growth of moulds. Bread containing high water activity spoils faster. Based on water activity, baked products can be divided into three groups. (a) low moisture baked products (a_w <0.6); (b) intermediate moisture products (a_w 0.6–0.85); (c) high moisture products (a_w 0.94–0.99) (Smith, Daifas, El-Khoury, Koukoutsis, & El-Khoury, 2004). The water activity of bread ranges from 0.80 to 0.98 (Forneck, Seger, Miklus, & Tangprasertchai, 2002; Hager et al., 2012; Troller, 2012). There is a relationship between water activity and ERH (ERH=a_w×100). Methods used to measure water activity are based on the assessment of ERH. The most common testing method for a_w is instrumental measurement using water activity meter, which is accurate, fast and easy to use (Rahman, 2007).

2.6.5 Growth of spoilage microorganisms

Microbiological spoilage is often the main factor restraining the shelf-life of bread. Bread, as high and intermediate moisture bakery product, provides ideal conditions for growth of microorganisms. Microbiological spoilage can be divided into bacteria, yeast and mould spoilage (Cauvain & Young, 2009).

The major bacterial problem in bread is "ropiness," caused by *Bacillus subtilis*, spore-forming bacteria. 'Ropey' bread releases a rotten fruit odour, and breadcrumb becomes discoloured and sticky, due to protein and starch degradation during growth of the bacteria (Pepe, Blaiotta, Moschetti, Greco, & Villani, 2003). *Pichia burtonii* (chalk mould) is the yeast that is mainly responsible for the spoilage of bread. The yeast forms a 'white patch", as growth spreads on surfaces of bread (Cauvain, 2015). Most moulds can grow on bread, of which $a_w \geq 0.8$. A study by Legan & Voysey (1991) reported that about 60% of spoilage of bakery products and their ingredients was attributed to growth of moulds. Apart from staling, mould growth on surface of bread is one of the biggest factors affecting the shelf-life (Legan, 1993; Latou, Mexis, Badeka, & Kontominas, 2010).

Table 3 Spoilage types for typical water activity (a_W) levels

Water activity	Spoilage types
0.90-0.99	Bacterial spoilage,e.g.'rope', mould growth and 'chalk moulds'
0.90-0.95	Mould and yeast, bacterial spoilage, e.g.'rope'
0.8-0.89	Moulds and yeast

Source: Cauvain & Young, 2009.

2.6.6 Texture

Texture is an important quality attribute used to assess food quality and acceptability (Bourne, 2002). It is also used to monitor product quality during transportation and storage. Textural characteristics of food can be evaluated by descriptive sensory or instrumental analyses, which are referred as subjective or objective methods, respectively. Descriptive sensory evaluation requires larger numbers of people and time (Stone, Bleibaum, & Thomas, 2012). Thus, the high expense, complexity, and strict requirements of sensory evaluation encourage the development of instrumental texture analysis. Application of texture

instruments to achieve valuable texture data is to complement consumer sensory evaluation (Zheng, Sun, & Zheng, 2006; Chen & Opara, 2013). A combination of subjective sensory and objective instrumental measurements can generate more credible results (Szczesniak, 1987). Correlation and cross-referencing can help instrumental analysis to provide more information about the product (Brady & Mayer, 1985; Gåmbaro et al., 2002). The decision for choosing any particular instrument and technology depends on cost and availability of equipment as well as product properties.

For bakery products, important texture characteristics are firmness (hardness), springiness, and crispness. During storage, firmness of bread crumb increases and springiness decreases due to starch retrogradation when moisture is transferred from starch to protein (Kim & d'Appolonia, 1977; Kulp, Ponte, & D'Appolonia, 1981). For bread crust, crispness decreases while toughness increases as moisture migrates from crumb to crust (Gray & Bemiller, 2003). In consideration of flatbread, such as tortilla, firmness and brittleness increase and rollability decreases during storage due to moisture loss to the air and retrogradation of starch (Bourne, 2002; Bejosano et al., 2005). The primary parameters used to describe bread texture are shown in Table 4.

Table 4 Relationship between texture parameters and popular nomenclatures

Primary parameters	Secondary parameters	Popular terms
Hardness		Soft firm hard
Cohesiveness	Brittleness	Crumbly crunchy brittle
	Chewiness	Tender chewy tough
	Gumminess	Short mealy pastry gummy
Elasticity		Plastic elastic
Adhesiveness		Sticky tacky gooey

Source: Szczesniak, 1963.

2.6.6.1 Evaluation of texture of flatbread using the objective method

Texture profile analysis (TPA)

The TPA was developed to imitate the conditions of food during chewing. The analysis mimics the chewing motion of teeth with two bites. The results are expressed by a force-time curve (Figure 1). Textural parameters (Table 5), which correlate with sensory evaluations, can be calculated (Meullenet, Lyon, Carpenter, & Lyon, 1998). TPA is widely used in fermented

loaf bread which can also predict the sensory texture (Gåmbaro et al., 2002; Wang, Rosell, & de Barber, 2002).

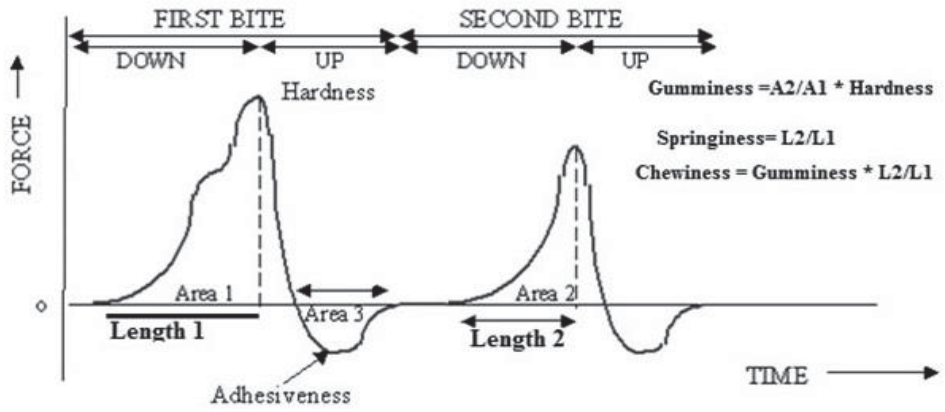

Figure 1 Typical curve of TPA

Source: Banjare, Kumar, Goel, & Uprit (2015).

Table 5 Descriptions of mechanical parameters of TPA

Mechanical parameter	Measured variable	Definition
Hardness	Force	The height of force peak on the first bite
Cohesiveness	Ratio	The ratio of positive force areas under the two bites
Springiness	Distance	The significant break in the curve on the first bite
Adhesiveness	Work	The negative force area of the first bite
Fracturability (brittleness)	Force	The force of significant on the first bite
Chewiness	Work	Hardness ×cohesiveness × springiness
Gumminess	Force	Hardness × cohesiveness

Source: Bourne, 2002.

Three-point bending test

The three-point bending test measures fracture and break strength of food (Hecke, Allaf, & Bouvier, 1995; Chen & Opara, 2013) (Figure 2). The test detects small differences in structure and mechanical resistance in the application of flatbread (Marzec & Lewicki, 2006). Rollability and flexibility were detected when the three-point bending test was used to measure the texture of tortilla. In studies on tortilla (Suhendro, Almeida-Dominguez, Rooney, Waniska, & Moreira, 1998; Chen & Opara, 2013), strips (tortilla) were bent to a 40°-angle and the force required to bend the strips of sample was used to analyse tortilla texture.

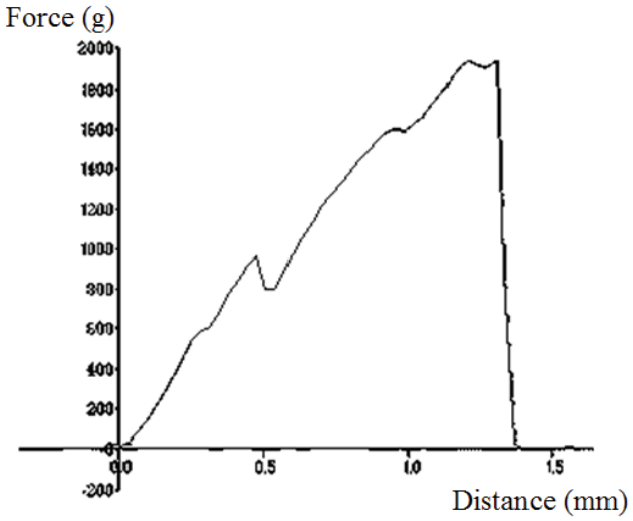

Figure 2 Typical curve of three-point bending test

Source: Retrieved from http://textureanalysisprofessionals.blogspot.co.nz/2015/04/texture-analysis-in-action-three-point.html

The two-dimensional test

In flatbread, the two-dimensional test is widely used to determine firmness and extensibility (Wang & Flores, 2000; Alviola, Waniska, & Rooney, 2008) (Figure 3). The texture analyser with tortilla/pastry burst rig (Figure 4), which is specially designed for analysing texture of thin sheet samples was widely used to analyse texture of flatbread (Gallagher, 2009; Forman & Evanson, 2010). The upper and lower plates are used to punch four holes to hold the sample in the plate. The sample is then extended and ruptured using a spherical probe. Firmness of tortilla made with bean flour was determined by a similar test with a TA.XT2 Texture Analyser equipped with a cylindrical probe (Anton et al., 2008). The rupture distance from two-dimensional extensibility test of tortilla correlated most strongly with subjective rollability ($r = 0.77$) which indicated texture parameter measurements had a potential to replace the subjective tests as primary methods for tortilla quality (Alviola & Awika, 2010). Strong correlations and factor analysis have shown changes occurring in flour tortillas during staling can be estimated better by subjective rollability, sensory evaluation, and 2-dimensional extensibility test than by other methods (bending, 1-dimensibility extensibility, puncture tests, and stress relaxation method) (Bejosano et al., 2005). The 2-dimensional extensibility test is useful to estimate sensory properties of flour tortillas.

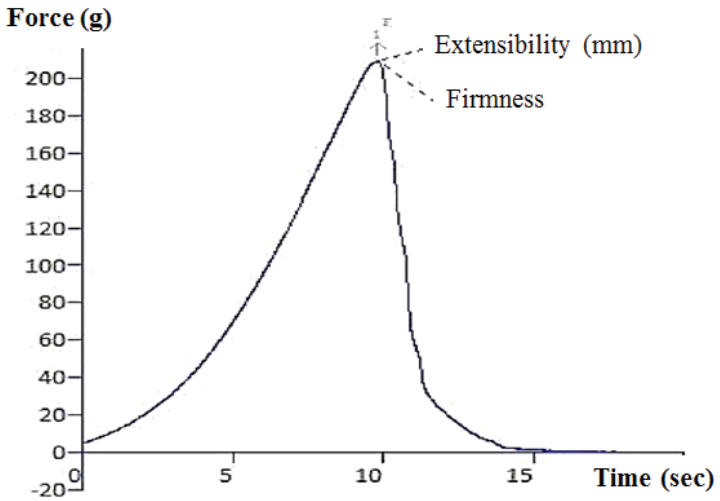

Figure 3 Typical curve of two-dimensional extensibility

Source: Retrieved from http://textureanalysisprofessionals.blogspot.co.nz/2015/03/texture-analysis-in-action.html

Figure 4 Tortilla/pastry burst rig

Source: Uthayakumaran and Lukow (2005).

2.6.6.2 Rollability test of flatbread using the subjective method

Rollability test is widely used to assess flatbread due to its simplicity and intuitiveness (Pascut, Kelekci, & Waniska, 2004; Abu-Ghoush et al., 2008; Alviola & Waniska, 2008; Cevoli, Gianotti, Troncoso, & Fabbri, 2015). Rollability reflects the way that flatbread and wrapped fillings are handled prior to consumption. Rollability by 'subjective method' describes the ability of flatbread to roll around a dowel and it evaluates the extent of breaking when flatbread is rolled (Friend, Waniska, & Rooney, 1993).

As the target of this study was to make wrap bread, high rollability of the products was of utmost importance and it was the most significant attribute used to determine the quality of GFW. However, rollability test is a subjective method to analyse rollability of flatbread. The analysis result of rollability test differs between testers (Akdogan, Tilley, & Chung, 2006). For consistency, rollability of flatbread should be tested by the same individual during shelf-life (Joseph, 1999; Pascut et al., 2004).

2.6.7 Sensory evaluation and consumer acceptance

Sensory evaluation comprises all the methods used to evoke, measure, analyse and interpret reactions to characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Meilgaard, Carr, & Civille, 2006). The test methods can be divided into three types according to purpose: discrimination tests, descriptive tests, and affective tests. In each type, there are several available methods. Selection of appropriate method used in sensory analysis is based on the properties of food and specific purposes of assessment (Brady & Mayer, 1985).

During product development, acceptance test is necessary to screen products under consumer insights. The liking and preference for a product can be measured using affective tests (Verbeke, 2005). Paired comparison and the 9-point hedonic rating scale are frequently used in affective tests. Paired comparison can determine the preference between two samples while the 9-point scaling method measures the extent of liking or acceptance (Lawless & Heymann, 2010).

The 9-point scaling method is probably the most used sensory method (Yeh et al., 1998; Stone et al., 2012). The affective method (9-point hedonic scaling) is easily understood by consumers and it has been demonstrated to be reliable and valid (Rosas-Nexticapa, Angulo, & O'mahony, 2005; Lim, 2011). The 9-point scale comprises nine verbal categories ranging from ‘dislike extremely’ to ‘like extremely’ (O'Mahony, 1986; Giménez et al., 2007). For subsequent quantitative and statistical analysis, the verbal categories are generally converted to numerical values: ‘like extremely’ as ‘9’, ‘dislike extremely’ as ‘1’ with respective intermediates. Using this scale provides more information about the product. Mean values, variance and distribution can be used to analyse the data. Statistical analysis, such as

ANOVA, can provide valuable information about product differences (Villanueva, Petenate, & Da Silva, 2000; Hein, Jaeger, Carr, & Delahunty, 2008; Lawless & Heymann, 2010).

Chapter 3 Materials and Methods

3.1 Introduction

In the manufacture of bread, baking conditions and formulations are vital to develop products with the correct physical, chemical and sensory qualities (Therdthai, Zhou, & Adamczak, 2002; Surdyk, Rosen, Andersson, & Åman, 2004; Shittu, Raji, & Sanni, 2007). This study aimed to determine suitable baking conditions (baking temperature and time) and formulations to produce gluten-free wrap bread.

Experiments for the development of GFWs were conducted in four phases. In phases 1 and 2, nine formulations were developed and each formulation consisted of a 2×2 completely randomized block design with two temperatures and two baking times. Baking information obtained in phases 1 and 2 of the project was optimised and then applied in phases 3 and 4. In phase 3, another nine formulations optimised using Taguchi method were developed (Roy, 2010; Khoshakhlagh, Hamdami, Shahedi, & Le-Bail, 2014). In phases 1, 2 and 3, physical characteristics of GFWs were analysed. Three promising formulations obtained from phase 3 were further evaluated in phase 4. In phase 4, consumer acceptance and physical characteristics of GFWs were investigated to select products with potential for further development.

3.2 Description of basic formulation and production of GFWs

3.2.1 Ingredients used in basic formulations

The ingredients in each formulation (Appendix A) used to make GFW in four phases were selected based on their functionality and nutritional properties as discussed in section 2.4. The ingredients were sourced and supplied by Venerdi Ltd (Auckland, New Zealand), a commercial gluten-free bread company. Basic GFW formulations of the four phases of the project (Table 6) were based on preliminary work conducted by Venerdi Ltd, Auckland (Tim Granger, Personal Communication, 12 May, 2015) and information from previous studies (Tangkanakul et al., 1995; Miyazaki et al., 2005; Miñarro et al., 2012; Winger et al., 2014).

Table 6 Basic formulation of GFW

Ingredients		% flour
Composite flour	Modified tapioca starch	60.00
	Hi-Maize starch	12.00
	Chickpea flour	8.00
	Coconut flour	17.00
	Psyllium	3.00
	Total	**100.00**
Lecithin		0.10
Salt		1.00
Baking powder		1.00
Rice syrup		4.00
Coconut oil		Variable
Guar gum		Variable
Xanthan gum		Variable
CMC		Variable
Water		123.30

Note: Details of ingredients listed in Appendix B

3.2.2 Production of GFW

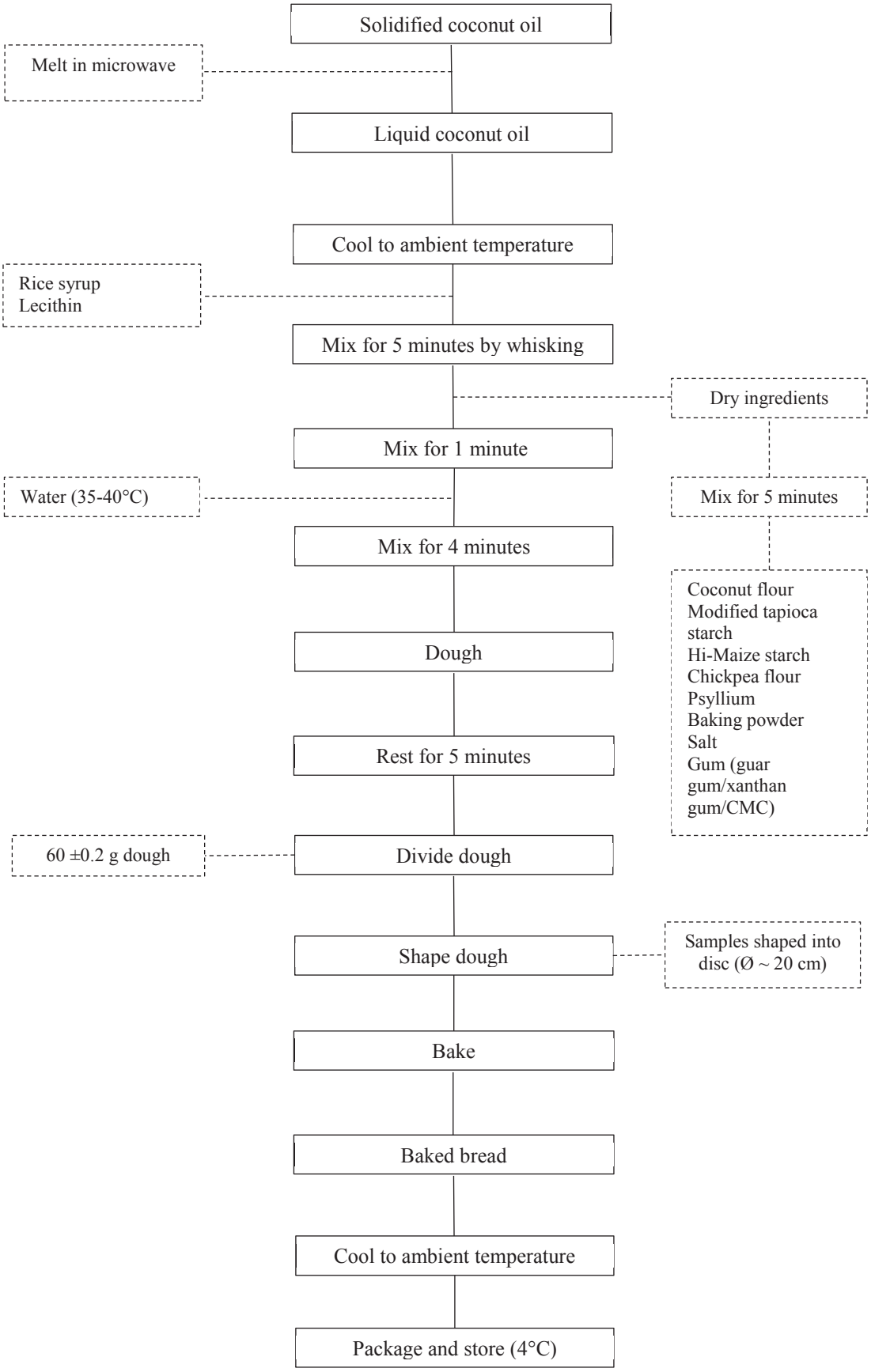

Figure 5 Laboratory scale production of GFW

Dough mixing

The preparation of gluten-free wrap bread (Figure 5) was based on the method by Mohammadi et al.(2014) with minor modifications and information supplied by Vernedi Ltd (Tim Granger, Venerdi Products Ltd, Personal communication, 1 May, 2015).

Each of the dry ingredients (Appendix A) was weighed using a Sartorius top pan balance (CP4202s, Goettingen, Germany) and then mixed in an 8-L stainless bowl of a dough mixer (Delta 8L Planetary Mixer, Delta Faucet, New Zealand). The dry ingredients were premixed at speed setting 1 using a flat beater for 5 min. Solidified coconut oil was measured according to each formulation (Appendix A) and heated in a microwave (RMS510TS, Menumaster, Norfolk, England) until melted. Liquid coconut oil was allowed to cool to ambient temperature (20°C). The liquid coconut oil was then mixed with lecithin and rice syrup in a 1- L stainless steel mixing bowl and then mixed by whisking using a stainless egg whisker for 5 min (Appendix A). The liquid mix was added to mixed dry ingredients in an 8 L-stainless bowl. The ingredients in the bowl were mixed using the dough mixer set at speed 1 for 1 min. Then potable water (35-40°C) (Appendix A) was added slowly to the mixture and further mixed at speed 1 for 2 min; the speed was increased to 2 for 2 min to form a homogeneous dough.

Dough resting, dividing and shaping

After mixing, the formed dough was covered with a clean damp kitchen towel and allowed to rest for 5 min at ambient temperature (20°C). Resting allowed dough relaxation and prevented the drying of the surface (dough) which could lead to the formation of a ‘damp skin’ (Qarooni, 1996). After resting, the dough was more elastic. The rested dough was divided into small units of dough balls weighing 60±0.2 g. Forty-five to fifty dough balls (units) were produced per 2.73 kg dough. The dough balls were placed in separate plastic containers with lids to retain moisture (Winger et al., 2014). Each dough ball was slightly flattened by hand and then formed into a thin and round shape (Ø ~ 20 cm, thickness ~ 2 mm) using a roller (Figure 6).

Figure 6 Disc dough (Ø ~ 20 cm, thickness ~ 2 mm)

Baking and cooling

The GFW shaped dough units were transferred onto a baking tray and then placed in a baking oven (Turbofan 32Max, Moffat Pty Ltd, New Zealand) preheated at a selected temperature. The dough was baked at selected baking times according to different baking conditions (section 3.3.1, 3.4.1, and 3.5.1). Baked GFWs were cooled to ambient temperature (20°C) on a rack.

Packaging and storage

After cooling to ambient temperature, GFWs were packed individually in transparent polyethylene (PE) zipped bags (250×350 mm, 45 µm thickness) (Pams, New Zealand). The bags were sealed and placed at the same level in a 4°C refrigerator (TME1500 3-Door Chiller Remote, Skope Ltd, Auckland, New Zealand) for storage.

3.3 Phases 1 and 2: Initial selection of gluten replacers and baking conditions

3.3.1 Experimental design

Phase 1

The formulations for gluten-free breads used in phase 1 comprised a basic set of ingredients (Table 6) with 4 levels of xanthan gum or guar gum as replacements for gluten. The breads were baked at 200 or 220°C for 2 or 4 min respectively to pale or yellowish crust.

Table 7 Experimental design used in phase 1

Sample	Baking condition	Baking temp. (°C)	Baking time (min)	Formulation	Xanthan gum (%)	Guar gum (%)	CMC (%)	Coconut oil (%)
S1	a1	200	2	A1	0.00	1.00	0.00	10.00
S2				A2	1.00	0.00	0.00	10.00
S3				A3	1.50	0.00	0.00	10.00
S4				A4	0.60	0.60	0.00	10.00
S5	a2	200	4	A1	0.00	1.00	0.00	10.00
S6				A2	1.00	0.00	0.00	10.00
S7				A3	1.50	0.00	0.00	10.00
S8				A4	0.60	0.60	0.00	10.00
S9	a3	220	2	A1	0.00	1.00	0.00	10.00
S10				A2	1.00	0.00	0.00	10.00
S11				A3	1.50	0.00	0.00	10.00
S12				A4	0.60	0.60	0.00	10.00
S13	a4	220	4	A1	0.00	1.00	0.00	10.00
S14				A2	1.00	0.00	0.00	10.00
S15				A3	1.50	0.00	0.00	10.00
S16				A4	0.60	0.60	0.00	10.00

Notes: temp. = temperature; CMC = carboxymethyl cellulose; n = 16.

Phase 2

Table 8 Experimental design used in phase 2

Sample	Baking condition	Baking temp. (°C)	Baking time (min)	Formulation	Xanthan gum (%)	Guar gum (%)	CMC (%)	Coconut oil (%)
Q1				B1	0.60	0.60	0.00	10.00
Q2				B2	0.50	0.50	0.00	10.00
Q3	b1	230	2	B3	0.50	0.50	0.00	12.00
Q4				B4	0.50	0.50	0.20	10.00
Q5				B5	0.50	0.50	0.20	8.00
Q6				B1	0.60	0.60	0.00	10.00
Q7				B2	0.50	0.50	0.00	10.00
Q8	b2	230	4	B3	0.50	0.50	0.00	12.00
Q9				B4	0.50	0.50	0.20	10.00
Q10				B5	0.50	0.50	0.20	8.00
Q11				B1	0.60	0.60	0.00	10.00
Q12				B2	0.50	0.50	0.00	10.00
Q13	b3	240	2	B3	0.50	0.50	0.00	12.00
Q14				B4	0.50	0.50	0.20	10.00
Q15				B5	0.50	0.50	0.20	8.00
Q16				B1	0.60	0.60	0.00	10.00
Q17				B2	0.50	0.50	0.00	10.00
Q18	b4	240	4	B3	0.50	0.50	0.00	12.00
Q19				B4	0.50	0.50	0.20	10.00
Q20				B5	0.50	0.50	0.20	8.00

Notes: temp. = temperature; CMC = carboxymethyl cellulose; n = 20.

Hence four formulations of bread were baked under two baking temperatures and two baking times resulting in sixteen GFW treatments (Table 7). The GFW's made in phase 2 were based on the results of phase 1. Five formulations (B1-B5) with variable levels of xanthan gum, guar gum, CMC, and coconut oil were used. Each formulation was baked for either 2 or 4 min at 230 or 240 °C. Hence five formulations, two baking temperatures and two baking times resulted in twenty GFW treatments (Table 8).

3.3.2 Characterization of GFWs

Phase 1

After baking (day 0), the ready-to-eat GFW samples (S1-S16) were weighed to determine baking weight loss. After cooling, subjective rollability test were conducted (day 0) using a three centimetre (3-cm) diameter dowel (Ø = 3cm) and 1-cm diameter dowel (Ø = 1 cm). All

the analyses were conducted in triplicate.

Phase 2

Baking weight loss was immediately measured in bread samples (Q1-Q20) after baking (day 0). Colour measurement, water activity and subjective rollability test were determined after cooling to ambient temperature (day 0). 1-cm diameter dowel was used for the subjective rollability test in this phase. Subjective rollability test and examination of visible growth of moulds were conducted on GFW samples in phase 2 during storage (4 °C) at days 4, 7, 14, 21 and 28. All the analyses and tests were done in triplicate.

3.3.2.1 Measurement of baking weight loss

Baking weight loss is an index of moisture loss during baking (Ozge Keskin, Sumnu, & Sahin, 2004). It was determined by obtaining the difference between the initial weight of the dough (wet) and weight of the baked bread (heated) immediately after removal from the oven (Ozmutlu, Sumnu, & Sahin, 2001). The weight of GFW (wet dough) was weighed (W_d) using a top pan balance (CP4202s, Sartorius, Goettingen, Germany). The wet dough was then baked under specified conditions (baking temperature and baking time). After baking, the GFW were weighed immediately (W_b). The baking weight loss (BWL) of the GWF was calculated using Equation 1 (Mariotti et al., 2013):

$$BWL(\%) = (W_d - W_b) \times 100/W_d \quad \text{Equation 1}$$

where,

BWL = *Baking weight loss (%);*

W_d = *Weight of wet dough (g),*

W_b = *Weight of baked GFW bread (g)*

3.3.2.2 Measurement of colour

Colour (CIE L* a* b* colour space) of the baked GFWs was measured using a Minolta CR-300 model chroma meter (Japan). CIE L*a*b* is a colour space proposed by the International

Commission on Illumination (CIE) in 1976 (Tkalcic & Tasic, 2003). It describes all the colours visible to the human eye and was created to serve as a device-independent model to be used as a reference. Chroma meter measures the surface colour by illuminating the site with a pulse of flight of defined colour from a xenon arc lamp (Muizzuddin, Marenus, Maes, & Smith, 1990). In this colour system, colour is described by three coordinates which are L*, a*, and b*. L* describes the lightness, +a* redness, -a* greenness, +b* yellowness, and -b* blueness as shown in Figure 7.

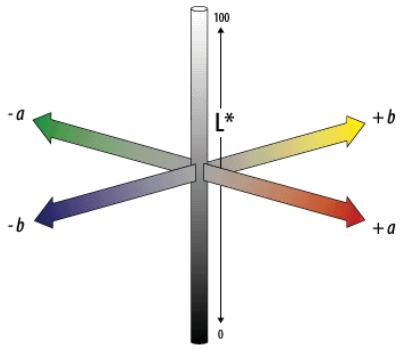

Figure 7 CIE L* a* b* colour model

Source: Retrieved from http://dba.med.sc.edu/price/irf/Adobe_tg/models/cielab.html

Colour measurement of baked GFWs was based on the method of Izydorczyk et al. (2008) and also following the instructions of the supplier of the equipment. A Minolta white calibration plate (L* = 97.59, a* = −5.00, b* = +6.76) was used to standardize the equipment prior to colour measurements. After calibration, a sample of GFW (20°C) was placed flat on a standard black background (Minolta CR-300 model chroma meter，Japan). The colour measurements were recorded. After three consecutive colour measurements were completed, L*, a*, and b* values were obtained. Each GFW was measured at six different positions and mean values were calculated and recorded. Using L*, a*, b*, the whiteness index was calculated based on equation 2 (Hsu, Chen, Weng, & Tseng, 2003; Borsuk, et al., 2012).

$$Whiteness\ index = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad \text{..... Equation 2}$$

3.3.2.3 Measurement of water activity

The method of Pourfarzad at al. (2011) was used and modified to measure water activity. Water activity was measured using a Novasina AW SPRINT-TH 500 instrument at 20±1 °C (Axair Ltd., Pfaanffikon, Switzerland). Before measuring water activity of samples, the equipment was calibrated with SAL-T Standards (Humidity 90%). Test samples (Ø = 15 mm) were prepared from the centre of random GFW samples. The test portion was placed in a sample dish (40 mm diameter × 12 mm deep) supplied with the equipment.

3.3.2.4 Subjective rollability test

The method of Barros, Alviola, and Rooney (2010) was used to conduct the subjective rollability test described in this section. GFWs were wrapped around a wooden dowel (Ø=1 or 3 cm) at room temperature (20°C) and evaluated subjectively using a 1 - 5 point scale (1-unrollable； 2-cracking and imminent breaking on both surfaces; 3-cracking and, easily breaking beginning on one surface; 4-signs of cracking but no breaking; 5-no cracking, very flexible). GFWs were considered unacceptable when the rollability score with 1-cm diameter dowel was below 3 (Cevoli et al., 2015; Tuncil, Jondiko, Tilley, Hays, & Awika, 2016). Each GFW was wrapped and evaluated twice to obtain a mean value, which was recorded.

3.3.2.5 Examination of visible mould growth

The GFWs were checked for visible mould growth according to El-Khoury (1999) and Kamaljit, Amarjeet, and Pal (2011). When any visible mould growth appeared, the shelf-life trial was terminated and results recorded.

