Development of a Gluten-free Commercial Bread

by

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Attestation of Authorship

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Signed …….

Date …….

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Abstract

Background

Because of coeliac disease, some individuals cannot tolerate the protein gliadin present in the gluten fraction of wheat flour. From a commercial perspective, there is a need for the development of gluten-free bread with texture and flavour properties similar to the conventional wheat flour loaf. In the context of bread, the gluten component of wheat has a crucial role in stabilising the gas-cell structure and maintaining the rheological properties of the bread. The absence of gluten results in liquid batter rather than pre-baking dough, yielding baked bread with a crumbling texture, poor colour and other post-baking quality defects. The liquid batter cannot be processed on the existing production line of baking industry.

Aim

The aim is to develop a gluten-free white loaf with similar quality characteristics to that of standard white bread on the existing processing lines at Quality Bakers New Zealand. Within this constraint, dough has to be produced with handling and moulding properties similar to those of conventional wheat flour loaves. This research focused on finding and implementing the gluten substitutes for the development of gluten-free high quality commercial bread.

Methods

In this research, the independent variables were conventional wheat flour (the most basic control), other gluten-free flours from a variety of sources, starches, supplementary proteins, hydrocolloids such as hydroxypropylmethylcellulose (HPMC), hydrophilic psyllium husk, and enzymes such as microbial transglutaminase, glucose oxidase, lipase and fungal α-amylase. These ingredients were trialled in different combination and composition to produce a dough having ability to trap the carbon dioxide gas during proofing and baking to get high specific volume bread suitable for the Quality Bakers' product range.

After an essentially 'shotgun' approach to formulations, the research narrowed to a systematic and progressive variation of ingredients and their composition to develop workable commercial models. Ingredients and their compositions were manipulated according to the outcomes of the trials and their contribution in the formulations. The dependent variables included standard bakery rheological properties based on dough stickiness, dough extensibility, oven spring, bread specific volume, bread sliceability, and bread staling.

A gelation system of the lower-temperature-stable hydrocolloid psyllium husk, the heat-stable hydrocolloid hydroxypropylmethylcellulose, maize starch, and potato starch was created to form industrial processable dough having ability to entrap carbon dioxide gas produced during proofing and initial phase of baking. Microbial transglutaminase was used to increase the cross linking of protein present in flours and supplemented for enhancing the dough-like structure and its gas entrapping abilities.

Results

A formulation has been discovered by this research for the development of high quality gluten-free commercial bread. The formulated bread has similar quality characteristics to that of standard white bread and can be produced on existing processing lines at Quality Bakers.

Conclusion

Industrial processable gluten-free bread with similar quality characteristics to that of standard white bread can be formulated by using a specific combination of soy flour, maize starch, potato starch, yoghurt powder, milk protein, HPMC (K4M), psyllium husk, microbial transglutaminase, lipase, and fungal α-amylase. The significance of this research is mainly commercial and the insights gained may extend to other bakery items that could be used by coeliacs.

Chapter 1 Introduction

1.1 Adverse reaction of food, food allergy and intolerances

Some people encounter an adverse physiological reaction when they eat certain foods. The typical human diet contains thousands of potentially adverse biochemicals, and several mechanisms in the gastrointestinal tract are involved to prohibit the absorption of these substances into systemic circulation. These biochemicals are expelled by a combination of non-immune and immune means. Non-immune factors include normal gastric function where acid and pepsin digestion limits the entrance of adverse biochemicals to the small intestine, and intestinal secretion of glycoproteins and glycolipids inhibits the attachment of these substances to the epithelial surface. However, the absorption of adverse biochemicals does occur in healthy humans and in increased amounts in certain disease states (Robertson & Wright, 1987) and causes adverse reactions.

These adverse reactions of food are distinguished in toxic and non-toxic reactions (Figure 1). The toxic reactions occur in all exposed human subjects and are not included under food allergy and intolerance reactions, since they do not depend on individual susceptibility but on their direct toxic effects.

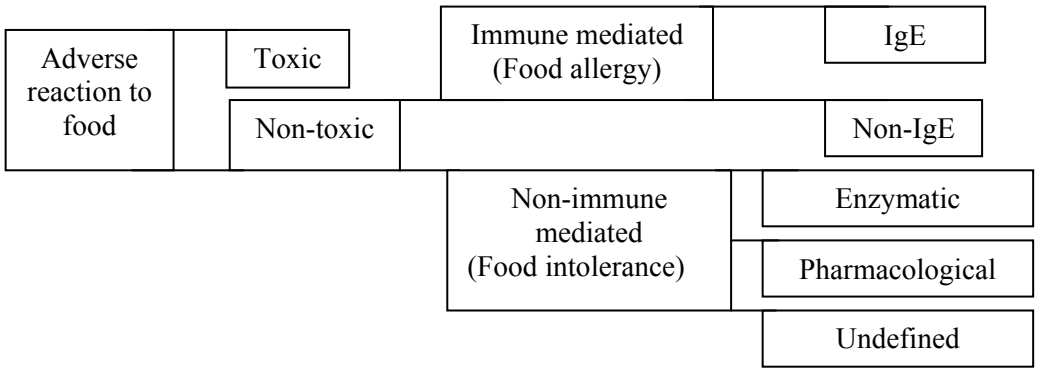


Figure 1 Adverse reaction of foods, redrawn from Ortolani (2006)

In contrast, non-toxic adverse food reactions occur only in susceptible individuals and are imperfectly divided in immune-mediated and non-immune-mediated reactions (Ortolani & Pastorello, 2006). The term food allergy is generally used for immune-mediated reactions, whereas non immune-mediated reactions are generally classified as food intolerances (Ispano et al., 1998) and refer to any adverse reaction to food irrespective of the (non-immune) mechanism (Robertson & Wright, 1987). Food allergies and food intolerances may be caused by several factors including heredity, gut permeability, an overly sensitive immune system, poor digestion, or an excessive exposure to a limited number of foods.

Food allergic reactions are further subdivided into IgE (immunoglobulin E)-mediated and non-IgE-mediated (Ortolani & Pastorello, 2006). Only IgE-mediated reactions have been generally acknowledged as causing food allergies, and can involve more than one target organ and are noticeable by the typical symptoms of allergies, including rapid onset, oral allergy syndrome, urticaria-angioedema, eczema, asthma, gastrointestinal symptoms, and occasionally anaphylactic shock. In contrast, non-IgE-mediated reaction involve other immunoglobulins, immune complexes, and cell-mediated immunity (Ispano et al., 1998).

The non-immune-mediated food intolerances are respectively classified as enzymatic, pharmacological, and undefined (Figure 1), on the basis of an enzymatic defect, the effect of pharmacological substances present in food, and the reactions non-classifiable by any known mechanism (Ortolani & Pastorello, 2006).

The parts of the food responsible for the allergic reactions are usually proteins and termed allergens. The most common food allergens, which are responsible for up to 90 % of all allergic reactions, are the proteins in cow milk, eggs, peanuts, wheat, soy, fish, shellfish and tree nuts (Sicherer & Sampson, 2006). Allergic reactions vary between subjects and exposure event from mild, such as a skin rash, to a life-threatening anaphylactic shock. The best way to manage a diagnosed food allergy is to avoid foods that contain the particular allergen (Marinho, Simpson, & Custovic, 2006).

1.2 Wheat sensitivity and coeliac disease

Wheat sensitivity and coeliac disease are two distinct phenomenona. There are four classes of protein in wheat: albumin, globulin, gliadin, and glutenin. In some people, albumin and globulin act as allergens and activate their immune system. Such individuals are called wheat sensitive and have an IgE-mediated response to these wheat proteins. (The other two proteins may or may not elicit IgE-mediated responses in those subjects.) Wheat sensitivity is rare (Scibilia et al., 2006) and is usually diagnosed in early childhood. These individuals must avoid wheat but may consume barley, rye and oats etc.

The pathogenesis of coeliac disease involves environmental, genetic, and immunological factors. The complex of glutenin and gliadin protein is called gluten and its ingestion act as an environmental factor to cause coeliac disease. Coeliac disease and gluten-sensitive enteropathy are equivalent names for an affliction causing severe damage to the gut of affected subjects unless gluten is eliminated from the diet (Ciclitira, Ellis, & Lundin, 2005). Coeliac disease is usually a lifelong inflammatory disease of the proximal small intestine

caused by exposure to gluten (Clot & Babron, 2000). It causes damage to the mucosa of the proximal small intestine with damage gradually decreasing in severity towards the distal small intestine, although in severe cases the lesion extends to the ileum and colon (Ciclitira & Moodie, 2003). The disease is caused by an unusual body defence response to gluten. Individuals who have the disorder produce antibodies to ingested gluten, and these injure villi cells in the small intestine (Hamer, 2005), which are involved with nutrient absorption. The jejunal mucosa in coeliac disease may be flat and featureless but usually presents a mosaic pattern caused by the intersection of deep depressions leaving elevated mounds (Ciclitira & Moodie, 2003). This leads to poor absorption of nutrients including iron, folic acid, calcium, and fat-soluble vitamins. Hence, the alternative name for the condition is the more descriptive gluten-sensitive enteropathy.

Coeliac disease has a genetic basis. The incidence in first-degree relatives (parents, siblings) is about 10-15 % if a parent or sibling has coeliac disease. The existence of a genetic predisposition was suggested by the observation of a disease prevalence of 10 to 15 % among first-degree relatives of probands (People who have the disorder under investigation in a family history study) and a high concordance (the presence of the same trait in both members of a pair of twins) rate of 70 % in identical twins, compared with 20 % in dizygotic twins (Clot & Babron, 2000). The human leukocyte antigen-DQ (HLA-DQ) and CTLA4 genes are implicated in coeliac disease. Most coeliacs (90 %) carry the HLA-DQA1*05 and HLA-DQB1*02 genes that code for HLA-DQ2 protein. This protein plays an important role in the sequence of events that lead to intestinal damage. The fact that the presence of HLA-DQ2 does not predict well for coeliac disease by itself, it is expected that the sensitivity to develop coeliac disease is the result of several genes (Hamer, 2005).

Coeliac disease is not IgE-mediated, and therefore often not classified as an allergy, but in terms of Figure 1 it clearly is and a non-IgE response. With an IgE-mediated allergy, at least two binding sites must be present on the epitope, whereas in coeliac disease only a single binding peptide is sufficient (Hamer, 2005). Moreover, the onset of intestinal damage symptom of coeliac disease is not immediate, and clearly differs from typical IgE-type responses that occur within an hour or so of exposure to an allergen (Hamer, 2005). Wheat sensitivity reactions can give rise to a range of clinical manifestations that can be immediate and/or delayed, and their severity can vary from mild to life-threatening depending upon the type of allergic protein involved and its sensitivity to the individual. Typical immediate symptoms include oropharyngeal symptoms, urticaria, angioedema, atopic dermatitis flare, rhinitis, asthma, gastrointestinal symptoms, and anaphylaxis (Scibilia et al., 2006).

Symptoms of coeliac disease may include, indigestion, abdominal pain, bloating and gas production, bulky fatty bowel motions that are some times pale and offensive smelling, failure to thrive, vomiting, muscle wasting, signs of hypoproteinaemia including possible ascites, general irritability and unhappiness. Diarrhoea may be severe, especially with intercurrent infection (Ciclitira & Moodie, 2003). However, signs of intestinal malabsorption, such as chronic diarrhoea, weight loss, abdominal distension and anaemia are more common (Catassi, Fornaroli, & Fasano, 2002). Other symptoms includes muscle cramps due to low calcium levels, slowed growth rate in children, and blistering, itchy or painful rashes particularly about the knees, elbows, buttocks, back (dermatitis herpetiformis). In advanced (untreated) conditions, nervous system damage can result, including numbness and 'pins and needles' in limbs, changed behaviour, irritability and depression. In adults, the disease often present in a milder form with non-specific symptoms such as fatigue, vague abdominal pains, intermittent diarrhoea. Deficiency of lactase can also occur due to damage of intestinal mucosa, so leading to lactose intolerance. Untreated coeliac disease is associated with long-term risks such as osteoporosis, anaemia and gastrointestinal malignancy (Hamer, 2005). Women with untreated coeliac disease are at an increased risk of suffering from miscarriages and mothers are at increased risk of having low birth weight children (Ciclitira & Moodie, 2003).

Histological recovery to a gluten-free diet is variable and incomplete in a substantial subgroup of patients and recovery is inversely correlated to the degree of initial mucosal pathology (Mulder & Cellier, 2005). Ciclitira and Moodie (2003) showed that approximately 70 % of patients showed noticeable clinical improvement within two weeks, and the improvement in both symptoms and intestinal function preceded the histological improvement. They further found that intestinal permeability improves within two months of starting a gluten-free diet, but a measurable improvement in the histology usually requires a gluten-free diet for at least three to six months and even then may remain incomplete.

1.3 Gluten composition

Gluten is found in triticale, modern wheat varieties – and derivatives like pasta, semolina, farina – and to a lesser extent in rye, barley, and many of their derivatives like, malt flavouring and malt extract (Thompson, 2003). The storage proteins of these cereals are in the endosperm and are classified as two major groups: the ethanol soluble fraction (termed prolamins) and ethanol insoluble (termed glutenins). Prolamins from wheat, rye, barley, and oats are termed gliadin, secalin, hordeins, and avenins respectively. Gliadins are single chained, extremely sticky when hydrated. They are rich in proline and glutamine and have a low level of charged amino acids. The amino acid compositions of glutenins are very similar

to those of gliadins, with high levels of glutamine and proline and low levels of charged amino acids (Van Der Borght, Goesaert, Veraverbeke, & Delcour, 2005).

1.4 The adverse reaction mechanism in coeliac disease

The fact that many gliadin peptides have bulky hydrophobic amino acids followed by a proline, the activity of intestinal peptidases such as pepsin and chymotrypsin is greatly inhibited. These peptides are resistant to degradation by all gastric, pancreatic, and intestinal brush border membrane proteases in the human intestine (van Heel & West, 2006). Under normal physiological circumstances, the intestinal epithelial barrier is almost impermeable to macromolecules like peptides. However, in coeliac disease there is and enhanced paracellular permeability across epithelium called 'leaky gut', a condition that would allow passage of macromolecules through the paracellular spaces (Clemente et al., 2003). The pathogenic component of gluten is believed to be a particular gliadin peptide that crosses the epithelial barrier by this leaky gut. Gliadin has further sub fractions (Ciclitira et al., 2005) that are termed as α, β, γ, and ω in decreasing order of mobility and gel electrophoresis (Battais et al., 2005; Ciclitira & Moodie, 2003).

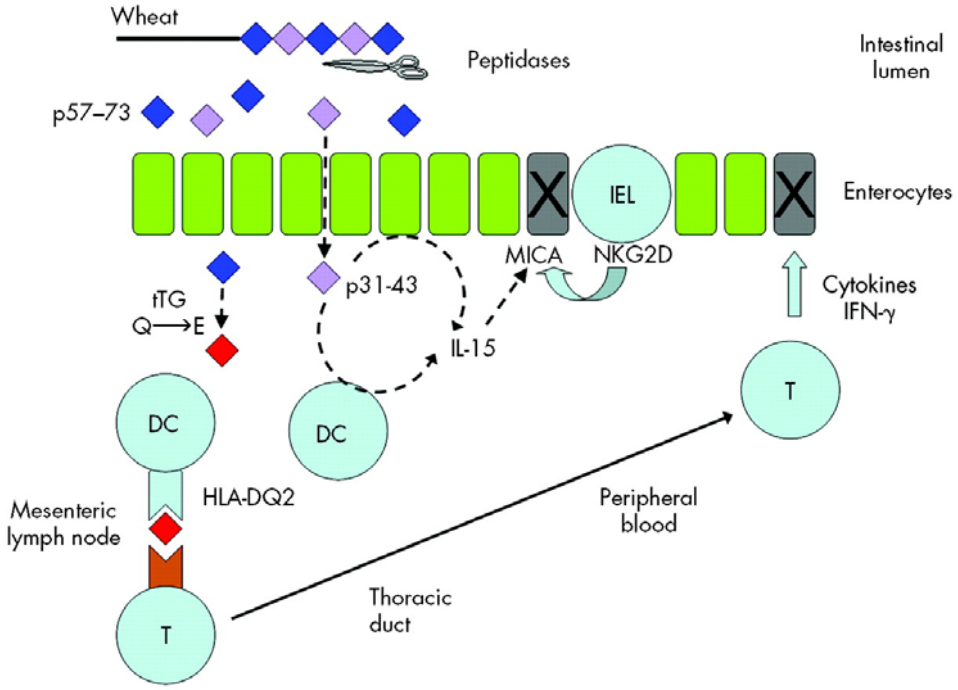


Figure 2 Pathogenic mechanisms of gliadin from Van Heel & West (2006). The meaning of T, DC etc. is explained in the text.