3.4 Phase 3: Screening of formulations using the Taguchi design

3.4.1 Experimental design

Taguchi design (Mertol, 1995) can determine the effect of factors on characteristic properties and optimise levels of the factors. One of the advantages of Taguchi design is less

experimental runs required to determine optimal conditions. Orthogonal arrays and ANOVA are used as the tools of analysis for the Taguchi deign. Orthogonal arrays can considerably reduce the number of experimental runs and ANOVA can estimate the effect of each factor on the characteristic properties of the samples (Unal & Dean, 1990). Experiments conducted by Taguchi design can predict values for optimising ingredient levels (Yang & Tarng, 1998; Oztop, Sahin, & Sumnu, 2007). Taguchi design been widely used in product/process optimisation (Otto & Antonsson, 1993; Jeyapaul, Shahabudeen, & Krishnaiah, 2005).

Preliminary results from phases 1 and 2 (section 4.1 and 4.2) were used to design experiments in this phase. Baking conditions were kept constant in this phase (baking temperature = 240°C, baking time = 2 min). Levels of coconut oil, CMC and xanthan-guar gum (mixtures of xanthan gum and guar gum, the ratio = 1:1) used in GFW formulations were optimised in this phase.

The standardised Taguchi-based experimental design uses an L9 (3^4) orthogonal array with four columns and nine rows (Zhang, Chen, & Kirby, 2007). The L9 (3^4) array has eight degrees of freedom with capacity to use up to four control factors, each at three levels. In this experiment, the L9 orthogonal array had four columns and three factors (coconut oil, CMC, xanthan-guar gum), with one column of the array being left empty. Orthogonality of array is not lost by letting one or more columns of the array remain empty (Mertol, 1995; Khoei, Masters, & Gethin, 2002). Thus, nine experimental treatments (formulations) were conducted, using a combination of levels for each control factor (coconut oil, CMC, xanthan-guar gum) as shown in Table 9. Levels of the three factors were selected based on previous experimental results (section 4.2). Nine formulations (C1-C9, Appendix A) and corresponding samples were developed in this phase.

Table 9 Taguchi L9 (3^4) orthogonal array applied in phase 3

Formulation/Treatment	Factor (%)[a]		
	Coconut oil	CMC	Xanthan-guar gum (1:1)
C1	8.00	0.10	0.80
C2	8.00	0.20	1.00
C3	8.00	0.30	1.20
C4	10.00	0.10	1.00
C5	10.00	0.20	1.20
C6	10.00	0.30	0.80
C7	12.00	0.10	1.20
C8	12.00	0.20	0.80
C9	12.00	0.30	1.00

Note: %[a] (w/w) Flour basis; Taguchi design generated by Minitab version 16 (State College, PA, USA)
Baking conditions were kept constant in this phase (baking temperature = 240°C, baking time = 2 min); CMC = carboxymethyl cellulose.

3.4.2 Characterization of GFWs in phase 3

In phase 3, GFW samples were analysed for bread weight loss immediately after baking, colour, water activity, subjective rollability test and two-dimensional extensibility test were done after cooling (day 0). Water activity, subjective rollability test, two-dimensional extensibility test, and mould counts were determined at days 7, 14 and 21 during storage of samples at 4°C. Testing methods were the same as described in section 3.3.2 except two-dimensional extensibility test. The two-dimensional extensibility test was conducted as described in 3.4.2.1. All the tests were done in triplicate.

3.4.2.1 Two-dimensional extensibility test

Firmness and extensibility were determined using the TA.XT Plus Texture Analyser (Stable Micro Systems, UK) equipped with the Tortilla/Pasty Burst Rig (TA.XT Plus, Stable Micro Systems, UK). The two parameters were determined according to the instructions of the equipment manufacturer and published information (Uthayakumaran & Lukow, 2005; De-Barros, 2009). The upper and lower holding plates were used to punch four holes to hold the GFWs tightly on the plate. GFWs were extended and ruptured using a 1-inch (2.5 cm) spherical probe. The settings of the texture analyser are shown in Table 10. Two parameters measured in the two-dimensional extensibility test were rupture distance and maximum rupture force. Rupture distance (mm) indicated the extensibility of samples and rupture force

(g) measured the firmness of samples (Bejosano et al., 2005; De-Barros, 2009).

Table 10 TA.XT plus Texture Analyser settings

Mode	Setting	Value
Measurement	Pre-Test Speed	5.00 mm/sec
	Test Speed	1.00 mm/sec
	Post-Test speed	5.00 mm/sec
	Target Mode	Distance
	Distance	30.0 mm
Trigger	Type	Auto(Force)
	Force	0.050 N

3.5 Phase 4: Sensory evaluation of optimised GFWs

3.5.1 Experimental design

In phase 4, three formulations (D1, D2 and D3, Table 11) were optimised using the Taguchi method in phase 3 (section 4.3); the GFW samples were subjected to consumer sensory evaluation to determine their acceptability. Simultaneously, physical tests of the three samples were conducted to determine their physical characteristics.

Table 11 Experimental design in phase 4

Formulation/Sample	Xanthan gum (%)	Guar gum (%)	CMC (%)	Coconut oil (%)
D1	0.50	0.50	0.30	8.00
D2	0.50	0.50	0.30	10.00
D3	0.50	0.50	0.30	12.00

Notes: Baking conditions were kept constant in this phase (baking temperature = 240°C, baking time = 2 min); CMC = carboxymethyl cellulose.

3.5.2 Characterization of GFWs in phase 4

In phase 4, ready-to-eat GFW samples were analysed immediately after baking (day 0) for baking weight loss and colour after cooling (day 0). During storage, water activity, subjective rollability test and two-dimensional extensibility test were conducted at days 1, 7, and 14 using the same methods described in section 3.4.2. All the physical analyses were conducted in triplicate. Sensory evaluation was done at days 1, 7, and 14 as described in section 3.5.2.1.

Samples were screened for microbiological safety as described in section 3.5.2.2 prior to sensory evaluation (FSANZ, 2001).

3.5.2.1 Sensory evaluation

Qualitative sensory evaluations of the GFWs were conducted for the parameters of appearance, texture, aroma, taste, and overall acceptability of the samples which were served plain. A 9-point hedonic scale with descriptive anchors was used to evaluate each parameter (1 = dislike extremely; 9 = like extremely). Sample products were considered acceptable by consumers if their mean scores of acceptability were at least 5 or higher (Lazaridou et al., 2007). Panellists were randomly recruited at Massey University Albany campus, who consisted of students, staff and guests included. The sensory panellists were given questionnaires (Appendix C) to record scores (degree of liking) during the tasting sessions.

GFW samples D1, D2 and D3 were evaluated on days 1, 7, and 14 in three separate sensory sessions. The samples were prepared with a round aluminium mould and shaped into round pieces of bread (Ø ~ 7 cm). The formed (shaped) samples were coded with 3 random digit numbers on paper plates, and served with bottled water at 20±1°C in sensory booths. Before being served with bread samples, panellists were asked to give their consent after reading the accompanying information (Appendix D). Then samples were served with questionnaires to indicate their liking of sample. Panellists were asked to cleanse their palate with water before tasting each sample. Three samples were served sequentially but randomly. Thirty (30) panellists participated in each sensory session. Sensory evaluation was conducted twice.

3.5.2.2 Microbiological analysis

Mould and yeast counts, and total aerobic plate counts in GFW samples were determined by the modified methods of Khoshakhlagh et al. (2014), and Saiz, Iurlina, Borla, and Fritz (2007), respectively.

GFW samples were cut into pieces measuring <1 mm thick. A sample, weighing 10.00±0.01 g was obtained using a top pan balance (PB 1502, Mettler Toledo, Columbus, USA). The

sample was then homogenized in 90 mL of sterile 0.1% peptone solution (Merck, Darmstadt, Germany) for 2 min in a sterile stomacher bag (LABPLAS, Quebec, Canada), giving a 10^{-1} dilution. Suitable serial dilutions of the sample were prepared up to 10^{-4}. Aliquots of 1 mL from each dilution of samples were plated on plate count agar (BD Diagnostics, Sparks, MD, USA) and yeast extract glucose chloramphenicol agar (Merck, Darmstadt, Germany) for total aerobic plate counts and, yeast and mould counts, respectively. The plated Petri dishes were incubated (IM 1000, Clayson Laboratory Apparatus Pty Ltd, Australia) at 35°C for 2 days to determine total aerobic plate count, and at 25°C for the yeast and mould counts (Tournas, Stack, Mislivec, Koch, & Bandler, 1998; Khoshakhlagh et al., 2014). After incubation, the results of the counts were expressed as colony forming units (CFU) per gram of GFW (Beuchat, Mann, & Gurtler, 2007). All materials and equipment used for microbiological analyses were sterile.

3.6 Data analysis

Data analyses were conducted using Minitab version 16 Statistical Software (Minitab Inc., State College, PA, USA) and Microsoft Excel version 14.0.0 (Santa, CA, USA). Kolmogorov-Smirnov tests (SPSS version 21, IBM, USA) were applied to determine the normality of the data. Normal data was analysed by analysis of variance (ANOVA) using Minitab ($p<0.05$). Kruskall-Wallis tests (SPSS) were used for tests of significance for non-parametric data. Descriptive statistical analysis was generated by Excel to calculate mean values and standard deviations/standard errors of the mean for each treatment. All graphical presentations were generated by Microsoft Excel.

3.6.1 Phases 1 and 2

In phases 1 and 2, data were subjected to Minitab's factorial design analysis using the General Linear Model (GLM) and one-way of the analysis of variance (ANOVA) to determine the significance of varying formulation, baking temperature, baking time and storage time. Significant differences ($p<0.05$) between means were analysed using Tukey's multiple comparison test. Descriptive statistics were also used to analyse data on rollability score of samples during storage in phase 2.

3.6.2 Phase 3

In phase 3, the Taguchi design generated by Minitab was applied to optimise formulations. Taguchi design analysis, the GLM and one-way ANOVA of Minitab were used to determine the significance of varying levels of coconut oil, CMC, and xanthan-guar gum on the physical parameters of samples. Significant differences ($p<0.05$) between means were analysed using Tukey's multiple comparison test.

3.6.3 Phase 4

In phase 4, data of parameters of GFWs were analysed by the GLM and one-way ANOVA of Minitab except rollability scores and sensory data. Rollability and sensory data were not normally distributed, they were therefore analysed by non-parametric test of Kruskal-Wallis Test ($p<0.05$) (Basman & Köksel, 1999). Tukey's test was applied to the normal data to compare the significance of treatments at 95% confidence interval. Kruskall-Wallis test of SPSS was used to analyse non-parametric data.

Chapter 4 Results and Discussion

4.1 Results and Discussion: Phase 1

In phase 1, four combinations of guar gum and xanthan gum were compared for their effectiveness in replacing gluten in GFWs under four baking conditions. Sixteen GFW samples (S1-S16) were developed in phase 1. The baked breads were compared by baking weight loss and rollability tests using 1- and 3-cm diameter dowels. Baking weight loss defines the moisture loss during baking. Excessive water loss can result in dry and underweight products, which can consequently lead to poor sensory properties and packaging problems (Kotoki & Deka, 2010). Subjective rollability describes the ability of flatbread to roll around a dowel and it evaluates the extent of breaking when flatbread is rolled (Friend et al., 1993).

4.1.1 Baking weight loss

In this phase, baking time, baking temperature and formulation had significant effects on baking weight loss ($p<0.05$) (Appendix E-1). The results also showed interactions ($p<0.05$) between baking time and baking temperature on baking weight loss (Appendix E-1).

Baking weight loss of samples in phase 1 ranged from 20.0 to 30.7% (Figure 8). Irrespective of baking conditions (baking temperature and time), formulation A4 (0.6% xanthan gum, 0.6% guar gum) had the lowest mean baking weight loss (24.3±3.5%), followed by formulation A1 (1% guar gum) and A3 (1.5% xanthan gum). The highest loss was observed in formulation A2 (1.5% xanthan gum). To minimize baking weight loss of GFWs, formulation A4 (0.6% guar gum, 0.6% xanthan gum) demonstrated good potential for further development.

The results also indicated that the type of hydrocolloid (xanthan gum and guar gum) had an effect on baking weight loss (Figure 8). The presence of guar gum (1%) in formulation resulted in lower baking weight loss compared to xanthan gum (1%). This may be attributed to better swelling and higher water binding capacity of guar gum during hydration than

xanthan gum, which lead to hold larger water during baking (Sidhu & Bawa, 2002; Guarda et al., 2004; Shalini & Laxmi, 2007). The ability of hydrocolloids to bind water during baking has also been reported by Gambuś et al.(2001). However, when the level of xanthan gum was increased from 1% to 1.5%, baking weight loss of GFWs decreased. The reason for decreased baking weight loss may be attributed to the increase in swelling index caused by increased hydrocolloid concentration (xanthan gum) from 1 to 1.5%.

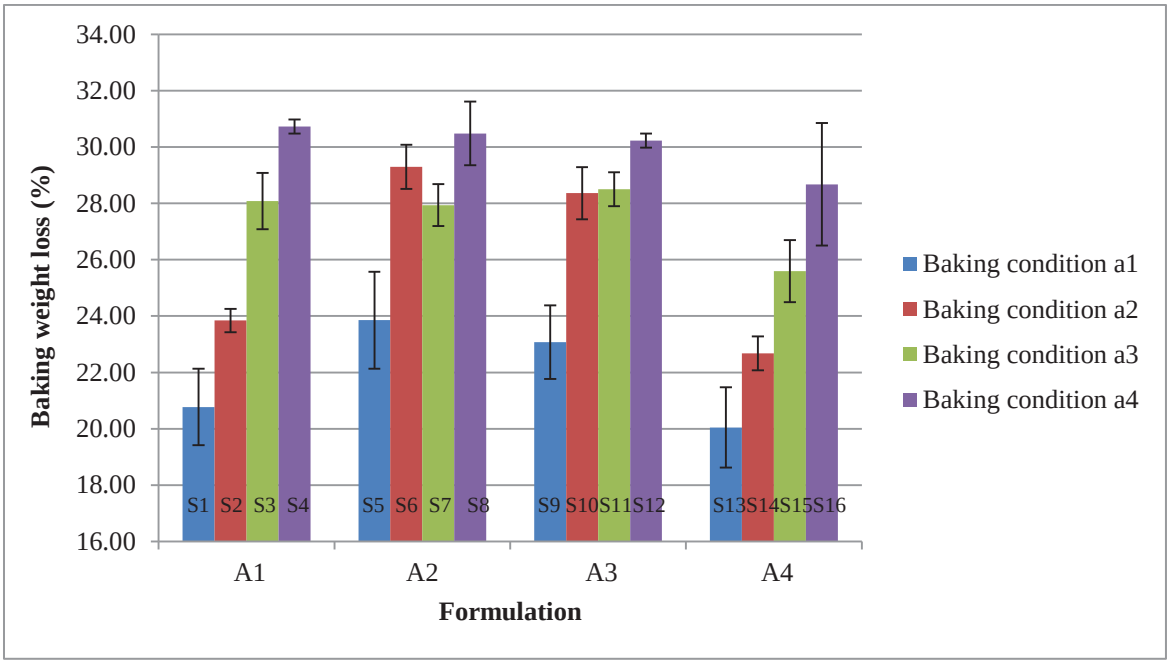

Figure 8 Baking weight loss (%) of four formulations of GFWs under four baking conditions in phase 1

Notes: Baking conditions: a1 = 200°C/2 min; a2 = 200°C/4 min; a3 = 220°C/2 min; a4 = 220°C/4 min. Formulations: A1 = 1% guar gum; A2 = 1% xanthan gum; A3 =1.5% xanthan gum; A4 =0.6% xanthan gum, 0.6% guar gum. Samples: S1, S2, S3, S4 = A1 under baking condition a1, a2, a3, a4 respectively; S5, S6, S7, S8 = A2 under a1, a2, a3, a4 respectively; S9, S10, S11, S12 = A3 under a1, a2, a3, a4 respectively; S13, S14, S15, S16 = A4 under a1, a2, a3, a4 respectively.
Error bars are standard error of three replications (SEM).

As expected, baking weight loss was affected by baking temperature and time (Figure 8). When the baking time or baking temperature was increased, baking weight loss increased. The effects of higher baking temperature and reduced baking time on baking weight loss depended on formulations shown in Figure 8. Baking weight loss increased in formulations A1 and A4 (S3> S2, S15> S14), when baking temperature / time (200°C/4 min) was changed respectively (220°C/2 min). Whereas in formulation A2 (1% guar gum), baking weight loss

of sample S6 was higher under baking condition a2 (200°C/4 min) than sample S7 under baking condition a3 (220°C/2 min). In formulation A3 (1.5% xanthan gum), baking weight losses of samples S10 and S11 were similar (28.4%, 28.5%).

4.1.2 Subjective rollability

Fifteen GFW samples (S1-S15) had a rollability score of 1 when evaluated with 1-cm diameter dowel while only one sample (sample S16) had a score of 2. Sample S16 was brittle and characterised by cracks on both surfaces when subjected to a 1-cm diameter dowel test. Overall, the results indicated that all the GFWs made during this phase had poor rollability or that the test used was not appropriate for assessing the rollability of the GFWs.

Rollability is a very important quality index for GFWs. As the 1-cm diameter dowel could not measure rollability of samples in phase 1 well and the rollability scores of most samples were too low to differentiate between each other, larger radius of a 3-cm diameter dowel was then used to evaluate rollability (Figure 9). Formulation, baking temperature and time had significant effects on the rollability scores of GFW samples using a 3-cm diameter dowel in phase 1 ($p<0.05$).

Rollability scores of GFWs using a 3-cm diameter dowel increased with increases in baking temperature and time (Figure 9). Rollability scores for 3-cm diameter dowel of all formulations (n = 4) under baking condition a4 (220°C/4 min) were the highest compared to the other baking conditions. The results indicated that high baking temperatures and long baking times resulted in better rollability of products. However, long baking times decrease production efficiency (Therdthai & Zhou, 2003), therefore further trials using higher temperatures were necessary and this formed the subject of the next phase (phase 2).

The presence of hydrocolloids in the formulation affected the rollability of GFWs (Figure 9). Overall, GFWs made with formulation A4 containing two hydrocolloids (0.6% xanthan gum and 0.6% guar gum) had the highest rollability while formulation A1 (1% guar gum) had the lowest (Figure 9). A mixture of xanthan and guar gum may partly explain the higher rollability of GFW samples (Tako & Nakamura, 1985). The higher viscosities of combined hydrocolloids may be due to the intermolecular interactions between xanthan and guar gum

which occurred between periphery of the side chains of the xanthan molecule and the backbone of the guar gum molecule (Tako, Teruya, Tamaki, & Ohkawa, 2010). In a study of rice gluten-free bread, addition of xanthan-guar gum improved dough structure and bread texture (Demirkesen et al., 2010a).

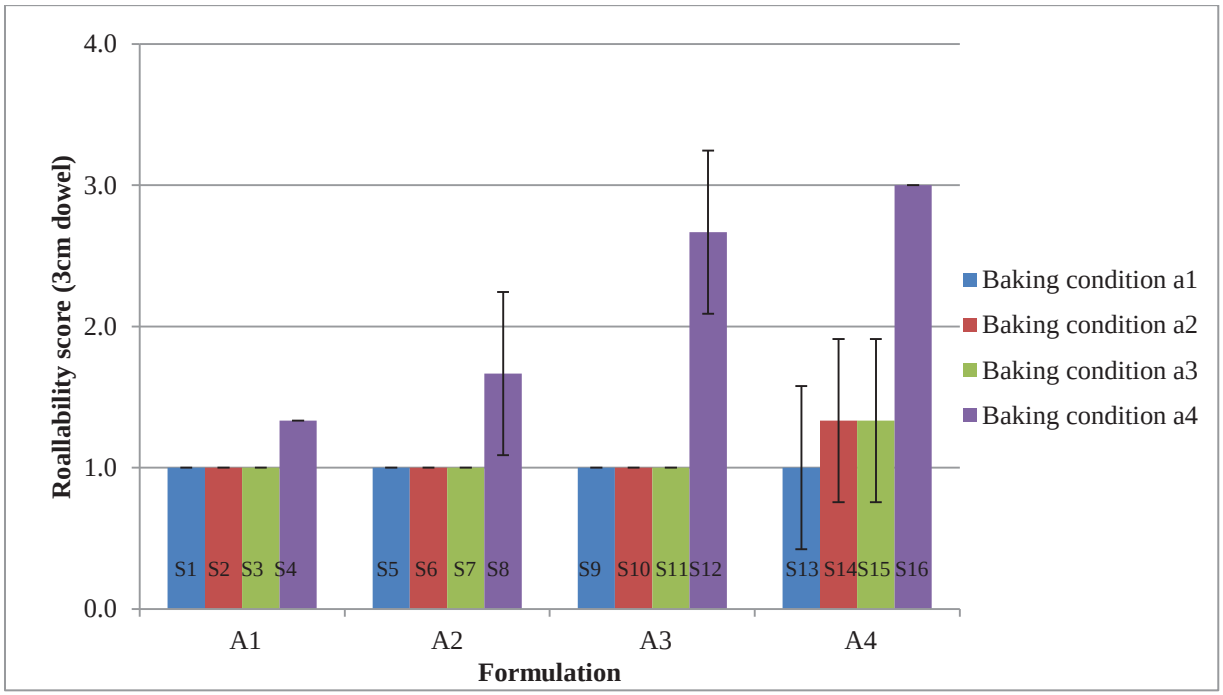

Figure 9 Rollability scores using a 3-cm dowel in four formulations of GFWs under four baking conditions in phase 1

Notes: Baking conditions: a1 = 200°C/2 min; a2 = 200°C/4 min; a3 = 220°C/2 min; a4 = 220°C/4 min. Formulations: A1 = 1% guar gum; A2 = 1% xanthan gum; A3 =1.5% xanthan gum; A4 =0.6% xanthan gum, 0.6% guar gum. Samples: S1, S2, S3, S4 = A1 under baking condition a1, a2, a3, a4 respectively; S5, S6, S7, S8 = A2 under a1, a2, a3, a4 respectively; S9, S10, S11, S12 = A3 under a1, a2, a3, a4 respectively; S13, S14, S15, S16 = A4 under a1, a2, a3, a4 respectively.
Error bars are standard error of three replications (SEM). Rollability scores: 1-5, with 1 as lowest and 5 highest.

When xanthan gum replaced guar gum at level of 1% in the formulation of GFWs (from formulation A1 to A2), rollability scores using 3-cm diameter dowel increased (Figure 9). The results suggested that xanthan gum had better effect on rollability of GFW than guar gum. This observation may be attributed to the structural properties of xanthan gum such as molecular weight, molecular shape and configuration, and chain length (García-Ochoa et al. 2000). When xanthan gum was used to replace gluten, it enhanced the elasticity and resistance to deformation of gluten-free doughs better than CMC, pectin and agarose (Abdel-Aal, 2009). A study by Acs, Kovacs, and Matuz (1996) on gluten-free bread using 1-3%

xanthan gum as substitute for gluten produced better quality crumb structure compared to guar gum. When the level of xanthan gum was increased from 1% to 1.5%, the rollability of GFWs was improved. Increased xanthan gum probably resulted in the increase of dough elasticity, thus improving rollability. In a previous study of rice based gluten-free bread, increase of xanthan gum (1% – 2%) produced better elastic properties of dough and bread crumb (Lazaridou et al., 2007).

4.1.3 Conclusion

The GFW containing xanthan-guar gum (1:1) in this phase had the least baking weight loss (25.6%) and the highest rollability score when fresh was 3 (on a scale of 1 - 5) after baking at 220°C/4 min. Therefore, the main objective of the next phase was to improve on the rollability of baked GFW.

4.2 Results and Discussion: Phase 2

In phase 1, formulation A4 containing a combination of hydrocolloids (xanthan gum and guar gum) had the lowest baking weight loss and the highest rollability. Thus, one of the objectives of phase 2 was to optimise the formulation with xanthan-guar gum (xanthan gum: guar gum=1:1). Phase 2 also investigated the addition of CMC as it is reported to improve the rollability of tortilla (Friend et al., 1993) and baking weight loss of gluten-free flatbread (Mohammadi et al., 2014). The effect of coconut oil on GFWs was also investigated in this phase to improve rollability of GFWs (Maleki et al., 1981; Gujral & Gaur, 2002). Meanwhile, in phase 2 baking temperature would be increased to achieve higher rollability of GFWs. Samples of GFWs produced in phase 2 were analysed for baking weight loss, water activity and colour following baking. Rollability and mould growth of the GFWs were determined during storage.

4.2.1 Baking weight loss

Baking weight loss was variable among different baking conditions and different formulations in phase 2 (Figure 10). Formulation, baking temperature, and baking time affected baking weight loss ($p<0.05$) (Appendix E-2).

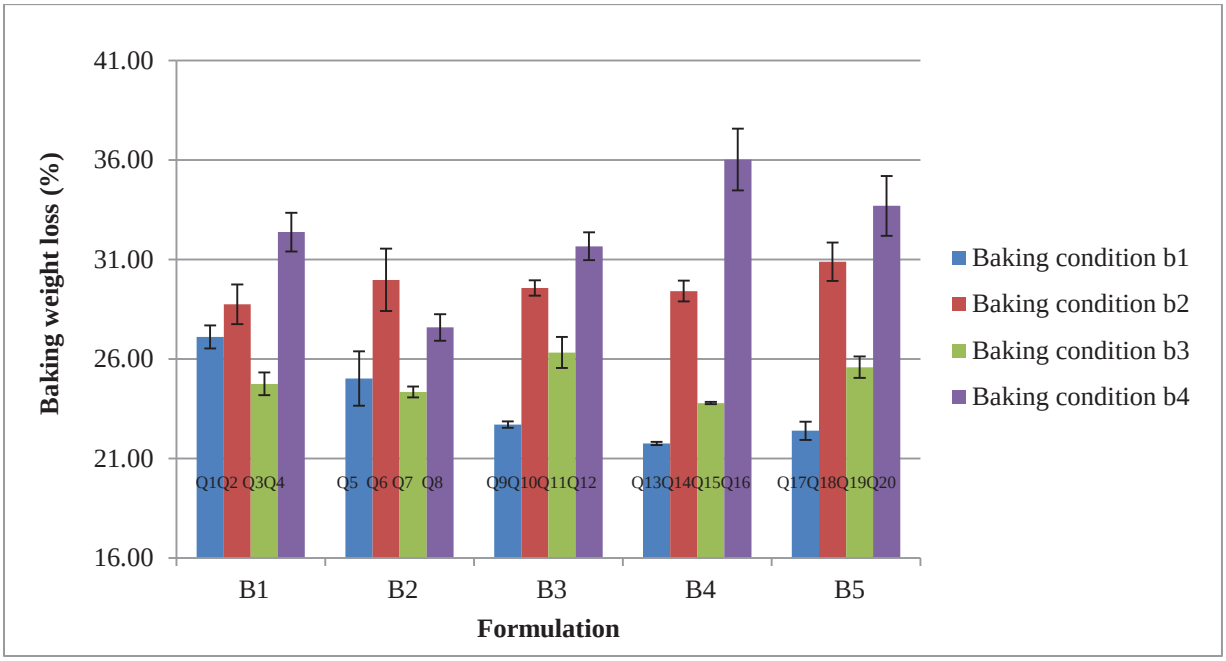

Figure 10 Baking weigh loss (%) of five formulations of GFWs under four baking conditions in phase 2

Notes: Baking conditions: b1 = 230°C/2 min; b2 = 230°C/4 min; b3 = 240°C/2 min; b4 = 240°C/4 min.
Formulations: B1 = 0.6% xanthan gum, 0.6% guar gum, 10% coconut oil; B2 = 0.5% xanthan gum, 0.5% guar gum, 10% coconut oil; B3 = 0.5% xanthan gum, 0.5% guar gum, 12% coconut oil; B4 = 0.5% xanthan gum, 0.5% guar gum, 0.2% CMC, 10% coconut oil; B5 = 0.5% xanthan gum, 0.5% guar gum, 0.2% CMC, 8% coconut oil.
Samples: Q1, Q2, Q3, Q4 = B1 under baking condition b1, b2, b3, b4, respectively; Q5, Q6, Q7, Q8 = B2 under b1, b2, b3, b4, respectively; Q9, Q10, Q11, Q12 = B3 under b1, b2, b3, b4, respectively; Q13, Q14, Q15, Q16 = B4 under b1, b2, b3, b4, respectively; Q17, Q18, Q19, Q20 = B5 under b1, b2, b3, b4 respectively.
Error bars are standard error of three replications (SEM).

Baking weight loss of GFW samples in phase 2 ranged from 21.8 to 36.0% (Figure 10). Formulation B2 containing xanthan gum (0.5%), guar gum (0.5%) and coconut oil (10%) had the lowest mean baking weight loss (26.7%) of four the baking conditions (baking temperature/time),while formulation B1 (0.6% xanthan gum, 0.6% guar gum and 10% coconut oil) had the highest (28.3%). Mean baking weight loss of formulations B3, B4 and B5 of four different baking conditions were similar. The results indicated that xanthan-guar

gum, CMC and coconut oil had apparent effects on baking weight loss. When xanthan-guar gum (xanthan gum: guar gum = 1:1) was decreased from 1.2 to 1%, mean baking weight loss decreased. The reason for the decrease in baking weight loss may be a result of increase in swelling index when xanthan-guar gum decreased. Our observation in this study was similar to Shittu, Aminu, and Abulude (2009), who reported an increase in swelling index with decreased xanthan gum. When coconut oil increased from 10 to 12% (formulations B2 to B3, respectively), mean baking weight loss increased probably due to the presence of higher amount of shortening (Maleki et al., 1981). During baking, the straight chain fatty acids of shortening align in the centre of starch helix which reduces water absorption and delays gelatinisation of starch, thereby impacting on the water loss (Pareyt et al., 2011; Wassell, 2014).

When xanthan gum replaced guar gum at level of 1% in the formulation of GFWs (from formulation A1 to A2), rollability scores using 3-cm diameter dowel increased (Figure 9). The results suggested that xanthan gum had better effect on rollability of GFW than guar gum. This observation may be attributed to the structural properties of xanthan gum such as molecular weight, molecular shape and configuration, and chain length (García-Ochoa et al. 2000). When xanthan gum was used to replace gluten, it enhanced the elasticity and resistance to deformation of gluten-free doughs better than CMC, pectin and agarose (Abdel-Aal, 2009). A study by Acs, Kovacs, and Matuz (1996) on gluten-free bread using 1-3% xanthan gum as substitute for gluten produced better quality crumb structure compared to guar gum. When the level of xanthan gum was increased from 1% to 1.5%, the rollability of GFWs was improved. Increased xanthan gum probably resulted in the increase of dough elasticity, thus improving rollability. In a previous study of rice based gluten-free bread, increase of xanthan gum (1% – 2%) produced better elastic properties of dough and bread crumb (Lazaridou et al., 2007).

4.2.2 Water activity

Formulation, baking temperature and time had significant effects on the water activity (a_w) of GFW samples ($p<0.05$) in phase 2 (Appendix E-2). Water activity in baked products is critical as it affects the growth of microorganisms leading to spoilage. When a_w is higher than 0.91, most bacteria will grow (Troller, 2012). The range of water activity obtained in this

study was similar to previous reports (Suhendro, Waniska, Rooney, & Gomez, 1995; Schmidt, & Fontana, 2008).

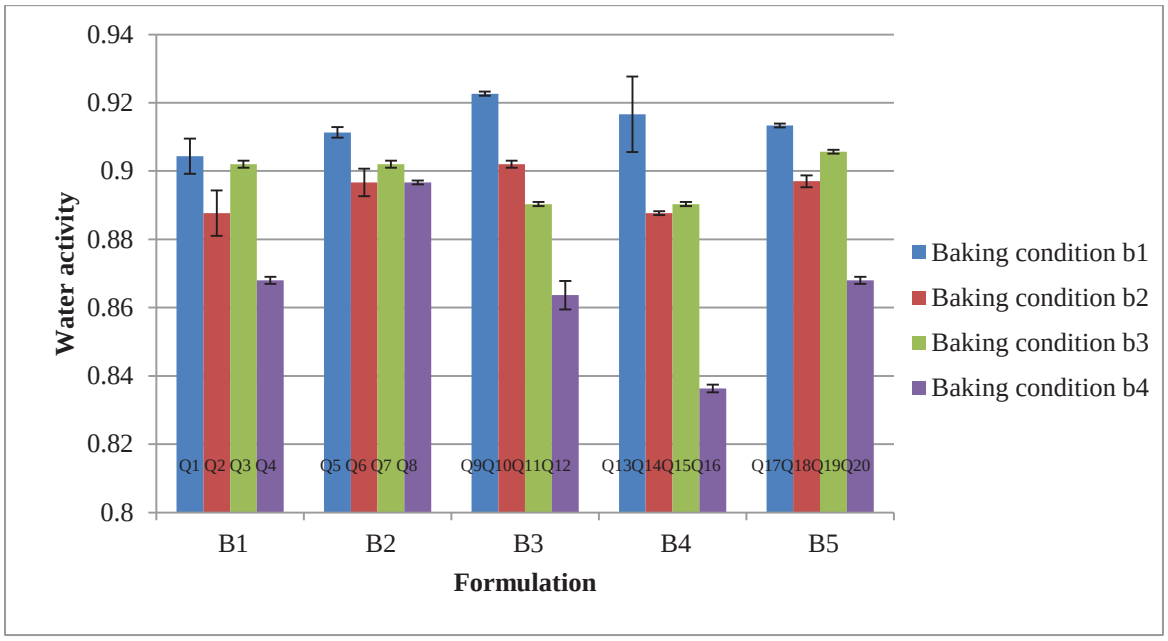

Figure 11 Water activity of five formulations of GFWs under four baking conditions in phase 2

Notes: Baking conditions: b1 = 230°C/2 min; b2 =230°C/4 min; b3 = 240°C/2 min; b4 = 240°C/4 min. Formulations: B1 = 0.6% xanthan gum, 0.6% guar gum, 10% coconut oil; B2 = 0.5% xanthan gum, 0.5% guar gum, 10% coconut oil; B3 = 0.5% xanthan gum, 0.5% guar gum, 12% coconut oil; B4 = 0.5% xanthan gum, 0.5% guar gum, 0.2% CMC, 10% coconut oil; B5 = 0.5% xanthan gum, 0.5% guar gum, 0.2% CMC, 8% coconut oil.
Samples: Q1, Q2, Q3, Q4 = B1 under baking condition b1, b2, b3, b4, respectively; Q5, Q6, Q7, Q8 = B2 under b1, b2, b3, b4, respectively; Q9, Q10, Q11, Q12 = B3 under b1, b2, b3, b4, respectively; Q13, Q14, Q15, Q16 = B4 under b1, b2, b3, b4, respectively; Q17, Q18, Q19, Q20 = B5 under b1, b2, b3, b4 respectively.
Error bars are standard error of three replications (SEM).

Water activity ranged from 0.84 to 0.92 (Figure 11). Samples made from formulation B4 had the lowest mean a_w of four baking conditions, while formulation B2 had the highest. Mean a_w of GFWs made from formulations B1, B3 and B5 were similar. Water activity in formulation B4 was lower than B2, and this may be attributed to the addition of CMC. According to Rosell, Rojas, and De Barber (2001), water activity was expected to increase due to the ability of hydrocolloids to hold water. However, in a later report by Lazaridou et al. (2007), presence of hydrocolloid (pectin, CMC, xanthan, agarose and β-glucan) did not affect water activity. This therefore suggested that the effect of hydrocolloids on water activity may be depended on type of gums and the synergistic effects of mixed gums.

Increase in baking time or baking temperature, resulted in lower a_w of GFWs (Figure 11). GFWs of all formulations under baking condition b4 (240°C/4 min) had the lowest a_w among the four baking conditions, whereas the highest was under baking condition b1. When baking temperature was increased and baking time decreased (from 230°C/4 min to 240°C/2 min), changes of a_w differed with formulations (Figure 11); a_w increased significantly from baking condition b2 to b3 with formulations B1, B2, B4 and B5; whereas, with formulation B3, a_w decreased.

4.2.3 Colour

Colour parameters (L*, a*, b*) differed ($p<0.05$) with baking conditions and formulations, (Appendix E-2). When baking time or baking temperature increased, L* (lightness) of GFW samples decreased (Figure 12). Similar results were reported by Shittu et al. (2007), where the L* value of bread crust reduced with increased baking time and temperature as well. This is expected because the rate of brown pigment formation of crust increases with temperature and time. GFWs under baking condition b4 (240°C/4 min) had the lowest L* value among the four baking conditions while b1 (220°C/2 min) had the highest. However, the range of L* values among all baking conditions was narrow with the exception of samples made from formulation B4. L* with formulation B4 was higher under baking condition b1 (230°C/2 min) and b2 (230°C/4 min) than other eighteen samples (Figure 12). Hence the bread with higher CMC and coconut oil had a lighter colour. It has been reported that shortening and hydrocolloid can impact on bread colour (Hartnett & Thalheimer, 1979; Shittu et al., 2009).

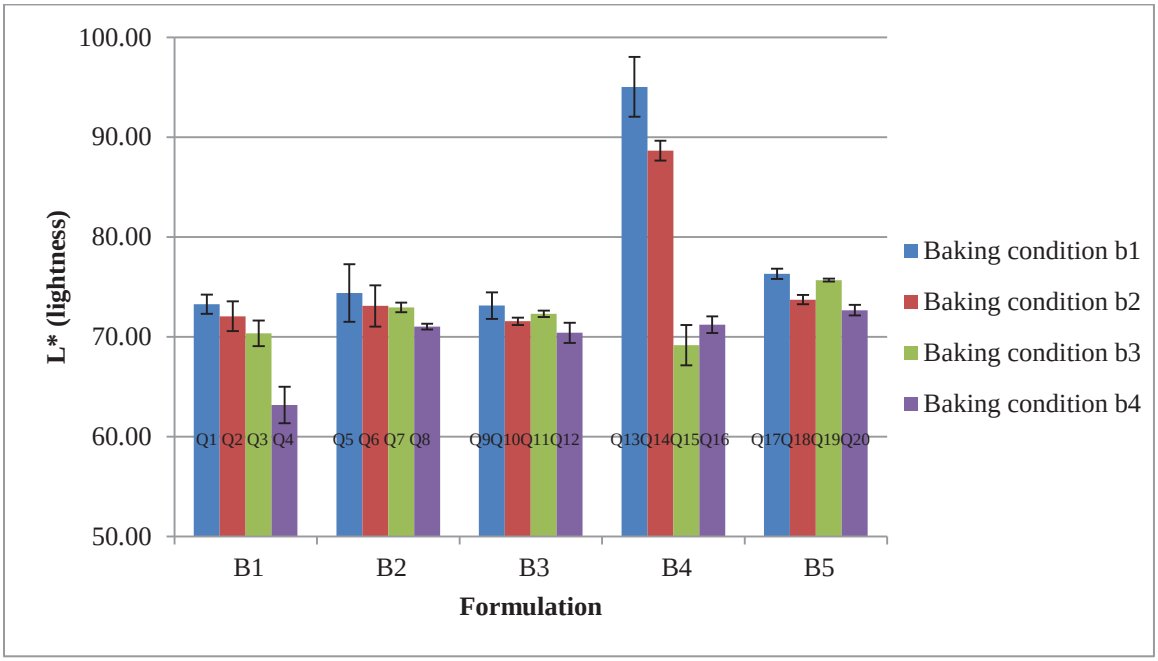

Figure 12 L* (lightness) of five formulations of GFWs under four baking conditions in phase 2

Notes: Baking conditions: b1 = 230°C/2 min; b2 =230°C/4 min; b3 = 240°C/2 min; b4 = 240°C/4 min. Formulations: B1 = 0.6% xanthan gum, 0.6% guar gum, 10% coconut oil; B2 = 0.5% xanthan gum, 0.5% guar gum, 10% coconut oil; B3 = 0.5% xanthan gum, 0.5% guar gum, 12% coconut oil; B4 = 0.5% xanthan gum, 0.5% guar gum, 0.2% CMC, 10% coconut oil; B5 = 0.5% xanthan gum, 0.5% guar gum, 0.2% CMC, 8% coconut oil.
Samples: Q1, Q2, Q3, Q4 = B1 under baking condition b1, b2, b3, b4, respectively; Q5, Q6, Q7, Q8 = B2 under b1, b2, b3, b4, respectively; Q9, Q10, Q11, Q12 = B3 under b1, b2, b3, b4, respectively; Q13, Q14, Q15, Q16 = B4 under b1, b2, b3, b4, respectively; Q17, Q18, Q19, Q20 = B5 under b1, b2, b3, b4 respectively.
Error bars are standard error of three replications (SEM).

Whiteness index indicates the closeness of the sample to the standard white colour (L*=100, a*= 0, b*= 0) (Ulziijargal, Yang, Lin, Chen, & Mau, 2013). Thus, higher whiteness index is associated with whiter bread. However, the consumer is more attracted by the brownness of bread (Lin, Liu, Yu, Lin, & Mau, 2009). The whiteness index of GFWs ranged from 59.35±1.70 to 82.65±0.88. The whiteness index decreased with increased baking time and temperature (Figure 13). Whiteness indices of two samples (Q13 and Q14) were higher than the other 18 samples (Figure 13). However, the differences in whiteness index were small and were within the range that would not be easily noticeable by the naked eye (Angioloni & Collar, 2013). The presence of higher levels of coconut oil and CMC in Q13 and Q14 may be responsible for the higher whiteness index. Chin, Rahman, Hashim, and Kowng (2010) reported that shortening (palm oil) used in bread-making affected colour. The whiter colour of GFWs may also be influenced by the presence of CMC, which has a whiteness index of

86.02 (Angioloni & Collar, 2011).

The colour of baked products is due to composite properties which are partly determined by the extent of Maillard reactions and bread structure (Scanlon & Zghal, 2001; Purlis & Salvadori, 2007). Overall, L* (lightness) and whiteness index of GFW samples decreased with increased baking time or baking temperature. L* (lightness) and whiteness index values of formulation B4 was higher ($p<0.05$) compared to other formulations. In this study, the colour parameters may be affected by functional properties of hydrocolloids on water distribution which impact on Millard reaction and caramelization during production of gluten-free bread (Mahmoud, Yousif, Gadallah, & Alawneh, 2013; Mezaize et al., 2009).

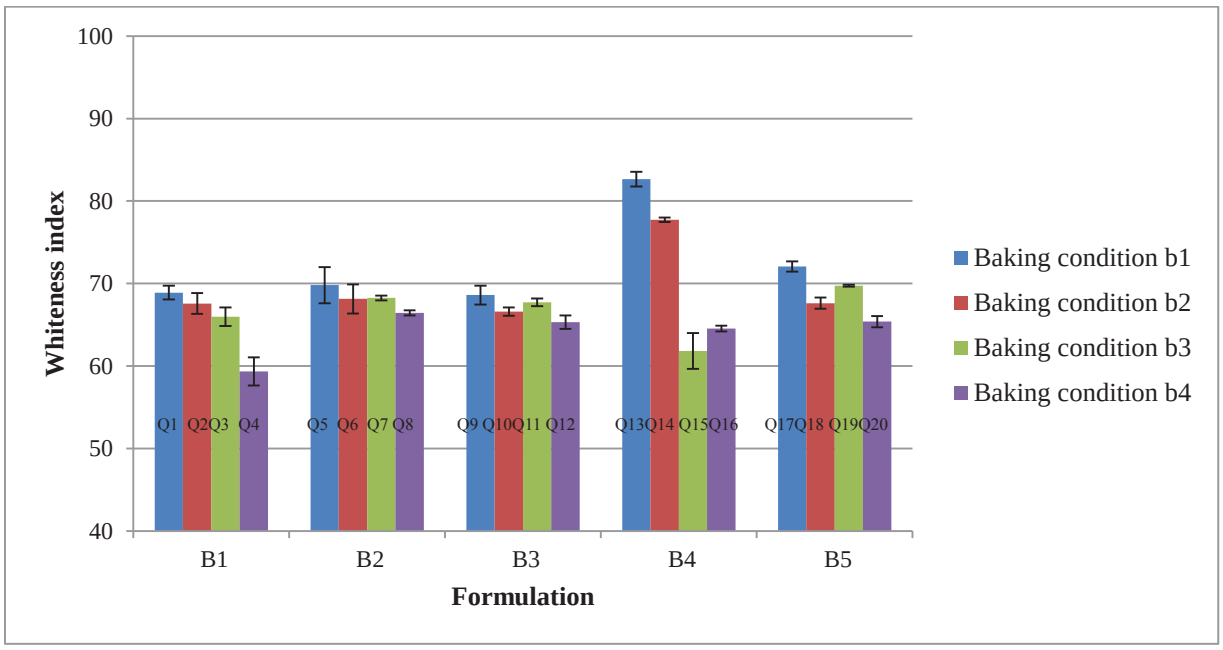

Figure 13 Whiteness indices of five formulations of GFWs under four baking conditions in phase 2

Notes: Baking conditions: b1 = 230°C/2 min; b2 = 230°C/4 min; b3 = 240°C/2 min; b4 = 240°C/4 min. Formulations: B1 = 0.6% xanthan gum, 0.6% guar gum, 10% coconut oil; B2 = 0.5% xanthan gum, 0.5% guar gum, 10% coconut oil; B3 = 0.5% xanthan gum, 0.5% guar gum, 12% coconut oil; B4 = 0.5% xanthan gum, 0.5% guar gum, 0.2% CMC, 10% coconut oil; B5 = 0.5% xanthan gum, 0.5% guar gum, 0.2% CMC, 8% coconut oil.
Samples: Q1, Q2, Q3, Q4 = B1 under b1, b2, b3, b4, respectively; Q5, Q6, Q7, Q8 = B2 under b1, b2, b3, b4, respectively; Q9, Q10, Q11, Q12 = B3 under b1, b2, b3, b4, respectively; Q13, Q14, Q15, Q16 = B4 under b1, b2, b3, b4, respectively; Q17, Q18, Q19, Q20 = B5 under b1, b2, b3, b4 respectively.
Error bars are standard error of three replications (SEM).

4.2.4 Subjective rollability during storage

Subjective rollability scores ranged from 2.5 to 4.5 (with 1 as lowest and 5 highest) using a 1-cm diameter dowel during storage (Table 12). The rollability of products was affected by composition of formulation, baking temperature and storage time, but not baking time ($p>0.05$) (Appendix E-2). Rollability scores markedly increased when baking temperature was increased from 230 to 240°C. However, rollability decreased during storage ($p<0.05$), which agrees with previous reports on tortilla (Kelekci, Pascut, & Waniska, 2003; Barros et al. 2010).

Table 12 [1]Mean subjective rollability scores of GFWs in phase 2 during storage for 28 days (4 °C)

Sample	Storage time					
	Day 0	Day 4	Day 7	Day 14	Day 21	Day 28
Q1	3.5 ± 0.0^{bcd}	3.2 ± 0.3^{def}	3.0 ± 0.0^{defg}	2.5 ± 0.5^{fgh}	2.5 ± 0.0^{efg}	-
Q2	3.7 ± 0.3^{bc}	3.3 ± 0.3^{de}	2.8 ± 0.3^{efg}	2.7 ± 0.3^{efgh}	2.5 ± 0.0^{efg}	2.5 ± 0.0^{cd}
Q3	3.7 ± 0.3^{bc}	3.7 ± 0.3^{bcd}	3.7 ± 0.3^{bcd}	3.5 ± 0.0^{bcd}	3.5 ± 0.0^{bc}	3.3 ± 0.3^{bc}
Q4	3.3 ± 0.3^{cd}	3.3 ± 0.3^{de}	3.0 ± 0.0^{defg}	2.8 ± 0.3^{defg}	2.8 ± 0.3^{cdefg}	2.7 ± 0.3^{c}
Q5	2.5 ± 0.0^{f}	2.5 ± 0.0^{f}	1.8 ± 0.3^{h}	2.0 ± 0.0^{h}	2.2 ± 0.3^{g}	1.3 ± 0.3^{e}
Q6	3.0 ± 0.0^{def}	2.8 ± 0.3^{ef}	2.8 ± 0.3^{efg}	2.7 ± 0.3^{efgh}	2.5 ± 0.0^{efg}	2.5 ± 0.0^{cd}
Q7	3.5 ± 0.0^{bcd}	3.2 ± 0.3^{def}	3.5 ± 0.0^{bcde}	3.3 ± 0.3^{cde}	3.0 ± 0.0^{cdef}	3.0 ± 0.0^{c}
Q8	3.2 ± 0.3^{cde}	2.8 ± 0.3^{ef}	2.7 ± 0.3^{fg}	3.0 ± 0.0^{cdefg}	2.8 ± 0.3^{cdefg}	2.7 ± 0.3^{c}
Q9	2.7 ± 0.3^{ef}	2.5 ± 0.0^{f}	2.5 ± 0.0^{gh}	2.3 ± 0.3^{gh}	2.3 ± 0.3^{fg}	1.7 ± 0.6^{de}
Q10	3.2 ± 0.3^{cde}	3.2 ± 0.3^{def}	3.0 ± 0.5^{defg}	3.0 ± 0.0^{cdefg}	2.5 ± 0.5^{efg}	2.5 ± 0.5^{cd}
Q11	3.5 ± 0.0^{bcd}	3.3 ± 0.3^{de}	3.3 ± 0.3^{cdef}	3.3 ± 0.3^{cde}	3.2 ± 0.3^{cde}	2.8 ± 0.6^{c}
Q12	3.7 ± 0.3^{bc}	3.5 ± 0.0^{cde}	2.8 ± 0.3^{efg}	3.0 ± 0.0^{cdefg}	2.8 ± 0.3^{cdefg}	2.8 ± 0.3^{c}
Q13	3.2 ± 0.3^{cde}	3.7 ± 0.3^{bcd}	3.7 ± 0.3^{bcd}	3.5 ± 0.0^{bcd}	3.3 ± 0.3^{bcd}	-
Q14	4.0 ± 0.0^{ab}	3.7 ± 0.3^{bcd}	3.7 ± 0.3^{bcd}	3.5 ± 0.0^{bcd}	3.2 ± 0.3^{cde}	3.0 ± 0.0^{c}
Q15	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}	4.3 ± 0.3^{a}	4.3 ± 0.3^{a}
Q16	4.3 ± 0.3^{a}	4.3 ± 0.3^{ab}	3.7 ± 0.3^{bcd}	3.7 ± 0.3^{bc}	3.5 ± 0.5^{bc}	3.3 ± 0.3^{bc}
Q17	4.3 ± 0.3^{a}	4.2 ± 0.3^{abc}	3.7 ± 0.3^{bcd}	3.0 ± 0.0^{cdefg}	2.7 ± 0.3^{defg}	2.5 ± 0.0^{cd}
Q18	4.5 ± 0.0^{a}	4.3 ± 0.3^{ab}	4.0 ± 0.0^{abc}	3.2 ± 0.3^{cdef}	3.0 ± 0.0^{cdef}	2.7 ± 0.3^{c}
Q19	4.5 ± 0.0^{a}	4.3 ± 0.3^{ab}	4.2 ± 0.3^{ab}	4.2 ± 0.3^{ab}	4.0 ± 0.0^{ab}	4.0 ± 0.0^{ab}
Q20	4.5 ± 0.0^{a}	4.3 ± 0.3^{ab}	3.3 ± 0.3^{cdef}	3.3 ± 0.3^{cde}	3.3 ± 0.3^{bcd}	3.2 ± 0.3^{bc}

Notes: [1]mean (±SEM) of three replications;
Samples: Q1, Q2, Q3, Q4 = B1 under baking conditions b1, b2, b3, b4 respectively; Q5, Q6, Q7, Q8 = B2 under b1, b2, b3, b4, respectively; Q9, Q10, Q11, Q12 = B3 under b1, b2, b3, b4, respectively; Q13, Q14, Q15, Q16 = B4 under b1, b2, b3, b4, respectively; Q17, Q18, Q19, Q20 = B5 under b1, b2, b3, b4, respectively.
Within columns, means with different superscripts are significantly different (Tukey's test, $p<0.05$).
(-) = data not available. (At day 28, sample Q1 and sample Q13 had visible mould growth and rollability was not evaluated).

For all formulations (n = 5), samples under baking conditions b3 (240°C/2 min) and b4 (240°C/4 min) had higher mean rollability scores than the other two baking conditions during storage of the first three weeks (Table 13). Samples baked under condition b3 had the highest rollability scores while the lowest scores were under b1 (230°C/2 min). A steady decrease in

rrollability of samples under baking conditions b3 and b4 was observed during storage than under baking conditions b1 and b2 (Table 13). Descriptive statistics showed that rollability of samples under baking condition b3 was the most stable during storage (Table 13).

Table 13 Descriptive statistics of rollability during storage for 21 days (4°C) in phase 2

Sample	Mean	Standard deviation	Minimum	Maximum
Q1	2.9^{hij}	0.4	2.5	3.5
Q2	2.9^{ghi}	0.5	2.5	3.7
Q3	3.6^{cde}	0.1	3.3	3.7
Q4	3.0^{fghi}	0.3	2.7	3.3
Q5	2.1^{k}	0.4	1.3	2.5
Q6	2.7^{ij}	0.2	2.5	3.0
Q7	3.3^{defgh}	0.2	3.0	3.5
Q8	2.9^{hij}	0.2	2.7	3.2
Q9	2.3^{jk}	0.3	1.7	2.7
Q10	2.9^{ghij}	0.3	2.5	3.2
Q11	3.3^{defgh}	0.2	2.8	3.5
Q12	3.1^{efghi}	0.4	2.8	3.7
Q13	3.5^{cdefg}	0.2	3.2	3.7
Q14	3.5^{cde}	0.4	3.0	4.0
Q15	4.4^{a}	0.1	4.3	4.5
Q16	3.8^{bc}	0.4	3.3	4.3
Q17	3.4^{cdef}	0.8	2.5	4.3
Q18	3.6^{bcd}	0.8	2.7	4.5
Q19	4.2^{ab}	0.2	4.0	4.5
Q20	3.7^{bcd}	0.6	3.2	4.5

Notes: Samples: Q1, Q2, Q3, Q4 = B1 under baking conditions b1, b2, b3, b4 respectively; Q5, Q6, Q7, Q8 = B2 under b1, b2, b3, b4, respectively; Q9, Q10, Q11, Q12 = B3 under b1, b2, b3, b4, respectively; Q13, Q14, Q15, Q16 = B4 under b1, b2, b3, b4, respectively; Q17, Q18, Q19, Q20 = B5 under b1, b2, b3, b4, respectively.
Rollability scores: 1-5, with 1 as lowest and 5 highest.
Means with different superscripts are significantly different (Tukey's test, $p<0.05$).
Descriptive statistics of rollability were determined using data obtained from storage of GFW for 21 days (°C) as data at day 28 was unavailable.

Mean rollability of samples with formulation B1 during storage was higher than B2. There was probably insufficient mixture of xanthan gum and guar gum in B2 (1%) than in B1 (1.2%). Mixtures of xanthan gum and guar gum have been reported to improve rollability of corn tortilla (Platt-Lucero et al., 2010). The higher mean rollability of B3 than B2 may be attributed to increased level of coconut oil in the former formulation. Similar observations were reported by Maleki et al. (1981) and Gujral and Gaur (2002).

Samples made from formulations B4 and B5 had higher rollability than the other three formulations (B1, B2, B3) (Table 13). For samples with formulation B4 (0.5% xanthan gum,

0.5% guar gum, 0.2% CMC and 10% coconut oil), mean rollability scores ranged from 3.5 to 4.4 (Table 13). Meanwhile, with B5 (0.5% xanthan gum, 0.5% guar gum, 0.2% CMC and 8% coconut oil) rollability scores ranged from 3.4 to 4.2 (Table 13). The mean rollability scores of samples (Q13-Q20) with the two formulations (B4 and B5) during storage were >3 which is considered acceptable (Guo, Jackson, Graybosch, & Parkhurst, 2003). The high rollability of the products may be attributed to functional properties of CMC (Friend et al., 1993). The functions of CMC and xanthan-guar gum (1:1) in the formulation were probably synergistic (Mohammadi et al., 2014; Zhang & Kong, 2006). The slightly higher rollability of samples from B4 may be due to the higher concentration of coconut oil than with B5. This observation was consistent with the report by Gujral and Gaur (2002) when liquid oil was added to flatbread formulation. With respect to human health, consumption of oil adds calories of products (Heini & Weinsier, 1997). As hydrocolloids can replace part of shortening in bakery products, the coconut oil may need to be reduced for public health nutrition (Kaur, Singh, & Kaur, 2000).

4.2.5 Mould growth

Food products containing high water activity are generally susceptible to microbial spoilage (Rahman & Labuza, 1999). Baked bread is normally associated with growth of moulds, and some strains can be pathogenic (Smith et al., 2004). In phase 3, all the samples were mould-free during storage (4°C) for 21 days. However, after 4 weeks, only two samples (Q1 and Q13) had visible mould growth characterized by grey green spots (Liu et al., 2011). Water activity in the baked GFWs ranged from 0.84 to 0.92. Samples (Q5, Q9, Q13 and Q17) made from formulations B2, B3, B4 and B5 respectively, had water activity slightly higher than 0.91 under baking condition b1. According to Troller (2012), baked bread is expected to have water activity lower than 0.91 to minimise microbial spoilage.

4.2.6 Conclusion

Using baking temperature of 240°C for 4 min increased baking weight loss and decreased water activity. Higher baking temperature increased rollability while baking time had no effect on baked GFWs. Presence of xanthan-guar gum and CMC in the formulations

increased rollability of GFWs during storage. All samples had no visible mould growth during storage (4°C) for three weeks.

4.3 Results and Discussion: Phase 3

Based on results of phase 2, the most promising products (made from formulation B4) contained 1% xanthan-guar gum, 0.2% CMC and 10% coconut oil under baking condition b3 (240°C/2 min). However, the effect of each tested ingredient (coconut oil, CMC and xanthan-guar gum) was not investigated. Therefore, the main of objective of phase 3 was to optimise the tested ingredients in the basic formulation. The Taguchi method was thus used to optimise levels of the test ingredients in nine formulations. In this phase, the baked products were compared by measuring baking weight loss, colour, water activity, objective texture and subjective rollability.

4.3.1 Baking weight loss

Baking weight loss of samples, which ranged from 24.1 to 30.8% (Figure 13), was affected by formulations ($p<0.05$). Formulation C6 had the lowest baking weight loss while C1 had the highest. Baking weight loss for samples C2 and C4 was not different ($p<0.05$), whilst the baking weight losses for samples C3, C5, C6, C7, C8 and C9 were comparable ($p<0.05$).

Effects of test ingredients (coconut oil, CMC, xanthan-guar gum) levels on baking weight loss are shown in Figure 15. When coconut oil was increased from 8 to 10%, baking weight loss significantly decreased from 27.6 to 25.1%, while further increase of the oil (10 to 12%) in the formulation did not affect baking weight loss ($p<0.05$). Similar results were reported by Ozmutlu et al. (2001) when shortening was increased from 8 to 16%. High decrease of baking weight loss was observed when xanthan-guar gum was increased from 0.8 to 1.2%. This result may be associated with the water retention capacity of hydrocolloids in GFWs thereby preventing water loss (Guarda et al., 2004). With increased levels of CMC (0.1 to 0.2%), high decrease in baking weight loss (27.2 to 25.5%) was also observed. The results were similar to a study on gluten-free flatbread in which CMC decreased moisture loss during baking (Mohammadi et al., 2014). However, no marked changes in baking weight loss were

observed when CMC was increased from 0.2 to 0.3%.

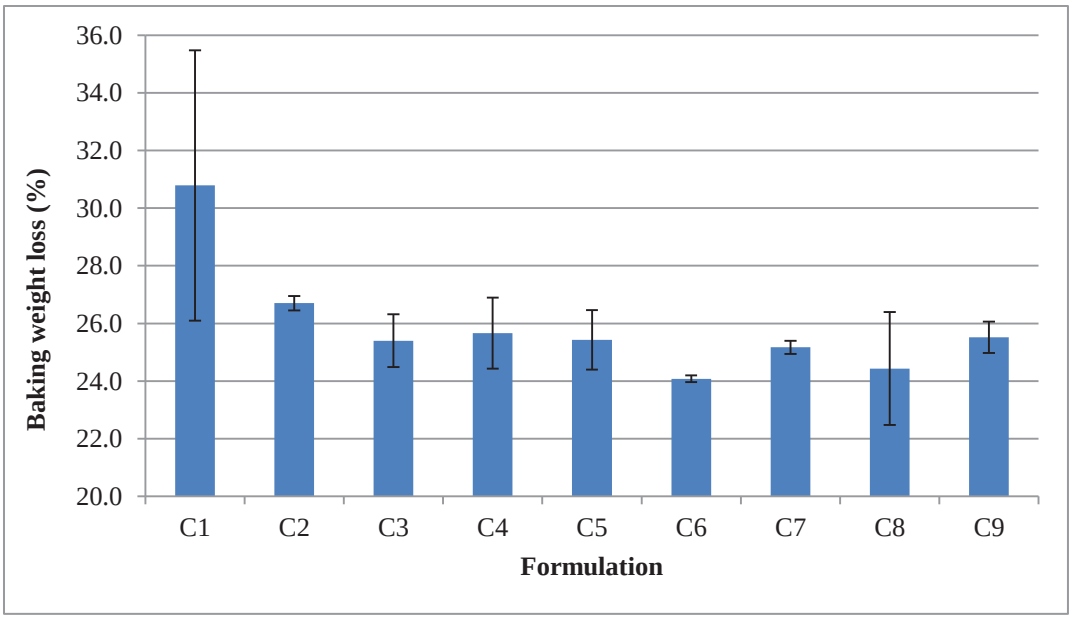

Figure 14 Baking weight loss (%) of GFWs for nine formulations in phase 3

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Error bars are standard error of three replications (SEM).

Results suggest that coconut oil affected baking weight loss of GFW samples ($p<0.05$), while the presence of xanthan-guar gum and CMC did not show any impact ($p>0.05$). Taguchi method showed that coconut oil had the highest impact on baking weight loss than the other two ingredients (coconut oil>CMC>xanthan-guar gum). Results showed that 12% coconut oil was suitable to produce GFW with optimal bread weight loss.

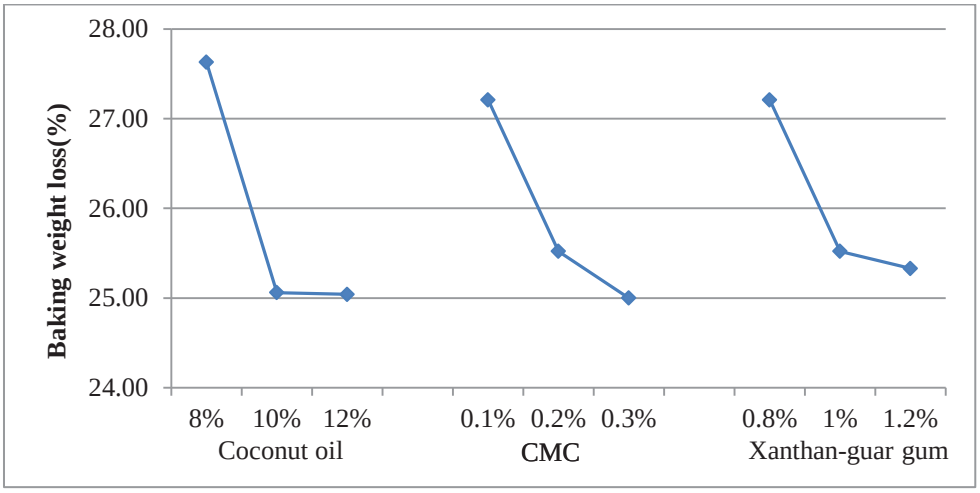

Figure 15 Main effects plot for mean baking weight loss (%)

4.3.2 Colour

L* (lightness) of samples in phase 3, ranged from 69.06 to 75.69 (Figure 16), was not significantly affected by formulations ($p>0.05$). Formulation C4 had the lowest L* while C1 had the highest. The L* value among the remaining seven (7) formulations was not different ($p<0.05$). Shalini and Laxmi (2007) reported similar results that lightness was not affected by hydrocolloids.