Gluten is partially digested in the intestinal tract but key coeliac-sensitive sequences are resistant to intestinal proteases. The mechanism of direct epithelial cytotoxicity in coeliac disease pathogenesis (Figure 2) involves a direct effect of a proline-rich gluten peptide α - gliadin, p31-43 (LGQQQPFPPQQPY) or p31-49, on the intestinal epithelium with in vivo

experiments (Sturgess et al., 1994), and in vitro in biopsy studies (Maiuri et al., 2003). The effects were observed in coeliacs within four hours, which is surprisingly rapid for what was initially thought to be a T cell (a type of leukocyte)-mediated response. More recent work demonstrated that this peptide appears to induce interleukin 15 (IL-15) production from enterocytes and dendritic cells (DC) (Maiuri et al., 2003). Interleukin 15 appears to induce expression of a stress molecule, MICA, on enterocytes and upregulates receptors (NKG2D) on intraepithelial lymphocytes. The interaction between enterocyte MICA and lymphocyte NKG2D results in the death of enterocytes (X), thus causing the villous atrophy (Meresse et al., 2004). Direct effects of gliadin on enterocytes may also increase intestinal permeability to macromolecules, including gliadin, through release of zonulin (a modulator of tight junction permeability, which is upregulated during the acute phase of coeliac disease) and effects on intracellular tight junctions (Clemente et al., 2003).

There is also an indirect mechanism that involves T cells. This mechanism in coeliac pathogenesis involves the crossing of gluten peptide (p57-73) through intestinal epithelial barrier, again by leaky gut, and reaching the lamina propria of the intestinal mucosa (Figure 2). This peptide is deamidated by tissue transglutaminase and is presented to T cells by a receptor (HLA-DQ2) on antigen presenting cells. Deamidation of the peptide enhances its recognition by HLA-DQ2/DQ8 in genetically predisposed subjects to initiate the cascade of autoimmune reactions responsible for production of cytokines (Clemente et al., 2003), that lead to damage of the intestinal epithelium (Hamer, 2005), the enterocyte, increased proliferation in the intestinal crypts and finally, severe damage to the intestinal mucosa architecture (Catassi et al., 2002).

1.5 Genuine and perceived sensitivity to gluten

Coeliac disease is a more common disorder in populations of European descent including those in Europe, Australia and North America (Ciclitira & Moodie, 2003). The highest reported prevalence of coeliac disease (one case in every 18 children) has been reported in an Arab people living in the Sahara desert (Catassi et al., 2002) followed by the Saharawi people from the Saharan region of Africa having one case in twenty people (Ciclitira & Moodie, 2003). Studies show coeliac disease to be a more common disorder than previously thought and possibly have the occurrence between 0.3 and 1 % in the general population of Europe and United States (Catassi et al., 2002).

The data collected by Bramwell et al. (2004) demonstrate that coeliac disease may be one of the most common chronic diseases in New Zealand, with a prevalence of 1:83 of the

Canterbury population for example. This prevalence is one of the highest recorded in the Western world (Bramwell, Robert , Chapman, Whitehead, & Burt, 2004). In the Canterbury region over the 30-year study period (1970-1999), 416 people were diagnosed as having coeliac disease. The overall incidence of newly recognised coeliac disease over this period was 2.2 per 100 000 per year. The cumulative incidence of childhood coeliac disease (0 to 12 years) over the 30-year period was 0.40 per 1000 births. The overall female to male ratio was 2.1:1, highest for those aged 30–39 (3.3:1), and lowest for those aged 0–19 years (1.4:1). For those aged 60 years and over the incidence was 1.15:1 (Bramwell et al., 2004). According to Coeliac Society of New Zealand, 2462 coeliac patients are registered members in New Zealand. The European origins of many New Zealand residents are consistent with the high occurrence of the disease.

Because of increasing recognition of new clinical patterns of presentation, the true prevalence is probably much higher than supposed (Southern Cross Healthcare, 2000). In the past, coeliac disease was regarded as only a childhood condition, which produced symptoms in very young children when gluten was introduced to their diet. At present, a large proportion of newly diagnosed coeliac are diagnosed as adults – usually in the 30-45 year age group (Auckland Allergy Clinic, 2006). Many have few or no problems during childhood but develop symptoms only when adults. In addition, the symptoms of coeliac disease can be minor or atypical and can even be clinically silent (Catassi et al., 2002).

There are also some consumers who avoid gluten because of a perceived intolerance, and others who are migrating to the market from so-called organic and natural foods and similar market segments. This shift – consisting mostly of white, middle-to upper-class consumers – is driven by the belief that certain major allergens and food components also play a role in exacerbating a wide range of other health conditions, from migraines to menstruation problems (Packaged Facts, 2006). Even some people without apparent symptoms remark on a new-experienced vitality and perceived well-being, thus coincidently conforming to the idea that in coeliac disease removal of gluten from the diet leads to ‘full clinical remission’(Mulder & Cellier, 2005). Some consumers also opt for gluten-free in the hope of preventing their young or unborn children from developing food allergies (Packaged Facts, 2006).

Although gluten-free products are largely bought by coeliac sufferers, very often the entire family of a coeliac will switch to gluten-free products primarily to avoid buying different versions of the same goods, but also as a perceived preventative step as coeliac disease is hereditary (Packaged Facts, 2006). But this remains a luxury of choice available only to those

able to afford it because gluten-free products are more expensive than wheat-containing equivalents. The high cost of gluten-free foods avert many coeliac sufferers from adhering precisely to their restricted diet, while most diagnosed coeliac are largely white, educated and at least middle-class citizens (Packaged Facts, 2006) and have access to good healthcare and are able to afford the higher cost of the products that comprise this market.

1.6 Food labelling requirements

Standard 1.2.3 Mandatory Warning and Advisory Statements and Declarations of the Australia and New Zealand Food Standards Code sets out mandatory advisory statements and declarations which must be made in relation to certain foods or foods containing certain substances. The provisions apply when substances are present in food as an ingredient; an ingredient of a compound ingredient; a food additive or component of a food additive; or a processing aid or a component of a processing aid. The mandatory declarations, warning statements and advisory statements are intended to provide consumers with sufficient information so that they can avoid potentially life-threatening allergic reactions to food or an ingredient in food (Australia New Zealand Food Authority, 2003). Allergens that must be declared on food labels include; cereals containing gluten and their products (e.g. wheat, rye, barley, and oats).

In respect of gluten-free standards, an older New Zealand Gluten Free Standard (NZ Food Regulations) has now been replaced with the Australia New Zealand Food Authority (ANZFA) Food Standard Code as set out in clause 16, Standard 1.2.8. In this standard, a claim that a food is gluten free must not be unless the food is free from detectable gluten, oats and malt (Australia New Zealand Food Authority, 2003).

1.7 Wheat and role of its constituents in bread formulation

The majority of bread is conventionally produced from wheat flour. Apart from its major constituent starch, wheat flour also contains many other types of substances of which the gluten, the non-starch polysaccharides, and the lipids are the most important in terms of their impact on the processability of the raw material and the quality of the final products. Wheat flour is the major ingredient in bread production and comprises of carbohydrate (70–75 %), water (about 14 %) and proteins (10–12 %). In addition, non-starch polysaccharides (2–3 %), in particular arabinoxylans, and lipids (2 %) are important minor flour constituents (Goesaert et al., 2005).

The proteins in wheat flour are particularly important in bread making. From a functional point of view, two groups of wheat proteins are distinguished: the non-gluten proteins, with either no or just a minor role in bread making, and the gluten proteins, with a major role (Goesaert et al., 2005). The non-gluten proteins (between 15 and 20 % of total wheat protein) mainly occur in the outer layers of the wheat kernel with lower concentrations in the endosperm. They are mostly monomeric physiologically active or structural proteins in the wheat kernel. They are genetically related to the major storage proteins in legumes and in the cereals oats and rice. The gluten proteins are the major storage proteins of wheat. They belong to the prolamin class of seed storage proteins and are insoluble in water or dilute salt solutions. Gluten proteins are found in the endosperm cells of the mature wheat grain where they form a continuous matrix around the starch granules (Van Der Borght et al., 2005).

Gliadins represent a highly polymorphic group of monomeric gluten proteins with molecular weights varying between 30,000 and 80,000 Da. Glutenins are a heterogeneous mixture of polymers with molecular weights varying from about 80,000 to several million Da (Van Der Borght et al., 2005). Glutenins are among the largest proteins found in nature. The quality of wheat flour is largely determined by gluten.

Gluten proteins are hugely important in bread making as they act as the main structure-forming proteins in bread and are responsible for the elastic and extensible properties of dough needed to produce good quality bread (Goesaert et al., 2005).

A range of chemical, biochemical and physical transformations occur throughout bread making process, which affect and are affected by the various flour constituents. These transformations are most important to get a final good quality baked product.

During dough preparation, the majority of the water added to make up the dough is absorbed by hydrophilic groups on the protein molecules on a roughly equal weight basis. Starch absorbs up to about 45 % of the added water (The actual water uptake by the starch in bread making depends on the extent of starch crystallite shearing that occurred in the earlier milling process (Scanlon & Zghal, 2001)).

During dough preparation, wheat flour is hydrated, and discrete masses of gluten protein are disrupted as a result of the mechanical energy input. This process is accompanied by a dramatic increase in the ‘extractability’ of the gluten proteins, where extractability means an aggregation of gluten that was previously evenly dispersed among starch granules. The gluten is transformed into a continuous cohesive viscoelastic gluten network (Goesaert et al.,

2005). The viscoelastic network encapsulates air, starch granules and other filler materials such as bran during dough mixing (Scanlon & Zghal, 2001). Glutenin polymers form a continuous network that provides strength (resistance to deformation) and elasticity to the dough. The glutenin molecule are linked by intermolecular disulfide bonds, so forming a network structure (Tronsmo, 2002). In contrast, the (monomeric) gliadins contain intramolecular disulfide bonds, giving the proteins a globular confirmation. They act as plasticisers of the glutenin polymeric system, and in this way provide plasticity and viscosity to wheat flour doughs. These properties of the gluten proteins allow wheat flour to be transformed into dough with suitable properties for bread making (Van Der Borght et al., 2005). These properties are unique to wheat and cannot be fully replicated by flours from cereals closely related to wheat such as barley and rye. This is due to the quality of the equivalent proteins in those grains and the lower concentrations (Goesaert et al., 2005).

Optimal gluten development by the mixing process is vital for the development of the ultimate desired crumb structure (Scanlon & Zghal, 2001). Too little or too much gluten extraction leads to denser crumb than that of desirable loaves due to the fact that little extraction will form insufficient gluten network to retain carbon dioxide. Higher dough strength increases loaf volume up to a certain limit, however, loaf volume is hindered if the doughs are too strong. The resulting aggregated gluten network plays a major role in retaining the carbon dioxide produced during fermentation and during the initial stages of baking to produce a light, aerated baked product (Van Der Borght et al., 2005). Gas retention properties in turn determine loaf volume and crumb structure of the resulting bread (Goesaert et al., 2005).

In the baking phase, the starch granules gelatinise and swell in response to the combination of heat, moisture and time. During this phase, changes occur in the gluten proteins that are probably a combination of changes in protein surface hydrophobicity, sulphydryl/disulphide interchanges and formation of new disulphide cross-links (Goesaert et al., 2005). As a result of these heat-induced changes as well as those of the starch, the typical foam structure of mixed and partially fermented dough is converted into the typical sponge structure of baked bread.

1.8 The New Zealand market for gluten-free starch-based products

Currently the majority of gluten-free products – around 50 percent – are sold in so-called health and natural food stores. Some 35 percent of sales in 2006 occurred through specialty

food website or catalogue purchases, with mainstream supermarkets coming in third with a 15 percent share of sales.

The majority of the gluten-free items including biscuits and muffins are available at Pack & Save, Woolworths, Foodtown and other major shopping outlets. But most of the gluten-free breads are made to order by their in-house bakeries due to the short shelf life but high cost of production. Some gluten-free breads are sold unsliced in vacuum packs in order to retain the moisture and slow staling. Most appear to be 'under baked' and dough-like piece, whereas others have a dense rock-like crumb texture. Prices start at $6 for a loaf with a height equal to only half that of regular wheat bread. Companies such as Buontempo Enterprises Pty, Dovedale, Healtheries, Bakels, Organic Bakeworks, South Flour, The Gluten Free Goodies Company, and Champion Flour are major suppliers of gluten-free bread premixes for baking (Manufactured Food Database, 2006). These premixes yield a batter[1] rather than dough, and are used in the home and in the limited commercial baking applications that occur in New Zealand. The breads are usually consumed shortly after baking due to the tendency to rapid staling.

1.9 Opportunities

Existing gluten-free products generally are denser than conventional loaves, have very poor shelf life properties. To give an example, a typical gluten-free bread is denser than a normal wheat bread (2.5–3 $L.kg^{-1}$ vs. 6–7 $L.kg^{-1}$, respectively) and becomes stale within 1–2 hours (Hamer, 2005). Moreover, the products are prepared from a batter and as such cannot be made on existing bread production equipment, which obviously represents a major capital outlay. Thus there is an opportunity for gluten-free bread in the market, if suitable dough could be developed. Moreover, there is currently no white gluten-free loaf available in the New Zealand market.

1.10 The aim of this research

The aim is to develop a gluten-free white loaf with similar quality characteristics to that of standard white bread that can be produced on existing processing lines at Quality Bakers. Within this constraint, dough has to be produced with handling and moulding properties similar to those of conventional wheat flour loaves. Thus, Quality Bakers wants to be a leader in a new market category.

[1] Batter is a thick or thin liquid mixture of flour and water

Chapter 2 Experimental approach, materials, equipment and basic methods

2.1 Introduction

Flours other than wheat, rye, barley and oats lack gluten and therefore, fail to form viscoelastic dough when they are kneaded with water in a conventional bread making process. They form a batter rather than a dough (Figure 3). The absence of gluten in these flours makes them suitable for gluten-free products but unsuitable for the production of dough, the product form for which industrial bread making process lines were developed. Moreover, the batters tend not to retain carbon dioxide gas during proofing and baking. Thus, the resulting bread has a low specific volume (a high density), and does not resemble wheat bread.

a. Batter from gluten free flours b. Dough of wheat flour

Figure 3 Batter and dough

Although there are countless gluten-free products in the market, the scientific literature was surprisingly brief on the systematic development of gluten-free breads with properties similar to those of conventional loaves. A literature search revealed a large number of references for bread texture but the literature surrounding gluten-free bread was more limited perhaps due to commercial secrecy. However, six research papers in particular appeared highly relevant. These research papers are: 'Combined use of ispaghula (the milled seed husk[2] of *Plantago*

[2] Also known as psyllium husk.

ovata) and HPMC to replace or augment gluten in breadmaking' (Haque & Morris, 1994); 'Crust and crumb characteristics of gluten free breads' (Gallagher, Gormley, & Arendt, 2003); 'Production of gluten-free bread using soybean flour' (Ribotta et al., 2004); 'Functionality of rice flour modified with a microbial transglutaminase' (Gujral & Rosell, 2004a); 'Improvement of the breadmaking quality of rice flour by glucose oxidase' (Gujral & Rosell, 2004b); and 'Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations' (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2006).

The scientific information from these research papers shows that a mixture of psyllium husk and HPMC proved to be an effective substitute for wheat gluten in bread-making, as these two materials stabilise gas cells over complementary ranges of temperature (Haque & Morris, 1994). Dairy powders with high protein contents (80-90 %) produced wheat starch-based gluten-free breads with good crust and crumb characteristics, and improved nutritional content (Gallagher et al., 2003). Good-quality gluten-free breads could be formulated from a mix of rice, cassava and active soybean flours (Ribotta et al., 2004). Rice flour is low in prolamins (2.5–3.5 %), and various hydrocolloids can be incorporated to produce bread from a batter of rice flour. HPMC has been found to be the most suitable hydrocolloid to produce rice bread (Gujral & Rosell, 2004a). The applications of rice flour can be broadened by covalently cross-linking the rice proteins, either intramolecularly or intermolecularly, to produce a stable network. This was achieved by using microbial transglutaminase and glucose oxidase in combination with 2 % HPMC (Gujral & Rosell, 2004a, 2004b). Hydrocolloids improve the rheological behaviour of the doughs, have pronounced effect on viscoelastic properties and resistance to deformation and strengthen doughs (Lazaridou et al., 2006; Rosell, Rojas, & Benedito de Barber, 2001).

Although the gluten-free breads developed in previous studies had some similarities to conventional wheat flour bread but there were some differences in colour, taste and texture as well. For example, gluten-free bread developed by using rice flour, hydrophilic psyllium husk and HPMC by Haque & Morris (1994) was whiter than wheat bread but had a characteristic rice taste. The gluten-free bread developed by Gallagher, McCarthy, Gormle, & Arend (2004) using wheat starch, gluten-free flour, milk powder, and milk proteins had, among other problems, a low specific volume, and an excessively dark crust due to Maillard reaction. Breads made with inulin or fish proteins exhibited similar (excess browning with inulin) or different (rapid staling) defects. The addition of cross linking enzymes and HPMC yielded acceptable rice bread as reported by Gujral & Rosell (2004a, 2004b) but failed to produce acceptable non-sticky dough for the industrial production.

Additionally the breads in previous researches were made from batter instead of dough. The batters transform into sticky paste when less water content are used. The sticky paste is not suitable for industrial production due to its stickiness and insufficient hydration of flours leading to tough mixture that fails to rise, whereas, the batter itself is unsuitable for industrial production due to its sticky and liquid state.

2.2 Starting points for this research

The collected information from the above research cannot therefore be immediately applied to commercial production because the ingredients are in the form of batter rather than a dough. Nonetheless, the information is instructive and provides a good starting point using ingredients such as HPMC, psyllium husk, dairy powders, rice, active soybean flours, starches, microbial transglutaminase, and glucose oxidase.