Indices of whiteness of GFW samples ranged from 62.56 and 70.77 in phase 3 (Figure 17). Formulations in phase 3 had significant effects on whiteness index of GFWs ($p<0.05$). Formulation C4 and C7 had the lowest whiteness index while C1 had the highest. Whiteness indices of the remaining 6 formulations were not different ($p<0.05$).

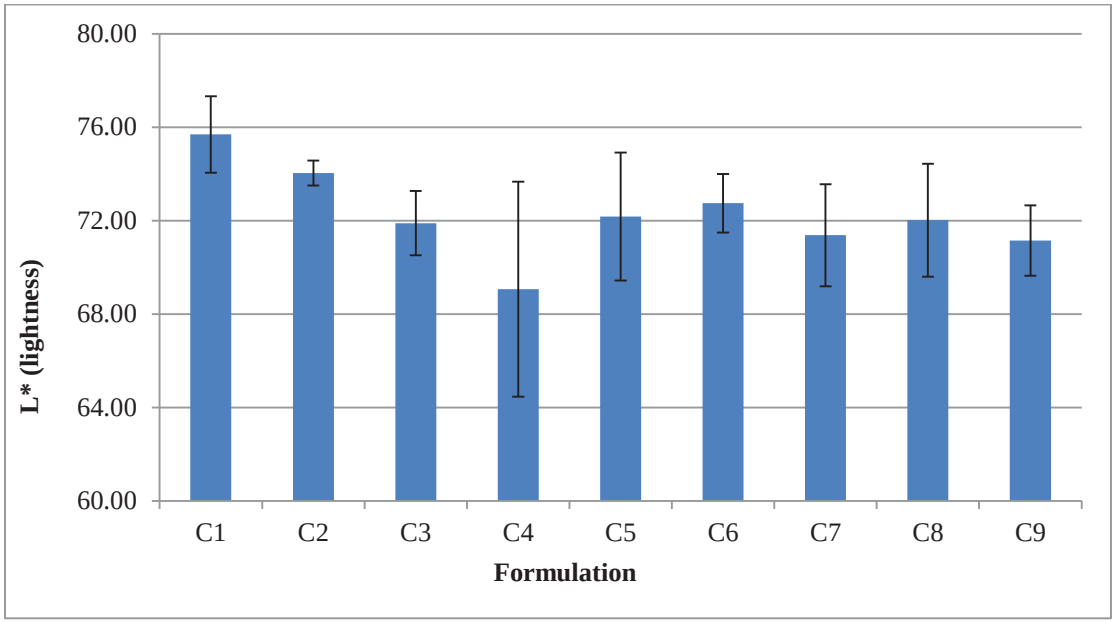

Figure 16 L* (lightness) of GFWs for nine formulations in phase 3

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Error bars are standard error of three replications (SEM).

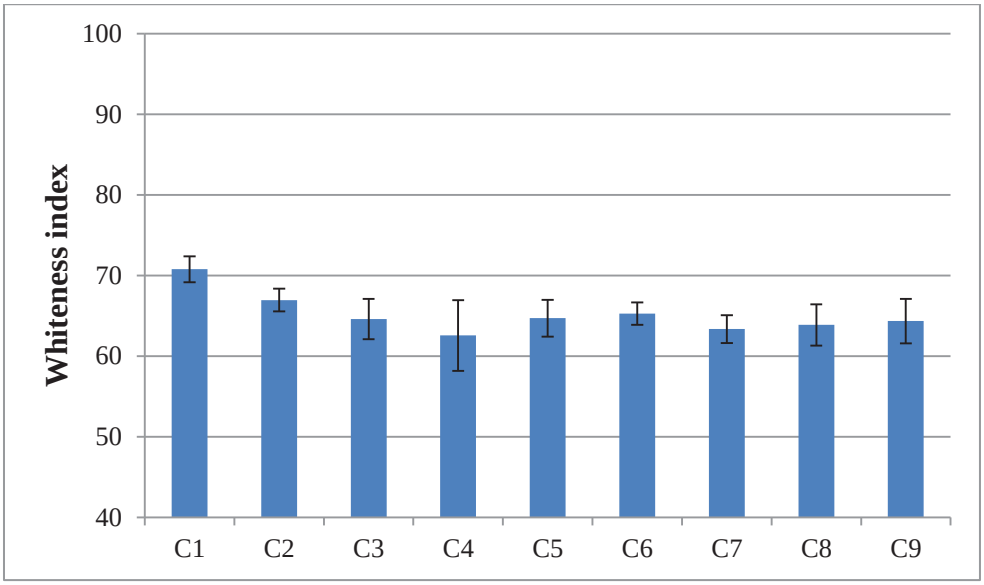

Figure 17 Whiteness indices of GFWs for nine formulations in phase 3

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Error bars are standard errors of three replications (SEM).

Effects of test ingredients (coconut oil, CMC, xanthan-guar gum) levels on whiteness index are shown in Figure 18. When coconut oil was increased from 8 to 10%, whiteness index decreased from 67.44 to 64.18 ($p<0.05$). This phenomenon may be caused by increased rate of heating of bread due to presence of high levels of coconut oil, thus leading to faster browning (Chin et al., 2010). Another possible reason was that the effect of carotene in coconut oil can generate yellowish-orange of baked products (Chin et al., 2010). Further increase of coconut oil in formulation from 10 to 12% slightly decreased whiteness index (64.18 to 63.86). Similar trends were also noted when levels of xanthan-guar gum were increased. When CMC was increased from 0.1 to 0.3%, whiteness index of samples slightly decreased (65.56 to 64.74).

Coconut oil affected whiteness index of GFW samples ($p<0.05$), while the presence of xanthan-guar gum and CMC did not show any significant effect ($p>0.05$). Based on results in Figure 18, it is assumed that high levels of coconut oil in formulation contributed to the brownness of the baked products. Meanwhile, low concentrations of coconut oil may result in a whiter product.

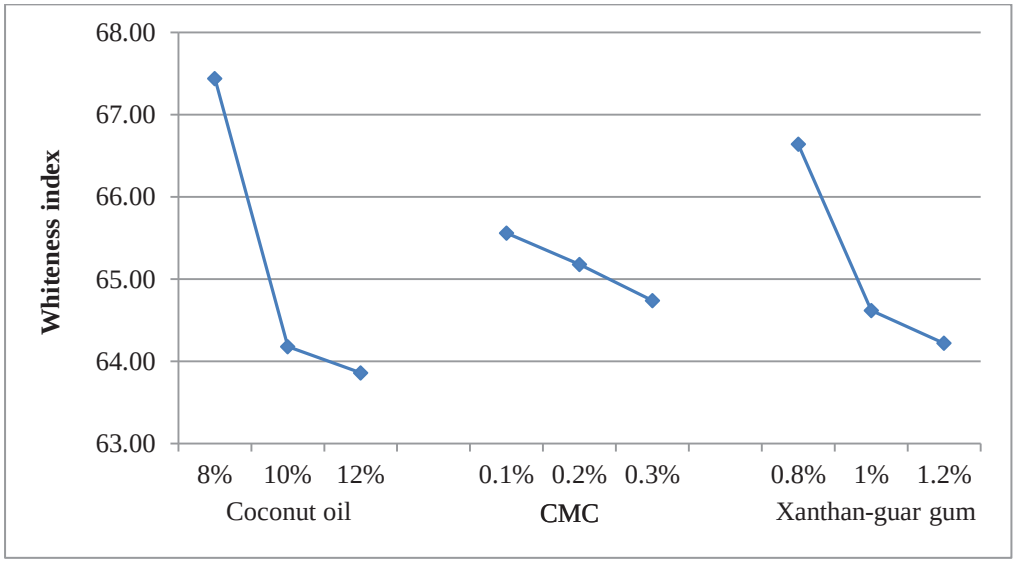

Figure 18 Main effects plot for mean whiteness index

4.3.3 Water activity during storage

Water activity was determined on nine GFWs samples during storage (4°C) for 21 days. In phase 3, water activity of samples ranged from 0.83 to 0.93 (Figure 19) and was affected by formulations ($p<0.05$). Results show that storage time did not the affect water activity of products ($p>0.05$) (Appendix E-3) in contrast with the report by Lazaridou et al. (2007) on gluten-free bread.

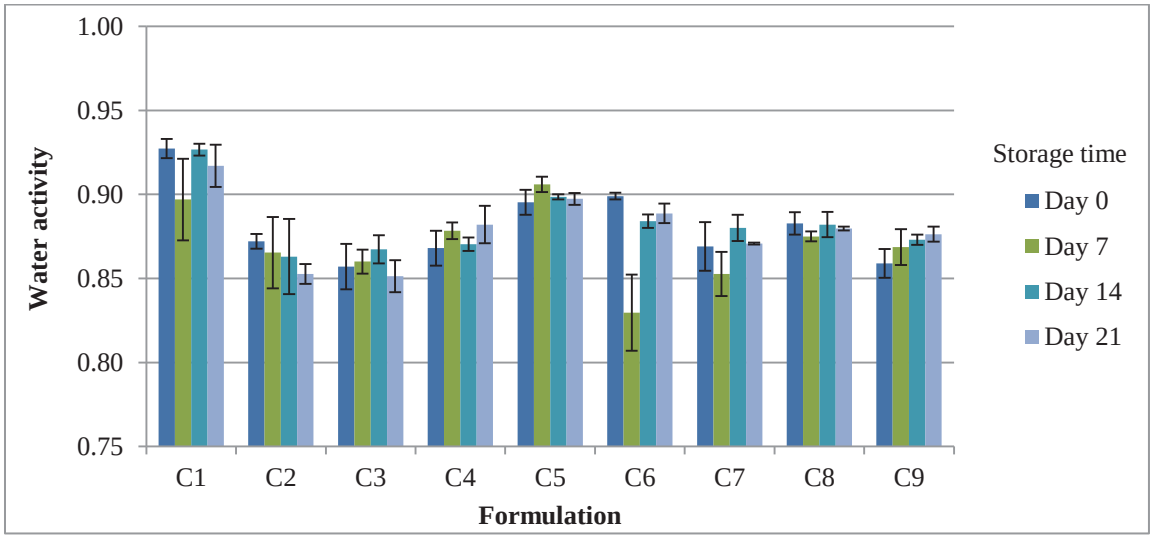

Figure 19 Water activity of GFWs for nine formulations during storage for 21 days (4°C)

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Error bars are standard error of three replications (SEM).

Formulations C1 and C5 had the highest mean water activity (0.92 and 0.90, respectively), whereas, formulation C3 had the lowest (0.86) during storage (Table 14). Mean water activity of samples in formulations C2, C4, C6, C7 and C9 was not significantly different ($p>0.05$), which was consistent with findings by Lazaridou et al. (2007).

Table 14 Descriptive statistics of water activity during storage for 21 days (4 °C) in phase 3

Sample	Mean	Standard deviation	Minimum	Maximum
C1	0.92[a]	0.01	0.90	0.93
C2	0.86[ab]	0.01	0.85	0.87
C3	0.86[c]	0.01	0.85	0.87
C4	0.87[ab]	0.01	0.87	0.88
C5	0.90[a]	0.00	0.90	0.91
C6	0.88[ab]	0.03	0.83	0.90
C7	0.87[ab]	0.01	0.85	0.88
C8	0.88[b]	0.00	0.88	0.88
C9	0.87[ab]	0.01	0.86	0.88

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Means with different superscripts are significantly different (Tukey's test, $p<0.05$).

Effects of test ingredients (coconut oil, CMC, xanthan-guar gum) on water activity are shown in Figure 20. Mean water activity slightly increased when coconut oil was increased from 8 to 10%. Further increase of coconut oil in formulations from 10 to 12% slightly decreased water activity from 0.88 to 0.87. When CMC was increased from 0.1 to 0.3%, water activity of samples decreased slightly (0.89 to 0.87). Similarly, water activity decreased from 0.89 to 0.87 with increased xanthan-guar gum (0.8 to 1%). Reduction in water activity may be due to competition of hydrocolloids with bread polymers such as proteins and starch (Schiraldi, Piazza, & Riva, 1996). However, further increase of xanthan-guar gum resulted in a minor increase of water activity.

Xanthan-guar gum and CMC significantly affected water activity of GFW samples ($p<0.05$) while the presence of coconut oil did not ($p>0.05$). Taguchi method showed that xanthan-guar gum had the highest impact on water activity (xanthan-guar gum>CMC>coconut oil) than the other two ingredients. With respect to water activity, results suggest that the optimal level for the test ingredients was 0.3% CMC and 1% xanthan-guar gum.

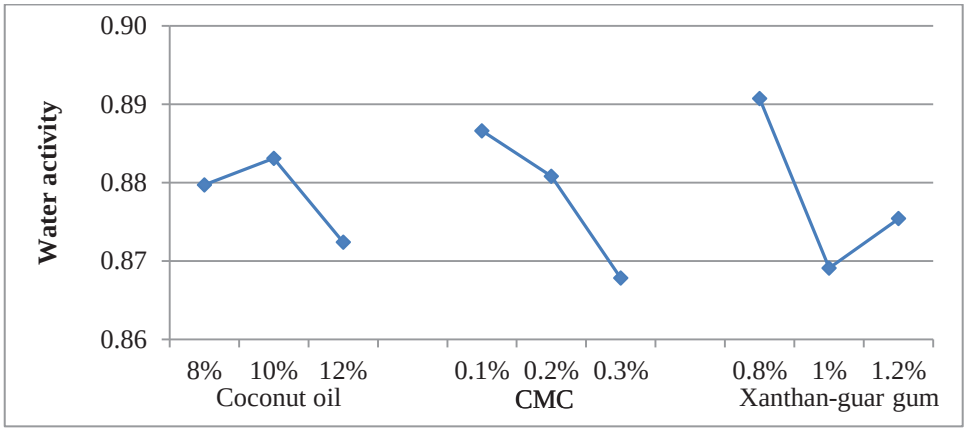

Figure 20 Main effects plot for mean water activity during storage

4.3.4 Subjective rollability during storage

Rollability test using a 1-cm diameter dowel was conducted on nine GFWs samples during storage (4°C) for 21 days. Rollability scores in phase 3 ranged from 2.7 to 5.0 (Figure 21); the scores were significantly affected by formulations and storage time ($p<0.05$) (Appendix E-3). During storage, rollability scores of all samples decreased which concurred with Kelekci et al. (2003).

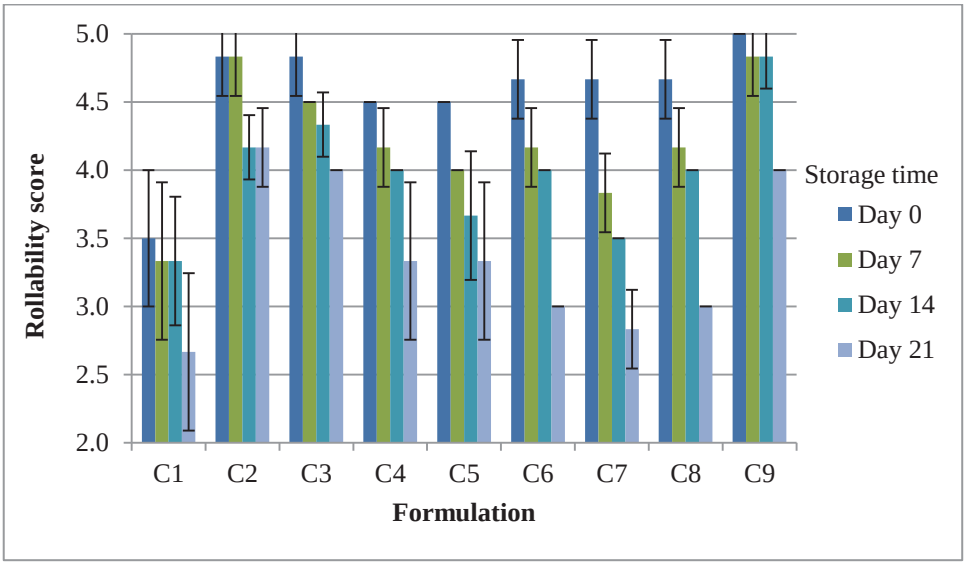

Figure 21 Rollability scores of GFWs for nine formulations during storage for 21 days (4°C)

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum.
Error bars are standard error of three replications (SEM); Rollability scores: 1-5, with 1 as lowest and 5 highest.

Mean rollability scores versus storage time for all formulations were >3 (Table 15). Samples made with formulation C9 had the highest mean rollability score (4.7), whereas, formulation C1 had the lowest (3.2). GFW samples made with six formulations (C2, C3, C4, C6, C8, and C9) had rollability score of >4 during storage. Rollability of samples in formulation C6, C7 and C8 was less stable than other six formulations during storage.

Table 15 Descriptive statistics of rollability scores during storage for 21 days (4°C) in phase 3

Sample	Mean	Standard deviation	Minimum	Maximum
C1	3.2^{d}	0.4	2.7	3.5
C2	4.5^{ab}	0.4	4.2	4.8
C3	4.4^{abc}	0.3	4.0	4.8
C4	4.0^{abc}	0.5	3.3	4.5
C5	3.9^{bcd}	0.5	3.3	4.5
C6	4.0^{abc}	0.7	3.0	4.7
C7	3.7^{cd}	0.8	2.8	4.7
C8	4.0^{abc}	0.7	3.0	4.7
C9	4.7^{a}	0.5	4.0	5.0

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum;

C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Rollability scores: 1-5, with 1 as lowest and 5 highest.
Means with different superscripts are significantly different (Tukey's test, $p<0.05$).
Descriptive statistics of rollability were determined using data obtained from storage of GFW for 21 days (°C).

Effects of test ingredients (coconut oil, CMC, xanthan-guar gum) levels on rollability are shown in Figure 22. When coconut oil was increased from 8 to 10%, mean rollability slightly decreased (4.0 to 3.9). Further increase of coconut oil in the formulations from 10 to 12% slightly increased rollability from 3.9 to 4.1. When CMC was increased from 0.1 to 0.3%, rollability of samples increased from 3.6 to 4.3, which was consistent with findings by Friend et al. (1993). When xanthan-guar gum was increased from 0.8 to 1%, rollability increased from 3.7 to 4.4. However, further increase of xanthan-guar gum resulted in minor decrease of rollability (4.4 to 4.0), suggesting that the optimum level may be 1%.

Xanthan-guar gum and CMC significantly affected rollability of GFW samples ($p<0.05$), while the presence of coconut oil did not ($p>0.05$). Taguchi method showed that CMC had the highest impact on rollability (CMC>xanthan-guar gum>coconut oil). Based on rollability results, the optimal levels for the test ingredients using Taguchi method were 0.3% CMC and 1% xanthan-guar gum.

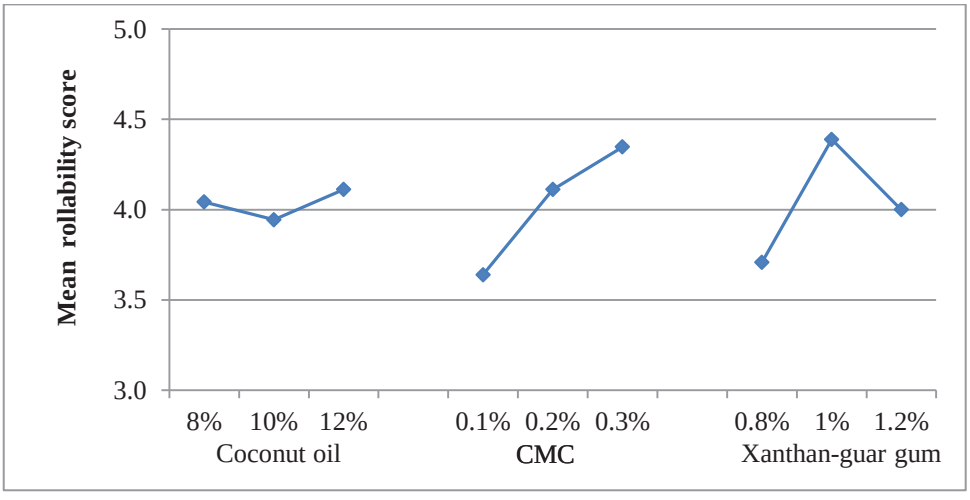

Figure 22 Main effects plot for mean rollability score during storage

(Rollability scores: 1-5, with 1 as lowest and 5 highest)

4.3.5 Objective textural properties of GFWs during storage

Objective texture test was conducted on nine GFWs samples during storage (4°C) for 21 days. The rupture distance and rupture force describes the extensibility and firmness of the flatbreads, respectively. Softer GFWs are reflected by smaller rupture force while longer rupture distance indicates more extensible flatbreads (De-Barros, 2009).

Rupture distance of GFW samples ranged from 5.48 to 13.97 mm (Figure 23) and was affected by formulations and storage time ($p<0.05$) (Appendix E-3). During storage, rupture distance of all samples decreased, which was similar to the report by Mao and Flores (2001).

Formulation C9 had the highest mean rupture distance (12.72 mm), whereas, formulation C7 had the lowest (8.55 mm) during storage (Table 16). Mean rupture distance for C2, C3 and C9 were >11 mm, higher than other formulations (<10 mm). Descriptive statistics indicated that rupture distance for C3 during storage was more stable than other formulations during storage (Table 16).

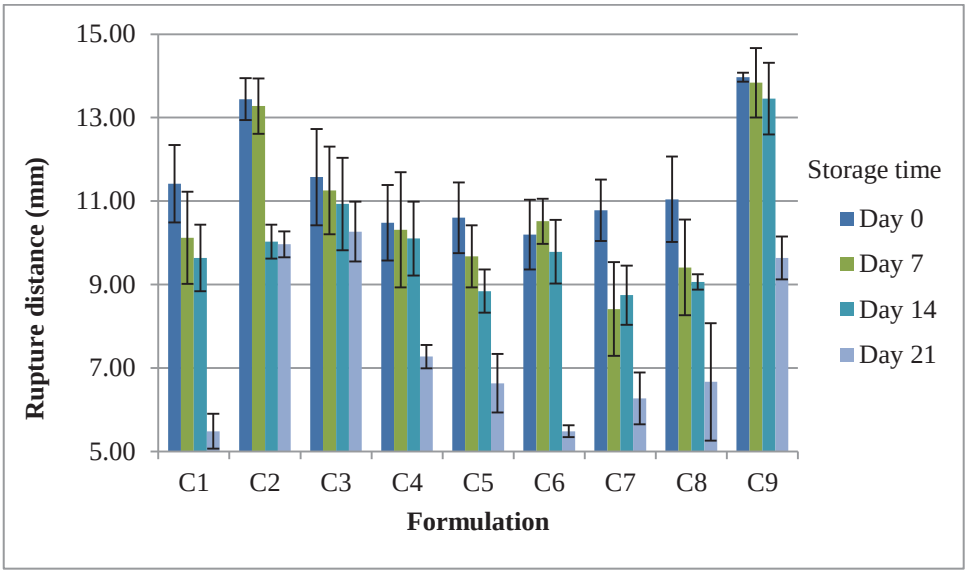

Figure 23 Rupture distance (mm) of GFWs for nine formulations during storage for 21 days (4 °C)

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Error bars are standard error of three replications (SEM).

Table 16 Descriptive statistics (mm) of rupture distance during storage for 21 days (4 °C) in phase 3

Sample	Mean	Standard Deviation	Minimum	Maximum
C1	9.16^{cd}	2.57	5.48	11.42
C2	11.68^{ab}	1.94	9.97	13.44
C3	11.01^{abc}	0.56	10.27	11.57
C4	9.54^{bcd}	1.52	7.27	10.48
C5	8.94^{cd}	1.70	6.63	10.60
C6	9.00^{cd}	2.36	5.48	10.52
C7	8.55^{d}	1.85	6.27	10.78
C8	9.05^{cd}	1.81	6.67	11.04
C9	12.72^{a}	2.07	9.64	13.97

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Means with different superscripts are significantly different (Tukey's test, $p<0.05$).

Effects of test ingredients (coconut oil, CMC, xanthan-guar gum) on rupture distance are shown in Figure 24. Rupture distance of samples decreased from 10.62 to 9.16 mm with increased coconut oil (8 to 10%), and decreased with further increases in oil to 12%. Increasing amounts of CMC resulted in increased rupture distance. An increase in the extensibility of wheat chapatti with CMC has also been reported (Gujral & Pathak, 2002). When xanthan-guar gum was increased from 0.8 to 1%, rupture distance increased from 9.07 to 11.31 mm. However, further increase of xanthan-guar gum (1.2%) led a decrease of rupture distance (9.45 mm). It has also been reported that additions of xanthan-guar gum at 1% (w/w) in gluten-free chapatti resulted in higher extensibility (Gujral & Rosell, 2004).

Coconut oil, xanthan-guar gum and CMC significantly affected rupture distance of GFW samples ($p<0.05$). Results using Taguchi method showed that xanthan-guar gum had the highest effect on rupture distance of GFWs (xanthan-guar gum>CMC>coconut oil). Regarding of rupture distance, the optimal levels for the test ingredients of GFWs was 8% coconut oil, 0.3% CMC and 1% xanthan-guar gum.

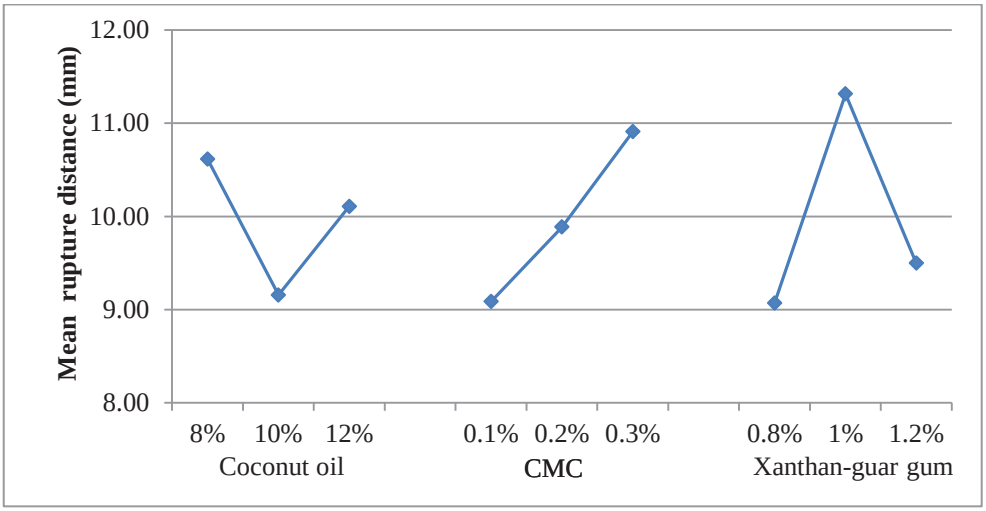

Figure 24 Main effects plot for mean rupture distance (mm) during storage

Rupture force ranged from 77 to 422 g during storage (Figure 25), which was significantly affected by formulations and storage time ($p<0.05$) (Appendix E-3). During storage, changes of rupture force differed in formulations.

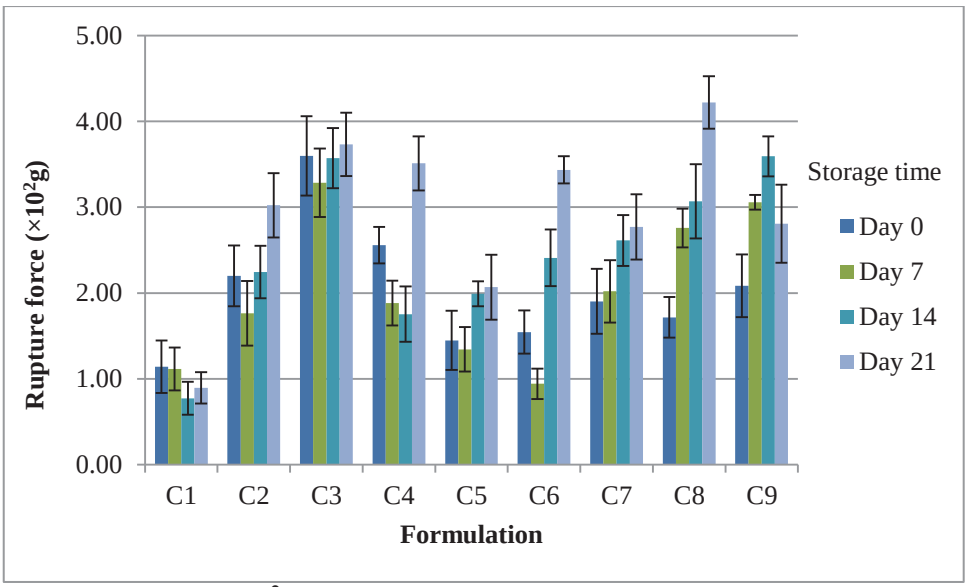

Figure 25 Rupture force ($\times 10^2$ g) of GFWs for nine formulations during storage for 21 days (4 °C)

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Error bars are standard error of three replications (SEM).

Formulation C3 had the highest mean rupture force (355 g), whereas, formulation C1 had the lowest mean rupture force (98 g) during storage (Table 17). Descriptive statistics showed that the firmness of samples in formulations C1 and C3 was more stable than other formulations during storage (Table 17).

Effects of test ingredients (coconut oil, CMC, xanthan-guar gum) levels on rupture force are shown in Figure 26. When coconut oil was increased from 8 to 10%, rupture force of samples decreased. Increasing the level of shortening may reduce the firmness of bread (Ghiasi, Hoseney, Zeleznak, & Rogers, 1984). However, further increases of coconut oil in formulation to 12% increased rupture force to 272 g. When CMC was increased from 0.1 to 0.3%, rupture force of samples increased. Therefore, the increase in CMC enhanced firmness of GFWs (Onyango, Unbehend, & Lindhauer, 2009). When xanthan-guar gum was increased from 0.8 to 1%, rupture force increased from 200 to 254 g. This result was probably attributed to strengthening effect of xanthan gum on bread structure which was consistent with previous reports (Lazaridou et al., 2007; Schober, Bean, & Boyle, 2007). However, rupture force did not change with further increases of xanthan-guar gum (1.2%). According to Sabanis and Tzia (2011), the firmness of gluten-free bread significantly increased when xanthan gum was increased from 0 to 1%. Further increases of the hydrocolloids did not significantly increase firmness.

Table 17 Descriptive statistics of rupture force ($\times 10^2$ g) during storage for 21 days (4 °C) in phase 3

Sample	Mean	Standard deviation	Minimum	Maximum
C1	0.98^{e}	0.18	0.77	1.14
C2	2.31^{bcd}	0.52	1.76	3.02
C3	3.55^{a}	0.19	3.28	3.73
C4	2.43^{bcd}	0.80	1.75	3.51
C5	1.71^{de}	0.37	1.34	2.07
C6	2.08^{cd}	1.08	0.94	3.43
C7	2.33^{bcd}	0.43	1.90	2.77
C8	2.94^{ab}	1.03	1.72	4.22
C9	2.88^{abc}	0.63	2.08	3.59

Notes: Formulation C1 = 8% coconut oil, 0.1% CMC, 0.8% xanthan-guar gum; C2 = 8% coconut oil, 0.2% CMC, 1% xanthan-guar gum; C3 = 8% coconut oil, 0.3% CMC, 1.2% xanthan-guar gum; C4 = 10% coconut oil, 0.1% CMC, 1% xanthan-guar gum; C5 = 10% coconut oil, 0.2% CMC, 1.2% xanthan-guar gum; C6 = 10% coconut oil, 0.3% CMC, 0.8% xanthan-guar gum; C7 = 12% coconut oil, 0.1% CMC, 1.2% xanthan-guar gum; C8 = 12% coconut oil, 0.2% CMC, 0.8% xanthan-guar gum; C9 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum. Means with different superscripts are significantly different (Tukey's test, $p<0.05$).