The present research focused on modifying and finding the combination of the different gluten-free flours, supplementary proteins, starch, hydrocolloids and enzymes to produce a dough having ability to trap the carbon dioxide gas during proofing and baking to get high specific volume bread suitable for the Quality Bakers' product range.

2.3 Materials and equipment

Table 1 lists the ingredients and equipment, and their suppliers, used in the development of gluten-free bread. The gluten-free flours listed in Table 1 contain various proportions of amylose and amylopectin, and differ in their gelation temperatures, viscosity, and tendency to retrograde. Gelation occurs when the crystalline and semicrystalline arrays of amylose and amylopectin held together by hydrogen bonds are disrupted by water and heat. Viscosity markedly increases on gelation. Retrogradation is the tendency of dispersed starch molecules to reassociate by hydrogen bonding into new crystalline arrays, and is responsible for staling and other phenomena in baked goods (Cui, 2004; Eliasson, 2004). Retrogradation is mostly a phenomenon of amylose. Amylose comprises about 1000 to 2000 D-glucose residues connected by α-(1─4) glycosidic bonds, whereas amylopectin is branched (Eliasson, 2004; Fennema, 1996). These flours also contain various quantities of (non-gluten) proteins that can be involved in the final bread structure (Figoni, 2004).

Starches, which can be purified from flour and from other carbohydrate sources, behave like flours when heated in water. They almost insoluble in cold water, but heating a mixture of starch and water leads to gelatinisation (Cui, 2004; Eliasson, 2004).

Table 1 Ingredients, equipment and suppliers

Ingredient and (product code)	Supplier
Rice flour (Remyflo R 200 T)	Invita N.Z. Ltd., Auckland, N.Z.
Soy flour (full fat)	Davis Trading Co. Ltd., Auckland, N.Z.
Potato starch (Novation 1900)	National Starch N.Z. Ltd., Auckland, N.Z.
Tapioca starch (National 7)	National Starch N.Z. Ltd., Auckland, N.Z.
Maize starch (Avon Maize starch)	Penford N.Z. Ltd., Auckland, N.Z.
Yoghurt powder (Ballantyne 28018)	Belletech International Ltd., Auckland, N.Z.
Rice protein (Remypro N80+)	Invita N.Z. Ltd., Auckland, N.Z.
Milk protein (Alacen 312)	Fonterra Co-operative Group Ltd., Auckland, N.Z.
Guar gum (NP 35)	Danisco N.Z. Ltd., Auckland, N.Z.
Xanthan gum (Grindsted Xanthan 80)	Danisco N.Z. Ltd., Auckland, N.Z.
Hydroxypropylmethylcellulose (Methocel K4M)	Swift N.Z. Ltd., Auckland N.Z.
Psyllium husk	Bronson & Jacobs Pty. Ltd., Auckland, N.Z.
Microbial transglutaminase (Activa TG-B)	Kerry Ingredients N.Z. Ltd., Auckland, N.Z.
Fungal α-amylase (Fungamyl 2500 SG)	Nutura N.Z. Ltd., Auckland, N.Z.
Glucose oxidase (Gluzyme M 10000 BG)	Nutura N.Z. Ltd., Auckland, N.Z.
Lipase (Lipopan FBG)	Nutura N.Z. Ltd., Auckland, N.Z.
Bakers' compressed yeast	New Zealand Food Industries Ltd., Auckland, N.Z.
D.Y.C. white vinegar	Goodman Fielder Ltd., Auckland, N.Z.
Canola oil, sugar, eggs, table salt	Woolworths New Zealand (local market)

Equipment	Supplier
Dough mixer (The Hobart Mixer, A-120)	Hobart Mfg. Co., Troy, Ohio, U.S.A.
Dough moulder (Supertex)	Baker Perkins Ltd., Peterborough, U.K.
Proofer	Manukau Sheetmetals (1984) Ltd., Auckland N.Z.
Baking oven (Rotel 2)	APV Moffat Ltd., Christchurch, N.Z.
Bread slicer (Ayres Jones)	Mono Equipment, Wales, U.K.
Precision incubator	Contherm Scientific Ltd., Petone, Wellington, N.Z.
Penetrometer	Stanhope-Seta Ltd., Surrey, U.K.
HunterLab colorimeter	ColorFlex, Hunter Associates, Virginia, U.S.A.
Aqua lab water activity meter	Formula Foods Corporation Ltd. Christchurch, N.Z.
Ohaus MB 45 moisture analyser	Ohaus Corporation, Florham Park, NJ, U.S.A.

Yoghurt powder is a dried yoghurt. Yoghurt is made from fermenting and coagulating milk by non-pathogenic bacillus family bacteria. They ferment milk sugar (lactose) into lactic acid, and lactic acid act on milk protein and give yoghurt its gel-like texture and characteristic tang.

According to manufacturer Remy Industries Belgium specification, Rice protein (Remypro N80+) is an 'all-natural', slightly brown rice protein concentrate that contains a minimum of 80 % rice protein. Remypro N80+ is insoluble in water, and is purportedly "non-allergenic with a superior amino acid profile, has a fine particle size, and a clean taste".

Alacen 312 is a whey protein concentrate. Whey protein is the name for a collection of globular proteins that can be isolated from milk whey, which is a by-product of cheese

production. It is a mixture of β-lactoglobulin (~65 %), α-lactalbumin (~25 %), and serum albumin (~8 %). According to the supplier specification, Alacen 312 is a form of concentrated whey protein containing 80 % protein with good emulsifying qualities.

Hydrocolloids is the name given to a wide range of water-dispersible polymers of monosaccharides, and in the present work includes guar gum, xanthan gum, HPMC, and hydrophilic psyllium husk. By virtue of their extended polymeric backbone, these hydrocolloids increase viscosity in aqueous suspension/solution and absorb water due to their hydrophilic nature (Lazaridou et al., 2006).

Guar gum is extracted from the seed of the leguminous shrub *Cyamopsis tetragonoloba*. It is a galactomannan consisting of (1–4)-linked β-D-mannopyranose backbone with branch points from their 6-positions linked to D-galactose. Guar gum is a thickener and stabiliser. It hydrates rapidly in cold water to give highly viscous pseudoplastic solutions (Chaplin, 2006).

Xanthan gum is a microbial desiccation-resistant polymer prepared commercially by an aerobic fermentation of glucose using *Xanthomonas campestris*. It is a carboxyl polyelectrolyte with a (1–4)-D-glucopyranose glucan backbone with trisaccharide side chains. It hydrates rapidly in cold water and is used as a thickener, stabiliser, emulsifier and foaming agent (Chaplin, 2006).

Hydroxypropylmethylcellulose is a unique and highly functional food gum. It comprises methylcellulose modified by attaching propyleneglycol ether groups to a fraction of the linked glucose molecules that comprise cellulose. A typical hydroxypropylmethylcellulose has between 1 and 2 mole of methoxy ($-OCH_3$) groups per glucopyranosyl unit, and between 0.02 and 0.3 mole of hydroxypropyl ($-OCH_2CHOHCH_3$) groups. It efficiently hydrates in cold water and, curiously, gels when heated. It also binds, clings, thickens and adds stability to food systems through multiple functional properties with a wide range of viscosities (Whistler & BeMiller, 1997).

About 85 percent of psyllium husk may consist of a single polysaccharide, comprising approximately 62 % D-xylose , 20 % L-arabinose, 9 % L-rhamose and 9 % D-galacturonic acid (Haque, Richardson, Morris, & Dea, 1993), and also contains approximately 15 % of non-polysaccharide material. The linear polysaccharide backbone is believed to comprise D-xylose residues, with single-sugar side chains of L-arabinose and D-xylose, and disaccharide side chains of L-rhamose and D-galacturonic acid. Any of the three side chain types may be

attached to either O-2 or O-3 of xylose in the polymer backbone (Haque, Morris, & Richardson, 1994).

According to the manufacturer's specification sheet (Urvesh Psyllium Industries, Gujarat, India), 99.9 % of the psyllium husk used in this study could pass through a 425μm wire mesh, and the swell volume was 79 $ml.g^{-1}$. On addition of water, psyllium husk immediately forms a gel. As will be reported later, this gel not only holds gas bubbles during proofing, but also gives dough-like consistency when added at the rate of more than 4 % in gluten-free flours. Being hydrophilic, psyllium husk holds the moisture in final finished baked bread (Gray & BeMiller, 2003). Addition of psyllium husk powder (2, 4, or 8 %) decreases the staling rate of bread as measured by compressibility (Gray & BeMiller, 2003). Moisture content remains constant and bread softness improves without increasing the possibility of microbial deterioration by the addition of psyllium husk.

Activa TG-B, microbial transglutaminase is a calcium ion-independent transferase with activity approximately 60 units g^{-1}. It catalyses the acyl transfer reaction between the γ-carboxyamide groups of peptide or protein bound glutaminyl residues and primary amines, including the ε-amino group of lysine residues in certain proteins. When transglutaminase acts on protein molecules, ε - (γ-glutamyl) lysine crosslinks are formed. In the absence of amine substrates, water act as the acyl acceptor and the glutamine residues are deamidated (Dickinson, 1997; Motoki & Seguro, 1998).

Fungal α-amylase acts on amylose and amylopectin to break α-(1-4) glycosidic bonds, eventually yielding D-glucose monomers (Eliasson, 2004). Glucose is metabolised by yeast during fermentation to produce carbon dioxide to raise the bread. Moreover, high molecular weight starches are converted into low molecular weight starches by the action of fungal α-amylase, which decreases the gelation temperature of starches.

Glucose oxidase catalyses the conversion of D-glucose, in the presence of oxygen, to D-gluconic acid and hydrogen peroxide (H_2O_2). The H_2O_2 oxidises the thiol groups of two proximate cysteine residues to form disulfide bonds (Gujral & Rosell, 2004b; Rasiah, 2005).

Lipase splits fat into glycerol and free fatty acids and retards staling in bread through interference with hydrogen bonding effects in retrogradation. The addition of 1, 3-specific lipases resulted in more uniform crumb structure and improved the crumb softness during storage. Furthermore, these lipases can replace shortening (edible fat) as a bread ingredient to

some extent. Lipase in combination with α-amylase markedly reduced retrogradation (Gray & BeMiller, 2003).

The equipment used is also listed in Table 1, and illustrated in Figures 4, 5 and 7. The preparation equipment is all bench-top scale but is nonetheless representative of equipment used in the Quality Bakers' production line.

The Hobart dough mixer has removable dough hook and mixing bowl (25 cm deep and 28 cm in diameter) and can be adjusted for three mixing speeds. The Supertex moulder have three pairs of sheeting rollers, each pair having an individually controlled variable gap, a rolling belt with side frames that can be adjusted to get required dimensions of dough piece. Moulding improves end product quality by controlling size, shape and length-to-width ratio. The supplier claims that the moulding process can increase volume, cell counts, improve crumb structure, and produces suitable shape that fills the can well, particularly important for sandwich producers

The Manukau Sheetmetal proofer is made of stainless steel and has adjustable shelves and front glass window. Both internal temperature and humidity is digitally displayed and can be controlled by external fitted regulators which control both dry and wet heating tubes in the proofer. The Rotel 2 baking oven has stainless steel internal chamber with two glass doors, circular rotating shelves, internal lightening and steaming systems. The circular rotating shelves ensure uniform heat exposure to every loaf. The Ayres Jones bread slicer is stainless steel slicer with 22 vertical blades set at 13 mm apart. The Precision incubator, which is used to hold baked bread at a set temperature prior to evaluation, has gentle fan-assisted air circulation to ensure optimum temperature stability throughout the chamber.

Stanhope-Seta Penetrometer (Figure 5 a.) was used for determination of crumb hardness. The principle of this equipment is the depth to which a set weight (445 g) of a given cross-sectional area (4.5 cm) penetrates multiple bread slices in given time (nine seconds). The head support, mounted on a vertical pillar, is adjusted such that the initially restrained plunger weight just touches the loaf surface. The plunger is released by a button-activated brake and begins to penetrate the bread. After nine seconds, the brake is reapplied, and the penetration depth, up to 40 mm, is read off the scale that had previously been set to zero. The precision is 0.1 mm. When tests are conducted over time, the penetrometer gives a good indication of staling rate. Hardness index (HI) was determined for the samples according to the method of Hayakawa and deMan (1982) as reported by Bourne (2002).

a. Hobart dough mixer

b. Supertex dough moulder

c. Manukau Sheetmetal proofer

d. Rotel 2 baking oven

e. Ayres Jones bread slicer

Figure 4 Equipment used in these trials

a. Stanhope-Seta Penetrometer

b. ColorFlex HunterLab colorimeter

Figure 5 Equipment used in these trials

The principle of the Hunter colorimeter is based on the concept of a colour space with the colour defined by three coordinates, L*, a*, and b* values (Coultate, 2002).

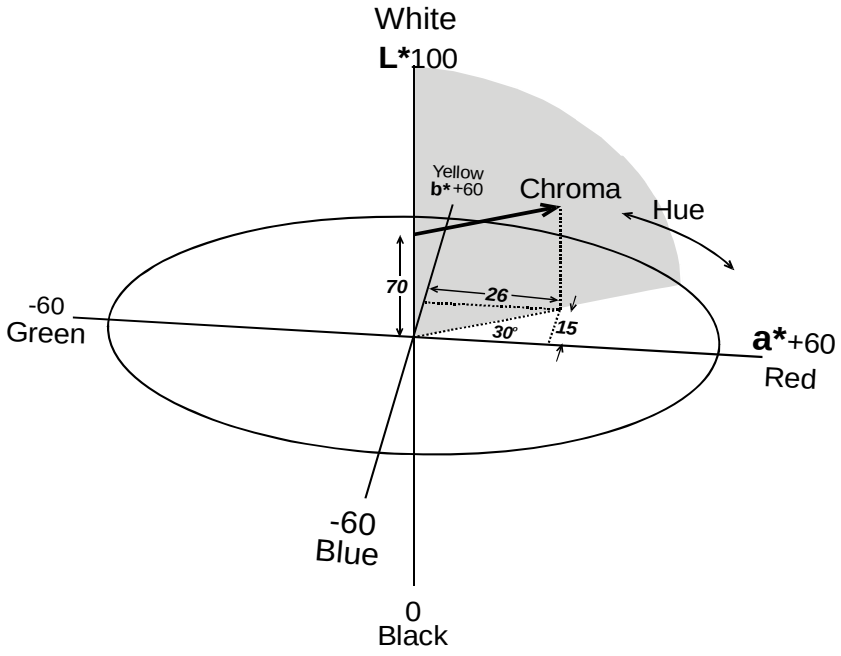


Figure 6 L* a* b* colour space, redrawn from Young & West (2001)

The vertical coordinate L* is lightness from 0 (total light absorbance and therefore completely black) through grey (50) to 100 (complete light reflectance); the horizontal coordinate a* is greenness/redness, from – 60 (green) through grey to + 60 (red); an

orthogonal horizontal coordinate b* is yellowness from – 60 (blue) to + 60 (yellow) (Figure 6). Hue angle refers to the gradation of colour within the visible spectrum of light. Hue angle is arctangent (b*/a*) determined by rotation about the a* and b* axes. Chroma or Saturation is the intensity of a specific hue: a highly saturated hue has a bright, intense colour, while a less saturated hue appears gentler. Chroma of a colour is determined by a combination of colour intensity and how much it is distributed across the spectrum of different wavelengths. Chroma is calculated as $\sqrt{(a^{*2} + b^{*2})}$ (Young & West, 2001).

a. Aqua Lab water activity meter b. Ohaus moisture analyser

Figure 7 Instruments for determination of water activity and moisture of breads

Water activities (a_w) of the gluten-free breads were determined during the five days storage at 25ºC with Aqua Lab series 3; model TE (Figure 7 a). It is a temperature-controlled water activity meter that allows having a temperature-stable sampling environment in the range of temperature from 15 to 40°C and range of water activity from 0.03 to 1.000 a_w with accuracy of ± 0.003 a_w.

Bread crust and crumb moisture was measured by Ohaus MB 45 moisture analyser (Figure 7 b). This instrument operates on the thermo-gravimetric principle. At the start of the measurement, the moisture analyser determines the weight of the sample; the sample is then quickly heated in aluminium tray (9 cm diameter) by the internal halogen dryer unit and moisture vaporises. During the drying operation, the instrument continuously determines the weight of the sample and displays the results. On completion of drying, results are displayed as % moisture contents, % solids, weight of solids left.

2.4 Basic methods for bread formulations

The selected ingredients were mixed in the dough mixer for three minutes at low speed and for four minutes at high speed. The dough temperature was 29 ± 1ºC after mixing. The dough was scaled into 780 g pieces and covered by cloth for 5 min at room temperature for resting. After resting the dough was moulded (26 cm in length) and proofed at 40ºC and 80 % relative humidity for 60 minutes in the baking can (27 cm long, 11 cm wide, and 10.5 cm deep) that had been the internally sprayed with canola oil as a release agent. Proofing is an industry expression for the step in creating yeast breads during which the yeast makes the bread rise.