Coconut oil, xanthan-guar gum and CMC significantly affected rupture force of GFW samples ($p<0.05$). Results using Taguchi method showed that CMC had the highest effect on rupture force (firmness) of GFWs (CMC>coconut oil>xanthan-guar gum).

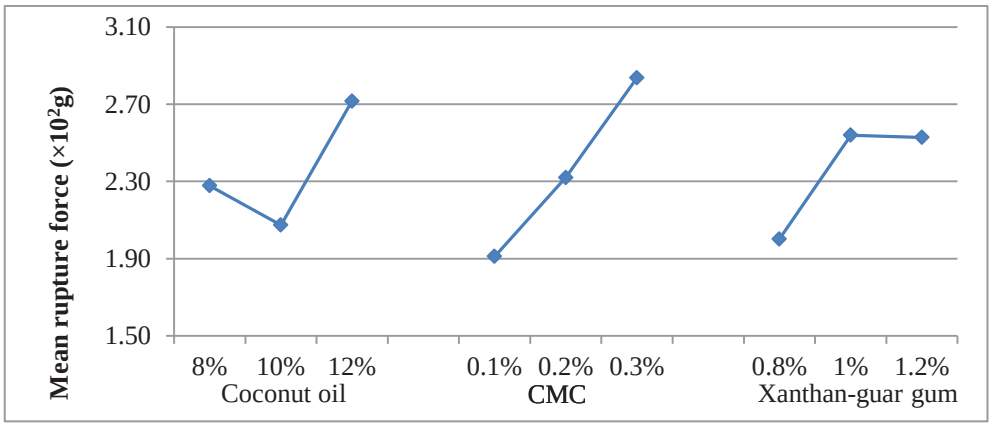

Figure 26 Main effects plot for mean rupture force ($\times 10^2$ g) during storage

4.3.6 Optimisation of formulations

In this phase, baking weight loss, colour parameters (L*, whiteness index), water activity, subjective rollability, rupture force and distance, (objective firmness and extensibility) of GFWs were analysed. Optimum levels for xanthan-guar gum and CMC were 1% and 0.3%, respectively. The presence of hydrocolloids in the products improved (increased) rupture distance and rollability. The level of coconut oil that minimized baking weight loss and maximized rupture distance was 12% and 8%, respectively. Thus three promising formulations made by three levels of coconut oil (8, 10, and 12 %) would be investigated in phase 4.

4.3.7 Conclusion

Statistical analysis showed that coconut oil had effect on baking weight loss and whiteness index of GFWs ($p<0.05$). CMC and xanthan-guar gum affected water activity and rollability of GFWs ($p<0.05$). All the three test ingredients affected rupture distance (extensibility) and rupture force (firmness) of GFW samples. Optimised ingredients in formulations using Taguchi method were 0.3% CMC, 1% xanthan-guar gum.

4.4 Results and Discussion: Phase 4

In this phase, three promising formulations from phase 3 shown in Table 18 were used to produce samples in Table 18. Sensory evaluation and physical tests were applied to analyse and compare products at day 1, 7 and 14 during storage (4°C).

Table 18 Test ingredients (%, w/w flour basis) of basic formulation used in phase 4

Sample	Coconut oil	CMC	Xanthan-guar gum
D1	8	0.3	1
D2	10	0.3	1
D3	12	0.3	1

Note: CMC = carboxmethyl cellulose.

4.4.1 Physical characteristics of GFWs

Physical characteristics of samples from three the formulations are shown in Table 19. Baking weight losses and whiteness indices ($p>0.05$) were similar among the samples. With respect to L* (lightness), D1 had the lightest colour while D3 had the darkest ($p<0.05$). Results suggest that water activity, subjective rollability, rupture distance and rupture force of GFW samples in phase 4 were affected by storage time ($p<0.05$, Appendix E-4). Overall, rollability, water activity and rupture distance (mm) decreased while rupture force ($\times 10^2$ g) increased during storage which may be attributed to moisture loss (Lazaridou et al., 2007). Rollability and water activity were similar among the three samples (Table 19). Meanwhile, rupture distance and rupture force of three samples were also similar at day 1. At days 7 and 14, the two parameters of D3 were higher than D1 and D2. D3 had more stable rupture distance than the other two samples. Extensibility and firmness of D3 was also higher than D1 and D2 during storage for two weeks (4°C).

Table 19 [1]Mean values for physical parameters of GFWs in phase 4

Parameter	Storage time	D1	D2	D3
BWL (%)	Fresh	26.1±2.3[a]	26.6±1.1[a]	25.5±0.9[a]
L*	Fresh	75.87±2.11[a]	74.52±1.25[ab]	72.14±2.54[b]
WI	Fresh	68.12±1.84[a]	67.25±1.78[a]	65.51±2.10[a]
Water activity	Day 1	0.87±0.00[a]	0.87±0.01[a]	0.88±0.00[a]
	Day 7	0.87±0.01[a]	0.87±0.00[a]	0.87±0.01[a]
	Day 14	0.86±0.00[a]	0.86±0.01[a]	0.86±0.00[a]
Rollability score	Day 1	5.0±0.0[a]	5.0±0.0[a]	5.0±0.0[a]
	Day 7	4.8±0.3[a]	4.8±0.3[a]	5.0±0.0[a]
	Day 14	4.8±0.3[a]	4.8±0.3[a]	4.8±0.3[a]
Rupture distance	Day 1	14.5±1.2[a]	13.7±0.9[a]	14.9±1.0[a]
	Day 7	12.0±1.5[b]	13.3±1.0[ab]	14.3±0.8[a]
	Day 14	12.5±1.0[a]	10.4±1.0[b]	13.8±1.9[a]
Rupture force	Day 1	221±31[a]	225±15[a]	205±18[a]
	Day 7	196±27[b]	191±11[b]	319±40[a]
	Day 14	232±26[b]	233±31[b]	335±30[a]

Notes: [1]mean (±SEM), (n=3). Within rows, mean value followed by different superscripts are significantly different (Tukey's test, $p<0.05$); Rollability data were not normally distributed, and was therefore analysed by Kruskal-Wallis' test ($p<0.05$); Baking weight loss, L* and whiteness index were measured in fresh samples only. The rest of the parameters were measured during storage for 14 days (4°C); BWL = baking weight loss; WI = whiteness index.

4.4.2 Sensory evaluation of GFWs

Results of consumer sensory evaluation of products in phase 4 are shown in Figure 27 to Figure 31. Mean sensory attribute of D1 decreased during storage (4°C) with overall acceptability of the products ranging from 6.4 to 5.9 (Appendix E-4). Mean overall acceptability of D2 decreased (6.4 to 5.5) during storage, with a slightly higher decrease from day 7 (6.2) to 14 (5.5). Mean appearance and mean texture of D2 also decreased during storage. Meanwhile, mean aroma and mean taste of D2 increased. Work done by El-Khoury (1999) and Rosado, Cassís, Solano, and Duarte-Vázquez (2005) on aroma and taste of flatbread reported similar results. Overall, mean acceptability of D3 slightly decreased from day 0 to day 7 (6.6 to 6.2) and then stabilised between days 7 and 14 (Appendix E-4), whereas appearance of D3 received the highest scores on day 7 during storage. Taste scores of D3 were similar between days 1 and 14, while at day 7 was slightly low. Overall, texture scores of all GFWs decreased during storage, which may be explained by decreased water activity, rupture distance and increased rupture force observed in Table 19. During storage, the texture of GFWs became brittle and hard, probably due to water loss which may have affected the sensory textural scores. The sensory (texture) of scores suggested that increased firmness of the products did not appeal to consumers.

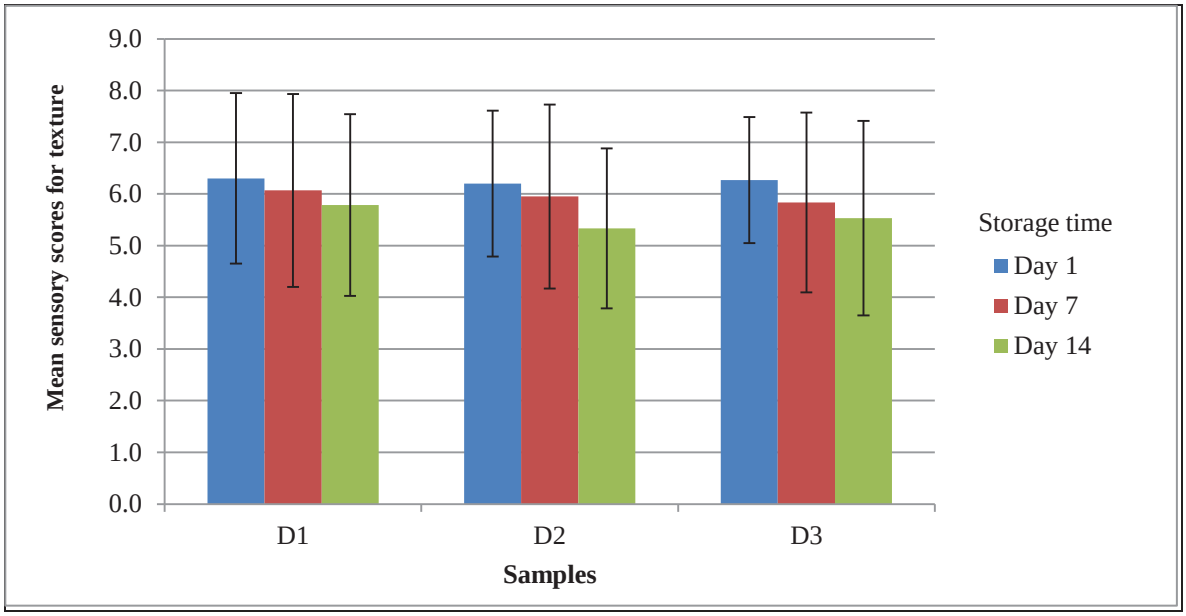

Figure 27 Texture of GFW samples during storage for 14 days (4°C) in phase 4

Notes: Formulation D1 = 8% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D2 = 10% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D3 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum.
Errors bars are standard deviation of 60 panellists.

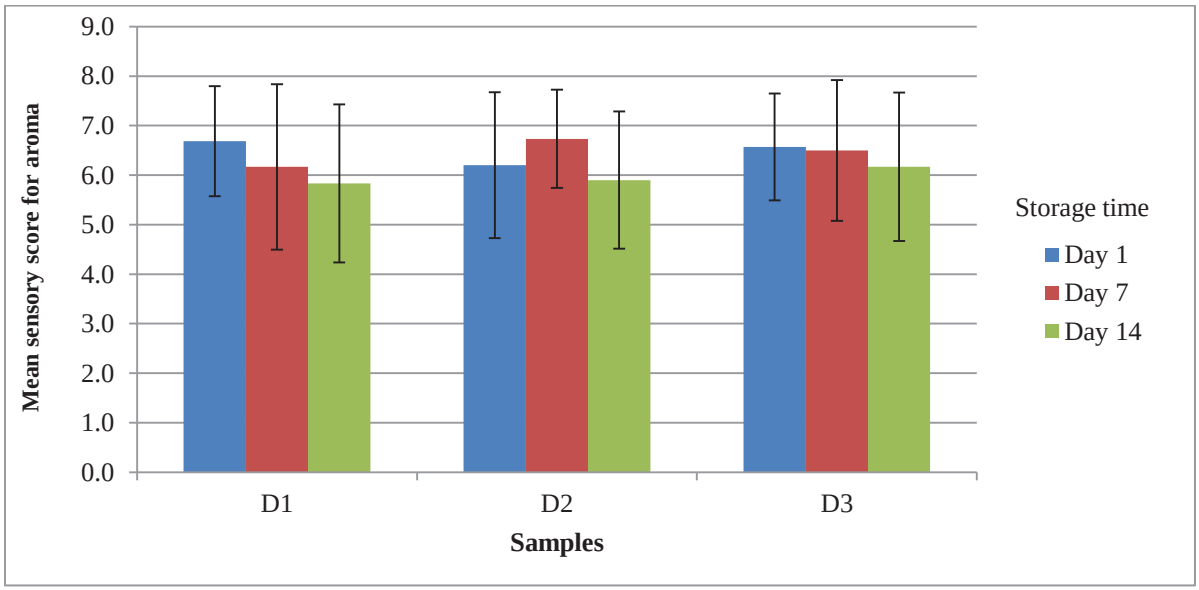

Figure 28 Aroma of GFW samples during storage for 14 days (4°C) in phase 4

Notes: Formulation D1 = 8% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D2 = 10% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D3 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum.
Errors bars are standard deviation of 60 panellists.

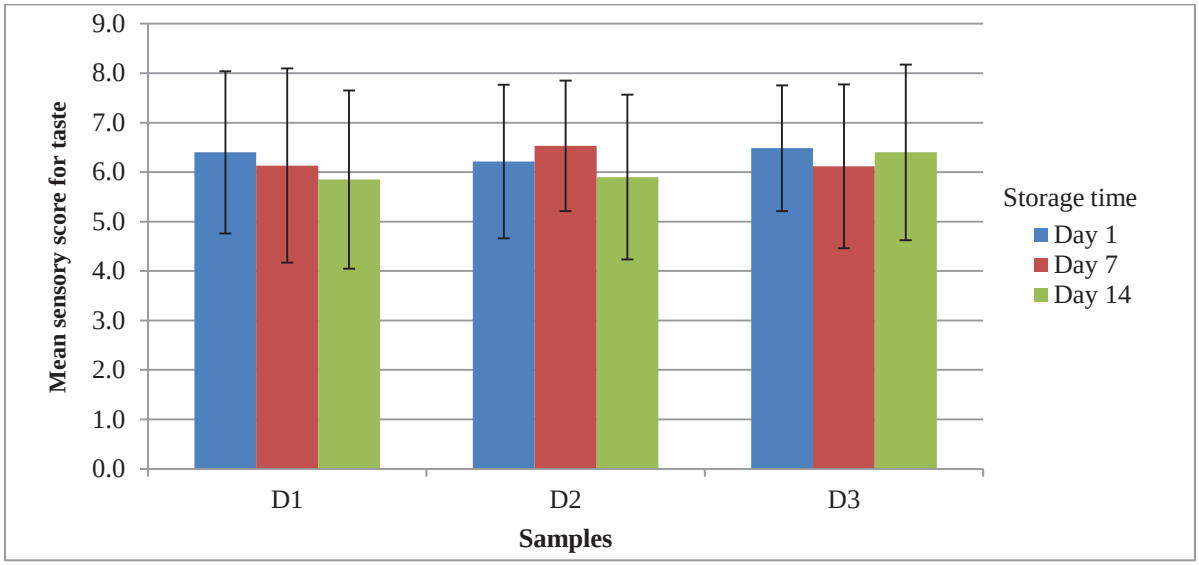

Figure 29 Taste of GFW samples during storage for 14 days (4°C) in phase 4

Notes: Formulation D1 = 8% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D2 = 10% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D3 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum.
Errors bars are standard deviation of 60 panellists.

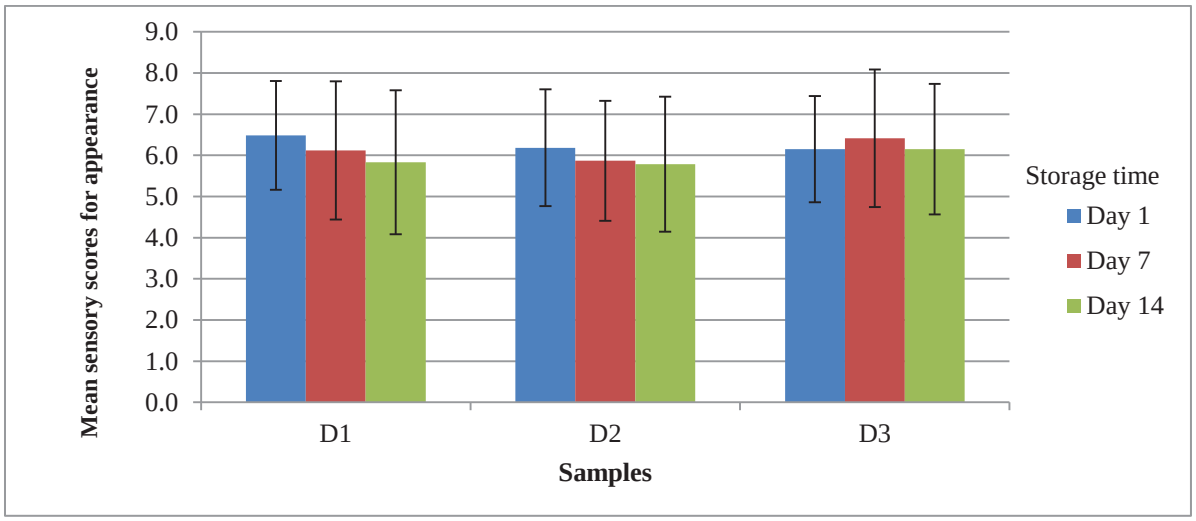

Figure 30 Appearance of GFW samples during storage for 14 days (4°C) in phase 4

Notes: Formulation D1 = 8% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D2 = 10% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D3 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum.
Errors bars are standard deviation of sensory scores for appearance of 60 panellists.

Of the three samples, D3 received the highest mean consumer sensory scores for overall acceptability (>6) during storage (Figure 31). A score of >6 on the 9-hedonic scale indicated that samples were well-accepted by consumer panellists (Munoz, 2013). Overall acceptability of D1 and D2 at days 1 and 7 was similar, however, the acceptability scores for the two

products were low (5.9 and 5.5, respectively) at day 14.

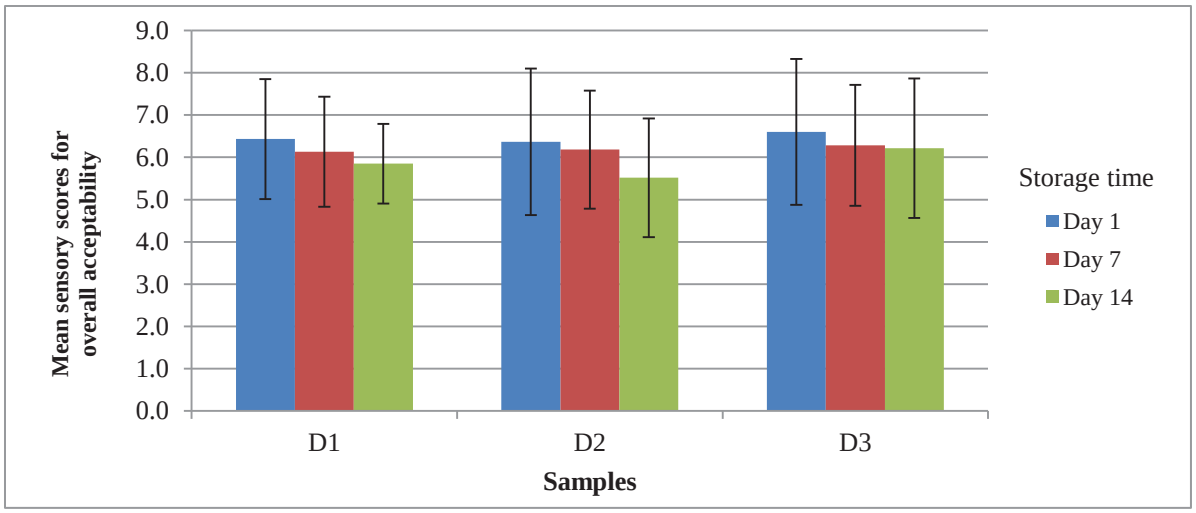

Figure 31 Overall acceptability of GFW samples during storage for 14 days (4°C) in phase 4

Notes: Formulation D1 = 8% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D2 = 10% coconut oil, 0.3% CMC, 1% xanthan-guar gum; D3 = 12% coconut oil, 0.3% CMC, 1% xanthan-guar gum.
Errors bars are standard deviation f sensory scores for overall acceptability of 60 panellists.

4.4.3 Conclusion

Sample D3 (12% coconut oil, 0.3% CMC and 1% xanthan-guar gum) received the highest overall acceptability sensory scores during storage. The sample had the most stable extensibility, high rollability and low baking weight loss.

Chapter 5 Overall Conclusions

Gluten-free wrap bread containing xanthan-guar gum, coconut oil and CMC baked at 240°C/2 min had high rollability and low water activity. The addition of the hydrocolloids in the basic formulations contributed to high extensibility (rupture distance) and firmness (rupture force). The products had low baking weight loss and low whiteness, presumably due to the presence of coconut oil. The physical characteristics of the bread samples mentioned here were stable during storage for 2 weeks at 4°C. The baked breads were well-accepted by consumer sensory panellists using hedonic rating scale.

Chapter 6 Recommendations

In this study, GFWs were stable for two weeks when stored in sealed polyethylene bags at 4°C. To extend shelf life and quality of GFWs, modified atmosphere packaging (MAP) is recommended. Previous reports have reported that using MAP (CO_2:N_2, 60:40) may extend the shelf life of flatbread up to 35 days at ambient temperature (El-Khoury, 1999).

Addition of yeast in the formulation of GFWs may contribute to the development of flavour and aroma of the bread (Thiele, Gänzle, & Vogel, 2002). One of the key factors in increasing the acceptability of the products is the significance of bread flavour and aroma (Maga & Pomeranz, 1974; Ahlborn, Pike, Hendrix, Hess, & Huber, 2005).

The level of shortening in the developed formulations of GFW was still relatively high. Inulin and Simplesse® (whey proteins) may be considered to replace part of coconut oil in formulation to reduce calories as reported by O'Brien, Mueller, Scannell, and Arendt (2003) and Zahn, Pepke, and Rohm (2010).

Honey powder is another ingredient that could replace rice syrup in the formulations of GFW. Tong et al., (2010) reported that honey powder contributed to softer texture and good appearance of bread when evaluated by consumer sensory evaluation.

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Appendix

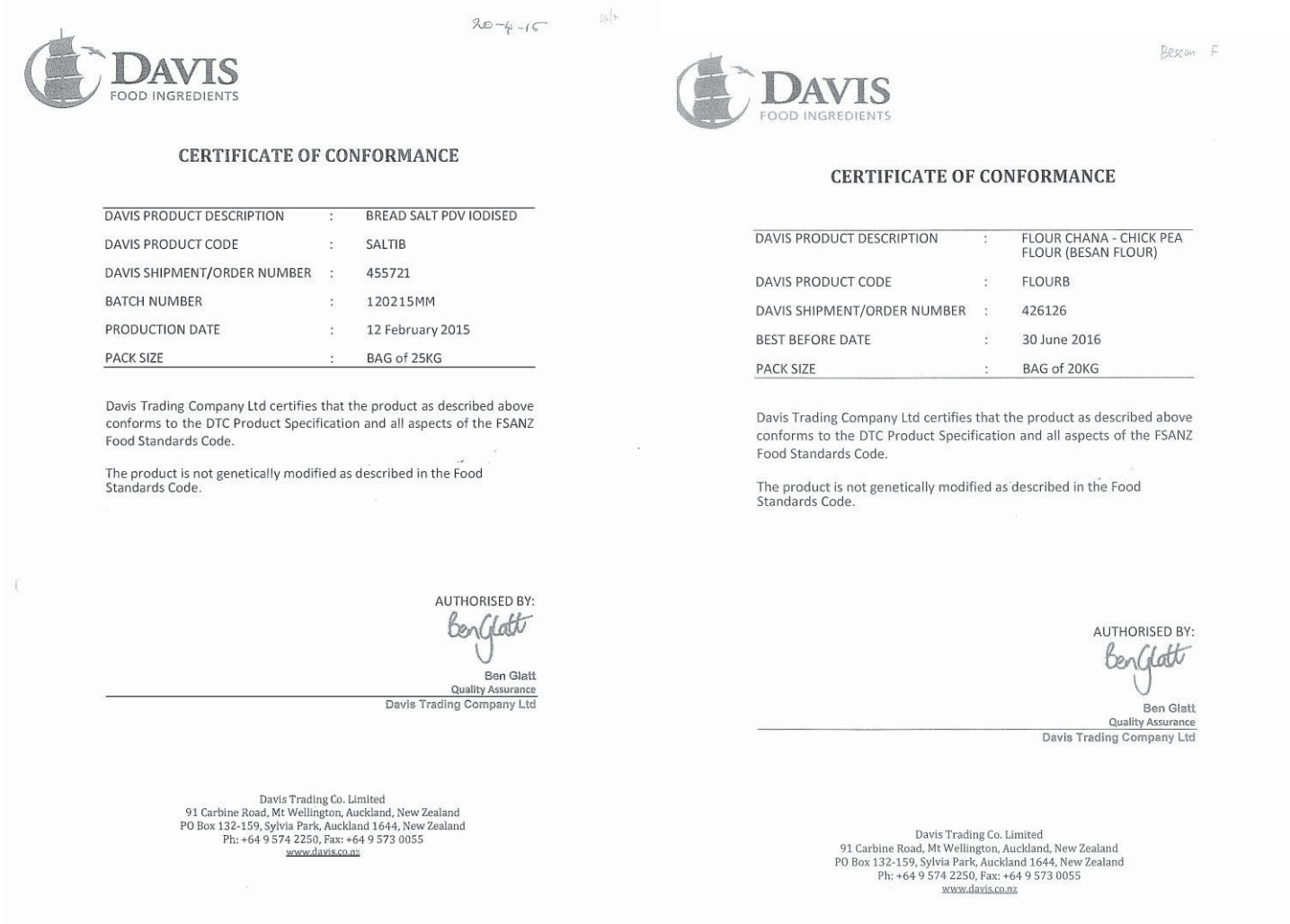
Appendix A

Table A-1 Formulation of GFWs in four phases

Formulation	Phase 1				Phase 2					Phase 3									Phase 4		
	A1	A2	A3	A4	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5	C6	C7	C8	C9	D1	D2	D3
Modified tapioca starch (%)	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Hi-Maize starch (%)	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	102.00	102.00	12.00	12.00	12.00	12.00
Chickpea flour (%)	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	80.0	80.0	8.00	8.00	8.00	8.00
Coconut flour (%)	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	170.0	170.0	17.00	17.00	17.00	17.00
Psyllium (%)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	30.0	3.00	3.00	3.00	3.00	3.00	3.00
Lecithin (%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Baking powder (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Xanthan gum (%)	0.00	1.00	1.50	0.60	0.60	0.50	0.50	0.50	0.50	0.40	0.50	0.60	0.50	0.60	0.40	0.60	0.40	0.50	0.50	0.50	0.50
Guar gum (%)	1.00	0.00	0.00	0.60	0.60	0.50	0.50	0.50	0.50	0.40	0.50	0.60	0.50	0.60	0.40	0.60	0.40	0.50	0.50	0.50	0.50
CMC (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.20	0.10	0.20	0.30	0.10	0.20	0.30	0.10	0.20	0.30	0.30	0.30	0.30
Oil (%)	10.00	10.00	10.00	10.00	10.00	10.00	12.00	10.00	8.00	8.00	8.00	8.00	10.00	10.00	10.00	12.00	12.00	12.00	8.00	10.00	12.00
Rice syrup (%)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Water (%)	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	123.30	1230.30	1230.30	123.30	123.30

Appendix B

Details of ingredients



DAVIS
FOOD INGREDIENTS

CERTIFICATE OF CONFORMANCE

DAVIS PRODUCT DESCRIPTION	:	BREAD SALT PDV IODISED
DAVIS PRODUCT CODE	:	SALTIB
DAVIS SHIPMENT/ORDER NUMBER	:	455721
BATCH NUMBER	:	120215MM
PRODUCTION DATE	:	12 February 2015
PACK SIZE	:	BAG of 25KG

Davis Trading Company Ltd certifies that the product as described above conforms to the DTC Product Specification and all aspects of the FSANZ Food Standards Code.

The product is not genetically modified as described in the Food Standards Code.

AUTHORISED BY:

Ben Glatt
Quality Assurance
Davis Trading Company Ltd

Davis Trading Co. Limited
91 Carbine Road, Mt Wellington, Auckland, New Zealand
PO Box 132-159, Sylvia Park, Auckland 1644, New Zealand
Ph: +64 9 574 2250, Fax: +64 9 573 0055
www.davis.co.nz

DAVIS
FOOD INGREDIENTS

CERTIFICATE OF CONFORMANCE

DAVIS PRODUCT DESCRIPTION	:	FLOUR CHANA - CHICK PEA FLOUR (BESAN FLOUR)
DAVIS PRODUCT CODE	:	FLOURB
DAVIS SHIPMENT/ORDER NUMBER	:	426126
BEST BEFORE DATE	:	30 June 2016
PACK SIZE	:	BAG of 20KG

Davis Trading Company Ltd certifies that the product as described above conforms to the DTC Product Specification and all aspects of the FSANZ Food Standards Code.

The product is not genetically modified as described in the Food Standards Code.

AUTHORISED BY:

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29-Apr-15 GELHC715

Modified Tapioca starch

GSL General Starch Limited

Tapioca starch and Modified starch Manufacturer

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

Tel : (662) 7322782
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 Bamrung)

28-Apr-15
H2 - M420

Ingredion

170 Epping Road Lane Cove NSW 2066
Locked Bacg 61 Lane Cove NSW 2066 Australia
Phone: 61 2 9911 1247 Fax: 61 2 9427 5131

Report No. 1504211
Date of Report Issue: March 04, 2015
Sales Order Number: 10199271

Customer Name:
INGREDION ANZ PTY LTD (NZ)
UNIT 5, 706 GREAT SOUTH RD,
PENROSE, AUCKLAND NEW ZEALAND
1061

CERTIFICATE OF ANALYSIS

This is to certify that the following batch has been tested and meets product specification

Product: **HI-MAIZE 1043 @25 KGS**
Batch code: **BEZ715** Use by date: **February 09, 2017**
Production Date: **February 09, 2015**

Test	Result	Units
Moisture	11.2	%
pH	5.7	
Sulphur Dioxide	< 5.0	mg/kg
Dietary Fibre	71.4	%
Sieve (Retained on 212 micron screen)	0	%
Standard Plate Count	3300	CFU/g
Yeast	< 10	CFU/g
Mould	90	CFU/g
E-Coli	Not Detected	MPN3
Salmonella	Negative	

This product does not require labelling as genetically modified food in accordance with Food Standard A18-foods produced using gene technology

L. Bagala

LORRAINE BAGALA
QUALITY CONTROL CO-ORDINATOR

30-3-15

DAVIS
FOOD INGREDIENTS

CERTIFICATE OF ANALYSIS

DAVIS PRODUCT DESCRIPTION	:	COCONUT FLOUR
DAVIS PRODUCT CODE	:	COCFL
DAVIS SHIPMENT/ORDER NUMBER	:	434403
BATCH NUMBER	:	71726
BEST BEFORE DATE	:	June 2015
DATE OF ANALYSIS	:	28 July 2014
PACK SIZE	:	BAG 20KG

ANALYSIS:

PARAMETER	RESULTS
Coliforms	<10 cfu/g
Total plate count	<10 cfu/g
Mould and Yeast	<10 cfu/g
E.Coli	Non-detected in 25 grams
Salmonella	Non-detected in 25 grams

AUTHORISED BY:

Stephanie Rigter

Stephanie Rigter

Quality Assurance
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AXIEO
16-4-15
CMC

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

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 Specification

Date of Product Spec:

Graphic of ingredient/product

Product Name/description	**Organic Refined, Bleached & Deodorized Coconut Oil**
Scientific Name of Product	***Cocos nucifera***
Legal Description/Suggested Labelling Description	**Organic Refined, Bleached & Deodorized Coconut Oil**
Code	**40718**
Country of Origin	**Philippines**
Bag markings (lot/dates)	**Organic Lot Code/Shift produced/Year produced/Month produced/Day produced**
Shelf life	**12 months**
Packaging	**190 kgs**
Storage	**Clean and dry, sealed.**
Processing Flow Diagram	Date:

1. Ingredient Declaration

Specify all ingredients including food additives in descending order, including percentage labeling of characterizing components or ingredients. Compound substances must specify all ingredients and additives present and the characterizing ingredient or component. Food additives must specify a functional class name or code number (e.g antioxidants (304, 306), Food acid (citric acid).

Ingredient Name	Percent of total %	Compound Substance Ingredients Full breakdown list of components in compound ingredient including additive code numbers	Characterising component %
ORGANIC Copra	100%		

1.1 PROCESSING AIDS

Specify all processing aids used in the manufacture of this product not otherwise declared in the ingredient list.

NAME OF PROCESSING AID	ADDITIVE NAME	PERMITTED USE AND CLASS NAME
None		

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2. INGREDIENTS TO BE DECLARED AS ALLERGENS OR SULPHITES

Please insert **YES** or **NO** to indicate if the product contains, or was manufactured using, any ingredient, additive or processing aid which has been derived from the following food sources. Highly processed derivatives must always be declared. Carefully assess compound ingredients for hidden allergens

ALLERGENS AND SULPHITES	YES/NO
Cereals containing gluten & their products (wheat, rye, barley, oats, spelt)	No
Crustacea & crustacean products	No
Fish and fish products (including mollusk with or without shells and fish oils)	No
Milk and milk products	No
Peanut and peanut products	No
Sesame Seeds and sesame seed products	No
Soybean and soybean products	No
Tree nuts and tree nut products	No
Sulphites, present **in** ingredients, additives or processing aids	No

2.1 COMPLETE ALL ROWS CORRESPINDING WITH "YES" DECLARATION AS PROVIDED IN 2.

ALLERGENIC SUBSTANCE	SOURCE NAME The allergenic food from which ingredient is derived (e.g. wheat)	DERIVATIVE NAME Ingredient, additive or processing aid (e.g maltodextrin)	PROPORTION (%) Derivative in product	 Protein in derivative	PROCESS Allergenic protein is removed?
Cereals containing gluten and their products (Wheat, rye, barley, oats, spelt & derived product eg. Wheat maltodextrin)					
Crustacea & crustacea products					
Egg & egg products					
Fish & fish products (including mollusk extract and fish oils)					
Milk & milk products					
Peanut & peanut products (including peanut oil)					
Sesame Seed & sesame seed products (including sesame seed oils)					
Soybean & soybean products(including soybean oils)					
Tree Nuts & tree nut products					

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2.2 All columns must be completed

ALLERGENIC SUBSTANCE	PRESENT IN SAME FACILITY YES/NO	PRESENT ON SAME LINE YES/NO	PRESENT IN THE FOOD YES/NO	SOURCE FOOD The allergenic food from which ingredient is derived (e.g. wheat)
Cereals containing gluten & their products	No	No	No	
Crustacea & crustacea products	No	No	No	
Egg & egg products	No	No	No	
Fish & fish products (inc mollusk & oils)	No	No	No	
Milk & milk products	No	No	No	
Peanuts & peanut products (inc peanut oil)	No	No	No	
Sesame Seeds & sesame products	No	No	No	
Soybeans & soybean products (inc soybean oil)	No	No	No	
Tree nuts & tree nut products	No	No	No	

3. NUTRIENTS AND CONSUMER INFORMATION CLAIMS

3.1 For nutritional information below, please specify **UNITS of measure**

Grams ☐ Milliliters ☐

Complete Nutrient Table below. Mandatory nutrients are highlighted in blue and bolded, others optional.

NUTRIENT	AVG Quantity per 100	
Energy	862	kJ
Protein, total	0	g
Fat, total	100	g
- Saturated	86.500	g
- Trans fat	0	g
- polyunsaturated	1.800	g
- monounsaturated	5.800	g
Cholesterol	0	mg
Carbohydrates	0	g
- sugars	0	g
Dietary Fibre, Total	0	g

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Sodium	0	mg
Potassium	0	mg

* Reference: US FDA Data

3.2 **Additional Nutrients – vitamins, minerals and other nutritive substances**

VITAMINS Specify which vitamin	AVG Quantity per 100

MINERALS Specify which mineral	AVG Quantity per 100

3.2 Please nominate the source used to provide the nutrition data in the tables above

Analytical – e.g Laboratory Tested ☐ Theoretical e.g by calculation ☐

3.3 SUITABILITY TO MAKE CERTAIN CLAIMS

Specify if the product is suitable for use in product intended for the following consumer uses

SPECIFY IF SUITABLE FOR....	Yes/No	HOW HAS THIS BEEN VALIDATED?	CERTIFICATE AVAILABLE YES/NO
Halal	Yes	Plant Audit	Yes
Kosher	Yes	Plant Audit	Yes
Organic	Yes	Plant Audit	Yes
Biodynamic			
Ovo-lacto-vegetarian			
Lacto-vegetarian			
Vegan			

PRODUCT SUITABILITY FOR....	Yes/No	SPECIFY PARTICULAR CLAIMS	HOW IS CLAIM VALIDATED?
"Free " Claims			
Sustainability Claims			
Any other claims			

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4. DURABILITY, PACKAGING AND SUPPLY CHAIN

4.1 SHELF-LIFE

4.1.1 Please complete the following details

	PRODUCT AS SUPPLIED Unopened pack or bulk container		PRODUCT-ONCE IN USE Bulk packs/containers	
Specify Shelf life	12	Months		
Temperature Control during storage	Is required?	YES. AMBIENT TEMPERATURE	Is required? Specify Range:	YES. AMBIENT TEMPERATURE
Temperature control during transport	Is required? No			
Specify any **OTHER** storage requirements				

4.1.2 Specify the type of coding on the bulk packaging:

PLEASE FIND ATTACHED

4.2 TRANSPORT

How is product transported and packaged:

IT IS PACKED EITHER IN FLEXITANKS OR ISOTANKS. SHIPPED IN CONTAINER VANS FOR FLEXITANK AND SHIPPED IN ISOTANKS

4.3 TRADE MEASUREMENT

4.3.1 Specify which method of trade measurement is used: **Calibrated Digital Scale**

4.3.2 What is the packaging size:

Diameter - 23 inches
Circumference - 72 inches
Height - 35 inches

4.3.3 Target fill (if applicable)
4.3.4 Drained Weight (if applicable)
4.3.5 Net Weight (if applicable)

4.4 TRACEABILITY

Please provide any general comments about the traceability coding used on the product i.e both the bulk bags, or the individual units and the outer cartons.

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Please specify the following where applicable

TRACEABILITY CODE	UNIT	CARTON/ BULK BAG/ OUTER (DRUM ETC)
Type of Primary Coding (Please TICK as appropriate	Date Code	Date Code
	Product Code	Product Code
	Batch Number	Batch Number
	Lot Number	Lot Number
Method of coding	Lot Coding System	Lot Coding System
Location of code	Label	Label
Number of characters in code	11 -14characters	11 -14characters
Example of coding format	4C 123 130403	4C 123 130403
Coding translation	4C- Organic Code/ 123 Shift produced/13- year produced/ 04 – month produced/ 03 – day produced	4C- Organic Code/ 123 Shift produced/13- year produced/ 04 – month produced/ 03 – day produced

4.5 PRODUCT PACKAGING

4.5.1 Are tamper evident controls included in this design? Yes/No
4.5.2 Has unit packaging been assessed for migration of substances into food? **Yes**
4.5.3 Provide a general description of unit packaging:

Cylindrical metal container Dimension:

Diameter - 23 inches
Circumference - 72 inches
Height - 35 inches

4.5.4 Complete the following table for questions related to packaging of unit packaging and/or shipper/drum/carton

PACKAGING		UNIT	OUTER/SHIPPER
TYPE	PACKAGING FORMAT		
Specify components/ materials used in packaging	Ceramic		
	Glass		
	Metal (drum)	**Cylindrical metal container**	**Mindanao Container Corporation**
	Paper/Cardboard		
	Packing Materials/Laminates		
	Plastics		
	% of total using recycled component		
Seal	What is the seal method	**Sealing equipment**	
Dimensions	Height (mm)		
	Width (mm)		
	Depth (mm)		

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4.6 PALLET CONFIGURATION

4.6.1 Gross Weight of loaded pallet	**860.15 kg**		
4.6.2 Stack height of loaded pallet	**44 inches**		
4.6.3 Specify the type of pallet	**Wooden**	Plastic	Other
4.6.4 What is the pallet pattern	**Column Stack**	Interlocking	
4.6.5 Number of:	units per shipper		shippers per pallet
			Layers per pallet

5.0 SPECIFICATION AND COMPLIANCE

Test methods are mandatory and must quote AOAC methods or recognized independent International standards. Where a supplier's internal test method is quoted a copy must be attached. Also state if Certificates of Analysis (C of A) or Certificate of Conformance (C of C) can be provided.

5.1 ORGANOLEPTIC SPECIFICATIONS

(Examples may include flavor, colour, aroma, texture etc)

TEST/PARAMETER	SPECIFICATION	TEST METHOD	AVAILABILITY	
			C of A	C of C
Odor	No smell	Organoleptic	Yes	Yes
Color	Yellowish	Lovibond Tintometer	Yes	Yes
Taste	Bland	Organoleptic	Yes	Yes

5.2 PHYSICAL SPECIFICATIONS

(Examples may include particle size, shape, specific gravity, metal detection, x-ray, foreign matter tolerances, physical defect tolerances etc as appropriate for the product)

TEST/PARAMETER	SPECIFICATION	TEST METHOD	AVAILABILITY	
			C of A	C of C

5.3 MICROBIOLOGICAL SPECIFICATIONS

(Examples may include standard plate count, APC, yeasts and moulds, coliforms, salmonella etc)

TEST/PARAMETER	SPECIFICATION	TEST METHOD	AVAILABILITY	
			C of A	C of C
Total Plate Count	cfu/g max.	BAM Online Ch.3, Sept 2002	YES	YES
Yeast and Molds	80 cfu/g max.	BAM Online Ch. 2 Sep 2002	Yes	Yes
E.Coli	Non detected in 25g	BAM Online Ch. 18, 2001	Yes	Yes
Salmonella	Non detected in 25g	BAM Online Ch. 5, 20011	Yes	Yes

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

5.4 CHEMICAL SPECIFICATIONS

(Examples may include pesticide residue screen, heavy metal screen, aflatoxin screen, salt, ph, acid, moisture, FFA's, PV's etc)

TEST/PARAMETER	SPECIFICATION	TEST METHOD	AVAILABILITY	
			C of A	C of C
Pesticide Residue	According to Codex	Gas Chromatography	Yes	Yes
Heavy Metal	According to Codex	Atomic Absorption Spectrometric Method ELISA	Yes	Yes
Aflatoxin		ELISA (Agri- Screen)	Yes	Yes
Free Fatty Acid	0.15 max.	AOCS Ca 5a-40	Yes	Yes
Peroxide Value	0 meq/kg max.	AOCS Cd 8-53	Yes	Yes
Moisture	5% max.	Air oven Method	Yes	Yes

6.0 ADDITIONAL INFORMATION

6.1 Additional manufacturing site information

When nominating product is supplied from more than one manufacturing site, the details provided must be applicable to product coming from any of the sites. For example, if particular allergens occur at only one site then the information provided on the form should identify that the allergens present even though batches of product made at other sites may be allergen free.

Company Name	
Site#1 Number/Street/Suburb	
Country/State	
Company Name	
Site#2 Number/Street/Suburb	
Country/State	

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

PROFILE PRODUCTS
Profile Holdings Ltd

PRODUCT SPECIFICATION SHEET

CUSTOMER: **Venerdi Limited** **Date:** 29/09/2014

PRODUCT: **Gluten Free Baking Powder**

PRODUCT CODE: 10923

DESCRIPTION: White free flowing powder. Free from foreign matter.

'NGREDIENTS: **Raising Agents (450, 500), Maize Starch**

AVERAGE NUTRITION

Nutrient		Per 100g
Energy (kJ)		1000
Protein (g)		0.1
Fat:	Total (g)	9.2
	Saturated (g)	1.1
Carbohydrate:	Total (g)	38.3
	Sugars (g)	0.0
Sodium (mg)		10600

PACKAGING: 10kg Polyethylene lined multi-wall paper bag

SHELF LIFE AND STORAGE: 12 months; stored sealed at ambient temperature protected from sunlight and moisture

SPECIFIC INGREDIENT INFORMATION

Is this product:	Yes or No
Suitable for Lacto-Ovo-Vegetarians? (does not contain animal meat or flesh, may contain dairy products or eggs)	**Yes**
Suitable for Lacto-Vegetarians? (does not contain animal meat, flesh or eggs, may contain dairy)	**Yes**
Suitable for Vegans? (does not contain animal derived products. E.g. ..eggs, gelatin, dairy)	**Yes**
Halal	**Suitable**
Kosher	**Suitable**

GMO Status: This product fully complies with Standard 1.5.2. of the joint ANZ Food Standards Code, and does not require a label indicating the presence of any GM ingredient.

ALLERGENS

Does the product contain any of the following:	Yes or No
Cereals containing gluten	**No**
Crustacea and their products	**No**
Egg and egg products	**No**
Fish and fish products	**No**
Milk and milk products	**No**
Peanuts and peanut products	**No**
Soybeans and soy products	**No**
Sulphites (>10ppm)	**No**
Sesame seeds	**No**
Tree nuts and tree nut products	**No**

ADVISORY STATEMENTS

Does the product contain any of the following:	Yes or No
Bee pollen	**No**
Aspartame	**No**
Quinine	**No**
Guarana	**No**
Caffeine	**No**
Propolis	**No**
Royal jelly	**No**
Polyols	**No**

Authorised by:
Product Development Coordinator: Sherlyn Ng

Please Note:
The information is provided in the belief that it is accurate within generally accepted standards and is provided for the information of qualified personnel. It does not constitute an offer by the manufacturer nor does the manufacturer warrant or guarantee its accuracy or completeness in describing the performance or suitability. Prospective users are encouraged to conduct their own tests and studies to determine the suitability for a particular purpose and specific applications. This specification is based on product at time of packing and there may be some variation at time of delivery.

Lecithin

AOT • All Organic Trading GmbH
Heisinger Straße 12
D-87437 Kempten

PRÜFBERICHT
Certificate of Analysis

All Organic Trading

Prüfgegenstand / product: **Sonnenblumenlezithin N kbA**
Sunflower lecithin N, organic

Chargen-Nr. /lot-no.: **LCGB 03/13**

Beginn/Ende der Untersuchung / start/end date of analysis: 13.08.2013 – 30.09.2013

Send email to supplier to request org cert.

Herkunft /origin: RU / IT
Produktion / production: IT
Produktions Datum / production date: 15.04.2013
MHD/bbd: 15.04.2015

Prüfergebnis / Test result:

Parameter	Einheit/unit	Resultat / result	Methode / method
Wassergehalt / Humidity	%	0,46	Karl Fischer
Säurezahl / acid value	mg/KOH/g	22,78	NGD Fa 3
Acetonunlöslichkeit / Acetone insolubilitiy	%	61,08	NGD Fa 7
Toluolunlöslichkeit / Toluol insolubilitiy	%	0,23	NGD Fa 6
Peroxidzahl / peroxide value	meqO_2/kg	n.d.	NGD Fa 4
Herstellerzertifikat / manufacture certificate (13.08.2013)			
Viskosität / Viscosity			DIN 53019
25°C Scherrate 0,2	mPas	12800	Kegelplatte 40 mm; 4°;25°C
Labor: DIL Nr. 13/770			
Allergene / Allergens			
Sojaprotein / Soy protein	%	nicht nachweisbar/ not dedectable	PCR Nachweisgrenze/ detection limit 0,01%
Labor: Institut Prof. Kurz GmbH Nr. L-5309/13-Z			
Schwermetalle / Heavy metals			
Arsen / Arsenic AS	mg/kg	<0,04	SOP M 1473, Mikrowelle o. Turbowave
Cadmium / cadmium Cd	mg/kg	0,03	DIN EN 15763 mod., SOP M 1474, ICP/MS
Quecksilber / Mercury Hg	mg/kg	<0,01	SOP M 2567, Feststoffanalysator
Blei / lead Pb	mg/kg	0,03	DIN EN 15763 mod., SOP M 1474, ICP/MS
Labor: SGS Institut Fresenius Nr. 1861150			

Geschäftsführer: Fabian Breisinger
Register-Gericht: Kempten HRB 8191
Gerichtsstand: Kempten (Allgäu)
Steuer.Nr.: 127/121/51860
Ust-Id-Nr.: DE813705446

Phone +49(0)831 5758-155
Fax +49(0)831 5758-159
info@a-o-t.com
www.a-o-t.com
DE-ÖKO-005

erstellt / created: ER:
geprüft und freigegeben: SV
checked and approved: SV

01.10.2013

Lecithin

AOT • All Organic Trading GmbH
Heisinger Straße 12
D-87437 Kempten

PRÜFBERICHT
Certificate of Analysis

All Organic Trading

Parameter	Einheit/unit	Resultat / result	Methode / method
Pesticide / pesticides			
Multi-Pestizidscreening / Multiresidue pesticide screening	mg/kg	negativ	ASU L 00.0034-34, GC Spekrum: Öl-Complete S
Labor: SGS Institut Fresenius Nr. 1861150			
Lösungsmittel Hexan / solvents hexane			
Hexan / hexane	mg/kg	<0,1	HS-GC/MS
Labor: SGS Institut Fresenius Nr. 1861402			

Mikrobiologische Analysen / microbiological Analyzes			
Aerobe mesophile Koloniezahl /Total plate count	KbE/g cfu/g	360	DIN EN ISO 4833/ ASU L 00.00-88/PCA/30°C/72h
Salmonellen / salmonella	in 25g	negativ	DIN EN ISO 6579mod. / ASU L 00.00-20 mod.
Schimmelpilze / moulds	KbE/g cfu/g	800	ASU L 01.00-37/YGC/25°C/72-120h
Hefen / Yeast	KbE/g cfu/g	<10	ASU L 01.00-37/YGC/25°C/72-120h
Coliforme Keime	in 0,1 g	negativ	ISO 4831/Laurylsulfat-Bouillon/37°C/24h/Brila-Bouillon/37°C/48h
Staphylocokken (Coag+)	KbE/g cfu/g	<10	DIN EN ISO 6888-2 ASU L 00.00-56/RPF/37°C/45h
E.Coli	KbE/g cfu/g	<10	DIN ISO 16649-2/ASU L 00.00-132/2,TBX/44°C/21h
Enterocokken	KbE/g cfu/g	<10	ASU L 06.00-32/CATC/37°C/48h
Labor: SGS Institut Fresenius Nr. 1861151			

Farbe			
Gardner Farbzahl		8,7	Beiersdorf General Test Method AP005-01
Labor: SGS Institut Fresenius Nr. 1861150			

GVO:			
T-NOS		negativ	Real-time PCR Screening
P-35S		negativ	Real-time PCR Screening
FMV		negativ	Real-time PCR Screening
Labor: SGS Institut Fresenius Nr. 1861150			

Analysenzertifikat entbindet den weiterverarbeitenden Abnehmer der Produkte nicht von der Qualitätsverantwortung.
Certificate of analysis does not release customers from their quality responsibility when processing our products. AOT Qualitätssicherung / Quality AssuranceMaschinell erstellt, daher ohne Unterschrift. Established by Computer, therefore no signature.

Geschäftsführer: Fabian Breisinger
Register-Gericht: Kempten HRB 8191
Gerichtsstand: Kempten (Allgäu)
Steuer.Nr.: 127/121/51860
Ust-Id-Nr.: DE813705446

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info@a-o-t.com
www.a-o-t.com
DE-ÖKO-005

erstellt / created: ER:
geprüft und freigegeben: SV
checked and approved: SV

01.10.2013

Hawkins watts Xanthan Gum

5-1-15

ADM

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
COPC: 9535

Manufacture date: 08 Mar 2014
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
PYRUVIC ACID	>1.5 %	1.5% MIN	NF/FCC
ASSAY	COMPLIES	4.2-5.0% CO2	NF/FCC
PH	7.1	5.5-9.1	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)

Shamrock
13-4-15

RICSYR

SHAFI GLUCO CHEM (Pvt) Ltd

Plot No: B-22 to 26 & B-38 to 42, Hub Industrial Trading Estate, Distt. Lasbella, Balochistan, Pakistan.
Ph: (92-853) 303719-21 Fax: (92-853) 303952. E-mail: sgc@glucochem.com Web: www.glucochem.com

CERTIFICATE OF ANALYSIS

QC4/07
ISSUE 2
Page 1 of 1

December 12, 2014

PRODUCT	CONVENTIONAL BROWN RICE SYRUP 60 DE
MANUFACTURING DATE	12 DECEMBER, 2014
EXPIRY DATE	12 OCTOBER, 2015
SPECIFICATION	AS PER STANDARD SPECIFICATION
BATCH NO.	RS12L14C
Contract#7127	PO#111777

DESCRIPTION	RESULTS
Dextrose Equivalent%	59.8
Brix%	80.6
pH	5.4
Ash%	<0.1

12-12-2014

Manager Quality Control

DAVIS THADANI
3-3-15

AnaZeal Analyticals & Research Pvt. Ltd.
GOVT. APPROVED TEST HOUSE

C-404, TTC Industrial Area,
Opp. JISL, MIDC, Pawane, Navi Mumbai - 400 705
Tel. (Dir.) 64511555 (Board) : 27633040 / 41 / 42 / 43
Fax : 2763 3044 e-mail : anazeal@gmail.com
NABL ACCREDITED LABORATORY
(Cert. No.: T2692, T2693)

AGMARK Approved

CERTIFICATE OF ANALYSIS

COA No.: ANZ/F/14/544 Date: 07/07/2014

PRODUCT	PSYLLIUM HUSK 95%, 60 MESH POWDER
CLIENT	AATISH INDUSTRIES, MUMBAI.
DATE OF RECEIPT	30/06/2014 QTY. RECD.: 500 GM
REF.NO.	AAI/IND/1344/2014-15 DATED: 26/06/2014
MARKS	533 BAGS (7995 KGS) PRODUCT OF INDIA PSYLLIUM HUSK 95% 60 MESH
P.O.NO.429371	PRODUCT CODE: PSYHS60 P.O.NO.:429371 BATCH NO.: AAI/714
AAI/EXP/796/2014-15	MFG.DATE: JUNE 2014 EXP.DATE : MAY 2016

RESULTS OF ANALYSIS

Test	Method	Limit / Specification	Results
Purity	USP 34	Min. 95.0%	95.09%
Botanical Characters	USP 34	Pale to medium buff colored with weak characteristic odors entire or broken epidermal cells are filled with mucilage. In surface view the epidermal cells appear polygonal mucilage stains red with ruthenium red and lead acetate.	Passes
Identification A	USP 34	Mounted in cresol, cell viewed microscopically are composed of polygonal prismatic cells having 4 to 6 straight or slightly wavy walls.	Passes
Identification B	USP 34	Mounted in alcohol and irrigated with water, viewed microscopically, the mucilage in the outer part of the epidermal cells swells rapidly and goes into solution.	Passes
Moisture	USP 34	NMT 12%	4.82%
Total ash	USP 34	NMT 4%	3.41%
Acid insoluble ash	USP 34	NMT 1.0%	0.59%
Light Extraneous matter	USP 34	NMT 5.0%	4.91%
Insect Fragments /25g	USP 34	NMT 400	33parts
Swell volume	USP 34	NLT40 ml/g	50ml/gm
Salmonella / 25 g	USP 34	Should be absent	Absent
E.coli / g	USP 34	Should be absent	Absent
Yeast and Mould / g	USP 34	Max. 1000	800
Total bacterial count / g	USP 34		2500

PERSON IN-CHARGE OF TESTING

1. The Results related only to the sample as tested.
2. Legal liability is limited to the valuation in the invoice for testing.
3. This certificate in full or part shall not be reproduced unless written permission is obtained from M/s. AnaZeal Analyticals & Research Pvt. Ltd.
4. Subject to Mumbai Jurisdiction.

This inspection / testing has been performed to the best of our ability and our responsibility is limited to proven negligence. This certificate, which is issued on conditions declared overleaf, reflects our findings at the time and place of inspection / testing and does not relieve parties from their contractual obligations. Samples will be retained by us for a period of thirty days only, unless specific instructions to the contrary are received.

Appendix C

Questionnaires of sensory elevation

Sample code 348

SENSORY EVALUATION OF GLUTEN-FREE WRAP BREAD

You will be given 3-coded samples of gluten-free wrap bread one by one.

Please taste the first 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 tasting.

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

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or 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 or 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 or 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 or 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 or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Overall Comment about the product: .....

Sample code 551

SENSORY EVALUATION OF GLUTEN-FREE WRAP BREAD

Please taste the second 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 tasting.

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

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or 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 or 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 or 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 or 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 or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Overall Comment about the product: .....

Sample code 269

SENSORY EVALUATION OF GLUTEN-FREE WRAP BREAD

Please taste the last 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 tasting.

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

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or 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 or 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 or 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 or 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 or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Overall Comment about the product: .....

Thank you

Appendix D

Information sheet and participant consent forms

INFORMATION SHEET

Introduction

I am Tianyi Yang, 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 wrap bread. You are invited to take part in a study that assesses the sensory characteristics of this wrap bread. The aim of this sensory evaluation is to evaluate the level of acceptance of the gluten-free wrap bread by potential consumers.

Participant involvement

The trial involves tasting and evaluating gluten-free wrap bread. Your participation will take 3 to 5 minutes. The wrap bread you will taste may contain ingredients which maybe harmful or cause allergic reactions with certain groups of people. You should not take part if you are allergic or may be affected by the any following ingredients: Kumara powder, maize starch, tapioca starch, coconut flour, rice flour, psyllium husk, bescan flour. 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 Technology in 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

- Tianyi Yang (Master student)- skygobt@hotmail.com
- 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".

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 agree to voluntarily participate in this study under the conditions set out in the Information Sheet

Signature:…… Date: ……..

Full Names (Printed):……Tianyi Yang…… ……..

Appendix E

Appendix E-1 Original data and statistical analysis of phase 1

Table E-1 Original data of phase 1

Formulation No.	Baking temperature (°C)	Baking time (min)	Sample	Baking weight loss (%)	Rollability (3-cm dowel)
A 1	200	2.00	S1	20.11	1.00
		2.00	S1	19.88	1.00
		2.00	S1	22.33	1.00
		4.00	S2	23.55	1.00
		4.00	S2	25.69	1.00
		4.00	S2	22.28	1.00
	220	2.00	S3	26.76	1.00
		2.00	S3	28.12	1.00
		2.00	S3	29.37	1.00
		4.00	S4	29.33	1.00
		4.00	S4	32.17	2.00
		4.00	S4	30.67	1.00
A 2	200	2.00	S5	23.38	1.00
		2.00	S5	24.16	1.00
		2.00	S5	24.02	1.00
		4.00	S6	28.61	1.00
		4.00	S6	29.13	1.00
		4.00	S6	30.15	1.00
	220	2.00	S7	27.16	1.00
		2.00	S7	27.69	1.00
		2.00	S7	28.96	1.00
		4.00	S8	30.15	2.00
		4.00	S8	30.11	1.00
		4.00	S8	31.18	2.00
A 3	200	2.00	S9	22.14	1.00
		2.00	S9	24.13	1.00
		2.00	S9	22.96	1.00
		4.00	S10	27.64	1.00
		4.00	S10	28.31	1.00
		4.00	S10	29.13	1.00
	220	2.00	S11	28.13	1.00
		2.00	S11	28.17	1.00
		2.00	S11	29.20	1.00
		4.00	S12	31.36	2.00
		4.00	S12	30.17	3.00
		4.00	S12	29.16	3.00

Formulation No.	Baking temperature (°C)	Baking time (min)	Sample No.	Baking weight loss (%)	Rollability (3-cm dowel)
A 4	200	2.00	S13	19.82	1.00
		2.00	S13	20.01	1.00
		2.00	S13	20.31	1.00
		4.00	S14	21.38	1.00
		4.00	S14	23.21	1.00
		4.00	S14	23.44	2.00
	220	2.00	S15	25.31	1.00
		2.00	S15	25.78	2.00
		2.00	S15	25.69	1.00
		4.00	S16	26.18	3.00
		4.00	S16	29.67	3.00
		4.00	S16	30.18	3.00

Statistical analysis of phase 1

General Linear Model: Baking weight loss, Rollability (3-cm diameter dowel) versus Baking temperature, Baking time, Formulation

Factor	Type	Levels	Values
Baking temperature(°C)	fixed	2	200, 220
Baking time (min)	fixed	2	2, 4
Formulation No.	fixed	4	1, 2, 3, 4

Analysis of Variance for **baking weight loss** (%), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Baking temperature(°C)	1	275.042	275.042	275.042	222.16
Baking time (min)	1	130.878	130.878	130.878	105.71
Formulation No.	3	101.450	101.450	33.817	27.31
Baking temperature(°C)*Baking time (min)	1	7.744	7.744	7.744	6.26
Baking temperature(°C)*Formulation No.	3	36.738	36.738	12.246	9.89
Baking time (min)*Formulation No.	3	2.758	2.758	0.919	0.74
Baking temperature(°C)*Baking time (min)* Formulation No.	3	8.324	8.324	2.775	2.24
Error	32	39.618	39.618	1.238	
Total	47	602.552			

Source	P
Baking temperature(°C)	0.000
Baking time (min)	0.000
Formulation No.	0.000
Baking temperature(°C)*Baking time (min)	0.018
Baking temperature(°C)*Formulation No.	0.000
Baking time (min)*Formulation No.	0.535
Baking temperature(°C)*Baking time (min)* Formulation No.	0.102
Error	
Total	

S = 1.11268 R-Sq = 93.43% R-Sq(adj) = 90.34%

Analysis of Variance for **Rollability** (3 cm dowel), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Baking temperature(°C)	1	4.0833	4.0833	4.0833	39.20	0.000
Baking time (min)	1	4.0833	4.0833	4.0833	39.20	0.000
Formulation No.	3	2.5000	2.5000	0.8333	8.00	0.000
Baking temperature(°C)*Baking time (min)	1	3.0000	3.0000	3.0000	28.80	0.000
Baking temperature(°C)*Formulation No.	3	1.4167	1.4167	0.4722	4.53	0.009
Baking time (min)*Formulation No.	3	1.4167	1.4167	0.4722	4.53	0.009
Baking temperature(°C)*Baking time (min)* Formulation No.	3	0.8333	0.8333	0.2778	2.67	0.064
Error	32	3.3333	3.3333	0.1042		
Total	47	20.6667				