After proofing, the can was baked for 26 min in an oven fitted with a carousel and maintained at 215ºC, with baking steam applied during the first minute. The specific volumes of breads were measured after 1 hour cooling at room temperature and then were cut in 13 mm thick slices by the slicer. The slices were packed in polythene bags and held at 25ºC in an incubator for further analysis that includes measures of crumb hardness, crust and crumb colour, water activity, and moisture (Table 2). The term 'crumb hardness' does not refer to hardness of isolated bread crumbs, but rather to the hardness of the bread as distinct from the crust. As bread stales the crumb becomes harder, but a particular bread can be hard because of the way it was made, but is not initially stale.

2.5 Methodology for evaluating dough and bread quality

The doughs were evaluated on the bases of their stickiness, and extensibility. The baked breads were evaluated on the bases of oven spring, moisture loss during baking, specific volume, overall crumb and crust colour, sliceability, staling and crumb hardness, water activity, and moisture. These terms are described in Table 2. All the evaluations were conducted at the Quality Bakers Test Bakery in East Tamaki, where only minimal objective methods were available. These were oven spring, specific volume (American Association of Cereal Chemists Approved Methods Committee, 2000), staling by penetrometer as described above, crumb and crust colour, water activity and moisture. The other evaluations were subjective, and were determined by the author and three experienced bakers who were always on site. The breads were informally evaluated for six quality attributes (taste, texture, appearance, softness, flavour, and crumb structure).

Oven spring is considered necessary and an indication of the proper strength of gas cell walls that entraps the gas and their elasticity by which they expand without collapsing due to increased yeast activity during initial phase of baking. Oven spring was determined by the

increase in height of the proofed dough on baking. The difference in height between the dough (proofed for 1 hour at 40ºC) and the bread after baking indicated the loaf volume change of the baked bread.

Table 2 Description of methods used to evaluate dough and bread quality

Quality attribute	Assessment type	Definition
Dough stickiness	Subjective	Dough stickiness is a composite characteristic resulting from the balance between adhesive and cohesive forces of a dough. Stickiness causes problems in commercial bakeries by choking production lines.
Dough extensibility	Subjective	Extensibility is the ability of dough to stretch without tearing. As rising itself is a form of stretching, doughs with the right amount of extensibility rise well.
Oven spring	Objective	Oven spring is the increase in volume of bread during the first few minutes of baking. High values indicate that the dough has good strength to retain the carbon dioxide.
Bread volume and specific volume	Objective	The bread volume was measured by rapeseed displacement method (American Association of Cereal Chemists Approved Methods Committee, 2000) and divided by weight to calculate specific volume.
Bread sliceability	Subjective	Bread sliceability is the ability of bread to be sliced as judged by achieving a fine cut that does not stick to the slicing blades.
Crust and crumb colour	Objective	Crust and crumb colour was determined by Hunter colorimeter.
Bread staling (sensory)	Subjective	Bread staling was also judged by sensory tests for loss of flavour and aroma. These attribute are the most noticeable detrimental changes of bread upon staling.
Bread staling (crumb hardness)	Subjective	Staling was also judged by the 'squeeze test', which is popular with consumers and gives the perception of freshness of bread, and reflects the textural properties of the crumb.
Bread staling (crumb hardness)	Objective	Staling was measured by determining the penetration depth of a defined weight into bread over a set time by penetrometer.
Water activity	Objective	Water activity was measured by a water activity meter.
Moisture	Objective	Moisture was measured by Ohaus moisture analyser.

Excessive moisture loss produces breads with firm, crumbling and dry bread crumb structure, which is inferior to eat and is not preferred by consumer. Therefore, the moisture loss (grams) during baking was measured. It was determined by deducting the weight of baked bread from the initial weight of the dough.

Available gluten-free breads in the market considerably differ in colour compared to ordinary wheat bread. Thus for evaluation of the colour of the crust and crumb of formulated gluten-free bread was done with ColorFlex HunterLab colorimeter (Figure 5 b) by taking hue, saturation, and lightness into account. Duran cylindrical glass dish containing samples was placed in the illuminant path of the instrument and was covered with a black cover. Daylight D65/10° illuminant/observer combination was selected to measure daylight colour that measures the colour in terms of L*, a*, b*. These L* (lightness), hue angle and chroma are independent values that theoretically describe all perceived light. The values were compared with that of control wheat bread.

Delta values of L*, a*, and b* were calculated as follows: ΔL* = L* gluten-free bread - L* control, Δ a* = a* gluten-free bread - a* control, and Δ b* = b* gluten-free bread - b* control.

Where + ΔL* means gluten-free bread is lighter than control wheat bread, - ΔL* means gluten-free bread is darker than control, + Δ a* means gluten-free bread is redder than control, - Δ a* means gluten-free bread is greener than control, + Δ b* means gluten-free bread is yellowier than control, and – Δ b* means gluten-free bread is bluer than control.

Preliminary sensory evaluation on Day 1 of the formulated gluten-free bread , control wheat bread and other two gluten-free bread available in market were performed by a panel of eight trained judges for five quality attributes (taste, texture, appearance, softness, and flavour) using a nine point scale. Key to bread score was: liked extremely 9, liked a lot 8, liked moderately 7, liked slightly 6, neither liked nor disliked 5, disliked slightly 4, disliked moderately 3, disliked a lot 2 and disliked extremely 1. Crumb structure of breads was rated by visual observation using a five point scale. Key to bread score was: satisfactory 5, questionable to satisfactory 4, questionable 3, questionable to unsatisfactory 2, and unsatisfactory 1. The highest score of satisfactory was for crumb with small holes and thin cell walls, whereas the lowest score of unsatisfactory was for crumb with large holes and thick cell walls. The overall acceptability score was determined by summing the score for each characteristic and computing the average. Data were statistically analysed using Minitab 14 software to perform two way analysis of variation. Significance was accepted at $P < 0.05$.

Storage temperature and moisture contents have an impact on the crumb texture. It is well known that cold temperatures induce the retrogradation of starch and thereby increase the staling rate. He and Hoseney (1990) concluded that bread with higher moisture content was significantly fresher and firmed at a slower rate than did the bread with lower moisture content. The overall picture of the crumb could be described as interpenetrated gels separated

by aqueous interphase which contain most of the low molecular weight solutes. This water is rather mobile and can facilitate mutual displacement of the incompatible gel phases, thus behaving as a plasticizer, and can enhance the crumb-to-crust migration of moisture (Gray & BeMiller, 2003). This local drying makes the walls of the crumb alveoli more rigid, while the concurrent moisture increase within the crust region is accompanied by a reduction of crispness.

After chosen storage times, breads (approximately 20 slices per bread) were taken out of the incubator and polyethylene bags. For further evaluations, the slices at both ends were discarded and the middle four were chosen for water activity and percent moisture (w/w) measurements. The remaining fourteen slices per bread were used for hardness measurements by penetrometer.

For hardness measurements by the penetrometer, slices were put under the plunger containing probe of a set weight (making total assembly weight 445 g) and a given cross-sectional area (4.5 cm) so that it penetrates toward the middle of multiple bread slices in nine seconds. The compression was aimed at the centre of the slices. The plunger was released by a button-activated brake so that it can penetrate the bread. Two runs of penetrometer measurements were performed per bread along the longitudinal axis by using seven slices per run from the chosen fourteen slices. The two readings of penetration depth were recorded from each type of bread per day. These readings were averaged to give a single penetration depth value for the loaf and hardness index (HI) was calculated, where HI = Weight of probe assembly (445g)/ penetration depth in mm (Bourne, 2002).

Water activity and moisture significantly decreases in gluten-free breads due to loss of moisture on storage. Because water activity and moisture is so important, it is just as important to be able to measure it accurately and quickly. Water activity and moisture affects the shelf life, texture, and flavour of bread. Water activity of the bread crumb for five subsequent days after 24 hours of baking was determined by Aqua lab water activity meter. One loaf was taken per day for the evaluation from each control and gluten-free bread stored at 25ºC in the incubator. Four middle slices were chosen from each bread per day. Five millimetres of crust was removed from the four slices in order to separate crumb. Water activity of crumb was measured at 25ºC on one slice of chosen four slices of each loaf per day.

The remaining three slices per bread per day were used for determination of percent moisture of crumb and crust. The crust and crumb from these three slices per bread per day

were grinded separately in small food processor for 35 seconds and were immediately wrapped in polyethylene film to avoid any moisture loss. Ohaus moisture analyser was adjusted for 160ºC heating temperature for seven minutes and five grams of pulverised sample was taken for each analysis. The sample was spread uniformly on the aluminium tray so that it can be exposed to heat uniformly and moisture can evaporate effectively. Before each analysis the sample tray was cleaned properly and was waited for 10 minutes so that the moisture analyser and sample tray can attain the ambient temperature.

To know what was useful, required comparison with a typical wheat-based loaf. Therefore, a control wheat bread was formulated under the same set of processing conditions as that of gluten-free bread. The ingredients, suppliers and formula of control wheat bread are listed in Table 3. During this study, mostly two kilograms of control wheat dough was made according to the formula mentioned in Table 3 and divided into the pieces having equivalent weight to the trial gluten-free dough. The first comparison was the volume (heights) achieved by trial gluten-free dough compared to control wheat dough of same weight during proofing and retaining it during baking. Although this comparison was not completely justified in terms of similar dough weights, as water contents in the gluten-free bread formulation was considerably high compared to control wheat bread, but nonetheless gave rough estimation of other quality attributes mentioned in Table 2.

Table 3 Control wheat bread ingredients, suppliers and formula

Ingredients	Supplier	Weight (g)
Wheat flour	Woolworths New Zealand (local market)	1000
Water	Manukau water Tap water	600
Salt	Woolworths New Zealand (local market)	20
Bakers' compressed yeast	New Zealand Food Industries Ltd., Auckland, N.Z.	28
D.Y.C. white vinegar	Goodman Fielder Ltd., Auckland, N.Z.	10
Fungal α-amylase	Nutura N.Z. Ltd., Auckland, N.Z.	0.1
Lipase	Nutura N.Z. Ltd., Auckland, N.Z.	0.1
Canola oil	Woolworths New Zealand (local market)	10

Chapter 3 Initial dough development

3.1 Initial experiments

In the first 24 trials different ingredients were trailed to develop gluten-free dough. The initially formulated sticky gluten-free dough retained the carbon dioxide gas partially during proofing and thus failed to rise. The baked breads were dense and heavy with no definite crumb structure (Figure 8). However, the initial trials pointed to the ingredients that might be useful in the development of gluten-free bread.

Figure 8 A dense bread with undeveloped gas cells

In the initial experiments, a 'shotgun' approach was developed to identify the basic function of ingredients and their contribution towards the formulations. The 'shotgun' approach showed that a basic mixture of selected flours, starches, yoghurt powder, proteins, hydrocolloids, microbial transglutaminase, fungal α-amylase, glucose oxidase, lipase, yeast, and whole eggs gave promising results for the production of gluten-free dough and, critically, in retaining some of the carbon dioxide gas. Therefore, these ingredients were progressively trialled in different combination along with other baking ingredients, to produce an acceptable non-sticky dough and bread. The details of all formulations and their individual outcome are in Appendix I. This chapter reports work with details of ingredients found to be pivotally useful.

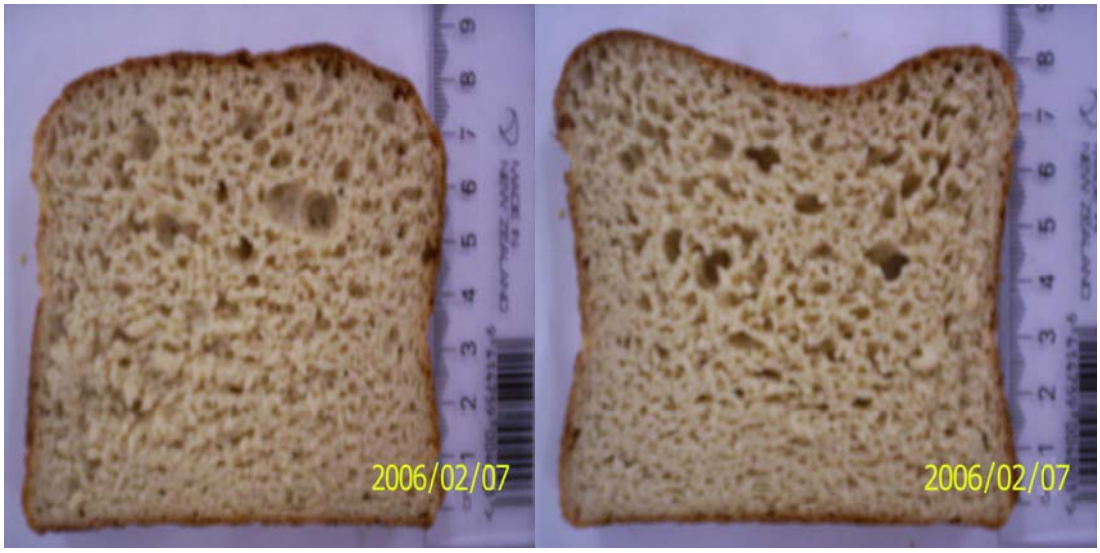
3.2 Preliminary results

A series of preliminary 24 formulations were trialled to narrow the possibilities. The basic mixture of the above mentioned ingredients produced the required good dough structure that could be processed on the existing equipment. In particular, psyllium husk and HPMC have water-holding and gel-forming capacities, which entrap carbon dioxide during proofing, where the maximum temperature is 40°C. According to the manufacturer's specification sheet (The Dow Chemical Company, Plaquemine, LA, USA), Methocel K4M ─ a type of HPMC does not form a strong gel until about 70°C is reached, it does have a viscosity at lower temperature by virtue of its hydrocolloid nature. Particularly Methocel K4M was used in this study due to its thermal gelation around 70-90ºC to form soft gel, compared to other types (E4M, E15, E50, F4M and F50) that thermally gels in the temperature range of 58 to 68ºC and form semi-firm gels, which greatly inhibit the oven spring during initial stage of baking and results in low specific volume and brittle crumb.

In different formulations, microbial transglutaminase enhanced the dough-like structure and its gas entrapping abilities, presumably due to increased cross linking. Glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide, which in wheat flour either, causes the formation of disulphide bonds between proteins or the tyrosine crosslinks (Gujral & Rosell, 2004b) and one molecule of water per disulphide bond as a by-product. Most of the literature reported that the improvements are related to crumb texture and strength, and product volume has not been observed to increase with glucose oxidase treatment. Rasiah, Sutton, Low, Lin, & Gerrard (2005) reported that glucose oxidase showed only small macroscopic affects in baked products. Moreover produced water as a by-product reduced the viscosity of the dough. Therefore, in the initial experiments glucose oxidase did not prove to be as good as microbial transglutaminase. Glucose oxidase, when used concurrently with microbial transglutaminase showed negative effects. This may be due to the direct or indirect oxidation of –SH group(s) at the active site of microbial transglutaminase. Microbial transglutaminase gave more favourable results than that of glucose oxidase; therefore the trials other than first 24 were solely conducted by incorporating microbial transglutaminase.

In experiments with microbial transglutaminase as an ingredient, gas was retained in the dough during proofing (Figure 9 a), rising to the height achieved by the control wheat dough (data not shown).

a. After proofing b. After baking



c. Two slices of the bread in b

Figure 9 Preliminary results. The bread retained the gas during proofing (a.) but collapsed during baking (b.). Slicing revealed large holes and an irregular crumb structure (c.)

When used alone, psyllium husk, which maintained a gel state up to proofing temperatures, failed to entrap the gas at baking temperatures, presumably because the gel structure was lost. Thus the bread collapsed. When HPMC was included, the favourable structure was maintained to a certain extent at baking temperatures. Use of milk and rice protein concentrates at high concentration in the formulation produced strong cross-linked doughs with the best handling properties, but failed to rise on proofing due to increased dough firmness. Thus, although the psyllium husk and HPMC combination held gas at proofing temperatures, the gel structure was somewhat lost as baking progressed (Figure 9).

This was affected by the water content. As it increased the gel structure weakened, which increased the gas loss. While psyllium husk and HPMC in combination showed promise, the challenge was to identify a starch would gelatinise and entrap the escaping gas before the combination failed. Tapioca, potato and maize starch give the best results. The immediate goal was to find a combination of these ingredients that would gel when the psyllium-HPMC gel structure was failing, and moreover, would not collapse when the bread were cooled.

3.3 Developmental issues

The preliminary results of initial experiments indicated four developmental issues that had to be solved in stepwise manner for the production of a gluten-free commercial bread. The first issue was to make a gluten-free non-sticky visco-elastic dough, the second was to entrap the carbon dioxide gas during proofing to achieve acceptable volume, the third was to avoid the collapse of cell structure by keeping the gas cells intact during baking, and the fourth was to prevent the collapse during bread cooling.

These issues could be resolved by utilizing certain ingredients that play different role in the time wise manner during the normal bread making process. To resolve the first issue, the ingredients of choice were those that hold water, increase viscosity and elasticity, and cross-link the protein substrates (native or supplemented) during dough formulation. Using ingredients that generate a gel of reasonable strength to entrap gas during proofing (40ºC) and being extensible to inflation could resolve the second issue. The third and fourth issues could be resolved by using ingredients with an ability to increase their gel strength with increasing processing temperature during initial stage of baking to keep the gas cells intact, and to finally transform into soft matrix of gas bubbles in the last stage of baking.

3.4 Ingredient selection

After reading the literature on the gelation properties of a range of flours, starches and hydrocolloids, and the preliminary results of initial experiments, the following ingredients were selected for more systematic work: rice flour, soy flour (full fat), potato starch, tapioca starch, maize starch, guar gum, xanthan gum, HPMC (K4M), psyllium husk, bakers' yeast, vinegar, canola oil, salt, sugar, microbial transglutaminase, lipase and α-amylase. To simulate the gluten protein network, supplementary rice protein, milk protein, whole egg and yoghurt powder were selected and crosslinked by microbial transglutaminase enzyme in gluten-free dough.

Chapter 4 Further development

4.1 Introduction

In the previous chapter, a series of experiments pointed to a short list of ingredients, which in several combinations gave promising results. This chapter describes trials where these ingredients were systematically manipulated to develop the desired loaf.

The formulations were developed with ingredients listed in section 3.4. In changing ratios of ingredients from the list of 22, the fewer that are changed, and the fewer the changes, the simpler the work. To this end the levels of α-amylase and lipase were held constant at the rate of 0.05 g each per formulation, while other ingredients were varied. It was felt that these two ingredients would not be directly involved in specific volume, which is arguably the most difficult attribute to optimise. This is because existing commercial gluten-free breads tend to suffer from a low specific volume.

The development proceeded in roughly four phases starting at trial 25. In Phase 1 (trials 25 to 35) a successful machine-processable dough was developed which was capable of holding the gas during proofing and in achieving the required volume during proofing. The bread developed in this phase suffered from minor escape of gas during baking which led to collapse of the bread to a minor extent. This resulted in low specific final volume of the bread and this problem was addressed in subsequent trials in Phase 2 (trials 36 to 54). In this second phase the problem of baking collapse was solved but the breads tended to show minor side-and-surface collapse during cooling. This problem was addressed in Phase 3 (trials 55 to 101). The bread produced in this phase showed good oven spring, did not collapse while baking or cooling, and achieved a specific volume comparable to control wheat bread. In Phase 4 (trials102 to 124), the formulation was further refined to increase the moisture retention and to reduce the bread staling rate. Trial 124 was the final recipe in the scope of this development. All trials are listed in Appendix I with their individual outcome and only significant, often successful, experiments are discussed in this chapter.

4.2 Presentation of data

A graphical presentation method is used, where the progressive change in formulation is shown as change in percent composition. To cope with the issue of scale, the ingredients were categorised as major and minor variables according to their contributed percent composition in the formulation. The major category variables were rice flour, maize starch, psyllium husk, and egg whereas other remaining ingredients listed in Table 1 were considered

as minor category variables. In calculating the percent composition of these ingredients, the amount of water used in the formulations was excluded, as the relative percent compositions of other ingredients were considered more important. Moreover the water content was highly variable in the formulations and adversely affected the interpretation of the percent composition change of other more structural ingredients and their graphical presentation. Water addition was dictated by the percent composition of hydrocolloids and egg. A higher percent of hydrocolloids dictated more water addition, whereas more egg – with its high moisture content – dictated less water.

4.3 Phase 1: development of mouldable, non-sticky dough

In trials 25 to 35, a mouldable, non-sticky dough was developed capable of entrapping the carbon dioxide during proofing. The aim was to attain a height equal to the height of control wheat bread.

The starting point bread, developed in Chapter 3, had a non-sticky dough structure, and could be processed on an existing bread production line. However it was deficient in other ways. In trial 25, the otherwise manageable dough collapsed on baking due to the loss of the psyllium husk gel structure. It was decided to increase the proportion of proteins and non-starch hydrocolloids at the expense of rice flour. The hypothesis was that an increase in protein content would strengthen the dough by introducing more protein crosslinks to entrap the gas, while increased concentrations of psyllium husk and HPMC would enhance gel strength and moisture retention in dough.

Thus, proportion of rice flour was decreased while those of yoghurt powder, rice protein, egg protein, milk protein, psyllium husk, HPMC, bakers' yeast, and sugar were increased (Figure 10 and Figure 11). The proportions of soy flour and maize starch were kept constant, and were used at the rate of 10 g and 32 g respectively in each formulation during this phase. The formulations containing xanthan gum were stickier than those containing guar gum due xanthan's rapid hydration in cold water. Therefore, the composition of guar gum was increased, while that of xanthan gum was gradually decreased and totally eliminated after trial 34. The percent composition of bakers' yeast and sugar were both slightly increased in this phase to compensate for gas loss in the initial stage of baking.

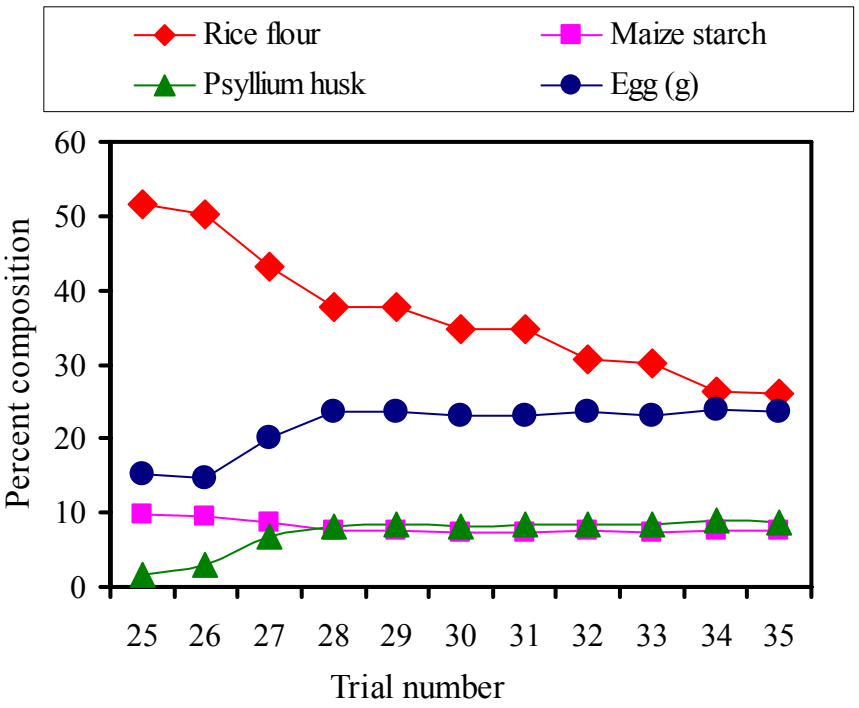


Figure 10 Percent composition changes in major variables in Phase 1

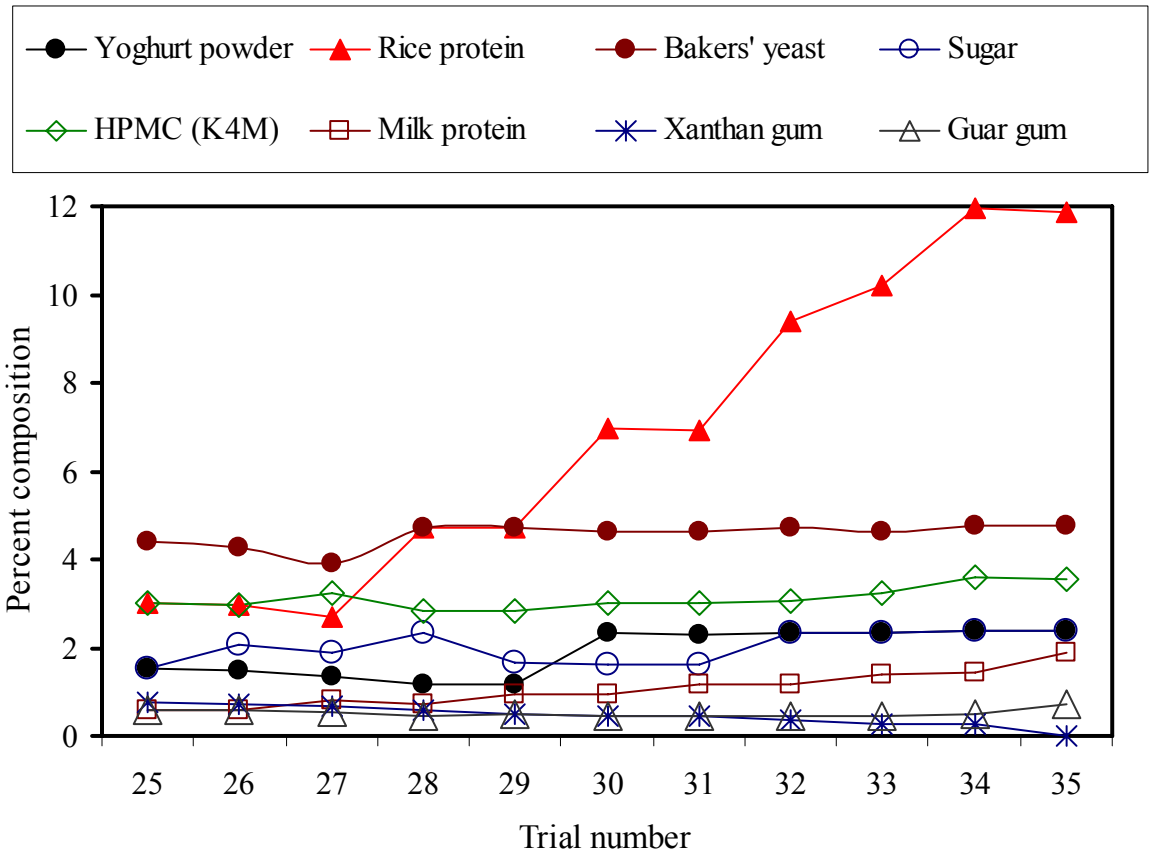


Figure 11 Percent composition changes in minor variables in Phase 1

As the proportions of the strongly water-binding non-starch hydrocolloids; psyllium husk and HPMC were increased in the formulations, more water was needed to produce acceptable

non-sticky viscoelastic dough. As the percent composition of the HPMC was increased, stickiness of dough increased, whereas psyllium husk reduced dough stickiness.

Increased hydrocolloids increased the dough viscoelasticity and further enhanced the gas entrapping capability of the dough during baking, and thus increased the bread volume during proofing. Increased proportions of proteins at the expense of rice flour increased moisture binding in dough, and reduced dough stickiness and the extent of baking collapse. However, no combination in this phase completely stopped the collapse on baking.

Therefore, the formulated breads at the end of this phase (trial 25 to 35) attained a volume comparable to control wheat bread during proofing, but exhibited minor collapse in structure during baking. The problem of baking collapse was addressed in Phase 2.

4.4 Phase 2: elimination of baking collapse

Yoghurt powder and guar gum were kept constant in Phase 2 (trials 36 to 54), and were used at the rate of 10 g and 4 g respectively in each formulation. Tapioca starch was untested in Phase 1, but was introduced in trial 37 as a new ingredient.

The proportions of maize starch, tapioca starch were gradually increased. The hypothesis was that starches with a low gelatinisation temperature would create a moisture-retaining gel at baking temperature, where the psyllium husk gel structure was failing and gas was being lost. The gel structure of psyllium husk is stable up to about 80°C (Haque et al., 1993), whereas, the gelation temperature of maize starch (62°C-80°C)[3] and tapioca starch (52°C-65°C) (Fennema, 1996) is lower than this psyllium husk gel stability point.

Therefore, the composition of maize and tapioca starch, soy flour, and psyllium husk were increased at the expense of rice flour (Figure 12 and Figure 13). These changes reduced dough stickiness and collapse on baking. Increased starch content resulted in more gas production as more fermentable monosaccharides became available for the yeast. Therefore, the composition of sugar and bakers' yeast were gradually decreased to avoid over-proofing of bread.

[3] From the initial temperature of gelatinisation to complete pasting (Fennema, 1996)

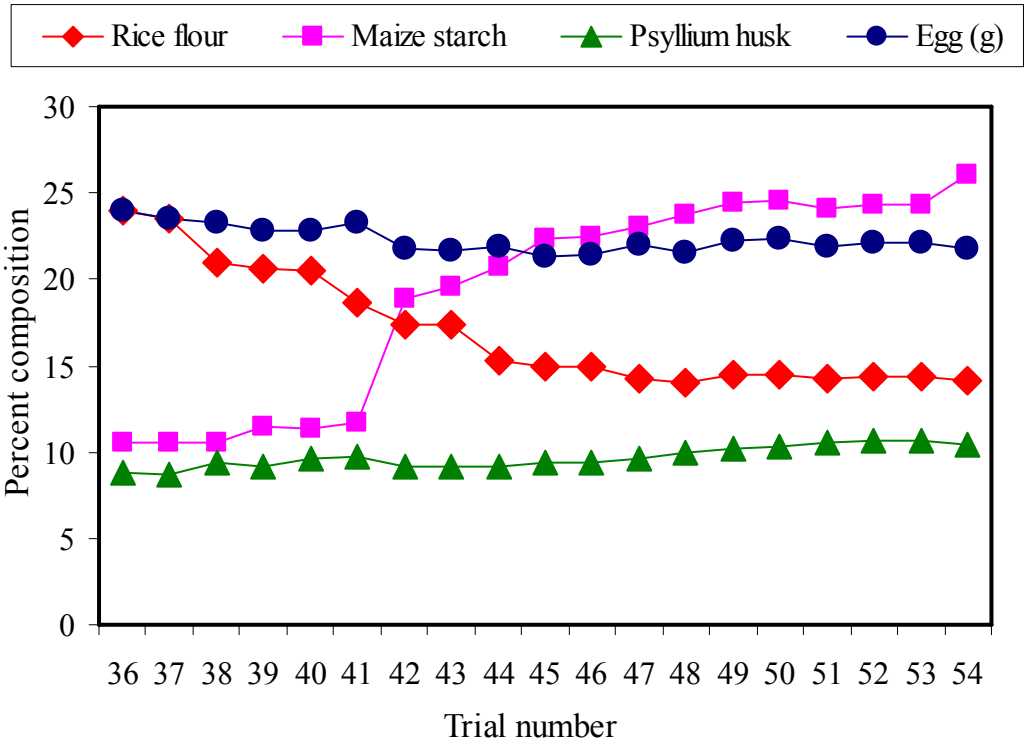


Figure 12 Percent composition changes in major variables in Phase 2

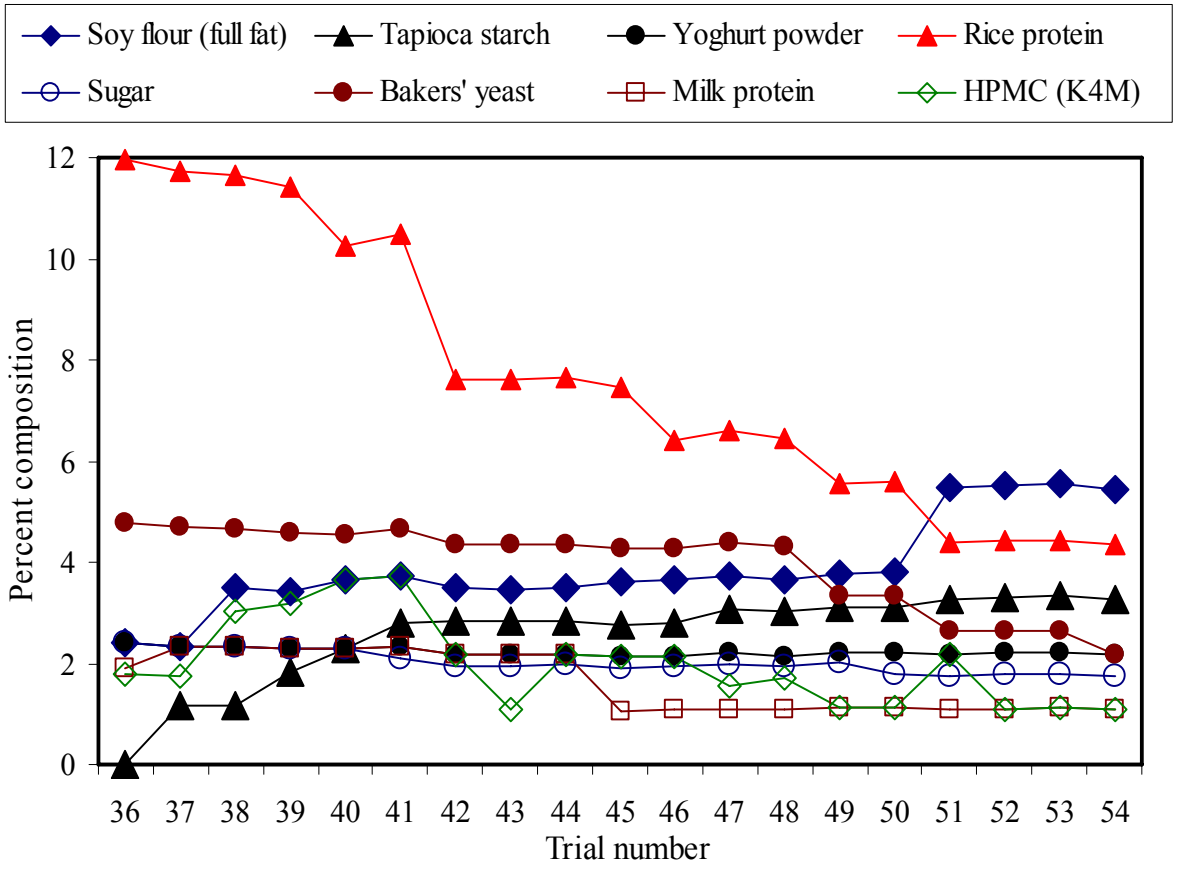
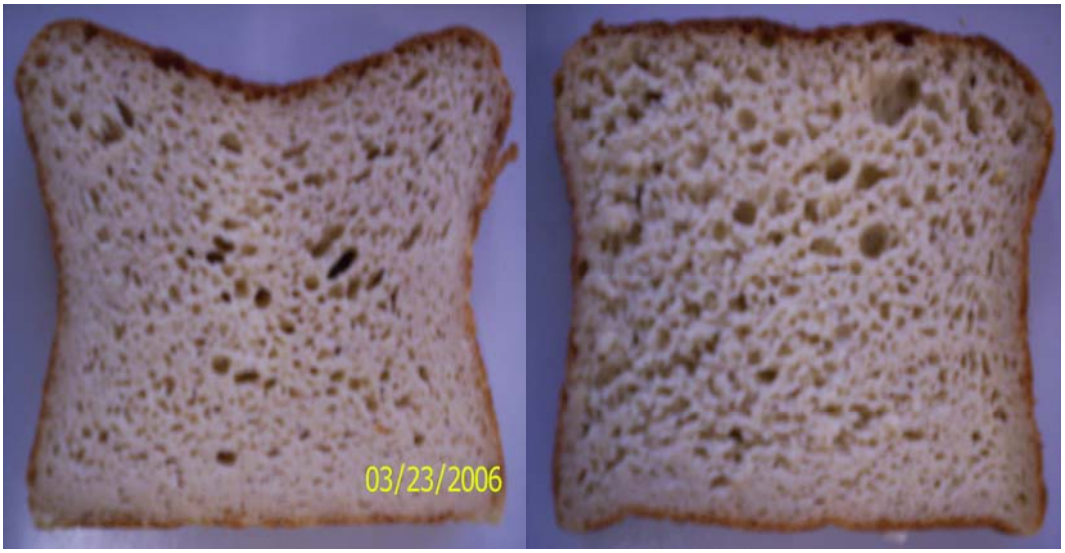