Appendix E-2 Original data and statistical analysis of phase 2

Table E-2 Original data of phase 2

Formulation	Baking temperature (°C)	Baking time (min)	Sample	Baking weight loss (%)	Water activity	L*	a*	b*	Whiteness index	Rollabilty					
										Day0	Day4	Day7	Day14	Day21	Day28
B1	230	2	Q1	26.69	0.90	73.61	-2.67	15.48	69.29	3.5	3	3	2.5	2.5	0
			Q1	26.9	0.90	72.19	-2.71	15.67	67.96	3.5	3.5	3	3	2.5	0
			Q1	27.78	0.91	74.03	-2.61	15.83	69.47	3.5	3	3	2	2.5	0
		4	Q2	29.87	0.88	71.89	-2.39	16.27	67.43	3.5	3	3	2.5	2.5	2.5
			Q2	27.93	0.89	73.64	-2.43	16.28	68.92	4	3.5	3	3	2.5	2.5
			Q2	28.49	0.89	70.66	-2.41	16.21	66.39	3.5	3.5	2.5	2.5	2.5	2.5
	240	2	Q3	24.17	0.90	70.23	-1.95	16.48	65.92	4	4	4	3.5	3.5	3
			Q3	24.79	0.90	71.69	-1.98	16.52	67.16	3.5	3.5	3.5	3.5	3.5	3.5
			Q3	25.31	0.90	69.13	-1.92	16.56	64.92	3.5	3.5	3.5	3.5	3.5	3.5
		4	Q4	31.29	0.87	63.5	-1.35	16.96	59.73	3.5	3.5	3	3	3	3
			Q4	33.13	0.87	64.8	-1.4	17.12	60.83	3	3	3	2.5	2.5	2.5
			Q4	32.74	0.87	61.2	-1.37	17.32	57.49	3.5	3.5	3	3	3	2.5
B2	230	2	Q5	23.59	0.91	73.48	-2.11	15.1	69.41	2.5	2.5	2	2	2	1.5
			Q5	26.31	0.91	77.61	-2.13	16.4	72.16	2.5	2.5	2	2	2.5	1
			Q5	25.19	0.91	72.06	-2.13	15.76	67.85	2.5	2.5	1.5	2	2	1.5
		4	Q6	30.71	0.90	72.64	-2.22	16.75	67.84	3	3	3	3	2.5	2.5
			Q6	31.06	0.89	75.36	-2.18	16.91	70.04	3	2.5	2.5	2.5	2.5	2.5
			Q6	28.19	0.90	71.29	-2.19	17.03	66.55	3	3	3	2.5	2.5	2.5
	240	2	Q7	24.61	0.90	72.39	-2.19	16.13	67.95	3.5	3	3.5	3	3	3
			Q7	24.06	0.90	73.18	-2.18	16.37	68.50	3.5	3	3.5	3.5	3	3
			Q7	24.39	0.90	73.26	-2.19	16.87	68.31	3.5	3.5	3.5	3.5	3	3
		4	Q8	26.92	0.90	71.13	-1.89	16.51	66.69	3	3	3	3	3	2.5
			Q8	28.25	0.90	71.23	-1.9	16.89	66.58	3.5	3	2.5	3	2.5	2.5
			Q8	27.61	0.90	70.69	-1.93	16.91	66.11	3	2.5	2.5	3	3	3
B3	230	2	Q9	22.64	0.92	73.84	-2.13	15.81	69.36	2.5	2.5	2.5	2.5	2.5	1
			Q9	22.89	0.92	73.96	-2.29	16.39	69.15	2.5	2.5	2.5	2	2	2
			Q9	22.59	0.92	71.59	-2.3	16.03	67.30	3	2.5	2.5	2.5	2.5	2
		4	Q10	29.38	0.90	71.19	-2.69	17.63	66.12	3	3	3	3	3	3
			Q10	29.33	0.90	71.93	-2.51	16.92	67.13	3.5	3.5	2.5	3	2.5	2.5
			Q10	30.02	0.90	71.58	-2.67	17.49	66.52	3	3	3.5	3	2	2
	240	2	Q11	26.48	0.89	72.09	-1.18	15.94	67.84	3.5	3.5	3.5	3.5	3.5	3.5
			Q11	25.49	0.89	72.69	-1.13	16.39	68.13	3.5	3.5	3.5	3.5	3	2.5
			Q11	27.03	0.89	72.18	-1.19	17.32	67.21	3.5	3	3	3	3	2.5
		4	Q12	31.98	0.87	70.48	-1.12	17.69	65.57	3.5	3.5	3	3	3	3
			Q12	32.16	0.87	71.38	-1.31	18.32	65.99	4	3.5	2.5	3	2.5	3
			Q12	30.87	0.86	69.36	-1.29	18.03	64.43	3.5	3.5	3	3	3	2.5

Formulation No.	Baking temperature (°C)	Baking time (min)	Sample	Baking weight loss (%)	Water activity	L*	a*	b*	Whiteness index	Rollability					
										Day0	Day4	Day7	Day14	Day21	Day28
B4	230	2	Q13	21.85	0.89	93.34	-1.98	15.69	82.84	3	3.5	3.5	3.5	3.5	0
			Q13	21.69	0.89	93.28	-1.69	16.94	81.70	3.5	3.5	3.5	3.5	3	0
			Q13	21.77	0.89	98.49	-1.64	16.43	83.42	3	4	4	3.5	3.5	0
		4	Q14	29.37	0.89	87.63	-1.12	18.36	77.83	4	3.5	3.5	3.5	3.5	3
			Q14	29.97	0.89	89.63	-1.31	20.03	77.41	4	3.5	4	3.5	3	3
			Q14	28.93	0.89	88.69	-1.29	18.91	77.93	4	4	3.5	3.5	3	3
	240	2	Q15	23.73	0.93	67.69	-1.31	22.36	60.69	4.5	4.5	4.5	4.5	4.5	4.5
			Q15	23.84	0.92	71.48	-1.13	21.39	64.33	4.5	4.5	4.5	4.5	4.5	4.5
			Q15	23.81	0.91	68.33	-1.19	23.57	60.50	4.5	4.5	4.5	4.5	4	4
		4	Q16	36.52	0.84	70.32	-1.12	19.67	64.38	4.5	4.5	3.5	3.5	3	3
			Q16	34.29	0.84	71.35	-1.31	20.18	64.93	4	4	3.5	3.5	3.5	3.5
			Q16	37.27	0.84	71.98	-1.29	22.1	64.29	4.5	4.5	4	4	4	3.5
B5	230	2	Q17	22.55	0.91	75.81	-1.72	14.74	71.62	4	4	3.5	3	3	2.5
			Q17	21.89	0.91	76.28	-1.78	15.08	71.84	4.5	4	4	3	2.5	2.5
			Q17	22.77	0.91	76.86	-1.98	14.21	72.77	4.5	4.5	3.5	3	2.5	2.5
		4	Q18	31.9	0.90	73.56	-1.23	19.35	67.21	4.5	4	4	3	3	2.5
			Q18	29.97	0.90	74.25	-1.32	18.28	68.39	4.5	4.5	4	3.5	3	2.5
			Q18	30.82	0.90	73.36	-1.28	18.96	67.28	4.5	4.5	4	3	3	3
	240	2	Q19	25.69	0.91	75.6	-1.36	17.6	69.88	4.5	4	4.5	4.5	4	4
			Q19	26.08	0.91	75.84	-1.41	18.32	69.65	4.5	4.5	4	4	4	4
			Q19	25.01	0.91	75.62	-1.69	17.85	69.74	4.5	4.5	4	4	4	4
		4	Q20	32.45	0.87	73.21	-1.19	20.69	66.13	4.5	4	3	3	3	3
			Q20	33.29	0.87	72.18	-1.32	21.54	64.79	4.5	4.5	3.5	3.5	3.5	3
			Q20	35.37	0.87	72.64	-1.28	21.39	65.25	4.5	4.5	3.5	3.5	3.5	3.5

Statistical analysis of phase 2

General Linear Model: Baking weight loss, Water activity, L*, whiteness index versus Formulation, Baking temperature and baking time

Factor	Type	Levels	Values
FormulationB No.	fixed	5	1, 2, 3, 4, 5
Baking temperature °C)	fixed	2	230, 240
Baking time (min)	fixed	2	2, 4

Analysis of Variance for **Baking weight loss** (%), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Formulation No.	4	17.421	17.421	4.355	5.77	0.001
Baking temperature °C)	1	51.504	51.504	51.504	68.26	0.000
Baking time (min)	1	656.638	656.638	656.638	870.25	0.000
Formulation No.* Baking temperature °C)	4	64.072	64.072	16.018	21.23	0.000
Formulation No.*Baking time (min)	4	73.315	73.315	18.329	24.29	0.000
Baking temperature °C)* Baking time (min)	1	7.218	7.218	7.218	9.57	0.004
Formulation No.* Baking temperature °C)* Baking time (min)	4	39.509	39.509	9.877	13.09	0.000
Error	40	30.182	30.182	0.755		
Total	59	939.858				

S = 0.868642 R-Sq = 96.79% R-Sq(adj) = 95.26%

Analysis of Variance for **Water activity**, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FormulationB No.	4	0.0023776	0.0023776	0.0005944	49.40	0.000
Baking temperature °C)	1	0.0070200	0.0070200	0.0070200	583.38	0.000
Baking time (min)	1	0.0097538	0.0097538	0.0097538	810.56	0.000
FormulationB No.* Baking temperature °C)	4	0.0026861	0.0026861	0.0006715	55.80	0.000
FormulationB No.*Baking time (min)	4	0.0015057	0.0015057	0.0003764	31.28	0.000
Baking temperature °C)* Baking time (min)	1	0.0005460	0.0005460	0.0005460	45.38	0.000
FormulationB No.* Baking temperature °C)* Baking time (min)	4	0.0005817	0.0005817	0.0001454	12.09	0.000
Error	40	0.0004813	0.0004813	0.0000120		
Total	59	0.0249522				

S = 0.00346891 R-Sq = 98.07% R-Sq(adj) = 97.15%

Analysis of Variance for **L***, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Formulation No.	4	886.46	886.46	221.61	111.76	0.000
Baking temperature °C)	1	582.07	582.07	582.07	293.53	0.000
Baking time (min)	1	93.95	93.95	93.95	47.38	0.000
Formulation No.* Baking temperature °C)	4	943.47	943.47	235.87	118.94	0.000
Formulation No.*Baking time (min)	4	13.39	13.39	3.35	1.69	0.172

Baking temperature °C)*	1	0.17	0.17	0.17	0.08	0.772
Baking time (min)						
Formulation No.*	4	80.47	80.47	20.12	10.15	0.000
Baking temperature °C)*						
Baking time (min)						
Error	40	79.32	79.32	1.98		
Total	59	2679.3				

S = 1.40819 R-Sq = 97.04% R-Sq(adj) = 95.63%

Analysis of Variance for **Whiteness index**, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
FormulationB No.	4	254.714	254.714	63.678	53.35
Baking temperature (°C)	1	455.064	455.064	455.064	381.28
Baking time (min)	1	108.498	108.498	108.498	90.91
FormulationB No.*	4	532.049	532.049	133.012	111.45
Baking temperature (°C)					
FormulationB No.*Baking time (min)	4	24.782	24.782	6.196	5.19
Baking temperature (°C)*	1	0.525	0.525	0.525	0.44
Baking time (min)					
FormulationB No.*	4	64.424	64.424	16.106	13.49
Baking temperature (°C)*					
Baking time (min)					
Error	40	47.740	47.740	1.194	
Total	59	1487.796			

Source	P
FormulationB No.	0.000
Baking temperature (°C)	0.000
Baking time (min)	0.000
FormulationB No.*	0.000
Baking temperature (°C)	
FormulationB No.*Baking time (min)	0.002
Baking temperature (°C)*	0.511
Baking time (min)	
FormulationB No.*	0.000
Baking temperature (°C)*	
Baking time (min)	
Error	
Total	

S = 1.09248 R-Sq = 96.79% R-Sq(adj) = 95.27

One-way ANOVA: Baking weight loss (%) versus Sample No.

Source	DF	SS	MS	F	P
Sample No.	19	909.677	47.878	63.45	0.000
Error	40	30.182	0.755		
Total	59	939.858			

S = 0.8686 R-Sq = 96.79% R-Sq(adj) = 95.26%

Grouping Information Using Tukey Method

Sample No.	N	Mean	Grouping
16	3	36.027	A
20	3	33.703	A B
4	3	32.387	B C
12	3	31.670	B C D
18	3	30.897	C D E
6	3	29.987	C D E F
10	3	29.577	D E F G
14	3	29.423	D E F G
2	3	28.763	E F G H
8	3	27.593	F G H I
1	3	27.123	G H I J
11	3	26.333	H I J K
19	3	25.593	I J K
5	3	25.030	I J K L
3	3	24.757	J K L
7	3	24.353	K L M
15	3	23.793	K L M
9	3	22.707	L M
17	3	22.403	L M
13	3	21.770	M

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample No.
Individual confidence level = 99.95%

General Linear Model: Rollability versus Formulation, Baking temperature, baking time and storage time .

Factor	Type	Levels	Values
FormulationB No.	fixed	5	1, 2, 3, 4, 5
Baking temperature °C)	fixed	2	230, 240
Baking time (min)	fixed	2	2, 4
Storage time (day)	fixed	5	0, 4, 7, 14, 21

Analysis of Variance for Rollability score, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FormulationB No.	4	58.9917	58.9917	14.7479	107.31	0.000
Baking temperature °C)	1	18.5008	18.5008	18.5008	134.61	0.000
Baking time (min)	1	0.3008	0.3008	0.3008	2.19	0.140
Storage time (day)	4	17.5500	17.5500	4.3875	31.92	0.000
Error	289	39.7192	39.7192	0.1374		
Total	299	135.0625				

S = 0.370724 R-Sq = 70.59% R-Sq(adj) = 69.57%

One-way ANOVA: Rollability score versus Sample No.

Source	DF	SS	MS	F	P
Sample No.	19	91.263	4.803	30.71	0.000
Error	280	43.800	0.156		
Total	299	135.063			

S = 0.3955 R-Sq = 67.57% R-Sq(adj) = 65.37%

Grouping Information Using Tukey Method

Sample No.	N	Mean	Grouping
15	15	4.4667	A
19	15	4.2333	A B
16	15	3.9000	B C
18	15	3.8000	B C D
20	15	3.7667	B C D
14	15	3.6000	C D E
3	15	3.6000	C D E
17	15	3.5667	C D E F
13	15	3.4667	C D E F G
11	15	3.3333	D E F G H
7	15	3.3000	D E F G H
12	15	3.1667	E F G H I
4	15	3.0667	F G H I
2	15	3.0000	G H I
10	15	2.9667	G H I J
1	15	2.9333	H I J
8	15	2.9000	H I J
6	15	2.7667	I J
9	15	2.4667	J K
5	15	2.2000	K

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample No.

Individual confidence level = 99.95%

One-way ANOVA: Subjective rollability at day 0 versus Sample No.

Source	DF	SS	MS	F	P
Sample No.	19	22.5792	1.1884	28.52	0.000
Error	40	1.6667	0.0417		
Total	59	24.2458			

S = 0.2041 R-Sq = 93.13% R-Sq(adj) = 89.86%

Grouping Information Using Tukey Method

Sample No.	N	Mean	Grouping
20	3	4.5000	A
19	3	4.5000	A
18	3	4.5000	A
15	3	4.5000	A
17	3	4.3333	A
16	3	4.3333	A
14	3	4.0000	A B
12	3	3.6667	B C
3	3	3.6667	B C
2	3	3.6667	B C
11	3	3.5000	B C D
7	3	3.5000	B C D
1	3	3.5000	B C D
4	3	3.3333	C D
13	3	3.1667	C D E
10	3	3.1667	C D E
8	3	3.1667	C D E
6	3	3.0000	D E F
9	3	2.6667	E F
5	3	2.5000	F

Means that do not share a letter are significantly different
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample No.

Individual confidence level = 99.95%

One-way ANOVA: Subjective rollability at day 4 versus Sample No.

Source	DF	SS	MS	F	P
Sample No.	19	22.7667	1.1982	17.97	0.000
Error	40	2.6667	0.0667		
Total	59	25.4333			

S = 0.2582 R-Sq = 89.52% R-Sq(adj) = 84.53%

Grouping Information Using Tukey Method

Sample No.	N	Mean	Grouping
15	3	4.5000	A
20	3	4.3333	A B
19	3	4.3333	A B
18	3	4.3333	A B
16	3	4.3333	A B
17	3	4.1667	A B C
14	3	3.6667	B C D
13	3	3.6667	B C D
3	3	3.6667	B C D
12	3	3.5000	C D E
11	3	3.3333	D E
4	3	3.3333	D E
2	3	3.3333	D E
10	3	3.1667	D E F
7	3	3.1667	D E F
1	3	3.1667	D E F
8	3	2.8333	E F
6	3	2.8333	E F
9	3	2.5000	F
5	3	2.5000	F

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample No.
Individual confidence level = 99.95%

One-way ANOVA: Subjective rollability at day 7 versus Sample No.

Source DF SS MS F P
Sample No. 19 22.5167 1.1851 17.78 0.000
Error 40 2.6667 0.0667
Total 59 25.1833

S = 0.2582 R-Sq = 89.41% R-Sq(adj) = 84.38%
Grouping Information Using Tukey Method

Sample No.	N	Mean	Grouping
15	3	4.5000	A
19	3	4.1667	A B
18	3	4.0000	A B C
17	3	3.6667	B C D
16	3	3.6667	B C D
14	3	3.6667	B C D
13	3	3.6667	B C D
3	3	3.6667	B C D
7	3	3.5000	B C D E
20	3	3.3333	C D E F
11	3	3.3333	C D E F
10	3	3.0000	D E F G
4	3	3.0000	D E F G
1	3	3.0000	D E F G
12	3	2.8333	E F G
6	3	2.8333	E F G
2	3	2.8333	E F G
8	3	2.6667	F G
9	3	2.5000	G H
5	3	1.8333	H

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample No.
Individual confidence level = 99.95%

One-way ANOVA: Subjective rollability at day 14 versus Sample No.

Source DF SS MS F P
Sample No. 19 19.9833 1.0518 19.42 0.000
Error 40 2.1667 0.0542
Total 59 22.1500

S = 0.2327 R-Sq = 90.22% R-Sq(adj) = 85.57%

Grouping Information Using Tukey Method

Sample No.	N	Mean	Grouping
15	3	4.5000	A
19	3	4.1667	A B
16	3	3.6667	B C
14	3	3.5000	B C D
13	3	3.5000	B C D
3	3	3.5000	B C D
20	3	3.3333	C D E
11	3	3.3333	C D E
7	3	3.3333	C D E
18	3	3.1667	C D E F
17	3	3.0000	C D E F G
12	3	3.0000	C D E F G
10	3	3.0000	C D E F G
8	3	3.0000	C D E F G
4	3	2.8333	D E F G
6	3	2.6667	E F G H
2	3	2.6667	E F G H
1	3	2.5000	F G H
9	3	2.3333	G H
5	3	2.0000	H

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample No.
Individual confidence level = 99.95%

One-way ANOVA: Subjective rollability at day 21 versus Sample No.

Source DF SS MS F P
Sample No. 19 17.6667 0.9298 13.13 0.000
Error 40 2.8333 0.0708
Total 59 20.5000

S = 0.2661 R-Sq = 86.18% R-Sq(adj) = 79.61%

Grouping Information Using Tukey Method

Sample No.	N	Mean	Grouping
15	3	4.3333	A
19	3	4.0000	A B
16	3	3.5000	B C
3	3	3.5000	B C
20	3	3.3333	B C D
13	3	3.3333	B C D
14	3	3.1667	C D E
11	3	3.1667	C D E
18	3	3.0000	C D E F
7	3	3.0000	C D E F
12	3	2.8333	C D E F G
8	3	2.8333	C D E F G
4	3	2.8333	C D E F G
17	3	2.6667	D E F G
10	3	2.5000	E F G
6	3	2.5000	E F G
2	3	2.5000	E F G
1	3	2.5000	E F G
9	3	2.3333	F G
5	3	2.1667	G

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample No.

Individual confidence level = 99.95%

One-way ANOVA: Subjective rollability at day 28 versus Sample No.

Source DF SS MS F P
Sample No. 19 68.3125 3.5954 43.14 0.000
Error 40 3.3333 0.0833
Total 59 71.6458

S = 0.2887 R-Sq = 95.35% R-Sq(adj) = 93.14%

Grouping Information Using Tukey Method

Sample No.	N	Mean	Grouping
15	3	4.3333	A
19	3	4.0000	A B
16	3	3.3333	B C
3	3	3.3333	B C
20	3	3.1667	B C
14	3	3.0000	C
7	3	3.0000	C
12	3	2.8333	C
11	3	2.8333	C
18	3	2.6667	C
8	3	2.6667	C
4	3	2.6667	C
17	3	2.5000	C D
10	3	2.5000	C D
6	3	2.5000	C D
2	3	2.5000	C D
9	3	1.6667	D E
5	3	1.3333	E
13	3	0.0000	F
1	3	0.0000	F

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample No.

Individual confidence level = 99.95%

Appendix E-3 Original data and statistical analysis of phase 3

Table E-3 Original data of phase 3

Form	BWL (%)	L*	a*	b*	WI	Water activity				Rollability				Rupture force (g)				Rupture distance (mm)			
						Day0	Day7	Day14	Day21	Day0	Day7	Day14	Day21	Day0	Day7	Day14	Day21	Day0	Day7	Day14	Day21
C1	29.37	74.33	-1.93	17.2	69.04	0.93	0.92	0.92	0.91	4	3	3	3	135.36	136.25	99.17	75.27	11.86	10.08	9.82	5.56
	29.72	75.24	-1.94	14.81	71.08	0.92	0.90	0.93	0.91	3	4	3	2	127.66	86.24	69.21	110.32	10.35	9.04	8.76	5.03
	29.53	77.5	-1.89	16.24	72.19	0.93	0.87	0.93	0.93	3.5	3	4	3	79.03	111.79	63.77	83.16	12.04	11.25	10.33	5.86
C2	23.36	73.63	-0.71	17.32	68.44	0.87	0.88	0.85	0.86	5	5	4	4.5	260.59	217.41	238.25	345.37	13.42	13.38	10.11	9.81
	23.32	73.83	-1.25	20.48	66.75	0.87	0.84	0.86	0.86	5	5	4.5	4	196.46	143.79	189.25	281.42	12.95	12.56	10.39	10.33
	23.02	74.64	-1.18	23.14	65.65	0.88	0.88	0.89	0.85	4.5	4.5	4	4	202.79	168.00	245.70	279.71	13.96	13.89	9.59	9.76
C3	22.00	72.6	-2.56	19.37	66.35	0.84	0.86	0.86	0.86	5	4.5	4	4	320.21	360.80	320.66	415.47	11.72	11.16	10.94	10.05
	21.94	72.77	-3.45	20.56	65.71	0.86	0.87	0.86	0.84	4.5	4.5	4.5	4	410.49	283.81	390.62	354.52	12.65	10.26	9.82	9.69
	21.97	70.31	-3.84	23.84	61.73	0.87	0.85	0.88	0.85	5	4.5	4.5	4	348.67	340.87	359.85	349.27	10.35	12.35	12.04	11.07
C4	25.89	64.37	-2.16	22.51	57.80	0.86	0.88	0.87	0.88	4.5	4	4	3	231.64	161.82	186.58	383.57	10.46	10.34	10.03	7.22
	26.47	69.26	-1.58	19.64	63.49	0.88	0.88	0.88	0.89	4.5	4	4	3	265.13	213.80	200.53	320.62	9.59	8.92	9.26	7.57
	26.06	73.56	-1.12	20.7	66.40	0.87	0.88	0.87	0.87	4.5	4.5	4	4	270.97	189.33	138.97	349.15	11.40	11.68	11.02	7.02
C5	28.68	72.87	-3.06	20.28	65.99	0.89	0.90	0.90	0.90	4.5	4	4	3	119.35	163.85	183.49	247.67	10.76	9.78	8.84	6.63
	28.70	69.16	-2.91	21.89	62.07	0.90	0.91	0.90	0.89	4.5	4	4	3	131.19	123.86	201.45	199.73	11.36	10.36	9.36	7.34
	28.75	74.5	-3.14	22.18	66.06	0.90	0.91	0.90	0.90	4.5	4	3	4	184.01	115.36	212.21	172.85	9.69	8.88	8.32	5.94
C6	26.48	73.63	-1.86	20.28	66.68	0.90	0.80	0.88	0.89	5	4	4	3	125.33	89.87	208.40	329.78	10.19	10.56	9.76	5.44
	26.39	71.31	-1.47	21.89	63.88	0.90	0.84	0.88	0.89	4.5	4	4	3	169.87	113.88	240.36	360.83	9.37	11.04	9.04	5.36
	26.36	73.3	-1.89	22.18	65.24	0.90	0.85	0.89	0.88	4.5	4.5	4	3	168.21	79.30	274.27	339.62	11.04	9.96	10.56	5.64
C7	23.83	73.46	-2.33	24.42	63.86	0.88	0.84	0.87	0.87	5	4	3.5	2.5	231.22	243.89	262.32	291.88	10.91	8.46	8.94	6.26
	23.70	71.57	-2.34	20.68	64.77	0.87	0.85	0.88	0.87	4.5	4	3.5	3	156.56	179.91	290.28	233.93	11.44	7.27	7.96	5.65
	23.75	69.1	-2.29	22.95	61.44	0.85	0.87	0.89	0.87	4.5	3.5	3.5	3	183.15	181.91	231.04	305.49	9.99	9.51	9.34	6.89
C8	26.31	73.52	-1.84	21.95	65.56	0.89	0.88	0.89	0.88	4.5	4	4	3	196.13	265.92	321.23	456.98	11.06	9.73	9.03	6.87
	26.19	73.31	-1.81	22.35	65.14	0.89	0.88	0.88	0.88	5	4.5	4	3	169.81	259.93	341.19	401.03	12.06	8.14	8.90	5.17
	26.15	69.23	-1.79	24.01	60.93	0.88	0.87	0.88	0.88	4.5	4	4	3	148.90	301.54	258.41	407.84	10.01	10.36	9.27	7.97
C9	24.52	72.16	-2.68	18.4	66.52	0.86	0.88	0.87	0.87	5	5	5	4	240.11	296.94	385.15	326.08	14.00	13.88	13.45	9.46
	24.53	69.42	-2.17	23.72	61.24	0.85	0.86	0.87	0.88	5	4.5	5	4	216.38	313.95	340.10	281.13	14.06	14.64	12.60	9.24
	24.18	71.86	-2.13	20.21	65.29	0.87	0.87	0.88	0.88	5	5	4.5	4	168.37	306.01	352.48	235.25	13.85	12.99	14.31	10.22

Note: Form = Formulation, BWL (%) = Baking weight loss (%), WI = Whiteness index.

Statistical analysis of phase 3

One-way ANOVA: BWL versus Formulation

Source	DF	SS	MS	F	P
Formulation	8	0.009354	0.001169	3.56	0.012
Error	18	0.005920	0.000329		
Total	26	0.015274			

S = 0.01814 R-Sq = 61.24% R-Sq(adj) = 44.01%

Grouping Information Using Tukey Method

Formulation	N	Mean	Grouping
1	3	0.30786	A
2	3	0.26703	A B
4	3	0.25663	A B
9	3	0.25516	B
5	3	0.25431	B
3	3	0.25401	B
7	3	0.25170	B
8	3	0.24436	B
6	3	0.24081	B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation
Individual confidence level = 99.75%

One-way ANOVA: L* versus Formulation

Source	DF	SS	MS	F	P
Formulation	8	82.74	10.34	1.94	0.115
Error	18	95.87	5.33		
Total	26	178.61			

S = 2.308 R-Sq = 46.33% R-Sq(adj) = 22.47%

Grouping Information Using Tukey Method

Formulation	N	Mean	Grouping
1	3	75.690	A
2	3	74.033	A B
6	3	72.747	A B
5	3	72.177	A B
8	3	72.020	A B
3	3	71.893	A B
7	3	71.377	A B
9	3	71.147	A B
4	3	69.063	B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation
Individual confidence level = 99.75%

One-way ANOVA: Whiteness index versus Formulation

Source DF SS MS F P
Formulation 8 142.52 17.82 2.96 0.027
Error 18 108.49 6.03
Total 26 251.02

S = 2.455 R-Sq = 56.78% R-Sq(adj) = 37.57%

Grouping Information Using Tukey Method

Formulation N Mean Grouping
1 3 70.770 A
2 3 66.946 A B
6 3 65.267 A B
5 3 64.706 A B
3 3 64.594 A B
9 3 64.350 A B
8 3 63.876 A B
7 3 63.356 B
4 3 62.563 B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation
Individual confidence level = 99.75%

One-way ANOVA: Water activity versus Formulation

Source DF SS MS F P
Formulation 8 0.033013 0.004127 18.89 0.000
Error 99 0.021623 0.000218
Total 107 0.054636

S = 0.01478 R-Sq = 60.42% R-Sq(adj) = 57.23%

Grouping Information Using Tukey Method

Formulation N Mean Grouping
1 12 0.91700 A
5 12 0.89929 A
8 12 0.87983 B
6 12 0.87533 B C
4 12 0.87467 B C
9 12 0.86925 B C
7 12 0.86808 B C
2 12 0.86325 B C
3 12 0.85892 C

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation
Individual confidence level = 99.80%

One-way ANOVA: Water activity versus Storage time

Source DF SS MS F P
Storage time 3 0.002508 0.000836 1.67 0.179
Error 104 0.052128 0.000501
Total 107 0.054636

S = 0.02239 R-Sq = 4.59% R-Sq(adj) = 1.84%

One-way ANOVA: Rollability versus Formulation

Source DF SS MS F P
Formulation 8 19.074 2.384 7.60 0.000
Error 99 31.063 0.314
Total 107 50.137

S = 0.5601 R-Sq = 38.04% R-Sq(adj) = 33.04%

Grouping Information Using Tukey Method

Formulation N Mean Grouping
9 12 4.6667 A
2 12 4.5000 A B
3 12 4.4167 A B C
4 12 4.0000 A B C
8 12 3.9583 A B C
6 12 3.9583 A B C
5 12 3.8750 B C D
7 12 3.7083 C D
1 12 3.2083 D

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation
Individual confidence level = 99.80%

One-way ANOVA: Rollability versus Storage time

Source DF SS MS F P
Storage time 3 20.618 6.873 24.21 0.000
Error 104 29.519 0.284
Total 107 50.137

S = 0.5328 R-Sq = 41.12% R-Sq(adj) = 39.43%

Grouping Information Using Tukey Method

Storage time N Mean Grouping
0 27 4.5741 A
7 27 4.2037 A B
14 27 3.9815 B
21 27 3.3704 C

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Storage time
Individual confidence level = 98.96%

One-way ANOVA: Rupture distance versus Formulation

Source DF SS MS F P
Formulation 8 207.48 25.93 7.56 0.000
Error 99 339.56 3.43
Total 107 547.04
S = 1.852 R-Sq = 37.93% R-Sq(adj) = 32.91%

Grouping Information Using Tukey Method
Formulation N Mean Grouping
9 12 12.724 A
2 12 11.677 A B
3 12 11.007 A B C
4 12 9.542 B C D
1 12 9.164 C D
8 12 9.046 C D
6 12 8.995 C D
5 12 8.938 C D
7 12 8.552 D
Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation
Individual confidence level = 99.80%

One-way ANOVA: Rupture distance versus Storage time

Source DF SS MS F P
Storage time 3 242.34 80.78 27.57 0.000
Error 104 304.70 2.93
Total 107 547.04
S = 1.712 R-Sq = 44.30% R-Sq(adj) = 42.69%

Grouping Information Using Tukey Method
Storage time N Mean Grouping
0 27 11.500 A
7 27 10.758 A B
14 27 10.065 B
21 27 7.519 C
Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sorage time
Individual confidence level = 98.96%

One-way ANOVA: Rupture force versus Sample

Source DF SS MS F P
Sample 8 531043 66380 15.45 0.000
Error 99 425218 4295
Total 107 956261

S = 65.54 R-Sq = 55.53% R-Sq(adj) = 51.94%

Grouping Information Using Tukey Method

Sample N Mean Grouping
3 12 354.60 A
8 12 294.08 A B
9 12 288.50 A B C
4 12 242.68 B C D
7 12 232.63 B C D
2 12 230.73 B C D
6 12 208.31 C D
5 12 171.25 D E
1 12 98.10 E

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 = 99.80%

One-way ANOVA: Rupture force versus Storage time

Source DF SS MS F P
Storage time 3 155270 51757 6.72 0.000
Error 104 800991 7702
Total 107 956261

S = 87.76 R-Sq = 16.24% R-Sq(adj) = 13.82%

Grouping Information Using Tukey Method

Storage time N Mean Grouping
21 27 294.00 A
14 27 244.63 A B
0 27 202.13 B
7 27 201.85 B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sorage time
Individual confidence level = 98.96%

Taguchi Design

Taguchi Orthogonal Array Design

L9(3**4)

Factors: 4
Runs: 9

Columns of L9(3**4) Array

1 2 3 4

Taguchi Analysis: BWL versus Oil, CMC, X&G
Response Table for Means

Level	Oil	CMC	X&G
1	0.2763	0.2721	0.2643
2	0.2506	0.2552	0.2596
3	0.2504	0.2500	0.2533
Delta	0.0259	0.0221	0.0110
Rank	1	2	3

Taguchi Analysis: L* versus Oil, CMC, X&G
Response Table for Means

Level	Oil	CMC	X&G
1	73.87	72.04	73.49
2	71.33	72.74	71.41
3	71.51	71.93	71.82
Delta	2.54	0.81	2.07
Rank	1	3	2

Taguchi Analysis: Whiteness index versus CCO, CMC, X&G
Response Table for Means

Level	CCO	CMC	X&G
1	67.44	65.56	66.64
2	64.18	65.18	64.62
3	63.86	64.74	64.22
Delta	3.58	0.83	2.42
Rank	1	3	2

Taguchi Analysis: Mean Water activity versus CCO, CMC, X&G
Response Table for Means

Level	CCO	CMC	X&G
1	0.8797	0.8866	0.8907
2	0.8831	0.8808	0.8691
3	0.8724	0.8678	0.8754
Delta	0.0107	0.0188	0.0217
Rank	3	2	1

Taguchi Analysis: mean Rollability versus CCO, CMC, X&G
Response Table for Means

Level	CCO	CMC	X&G
1	4.042	3.639	3.708
2	3.944	4.111	4.389
3	4.111	4.347	4.000
Delta	0.167	0.708	0.681
Rank	3	1	2

Taguchi Analysis: Rupture force versus CCO, CMC, X&G
Response Table for Means

Level	CCO	CMC	X&G
1	227.8	191.1	200.2
2	207.4	232.0	254.0
3	271.7	283.8	252.8
Delta	64.3	92.7	53.8
Rank	2	1	3

Appendix E-4 Original data and statistical analysis of phase 4

Table E-1 Original physical data of phase 4

Formulation	BWL (%)	L*	a*	b*	Whiteness index	Water activity			Subjective rollability			Rupture force (g)			Rupture distance (mm)		
						Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
D1	26.02	74.39	-1.21	19.87	67.56	0.87	0.88	0.86	5.0	5.0	5.0	229.36	200.65	200.68	12.86	12.66	13.39
D1	29.63	76.10	-1.57	20.28	68.62	0.87	0.88	0.86	5.0	4.5	5.0	223.57	213.27	209.32	14.65	12.29	12.70
D1	26.32	77.93	-1.17	19.67	70.41	0.88	0.87	0.85	5.0	5.0	4.5	200.37	182.15	239.07	15.70	10.35	11.04
D1	25.80	76.07	-0.99	20.21	68.66	0.87	0.88	0.86	5.0	5.0	5.0	239.09	199.18	218.74	13.43	13.77	13.66
D1	22.47	72.62	-1.41	21.96	64.87	0.87	0.88	0.86	5.0	4.5	5.0	258.91	229.38	254.96	15.45	13.03	12.43
D1	26.52	78.14	-1.10	22.55	68.57	0.88	0.86	0.85	5.0	5.0	4.5	171.99	151.47	266.31	15.20	10.00	11.88
D2	26.02	76.17	-1.21	19.07	69.45	0.87	0.87	0.87	5.0	5.0	5.0	200.68	198.26	250.17	13.45	12.37	10.56
D2	26.35	75.64	-1.03	21.31	67.62	0.86	0.86	0.87	5.0	5.0	5.0	220.17	187.57	231.78	14.04	13.59	9.87
D2	28.02	73.05	-0.98	23.87	63.99	0.87	0.86	0.85	5.0	4.5	4.5	223.72	185.48	208.45	12.55	12.60	9.95
D2	27.10	74.79	-1.39	20.21	67.66	0.88	0.86	0.87	5.0	5.0	5.0	223.12	206.04	268.65	13.69	12.33	12.15
D2	24.91	73.28	-1.13	18.65	67.40	0.86	0.87	0.86	5.0	5.0	5.0	237.26	196.73	255.45	15.29	14.45	10.61
D2	27.28	74.18	-0.96	19.90	67.38	0.88	0.87	0.86	5.0	4.5	4.5	245.05	173.82	185.92	13.02	14.55	9.29
D3	26.84	73.01	-1.78	21.65	65.35	0.88	0.86	0.86	5.0	5.0	5.0	177.56	326.85	300.50	13.15	13.70	16.11
D3	25.92	74.97	-2.11	19.23	68.37	0.88	0.88	0.86	5.0	5.0	4.5	204.94	285.81	313.20	15.77	15.06	11.91
D3	24.82	67.53	-1.76	19.64	62.01	0.87	0.88	0.86	5.0	5.0	5.0	197.40	383.64	375.27	15.97	14.27	14.23
D3	25.96	71.37	-1.80	20.39	64.81	0.88	0.87	0.86	5.0	5.0	5.0	200.48	283.67	313.25	15.03	13.59	14.64
D3	25.35	73.27	-2.37	20.19	66.42	0.88	0.87	0.86	5.0	5.0	4.5	220.27	290.47	351.03	15.26	15.57	14.66
D3	24.30	72.69	-1.69	20.01	66.10	0.88	0.87	0.86	5.0	5.0	5.0	229.36	345.34	355.35	14.36	13.58	11.06

Note: BWL (%) = Baking weight loss (%)

Table E-2 Original data of sensory evaluation

Formulation	Day1					Day7					Day14				
	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability
D1	3	2	4	3	3	1	1	2	2	2	6	6	6	6	7
D1	4	3	5	4	3	3	2	4	2	2	5	6	4	5	4
D1	5	3	5	4	4	3	3	4	2	4	7	7	6	5	6
D1	5	3	5	4	4	4	4	4	3	4	9	9	8	9	9
D1	5	4	6	5	4	4	4	5	3	4	6	7	5	6	6
D1	5	4	6	5	4	5	5	5	4	4	6	6	7	7	8
D1	6	5	6	6	5	5	5	5	5	6	7	7	7	6	6
D1	6	5	6	6	5	5	5	5	6	6	6	6	6	5	5
D1	6	5	6	6	6	5	6	5	6	6	7	8	8	8	8
D1	6	6	6	6	6	5	6	6	6	6	4	4	5	7	6
D1	6	6	6	6	6	6	7	6	6	6	4	5	4	5	4
D1	7	6	7	6	7	6	7	6	6	6	5	5	5	5	5
D1	7	6	7	6	7	6	7	6	6	7	8	8	5	5	8
D1	7	7	7	6	7	7	7	6	6	7	5	3	5	6	7
D1	7	7	7	6	7	7	7	6	6	7	2	3	3	2	3
D1	7	7	7	6	7	7	7	7	7	7	5	6	4	7	6
D1	7	7	7	7	7	7	7	7	7	7	9	7	9	9	9
D1	7	7	7	7	7	7	7	7	7	7	4	4	6	3	3
D1	8	7	7	7	7	7	7	7	7	7	7	7	5	7	7
D1	8	7	7	7	7	7	7	7	7	7	5	5	5	5	5
D1	8	8	7	7	7	7	7	7	7	7	5	4	4	4	4
D1	8	8	7	7	7	7	7	7	7	7	3	4	4	4	4
D1	8	8	8	7	7	7	8	7	8	7	5	6	5	6	6
D1	8	8	8	8	8	8	8	7	8	7	6	7	7	7	7
D1	8	8	8	8	8	8	8	7	8	8	3	2	3	3	4
D1	8	8	8	8	8	8	8	7	8	8	3	3	3	4	4
D1	8	8	8	9	8	8	8	8	8	8	5	5	5	6	5
D1	8	8	8	9	9	8	8	8	8	8	9	7	8	8	9
D1	8	9	9	9	9	8	8	8	8	8	8	7	8	6	7
D1	9	9	9	9	9	8	8	8	9	8	9	8	8	8	8
D1	6	3	6	6	5	7	6	6	4	5	7	6	6	5	5
D1	5	8	8	8	8	7	6	4	5	6	8	8	9	9	8
D1	6	7	6	6	6	4	5	6	6	6	8	9	8	9	8
D1	4	5	5	3	4	5	6	7	3	3	6	7	7	7	7
D1	7	7	6	4	6	6	7	6	5	6	3	4	4	4	4
D1	6	4	6	6	6	1	1	1	1	1	5	4	4	4	4
D1	7	7	9	9	8	7	7	5	7	7	4	6	5	4	5
D1	6	8	7	9	7	6	8	8	8	8	2	3	2	1	2
D1	7	6	7	6	6	6	6	8	9	8	5	6	5	7	6
D1	6	6	8	8	7	7	6	6	6	6	8	8	9	6	7
D1	7	7	7	8	7	8	3	4	4	4	7	4	7	4	5
D1	6	6	6	6	6	6	6	4	7	7	6	6	6	6	5

Formulation	Day1					Day7					Day14				
	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability
D1	3	6	6	8	7	5	4	6	5	5	8	4	5	4	5
D1	6	7	7	7	7	6	4	5	6	6	7	6	6	7	6
D1	6	6	5	4	5	6	6	6	6	6	6	3	6	6	5
D1	6	6	6	6	6	9	9	9	9	9	5	8	8	8	8
D1	6	6	5	3	5	3	3	3	3	3	6	7	6	6	6
D1	7	7	8	8	8	7	5	6	7	6	4	5	5	3	4
D1	6	7	7	8	8	6	4	6	6	5	7	7	6	4	6
D1	7	8	7	8	8	7	6	7	6	7	6	4	6	6	6
D1	6	7	6	7	6	7	7	6	7	7	5	4	5	5	3
D1	6	6	7	6	7	7	7	8	8	7	6	7	6	8	6
D1	8	8	8	9	8	6	7	7	8	7	5	4	6	6	3
D1	8	7	8	6	6	8	5	9	6	4	7	7	6	9	9
D1	5	7	6	4	6	6	8	8	8	7	7	8	6	7	6
D1	4	3	6	6	6	8	8	8	8	8	5	8	7	6	7
D1	8	7	6	5	6	6	8	9	8	8	4	4	5	6	5
D1	7	4	5	5	6	6	8	8	9	8	8	8	8	8	8
D1	7	6	6	5	5	6	6	6	6	6	5	4	6	5	5
D1	7	7	7	6	7	4	3	4	4	4	7	6	7	7	7
D2	6	6	5	7	6	8	7	8	8	8	3	2	4	2	3
D2	7	8	8	7	8	6	6	7	7	7	3	2	5	3	4
D2	8	8	8	9	8	7	7	7	7	7	4	3	5	4	4
D2	5	5	5	5	5	6	6	5	5	6	4	3	5	4	4
D2	4	5	4	4	4	8	7	7	8	7	4	3	5	4	4
D2	6	7	7	7	7	5	6	7	6	6	4	4	5	5	4
D2	7	8	7	6	6	5	6	7	6	6	5	4	5	5	4
D2	6	6	7	7	7	5	6	6	7	6	5	4	5	5	5
D2	5	6	6	6	6	6	8	8	7	7	5	4	5	5	5
D2	5	6	5	7	7	7	8	8	8	8	5	5	5	5	6
D2	6	7	9	5	6	6	7	6	7	7	5	5	5	6	6
D2	7	5	6	5	5	5	6	6	7	6	5	5	6	6	6
D2	7	5	6	7	6	6	8	6	7	8	6	5	6	6	6
D2	5	5	5	6	6	5	6	6	7	7	6	6	6	7	6
D2	6	8	7	8	8	3	3	8	8	6	6	6	6	7	6
D2	5	7	7	7	7	5	7	7	7	7	6	6	6	7	6
D2	3	3	8	8	6	6	8	7	8	8	6	6	6	7	6
D2	5	6	6	7	7	4	4	7	6	5	6	6	6	7	6
D2	6	8	6	7	8	7	8	7	8	8	6	6	6	7	6
D2	5	6	6	7	6	7	7	6	6	7	8	6	6	7	6
D2	6	5	5	6	5	8	8	7	8	8	7	6	7	8	6
D2	7	6	6	7	6	5	3	4	6	6	7	6	7	8	6
D2	6	5	4	5	6	9	7	7	7	8	7	6	7	8	6
D2	6	5	5	6	6	8	7	7	7	7	7	6	7	8	7
D2	5	5	6	6	5	6	8	7	7	7	7	7	7	8	7

Formulation	Day1					Day7					Day14				
	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability
D2	7	7	6	7	8	3	3	7	4	4	7	7	7	8	7
D2	7	5	6	4	5	5	5	6	5	5	7	7	7	8	7
D2	7	6	7	6	7	5	2	7	2	2	8	7	8	8	7
D2	7	6	7	6	7	7	5	7	6	6	8	7	8	8	8
D2	7	7	8	7	8	5	5	6	5	5	8	8	8	8	8
D2	6	7	6	6	7	3	2	5	4	2	6	5	5	4	4
D2	5	5	6	3	6	3	2	5	4	4	3	6	7	7	7
D2	8	9	5	5	6	4	3	5	5	4	4	4	5	5	4
D2	7	8	4	7	6	4	3	6	5	4	4	4	4	3	3
D2	7	7	7	7	7	4	4	6	5	4	3	4	5	6	4
D2	7	4	5	5	6	5	4	6	5	5	3	3	3	2	2
D2	8	7	7	8	7	5	4	6	5	5	6	5	5	6	5
D2	8	7	7	8	8	5	5	6	6	5	7	7	8	8	8
D2	7	7	8	7	7	5	5	6	6	5	7	6	6	6	6
D2	7	8	8	6	7	5	5	6	6	5	8	4	7	6	6
D2	6	6	6	6	7	5	5	6	6	6	6	7	4	5	6
D2	3	3	3	2	3	5	6	6	6	6	7	6	7	6	6
D2	7	7	7	4	6	5	6	6	6	6	6	6	5	6	6
D2	5	6	7	6	6	5	6	7	6	6	7	4	4	3	4
D2	5	5	5	5	5	6	6	7	6	6	9	9	9	9	9
D2	5	5	4	4	5	6	6	7	7	6	3	4	4	4	4
D2	9	9	9	9	9	6	6	7	7	6	5	3	6	7	5
D2	3	4	3	2	3	6	6	7	7	6	4	4	6	6	5
D2	7	6	5	6	6	6	6	7	7	6	6	6	6	6	6
D2	5	7	7	7	7	7	6	7	7	6	7	6	8	7	7
D2	5	4	4	5	5	7	7	7	7	7	4	6	6	6	5
D2	5	5	5	5	5	7	7	7	7	7	6	7	5	6	6
D2	5	6	7	7	7	7	7	7	8	7	8	5	9	6	4
D2	8	8	8	8	8	7	7	7	8	7	4	6	9	6	6
D2	4	4	4	4	4	7	7	8	8	7	6	6	6	4	4
D2	7	6	6	7	6	7	8	8	8	7	7	8	5	5	6
D2	7	7	6	7	6	7	8	8	8	7	8	8	6	7	8
D2	9	7	9	9	9	8	8	9	8	8	9	3	3	4	3
D2	8	8	8	8	8	8	9	9	8	8	5	5	5	5	5
D2	9	8	8	8	8	9	9	9	9	8	4	5	5	4	5
D3	6	5	5	5	5	6	7	7	8	7	3	3	4	4	4
D3	7	7	8	8	8	5	5	6	4	6	4	3	5	4	4
D3	4	3	4	4	5	8	9	7	6	7	4	3	5	5	4
D3	6	7	7	7	7	8	5	8	7	7	5	3	5	5	5
D3	6	6	6	6	6	7	7	6	7	7	5	4	5	5	5
D3	5	5	5	3	5	4	4	4	4	4	5	4	5	5	6
D3	5	6	5	6	6	9	7	7	7	8	5	6	5	5	6
D3	8	8	9	4	5	8	7	7	8	8	5	6	5	6	6

Formulation	Day1					Day7					Day14				
	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability
D3	7	6	6	5	6	6	5	7	7	7	5	6	5	6	6
D3	7	7	6	8	7	7	7	6	6	7	6	6	5	6	6
D3	8	5	6	6	6	8	7	7	8	8	6	6	5	6	6
D3	7	6	7	6	5	3	3	3	4	4	6	6	5	6	6
D3	6	6	7	7	7	8	8	8	8	8	7	6	6	6	6
D3	5	7	6	6	6	6	6	3	5	6	7	6	6	7	6
D3	8	7	8	8	8	3	3	3	4	4	7	6	6	7	6
D3	6	6	7	7	7	4	7	6	3	5	7	7	6	7	7
D3	7	7	7	7	7	9	9	9	8	9	7	7	5	7	7
D3	6	6	5	5	6	5	6	7	5	5	7	5	7	7	7
D3	8	7	7	8	7	7	7	5	8	7	7	7	7	7	7
D3	5	6	7	6	6	7	3	6	3	6	7	5	7	7	8
D3	5	7	6	6	6	5	6	6	6	6	7	8	7	8	8
D3	7	7	6	9	8	7	7	7	7	7	7	8	7	8	8
D3	6	6	6	7	7	5	6	6	7	7	7	5	7	8	8
D3	5	6	7	6	8	7	7	7	7	7	7	7	7	8	8
D3	5	7	5	6	7	3	3	3	4	4	8	8	8	8	8
D3	4	5	8	6	5	5	5	6	6	6	8	5	8	8	8
D3	4	4	6	5	6	6	6	6	6	6	8	8	8	8	8
D3	8	7	4	3	6	9	7	8	7	8	8	8	8	9	8
D3	7	6	7	6	6	9	5	8	8	8	9	8	9	9	8
D3	7	7	7	7	7	9	8	8	8	8	9	5	9	9	9
D3	6	5	7	8	8	3	1	4	2	4	5	5	5	4	4
D3	5	7	7	7	7	4	2	5	5	4	6	3	6	6	6
D3	3	3	8	8	6	4	3	5	2	4	4	4	5	4	5
D3	5	6	6	7	7	5	4	5	3	4	6	3	6	2	3
D3	6	8	6	7	8	5	4	6	4	5	4	3	4	3	3
D3	5	6	6	7	6	5	4	6	4	5	2	2	3	2	2
D3	6	7	6	7	7	5	4	6	5	5	4	4	5	6	6
D3	7	5	8	8	8	5	4	6	5	5	7	6	8	8	7
D3	6	8	8	7	7	6	5	6	6	5	6	7	8	7	7
D3	5	5	6	7	6	6	5	6	6	5	8	8	6	8	8
D3	5	6	7	6	6	6	5	6	6	5	8	8	8	9	8
D3	5	6	7	6	6	6	5	6	6	5	7	7	4	7	7
D3	8	5	7	8	7	6	5	7	6	5	7	6	7	6	6
D3	6	6	5	5	6	6	6	7	6	6	6	6	5	7	7
D3	7	7	7	7	7	6	6	7	6	6	6	3	6	4	4
D3	6	6	7	7	7	7	6	7	6	6	8	8	8	9	8
D3	8	7	8	8	8	7	6	7	6	6	2	3	4	4	4
D3	6	5	5	6	6	7	6	7	7	6	7	5	7	6	5
D3	7	7	7	5	6	7	6	7	7	6	8	6	6	6	6
D3	7	5	8	6	7	7	7	7	7	6	7	8	7	8	8
D3	6	7	7	7	7	7	7	8	7	7	7	8	8	8	8

Formulation	Day1					Day7					Day14				
	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability	Appearance	Texture	Aroma	Taste	Overall rollability
D3	4	4	5	6	5	8	7	8	7	7	6	7	8	6	6
D3	9	9	8	9	9	8	7	8	7	7	6	6	8	7	7
D3	8	8	8	7	7	8	7	8	7	8	7	3	9	8	4
D3	7	9	7	8	8	8	7	8	8	8	7	2	5	7	4
D3	6	7	7	6	7	8	7	8	8	8	4	3	7	8	7
D3	7	7	6	6	6	8	8	8	8	8	5	6	7	6	6
D3	4	7	7	7	7	8	8	8	8	8	7	9	5	9	9
D3	7	6	7	7	7	8	8	8	8	8	4	4	4	4	4
D3	7	7	6	6	6	8	8	8	8	8	5	4	4	4	5

Table E-3 Results of sensory evaluation

Parameter	Storage time	D1	D2	D3
Overall acceptability	Day 1	6.4±1.4	6.4±1.3	6.6±0.9
	Day 7	6.1±1.7	6.2±1.4	6.3±1.4
	Day 14	5.9±1.7	5.5±1.4	6.2±1.6
Texture	Day 1	6.3±1.6	6.2±1.4	6.3±1.2
	Day 7	6.1±1.9	6.0±1.8	5.8±1.7
	Day 14	5.8±1.8	5.3±1.5	5.5±1.9
Aroma	Day 1	6.7±1.1	6.2±1.5	6.6±1.1
	Day 7	6.2±1.7	6.7±1.0	6.5±1.4
	Day 14	5.8±1.6	5.9±1.4	6.2±1.5
Taste	Day 1	6.4±1.6	6.2±1.6	6.5±1.3
	Day 7	6.1±2.0	6.5±1.3	6.1±1.7
	Day 14	5.9±1.8	5.9±1.7	6.4±1.8
Appearance	Day 1	6.5±1.3	6.2±1.4	6.2±1.3
	Day 7	6.1±1.7	5.9±1.5	6.4±1.7
	Day 14	5.8±1.7	5.8±1.6	6.2±1.6

[1]mean (±SD), (n=60).

Statistical analysis of phase 4

One-way ANOVA: Baking weight loss versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation	2	0.000353	0.000176	0.73	0.496
Error	15	0.003602	0.000240		
Total	17	0.003955			

S = 0.01550 R-Sq = 8.92% R-Sq(adj) = 0.00%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
2.00	6	0.26616	A
1.00	6	0.26127	A
3.00	6	0.25533	A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: L* versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	42.88	21.44	5.16	0.020
Error	15	62.29	4.15		
Total	17	105.17			

S = 2.038 R-Sq = 40.78% R-Sq(adj) = 32.88%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
1.00	6	75.875	A
2.00	6	74.518	A B
3.00	6	72.140	B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: whiteness index versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	21.17	10.58	2.90	0.086
Error	15	54.82	3.65		
Total	17	75.99			

S = 1.912 R-Sq = 27.85% R-Sq(adj) = 18.24%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
1.00	6	68.117	A
2.00	6	67.250	A
3.00	6	65.509	A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Water activity at day1 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	0.0001080	0.0000540	1.97	0.174
Error	15	0.0004120	0.0000275		
Total	17	0.0005200			

S = 0.005241 R-Sq = 20.77% R-Sq(adj) = 10.21%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
3.00	6	0.876000	A
1.00	6	0.873000	A
2.00	6	0.870000	A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Water activity at day 7 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	0.0002080	0.0001040	2.84	0.090
Error	15	0.0005500	0.0000367		
Total	17	0.0007580			

S = 0.006055 R-Sq = 27.44% R-Sq(adj) = 17.77%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
1.00	6	0.873000	A
3.00	6	0.871000	A
2.00	6	0.865000	A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Water activity at day 14 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	0.0000480	0.0000240	0.78	0.478
Error	15	0.0004640	0.0000309		
Total	17	0.0005120			

S = 0.005562 R-Sq = 9.37% R-Sq(adj) = 0.00%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
2.00	6	0.862000	A
3.00	6	0.860000	A
1.00	6	0.858000	A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Rupture distance at day1 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	4.93	2.46	2.23	0.142
Error	15	16.57	1.10		
Total	17	21.50			

S = 1.051 R-Sq = 22.93% R-Sq(adj) = 12.66%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
3.00	6	14.920	A
1.00	6	14.548	A
2.00	6	13.672	A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Rupture distance at day 7 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	15.71	7.85	5.81	0.014
Error	15	20.26	1.35		
Total	17	35.97			

S = 1.162 R-Sq = 43.67% R-Sq(adj) = 36.16%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
3.00	6	14.297	A
2.00	6	13.315	A B
1.00	6	12.016	B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Rupture distance at day 14 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	34.66	17.33	9.42	0.002
Error	15	27.58	1.84		
Total	17	62.24			

S = 1.356 R-Sq = 55.69% R-Sq(adj) = 49.78%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
3.00	6	13.765	A
1.00	6	12.514	A
2.00	6	10.403	B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Rupture force at day1 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	1323	662	1.32	0.296
Error	15	7498	500		
Total	17	8821			

S = 22.36 R-Sq = 15.00% R-Sq(adj) = 3.67%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
2.00	6	225.00	A
1.00	6	220.55	A
3.00	6	205.00	A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Rupture force at day 7 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	63199	31600	38.33	0.000
Error	15	12365	824		
Total	17	75565			

S = 28.71 R-Sq = 83.64% R-Sq(adj) = 81.45%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
3.00	6	319.30	A
1.00	6	196.02	B
2.00	6	191.31	B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

One-way ANOVA: Rupture force at day 14 versus Formulation D No.

Source	DF	SS	MS	F	P
Formulation D No.	2	41878	20939	24.64	0.000
Error	15	12745	850		
Total	17	54622			

S = 29.15 R-Sq = 76.67% R-Sq(adj) = 73.56%

Grouping Information Using Tukey Method

Formulation D No.	N	Mean	Grouping
3.00	6	334.77	A
2.00	6	233.40	B
1.00	6	231.51	B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation D No.
Individual confidence level = 97.97%

General Linear Model: Water activity, rupture force, rupture distance ... versus Formulation D No., Storage time

Factor Type Levels Values
Formulation C No. fixed 3 1, 2, 3
Storage time fixed 3 1, 7, 14

Analysis of Variance for **water activity**, using Adjusted SS for Tests

Source DF Seq SS Adj SS Adj MS F P
Formulation C No. 2 0.0001053 0.0001053 0.0000527 1.53 0.226
Storage time 2 0.0016413 0.0016413 0.0008207 23.87 0.000
Error 49 0.0016847 0.0016847 0.0000344
Total 53 0.0034313

S = 0.00586353 R-Sq = 50.90% R-Sq(adj) = 46.90%

Analysis of Variance for **rupture force**, using Adjusted SS for Tests

Source DF Seq SS Adj SS Adj MS F P
Formulation C No. 2 58895 58895 29447 18.01 0.000
Storage time 2 22697 22697 11348 6.94 0.002
Error 49 80113 80113 1635
Total 53 161705

S = 40.4347 R-Sq = 50.46% R-Sq(adj) = 46.41%

Analysis of Variance for **rupture distance**, using Adjusted SS for Tests

Source DF Seq SS Adj SS Adj MS F P
Formulation C No. 2 32.920 32.920 16.460 9.29 0.000
Storage time 2 41.813 41.813 20.906 11.80 0.000
Error 49 86.788 86.788 1.771
Total 53 161.521

S = 1.33086 R-Sq = 46.27% R-Sq(adj) = 41.88%

Kruskal-Wallis Test: Rollability versus Formulation No.

Ranks				
	Storage time	**Formulation**	**N**	**Mean Rank**
Rollability	Day 1	D1	6	9.50
		D2	6	9.50
		D3	6	9.50
		Total	18	
	Day 7	D1	6	8.50
		D2	6	8.50
		D3	6	11.50
		Total	18	
	Day 14	D1	6	9.50
		D2	6	9.50
		D3	6	9.50
		Total	18	

Test Statistics[a,b]			
	Rollability		
	Day 1	Day 7	Day 14
Chi-Square	.000	2.429	.000
df	2	2	2
Asymp. Sig.	1.000	.297	1.000

a. Kruskal Wallis Test
b. Grouping Variable: Formulation

Kruskal-Wallis Test: Rollability versus Storage time

Ranks			
	Storage time	N	Mean Rank
Rollability	Day 1	18	32.50
	Day 7	18	26.50
	Day 14	18	23.50
	Total	54	

Test Statistics[a,b]	
	Rollability
Chi-Square	6.745
df	2
Asymp. Sig.	.034

a. Kruskal Wallis Test; b. Grouping Variable: Storage time

Kruskal-Wallis Test for sensory attributes

	Storage time	N	Mean Rank		
			D1	D2	D3
Appearance	Day 1	60	100.63	98.4	85.65
	Day 7	60	91.44	87.15	97.03
	Day 14	60	79.43	85.95	88.83
	Total	180			
Texture	Day 1	60	97.23	100.87	100.38
	Day 7	60	92.51	96.33	89.88
	Day 14	60	81.76	74.31	81.23
	Total	180			
Aroma	Day 1	60	105.33	88.14	94.88
	Day 7	60	90.3	108.74	95.93
	Day 14	60	75.88	74.62	80.7
	Total	180			
Taste	Day 1	60	96.79	90.18	92.67
	Day 7	60	93.18	100.68	85.21
	Day 14	60	81.53	80.64	93.63
	Total	180			
Overall acceptability	Day 1	60	98.63	102.02	96.84
	Day 7	60	92.81	97.83	87.17
	Day 14	60	80.06	71.66	87.49
	Total	180			

Test Statistics[a,b]

	Sensory attributes	Chi-Square	df	Asymp. Sig.
D1	Appearance	5.198	2	0.074
	Texture	2.889	2	0.236
	Aroma	10.013	2	0.007
	Taste	2.911	2	0.233
	Overall acceptability	4.147	2	0.126
D2	Appearance	2.172	2	0.338
	Texture	9.262	2	0.01
	Aroma	13.763	2	0.001
	Taste	4.644	2	0.098
	Overall acceptability	12.665	2	0.002
D3	Appearance	1.586	2	0.452
	Texture	4.229	2	0.121
	Aroma	3.363	2	0.186
	Taste	0.981	2	0.612
	Overall acceptability	1.404	2	0.496

a. Kruskal Wallis Test
b. Grouping Variable: Storage time

Kruskal-Wallis Test sensory attributes versus formulation

	Ranks			
Storage time	Sensory attributes	Formulation	N	Mean Rank
Day1	Appearance	D1	60	99.48
		D2	60	86.88
		D3	60	85.15
		Total	180	
	Texture	D1	60	95.77
		D2	60	86.92
		D3	60	88.82
		Total	180	
	Aroma	D1	60	97.25
		D2	60	80.44
		D3	60	93.81
		Total	180	
	Taste	D1	60	90.72
		D2	60	86.72
		D3	60	94.07
		Total	180	
	Overall acceptability	D1	60	91.13
		D2	60	86.18
		D3	60	94.19
		Total	180	
Day 7	Appearance	D1	60	92.44
		D2	60	79.56
		D3	60	99.50
		Total	180	
	Texture	D1	60	95.73
		D2	60	89.82
		D3	60	85.95
		Total	180	
	Aroma	D1	60	81.33
		D2	60	97.13
		D3	60	93.05
		Total	180	
	Taste	D1	60	88.86
		D2	60	96.43
		D3	60	86.21
		Total	180	
	Overall acceptability	D1	60	91.31
		D2	60	88.86
		D3	60	91.33
		Total	180	

Storage time	Sensory attributes	Formulation	N	Mean Rank
Day 14	Appearance	D1	60	87.28
		D2	60	85.76
		D3	60	98.46
		Total	180	
	Texture	D1	60	97.63
		D2	60	83.57
		D3	60	90.31
		Total	180	
	Aroma	D1	60	86.76
		D2	60	88.23
		D3	60	96.52
		Total	180	
	Taste	D1	60	84.13
		D2	60	86.09
		D3	60	101.28
		Total	180	
	Overall acceptability	D1	60	89.60
		D2	60	79.38
		D3	60	102.52
		Total	180	

Test Statistics[a,b]

Storage time	Sensory attributes	Chi-Square	df	Asymp. Sig.
Day 1	Appearance	2.844	2	0.241
	Texture	1.011	2	0.603
	Aroma	3.704	2	0.157
	Taste	0.629	2	0.73
	Overall acceptability	0.773	2	0.679
Day 7	Appearance	4.689	2	0.096
	Texture	1.113	2	0.573
	Aroma	3.173	2	0.205
	Taste	1.301	2	0.522
	Overall acceptability	0.094	2	0.954
Day 14	Appearance	2.197	2	0.333
	Texture	2.257	2	0.324
	Aroma	1.28	2	0.527
	Taste	4.017	2	0.134
	Overall acceptability	6.178	2	0.046

a. Kruskal Wallis Test
b. Grouping Variable: Formulation