Figure 13 Percent composition changes in minor variables in Phase 2

The proportion of soy flour was increased in Phase 2. It was observed that soy flour enhanced the water-binding capacity of the dough and softness of the bread. Breads with supplementary rice protein and whole egg were unacceptably too yellow and differed markedly from control wheat bread. Therefore, the composition of rice protein was reduced in a stepwise manner as it was affecting bread colour and was also yielding breads with a brittle crumb. Increase in psyllium husk increased desired cohesiveness of dough while HPMC at higher concentration increased undesired adhesiveness of the dough (data not shown).

The main outcome of these collective changes in composition was the creation of a dough capable of entrapping the carbon dioxide on proofing and baking to attain a height equal to the height of control wheat bread.

It is proposed, but not proven, that the increased composition of tapioca starch and maize starch gelatinised at a temperature where the resulting gel bridged the gap between the lower-temperature-stable psyllium husk gel and the heat-stable HPMC gel. In this way, the carbon dioxide and steam were trapped and the bread did not collapse on baking.



a. Bread with side and surface collapse b. Bread with irregular crumb

Figure 14 Quality defects of gluten-free bread prepared in Phase 2

Nonetheless, the bread suffered from other defects. These defect included, a sticky and gummy crumb as felt by the empirical squeeze test, and collapse from sides and surface during cooling (Figure 14 a). The latter phenomenon was due to the incomplete transition of

the proofed foam structure into baked sponge structure. Moreover, the breads had an irregular crumb structure (Figure 14 b) due to the collapse of gas cell structure.

4.5 Phase 3: elimination of surface and side collapse

The problems of minor side-and-surface collapse on cooling and stickier irregular crumb were addressed in the third phase (trials 55 to 101). This phase was also a deconstructive phase, to increase or optimise the use of ingredients that were performing well in their intended or likely intended roles, and to decrease or eliminate the use of selected ingredient with minor or contradictory functions in bread structure development. Potato starch was untested in Phase 1 and Phase 2, but was introduced in trial 67 in Phase 3 as a new ingredient. The cause of side-and-surface collapse and an irregular crumb structure was explored by the following simple gelation experiment:

Two grams each of maize starch, tapioca starch and potato starch were placed in three cups of a muffin tray; 2 ml of water was added to each starch and the mixture was heated in the baking oven for 5 minutes at 220°C. It was found that maize starch and potato starch formed dry, soft solid gels, whereas the tapioca starch was in liquid to semisolid state, although state was highly extensible.

It was thought that the tendency of tapioca starch to stay in the liquid gel form for longer before setting into a structure in the presence of high water contents in the formulation might be the cause of side-and-surface collapse. During cooling of the bread, the gas might escape from the gas cells due to its liquid to semisolid nature. This kind of problem was observed by other authors when tapioca starch was used in artificial flours made from dry wheat gluten (Chiharu, 1999).

High water contents were important in other ways. Generally, it was observed that whenever the water content was low, the breads did not rise to the height of control wheat bread, but neither did they collapse on baking or cooling. The specific volume of these breads was low because the doughs had a high viscosity, offering a high resistance to bubble expansion during proofing and the initial stage of baking. When the water quantities that the hydrocolloids could absorb were surpassed, the excess water promoted a dramatic decrease in dough viscosity and yielded sticky dough. However, the bread volume was increased during proofing. But this did not translate into high quality final loaf. The bread specific volume decreased for such formulations due to the collapse of gas cell structure during baking and cooling as shown in Figure 9 and Figure 14. Further increase in water contents was

unacceptable as the dough rheology was shifting from a sticky paste to a liquid batter. Moreover, the matrix was not capable of retaining the gas formed during proofing and gas cell collapse occurred.

In this phase, the proportions of maize starch, potato starch, HPMC, and yoghurt powder were progressively increased, while rice flour, soy flour, rice protein, egg, tapioca starch, psyllium husk were gradually decreased (Figure 15 and Figure 16). Tapioca starch had contradictory effects. In Phase 2 it was observed that the addition of tapioca starch and increase in maize starch stopped the baking collapse but tapioca starch appeared to be linked to the side-and-surface collapse on cooling. In Phase 3 it was found that as the proportions of tapioca starch and rice flour were decreased and those of maize starch and potato starch increased, this type of cooling collapse of bread was reduced. Moreover, rice flour tended to produce sticky dough and low height on proofing.

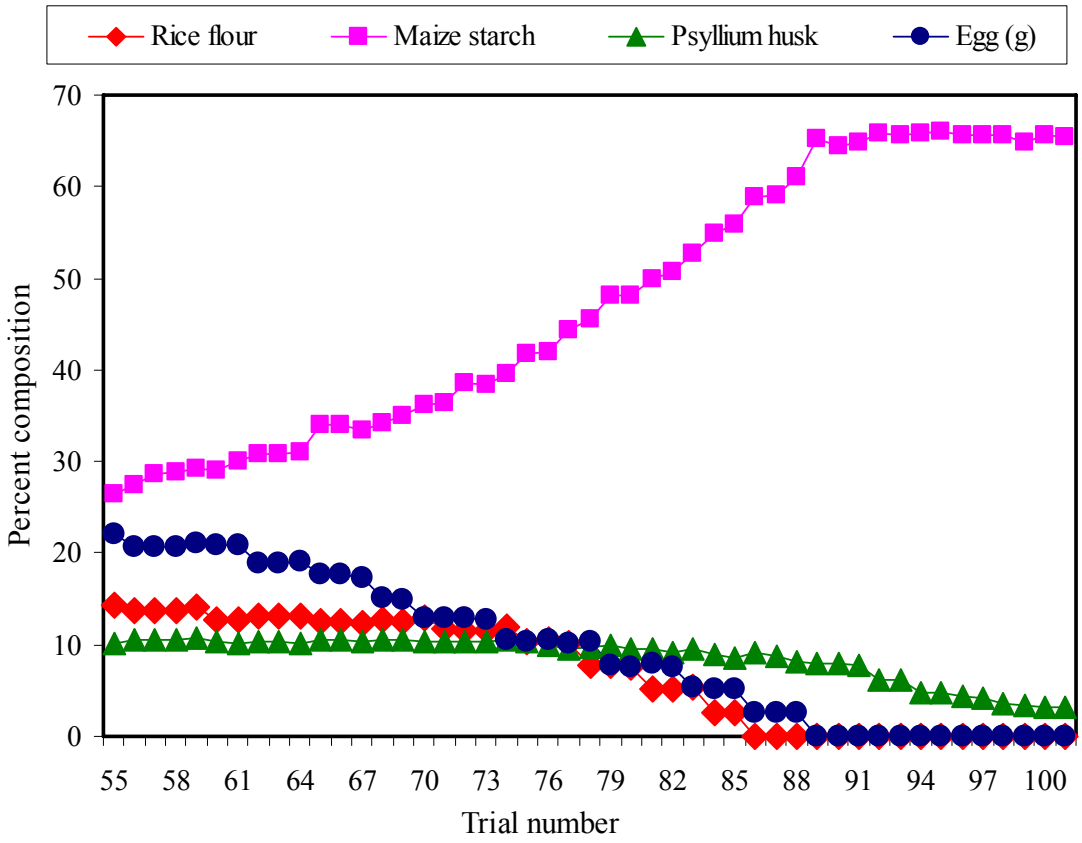


Figure 15 Percent composition changes in major variables in Phase 3

Tapioca starch appeared to produce an impermeable membrane (the starch granules fuse together forming a gas discontinuous system) which was extensible for a longer period than for other starches. This led to a larger loaf during proofing and baking but one that collapsed during cooling. Potato and tapioca starches have similar gelatinisation temperatures (~60°C)

(Fennema, 1996). However, the state of the gelatinised granules in bread was quite different for these two starches as illustrated by the simple gelation experiment described above. The gas cell membranes of tapioca starch breads appeared to be impermeable to gas, which was retained during proofing and baking but could not set into a non-contracting structure. Tapioca breads remained undesirably extensible during baking. On cooling, such loaves, with extensible gas cell walls, shrink due to negative internal pressure created by cooling. Thus, tapioca starch was deleted after trial 80 and rice flour after trial 85 (Figure 15 and Figure 16)

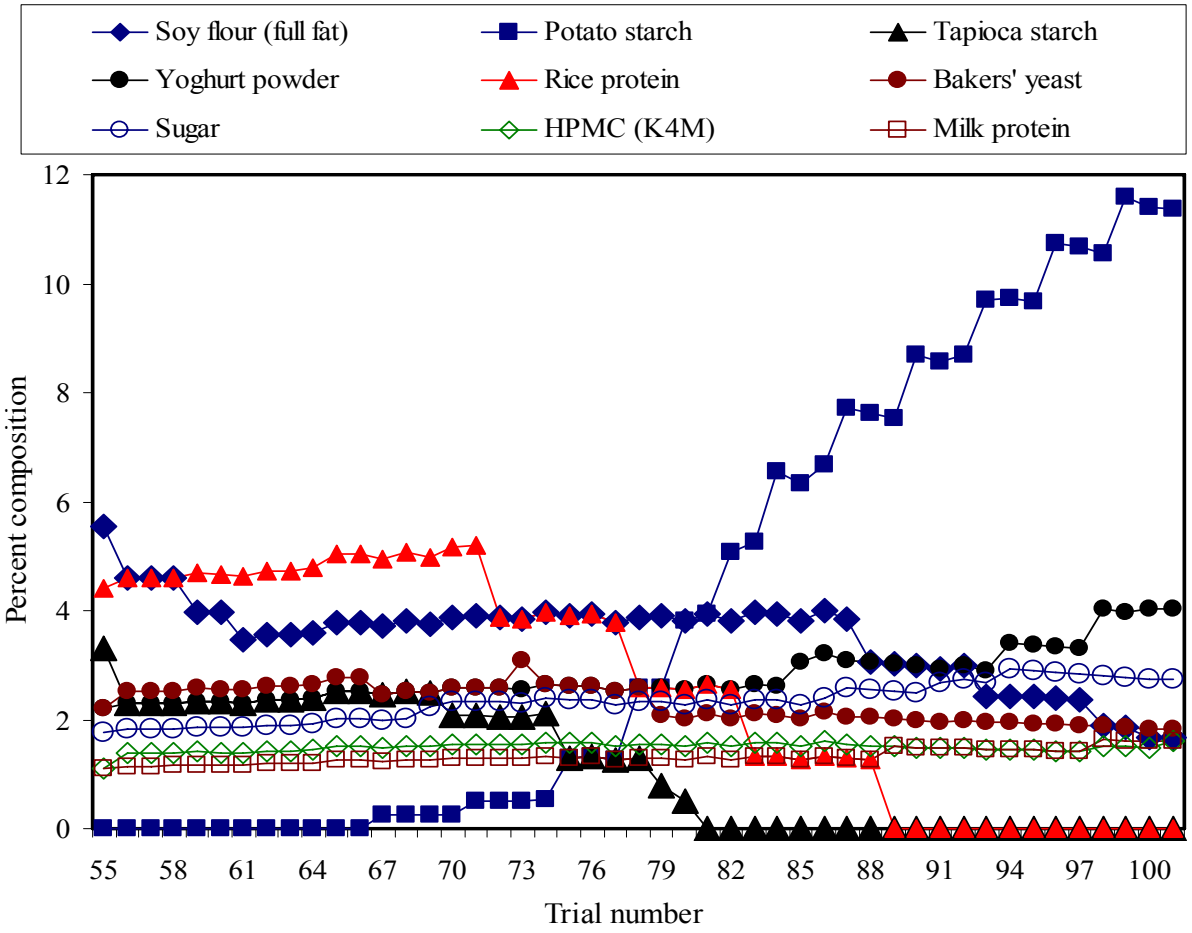
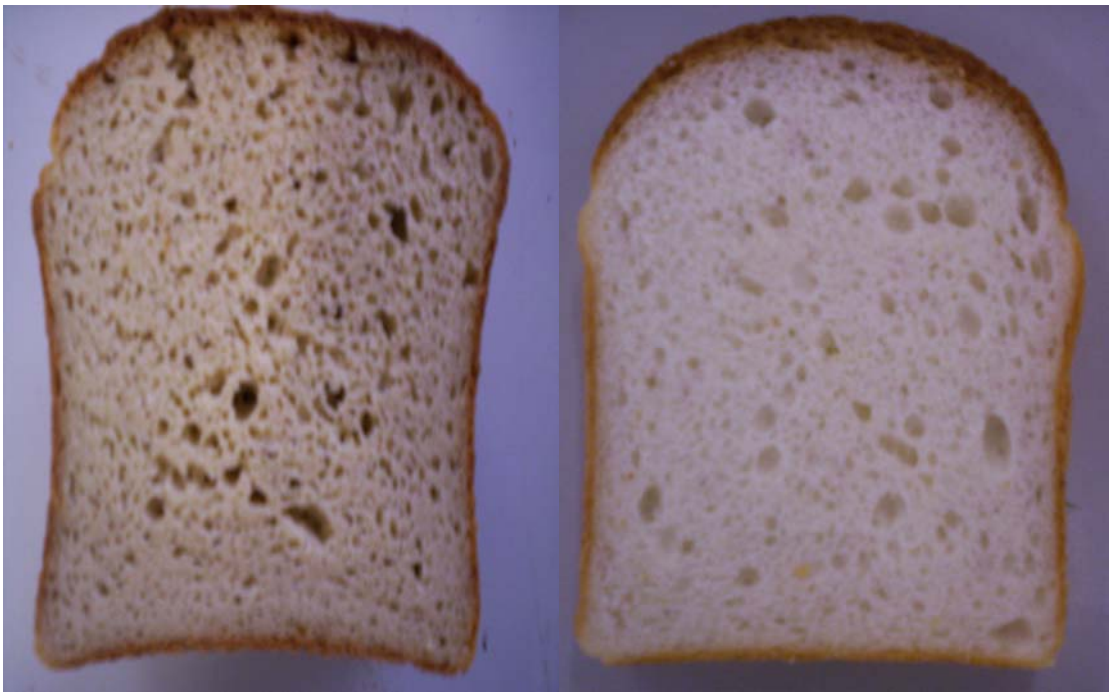


Figure 16 Percent composition changes in minor variables in Phase 3

The progressive increase in the concentration of maize starch, potato starch, yoghurt power, milk protein, sugar, the deletion of tapioca starch, and the decrease in rice flour, rice protein resulted in a continuous quality improvement in the breads. The collapse of bread structure during baking or cooling decreased and disappeared entirely when an optimised formulation (trial 88) was executed. At this point, the otherwise manageable dough was still slightly sticky; therefore guar gum was not used after trial 88. Trial 88 bread did not have any major quality defects, importantly having lost its prior gumminess in the squeeze test. However it had darker crust and irregular darker and yellowier crumb (Figure 17 a) that was not desirable.

The bread formulated in trial 88 was subsequently modified up to trial 101 to correct the irregular crumb and colour attributes. Phase 3 work up to trial 88 showed that lowering the proportions of psyllium husk and soy flour reduced the crumb yellowness (not shown). Further, lowering the proportion of rice protein and egg, and increasing HPMC reduced the bread crumb and crust darkness (not shown). Therefore, the proportion of psyllium husk and soy flour was decreased after trial 87. Rice protein and egg were deleted after trial 88, whereas milk protein was increased to roughly balance the protein content.



a. Formulated bread in trial 88 b. Formulated bread in trial 101

Figure 17 Significant improvements in Phase 3. (a) Bread without side or surface collapse but with a dark crust and crumb colour. (b) Bread with crust and crumb colour comparable to control wheat bread

By the end of Phase 3 (Trial 101) these changes yielded acceptable gluten-free bread (Figure 17 b) with crust and crumb colour comparable to the control wheat bread. Bread 101 was the starting point for further refinement in Phase 4, and was informally evaluated by staff on the bakery site before Phase 4 was begun.

The shelf life of Bread 101 was established by physical measures of retrogradation, called staling in the context of bread, after five days storage at 25ºC. Bread 101 was assessed on Day 5 by the three-member sensory panel for its firmness. The breads were found to be dry and firm at that time. The tendency to stale quickly was attributed to the high content of amylose-rich maize starch, which retrogrades rapidly (Fennema, 1996).

4.6 Phase 4: reduction of staling and moisture loss

In Phase 4, trials were conducted to reduce the bread staling rate and moisture loss during storage. The maize and potato starches were helpful in stopping baking and cooling collapse of gluten-free breads, but resulted in dry and firm crumb (as found in previous trials). As the Bread 101 was acceptable in other ways, refinement was needed only for moisture binding, moisture retention and reducing firmness of bread. These aims could possibly be achieved by increasing water content, increasing the composition of HPMC (Barcenas & Rosell, 2005, 2006; Guarda, Rosell, Benedito, & Galotto, 2004), psyllium husk (Park, 1997) to achieve moisture aims, and increasing canola oil content to reduce firmness.

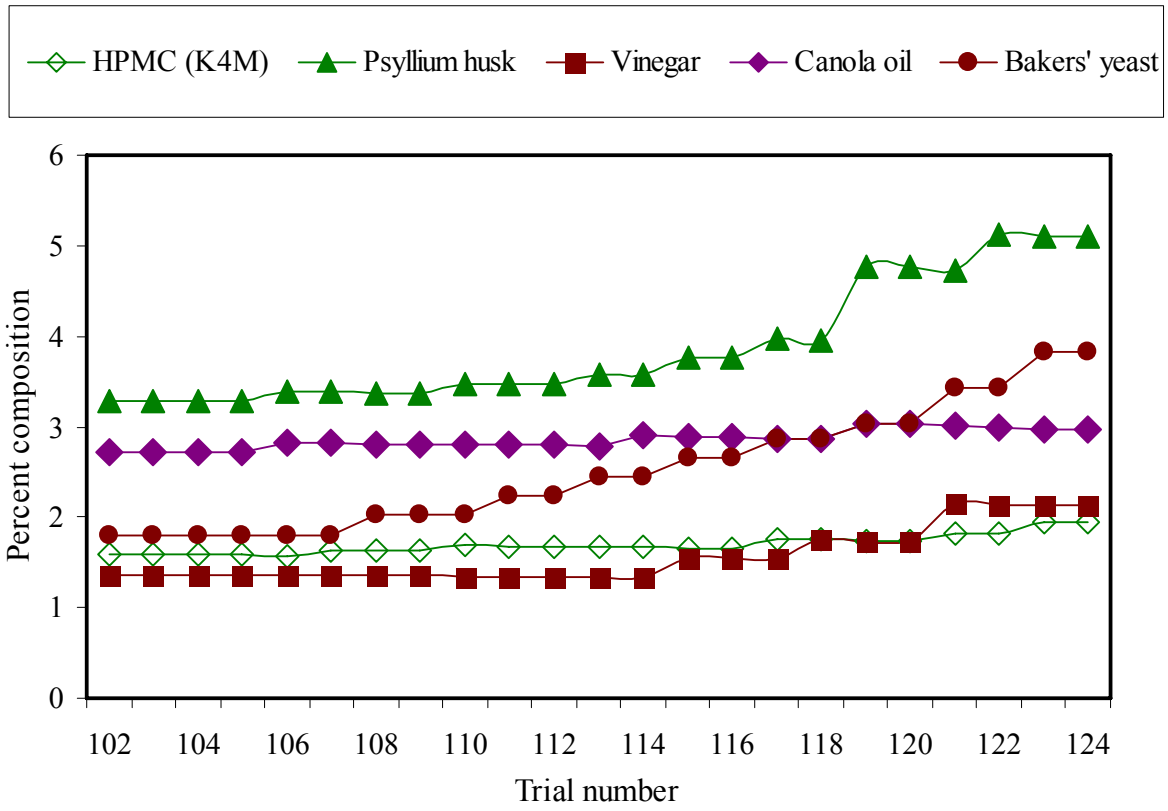


Figure 18 Refinement of formulation in Phase 4

Thus, the proportions of soy flour, maize starch, potato starch, yoghurt powder, milk protein and sugar were held constant, whereas, psyllium husk, yeast, vinegar, canola oil, and HPMC were varied (Figure 18). The proportion of microbial transglutaminase enzyme (as supplied[4]) was lowered in trial 106 and maintained at 0.5 g per formulation. Transglutaminase appeared to have two effects. When it was used at the rate of 0.5 to 1 g per formulation, it improved dough handling properties – presumably by cross-linking the protein fraction of dough – while at higher addition rates it decreased the specific volume. Below 0.5

[4] This has a claimed activity of approximately 60 units.g^{-1} as discussed in Chapter 2

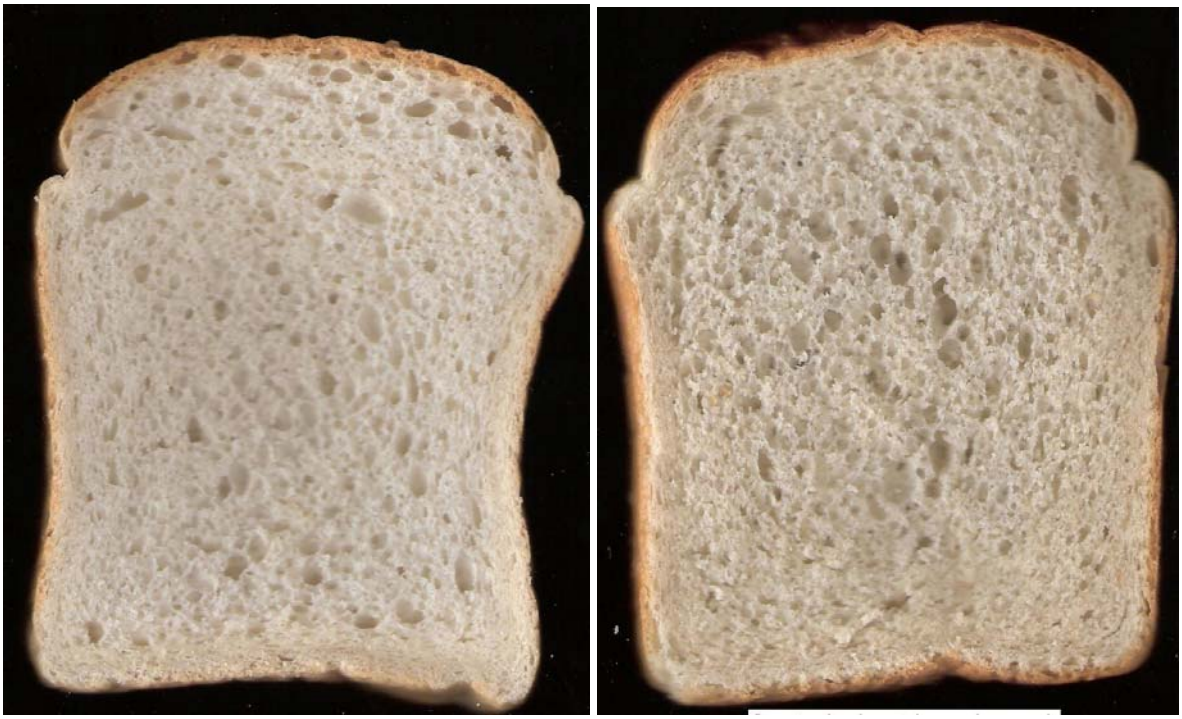
g per formulation, dough handling properties deteriorated, but bread specific volume increased and crumb strength decreased (data not shown). As the proportions of non-starch hydrocolloids; psyllium husk and HPMC were increased in the stepwise manner so as the proportions of water contents and bakers' yeast were increased. Water contents were increased to achieve moisture aims and to avoid the dough from becoming too sturdy due to increased hydrocolloids. Sturdy dough ultimately offers a high resistance to bubble expansion during proofing and the initial stage of baking and yields the breads with low specific volume. The proportion of bakers' yeast was increased in order to help bubble expansion by more gas production as increased hydrocolloids increased the viscosity of dough and retarded its expansion during proofing. The proportion of vinegar was increased in order to inhibit microbial or mould growth in the bread due to increased water contents.

In Phase 4, these collective changes refined the Phase 3 Bread 101 and yielded Bread 124 that had similar external and internal appearance to that of control wheat bread (Figure 19) and moist and soft crumb on storage compared to the all previous formulations. The formulation of Bread 124 was repeated for a number of times to check the consistency of results. Since the formulation for Bread 124 gave consistent results for all the quality attributes (data not shown), it was finalised and the specification was prepared (Table 4). The Bread 124 was objectively and subjectively compared with the control wheat bread for its quality attributes in next chapter. Table 4 lists the ingredients and their composition in the finalise recipe of the fully developed gluten-free loaf (Bread 124).

Table 4 Finalised recipe of gluten-free commercial bread, Bread 124

Ingredients	Amount per loaf (g)
Soy flour (full fat)	7.35
Potato starch	50
Maize starch	288
Yoghurt powder	17.7
Milk protein	7
HPMC (K4M)	9.12
Psyllium husk	24
Microbial transglutaminase	0.5
α-Amylase	0.05
Lipase	0.05
Bakers' yeast	18
Vinegar	10
Canola oil	14
Salt	9.5
Sugar	15
Water	355
Total	825

a. External appearance of Bread 124 b. External appearance of control wheat bread



c. Internal appearance of Bread 124 d. Internal appearance of control wheat bread

Figure 19 Visual appearance of Bread 124 and control wheat bread

Chapter 5 Attributes of the Bread 124

5.1 Introduction

In this chapter, the fully developed gluten-free bread, Bread 124, was objectively and subjectively compared with the control wheat bread (Table 3). The evaluations were for some of the variables listed in Table 2. These were oven spring, moisture loss during baking, loaf volume and specific volume, crust and crumb colour, crumb hardness, crumb water activity, and crumb and crust moisture contents, as well as preliminary sensory evaluation. In this chapter, the costs of ingredients are also described and compared with those of control wheat bread.

5.2 Preparation of breads for comparisons

A schematic diagram of bread preparation for these evaluations is shown in Figure 20.

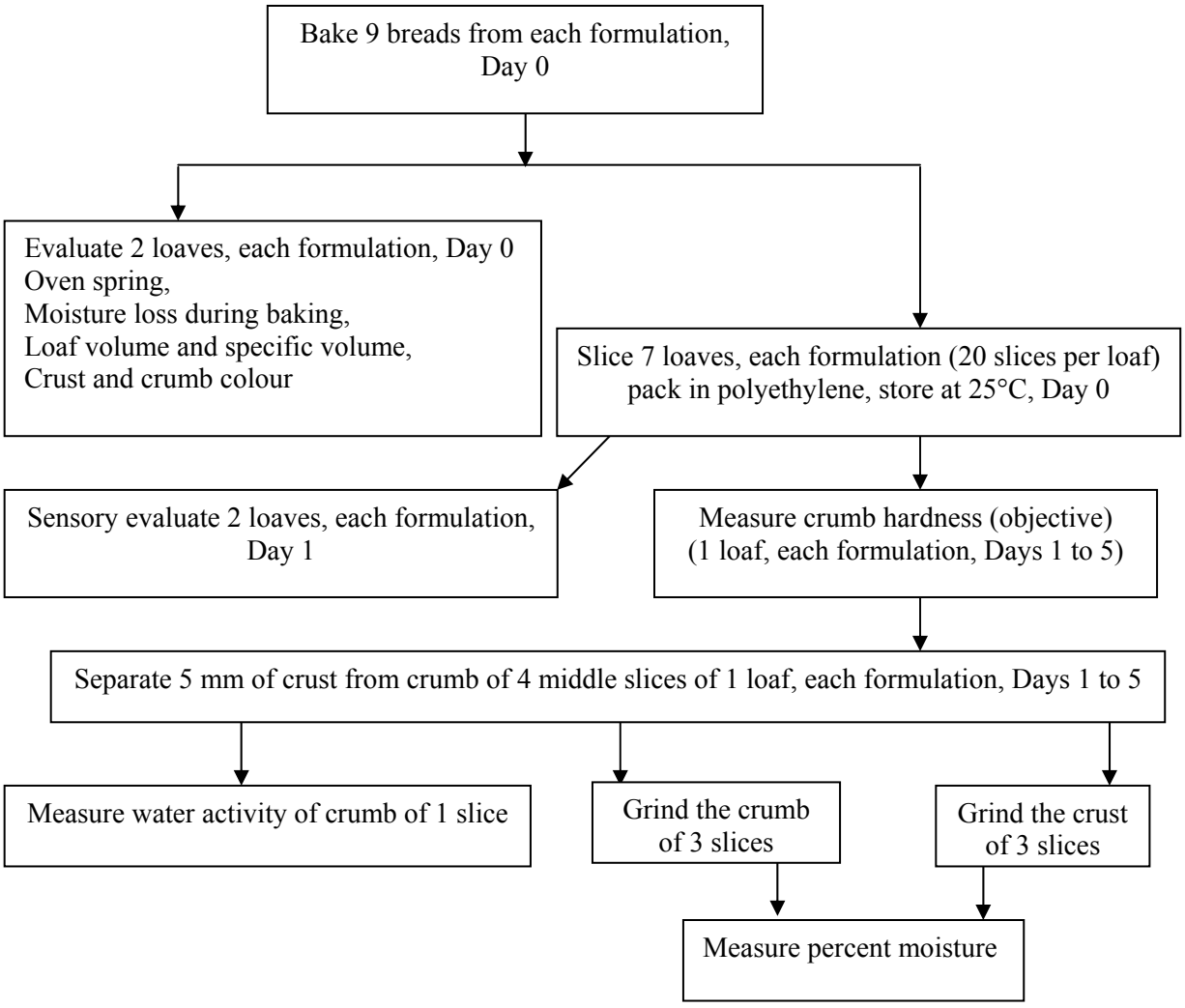


Figure 20 Schematic diagram of bread preparation for evaluation

Nine gluten-free loaves were made on Day 0 according to the finalised recipe (Table 4) and nine control wheat loaves were made according to the recipe shown in Table 3.

Analyses for oven spring, moisture loss during baking, loaf volume, loaf specific volume, bread crumb and crust colour (Table 2) were conducted on two loaves from each formulation (gluten-free, wheat) on Day 0 after the loaves had cooled to room temperature. Two loaves from each formulation were used for sensory analysis on Day 1. Evaluations for staling (crumb hardness), water activity of crumb and moisture contents of crumb and crust, were performed on remaining ten loaves on Days 1 to 5 requiring the remaining 10 loaves using one from each formulation each day.

Due to the limited production capability of equipment in test bakery, only 9 breads from each formulation were made and duplicates were used for evaluations. For evaluation on Day 0 only two loaves from each formulation were used, and the remaining breads were not subjected to these evaluations as this could affect the other evaluations by keeping breads out of packing.

5.3 Results and discussion from instrumental analysis

5.3.1 Evaluations on the day of baking (Day 0)

The oven spring of gluten-free bread and control wheat bread is shown in Table 5. Within the constraints of only duplicate determinations, the spring was the same for each formulation, 1.3 cm.

Table 5 Oven spring of gluten-free and control wheat bread

	Gluten-free bread		Control wheat bread	
Dough weight (g)	768	778	778	778
Height before baking (cm) (A)	10.1	10.5	10.2	10.2
Height after baking (cm) (B)	11.4	11.8	11.5	11.5
Oven spring (cm)= B-A	1.3	1.3	1.3	1.3

Moisture loss during baking was calculated after the baked breads had cooled to room temperature (Table 6).

Table 6 Moisture loss during baking

	Gluten-free bread		Control wheat bread	
Dough weight (g) (A)	768	778	778	778
Bread weight after baking (g) (B)	688	698	693	693
Moisture loss (g) = A-B	80	80	85	85
Percent moisture lost	10.4	10.3	10.9	10.9

The presence of high initial percent moisture and hydrocolloids in gluten-free breads retarded the moisture loss during baking compared to control wheat bread. In addition, the HPMC network formed during baking could act as a barrier to the gas diffusion, decreasing the water vapour losses, and thus increasing the final moisture content of the bread (Barcenas & Rosell, 2005). The moisture contents of baked gluten-free breads were slightly higher than that of control wheat bread.

Bread volume was measured by rapeseed displacement method (American Association of Cereal Chemists Approved Methods Committee, 2000) and the volume was divided by bread weight to calculate the specific volume (Table 7).

Table 7 Bread volume and specific volume

	Gluten-free bread		Control wheat bread	
Loaf weight (g) (A)	688	698	693	693
Loaf volume (cm^3) (B)	2660	2590	2670	2675
Specific volume ($cm^3.g^{-1}$) (B/A)	3.86	3.71	3.85	3.86

Within the limits of duplicates, the mean specific volume of control wheat bread was 3.85 $cm^3.g^{-1}$, slightly higher than that of gluten-free bread with the mean specific volume of 3.78 $cm^3.g^{-1}$. The specific volume of gluten-free bread developed in this study was comparable to control wheat bread and was higher than that of gluten-free breads developed by Haque, Morris, & Richardson, (1994) and Gujral & Rosell (2004a) with specific volumes of 2.8 $cm^3.g^{-1}$ and 2.5 $cm^3.g^{-1}$ respectively. Therefore, Bread 124 was an improvement.

After determination of specific volume, these four loaves (two from each formulation) were sliced and crust was separated from crumb for the colour determination with a HunterLab colorimeter (Table 2). L*, a*, b* values were recorded in triplicate and the mean calculated (Table 8) for lightness (L*), saturation, and hue angle. Table 8 also shows mean ΔL*, Δ a*, and Δ b* values which show how the crust and crumb colour of gluten-free bread and control wheat bread differ in terms of the primary colour values.

Table 8 Mean crust and crumb colour of gluten-free bread and control wheat bread

	Lightness L*		Saturation ($\sqrt{(a^2 + b^2)}$)		Hue angle (arctan b* /a*)		ΔL*[1]	Δ a*	Δ b*
	Gluten-free	Control	Gluten-free	Control	Gluten-free	Control			
Crust	55.5	47.3	35.6	33.3	1.16	1.09	8.2	-1.41	3.20
Crumb	78.1	74.0	15.7	19.0	1.55	1.54	4.1	-0.31	-3.32

[1] Values are gluten-free bread minus control wheat bread for the three primary colour values

The mean saturation, or chroma values of bread crust from gluten-free and control wheat breads were found to be closely similar (35.6 and 33.3, respectively), as were the hue angles (1.16 and 1.09, respectively). The saturation values of bread crumb of gluten-free bread and the control wheat bread were 15.7 and 19.0 and respectively whereas the value of hue angle was 1.55 and 1.54 respectively. The minor difference in these values suggests that both types of breads were not markedly different for crust and crumb colour appearance.

The mean ΔL* value for crust was 8.2, which shows that the gluten-free bread was slightly paler, but crumb colour was little affected (ΔL* value = 4.1). The mean Δ a* value for crust was -1.41, and that of crumb was -0.31. These values show that gluten-free bread was less red than control wheat bread. The mean Δ b* value for crust was 3.20, which show that the crust of gluten-free bread was slightly yellowier than the control, whereas it was -3.32 for crumb. Therefore, crumb of gluten-free bread was bluer than that of control wheat bread. The pale crust colour of gluten-free bread could result from the high moisture in gluten-free doughs, which would retard browning by diluting the concentrations of the sugar and amino acid reactants, and thus affecting the Maillard reaction.

5.3.2 Evaluations during storage

During storage important factors that can change are hardness of crumb[5], water activity (a_w), and moisture of the crumb and crust. Water is involved in the following changes in the bread system: drying out, and moisture equilibration between crumb and crust.

Drying of bread does not necessarily means it becomes stale, but may accelerate reactions leading to staling. Generally bread staling corresponds to increase in crumb hardness, loss of freshness in terms of flavour, texture, perceived moisture contents. The inverse relationship between moisture contents and staling rate has been confirmed by previous studies (Gray & BeMiller, 2003; He & C, 1990). Therefore, moisture content of the crumb is important considerations when studying bread staling.

Bread staling is responsible for significant financial losses to both consumers and bread producers. Therefore, the evaluations of these attributes were conducted for the determination of bread quality and susceptible changes during storage.

The bread staling (crumb hardness) was determined by the penetrometer and values are reported in Table 9.

[5] Commonly known as staling

Table 9 Bread staling (crumb hardness) by the penetrometer

	Penetration depth (mm)		Hardness index[1]	
Day	Gluten-free bread	Control wheat bread	Gluten-free bread	Control wheat bread
1	17.0	20.0	26.2	22.3
2	15.0	19.5	29.7	22.8
3	11.5	19.0	38.7	23.4
4	10.5	18.0	42.4	24.7
5	9.5	17.5	46.8	25.4

[1]Hardnes index = Weight of probe assembly (445g)/ penetration depth (mm)

From the hardness index values, it is evident that crumb hardness of gluten-free breads was higher than that of the control wheat bread, and it increased sharply during storage. The increase in crumb hardness of gluten-free breads was due to amylose-rich maize starch content that tends to retrograde (stale) faster than the wheat starch present in control wheat bread.

Initial percent moisture of control wheat bread was lower (approximately 59 %) than that of gluten-free bread (approximately 84 %). This was not exact percent initial moisture as compressed bakers' yeast was treated as solids but nonetheless contains 69 % moisture by weight.

Water activity (a_w) values for the bread crumb of both formulations during storage are given in Table 10. Due to moisture loss the water activity of control wheat bread decreased very slightly over five days (0.973 to 0.967), whereas in gluten-free bread it was higher and more stable (0.988 to 0.985), presumably due to moisture retention by added hydrocolloids.

Table 10 Water activity of bread crumbs

Days	Gluten-free bread	Control wheat bread
1	0.988	0.973
2	0.987	0.973
3	0.986	0.972
4	0.986	0.972
5	0.985	0.967

Rosell, Rojas, & Benedito de Barber (2001) similarly reported an increase of water activity as well of moisture retention due to the higher water holding capacity of the hydrocolloids.

Percent moisture of the bread crumb and crust of both formulations was determined by moisture analyser in triplicate over five storage days at 25ºC in closed polythene bags. The mean values are plotted in Figure 21.

During storage, the moisture content in the centre of the loaf decreases, while that in the external region increases. Transfer of moisture from one constituent of the bread crumb to another is generally accepted as a contributing factor in staling, possibly being responsible for the perceived dryness of stale bread. The migration of moisture from crumb to crust causes crust softening in wrapped bread. This changes the dry, crisp, pleasant texture of fresh crust into the soft, leathery, unpleasant texture of stale crust (Gray & BeMiller, 2003).

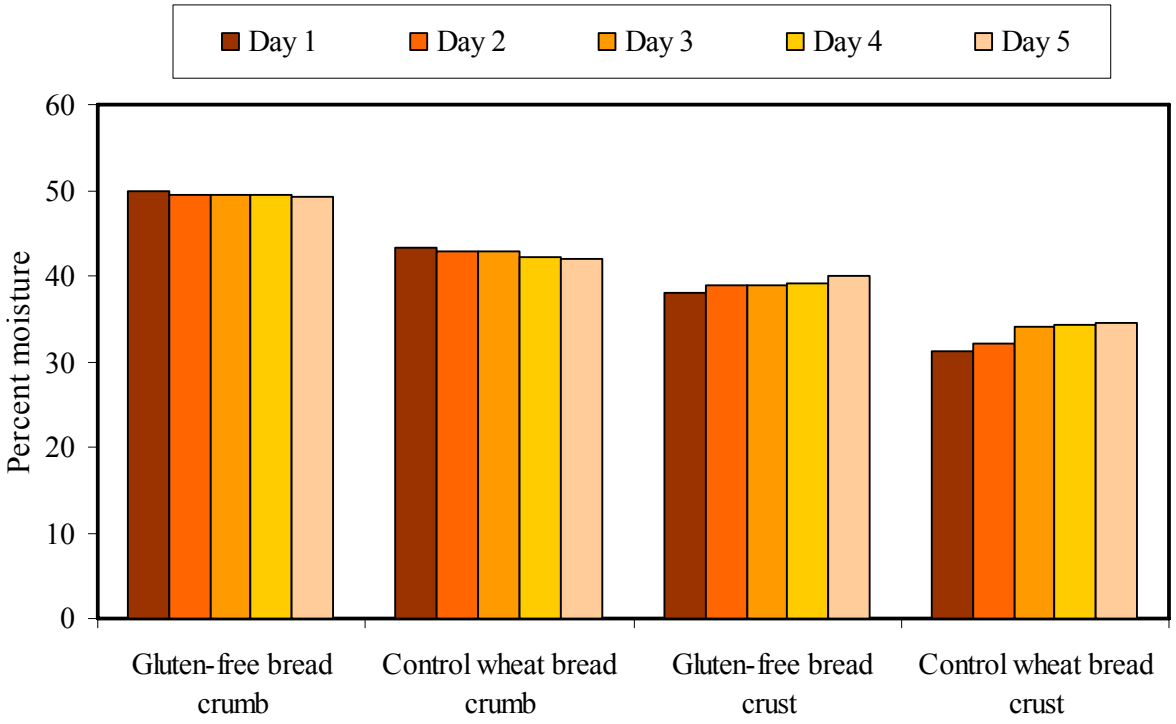


Figure 21 Percent crumb and crust moisture of breads (w/w)

Percent crumb moisture contents of gluten-free breads were clearly higher than those of control wheat bread and were maintained at an almost constant level, whereas in control wheat bread the percent moisture decreased more.

Likewise, percent crust moisture contents of gluten-free breads were significantly higher than those of control wheat bread. Percent crust moisture contents increased with the storage time in both formulations. This increase was more marked in the control wheat crust than gluten-free crust.

The behaviour and amount of decrease in percent crumb moisture and increase in percent crust moisture (moisture migration) is typical for wheat breads but is largely retarded in formulated gluten-free bread apparently due to the added hydrocolloids in the latter formulations, that bound the moisture in crumb and retarded its loss during storage.

These results were similar to those of Rosell, Rojas, & Benedito de Barber (2001) who also reported increased moisture retention by incorporating hydrocolloids. Although it is commonly found that higher moisture reduces the shelf life of bread by increasing the tendency for microbial or mould growth, no such problem was observed in the gluten-free bread. These observations were reviewed by Gray (2003), who reported that the addition of psyllium husk (2, 4 or 8 %) to wheat bread increased softness, decreased staling rate, and maintained moisture content without increasing the tendency for microbial deterioration.

5.4 Sensory evaluation

For this work, two loaves from each formulation and two other different gluten-free breads that are already available in the market were sensorially evaluated by eight experienced panellists on Day 1 (Table 11). The existing gluten-free breads were Dovedale rice bread and Venerdi Nice'n'Light. These retail at \$7.60 kg^{-1} and \$11.60 kg^{-1} respectively. The comparative retail price for Control wheat bread is about \$2.56 kg^{-1}.

Table 11 Mean panel scores and standard deviations for liking of sensory attributes by eight panellists for the formulated gluten-free bread, control wheat bread, and two commercial gluten-free breads

Attributes	Formulated gluten-free bread	Control wheat bread	Dovedale rice bread	Venerdi Nice'n'Light bread	Statistical effect of bread type
Crumb structure	4.75 ± 0.46a[1]	4.63 ± 0.74a	2.63 ± 1.30b	3.88 ± 0.99a	***
Taste	6.13 ± 1.96a	7.88 ± 0.99a	2.63 ± 1.60b	3.88 ± 2.23b	***
Texture	6.25 ± 1.83a	8.13 ± 0.83b	3.13 ± 1.81c	5.25 ± 2.31a	***
Appearance	7.88 ± 0.64a	7.75 ± 1.04a	3.63 ± 1.69b	5.75 ± 2.12c	***
Softness	7.75 ± 0.71a	8.25 ± 0.71a	2.63 ± 1.06b	4.50 ± 2.07c	***
Flavour	6.25 ± 2.05a	7.63 ± 1.30a	2.50 ± 1.60b	3.25 ± 1.75b	***

[1] Within rows means with dissimilar letter differs significantly ($P < 0.05$).
[2] *** = $P < 0.001$.

In the statistical analysis there was no significant panellist effect, so differences between panellists' assessments can be ignored.

There were no significant differences in liking of crumb structure, taste, appearance, softness and flavour between formulated gluten-free bread and the control wheat bread. However, the texture of control wheat bread was liked more (8.13 vs 6.25) (significance letters in row differ). For the appearance attribute, psyllium husk produced a brown speckling on the crust of formulated gluten-free bread which was liked by the panellists and was rated higher than that of the control wheat bread (7.88 vs 7.75). Liking of Dovedale rice bread was significantly lower than liking for formulated gluten-free and control wheat breads across all

attributes. The texture of Venerdi bread was statistically similar to that of formulated gluten-free bread, but the mean for Venerdi bread was numerically lower (5.25 vs 6.25). A similar result was obtained for crumb structure (3.88 vs 4.75).

The liking of crumb structure of formulated gluten-free bread had better score (4.75) than that of the control wheat bread (4.63). This might be due to added HPMC in gluten-free formulations, possibly because HPMC is not an ingredient in the Dovedale and Venerdi breads. The liking of softness of formulated gluten-free bread had significantly higher score (7.75) than that of Venerdi bread (4.50) and Dovedale rice bread (2.63). Similarly, the liking of flavour score was significantly higher for formulated gluten-free bread (6.25) than that of Venerdi bread (3.25) and Dovedale rice bread (2.50).

The improvement effect of HPMC on the sensory quality of bread could be due to its influence on the crumb structure that yields softer crumbs with small holes and thin cell walls. Significantly higher flavour scores of the formulated gluten-free bread compared to the Dovedale and Venerdi breads might be due to the heat stable gelation system of psyllium husk, HPMC and starches created in former and lacking in the latters. The gelation system appeared to be forming a heat stable gel network that entrapped the fermentation gas and flavours of the dough during proofing and in the initial stages of baking. The network largely formed of gel expanded during baking (oven spring) and consequently reduced the loss of gas and flavours and in turn bread specific volume was improved. These results were similar to those of Barcenas & Rosell (2005) who also obtained better sensory scores for all the quality characteristics by incorporating HPMC in wheat bread.

Overall, formulated gluten-free bread is much more like control wheat bread than existing commercial gluten-free breads.

5.5 Costing

For bread and many other foods, customers rely on receiving a product that is uniform in quality attributes and appearance, at a perceived reasonable price. Because the consumer demand for gluten-free products is driven by real and perceived health concerns, so-called natural, organic and health food companies are the leaders in producing gluten-free products for coeliacs and wheat-allergic consumers. They have to create baked foods and snacks that do not compromise the core focus of so-called healthy products. The sensory analysis of two leading gluten-free commercial breads revealed that these are of low standards in terms of many quality attributes, and differ significantly from ordinary wheat bread. It appears that

people who follow gluten-free diets are being 'punished' for their condition by having to consume inferior-tasting, and/or higher-priced baked breads. Nevertheless, the existing commercial gluten-free breads are costly due to the high cost of manual bread production, and expensive gluten-free ingredients. However, with established market dominance the existing suppliers of commercial gluten-free breads can and may well charge what the market will bear.

To enter this market, Quality Bakers might build strong ties with domestic and international coeliac communities through the supply of high quality acceptable gluten-free bread at a better price to fulfil their needs. Existing breads fail on quality and price.

The gluten-free bread formulated in this research can be processed on existing processing lines, is superior to other available gluten-free breads in the market, and is similar to the ordinary wheat bread for most quality attributes. Automated machine production would maintain the low manufacturing cost. A rough estimate of production costs was prepared (Table 12).

Table 12 Production cost of gluten-free and control wheat breads

	$\$.kg^{-1}$	Added amount (g) per loaf		Cost per formulation ($)	
Ingredients		Gluten-free bread	Control wheat bread	Gluten-free bread	Control wheat bread
Wheat flour	.66	0	495	0	0.327
Soy flour (full fat)	1.96	7.35		0.014	0
Potato starch	4.50	50		0.225	0
Maize starch	0.80	288		0.230	0
Yoghurt powder	7.92	17.7		0.140	0
Milk protein	8.59	7		0.060	0
HPMC (K4M)	25.50	9.12		0.233	0
Psyllium husk	7.31	24		0.175	0
Microbial transglutaminase	143	0.5		0.072	0
Alpha amylase	48	0.05	0.05	0.003	0.003
Lipase	270	0.05	0.05	0.014	0.014
Bakers' yeast	1.58	18	13.9	0.028	0.022
Vinegar	1.24	10	4.95	0.012	0.0061
Canola oil	1.79	14	4.95	0.025	0.0089
Salt	.990	9.5	9.89	0.010	0.010
Sugar	.991	15		0.015	0
Water	.0008	355	297	0.0003	0.0002
Labour				0.16	0.16
Packaging and distribution				0.22	0.22
Overhead costs				0.36	0.36
Total		825	825	2.0	1.13

The cost of ingredients in Table 12 to make a 825 g dough were $1.26 and $0.39 for gluten free and control bread, respectively, which represents a 223 % increase. When fixed costs are added, the total production cost is 77 % more expensive. While this still sounds a lot, in absolute terms it is only 87 cents. (Moreover, the cheapest existing gluten-free loaf, Dovedale – for which dough data are not available, was very much more expensive in percentage terms.)

Which ingredients contribute most to cost? In both formulations, alpha amylase, lipase and salt contributed the same cost as these were used at same level. In contrast, vinegar, canola oil and water were used at a higher level in the gluten-free formulation. Additionally, the cost of gluten-free bread production was raised by soy flour (full fat), potato starch, maize starch, yoghurt powder, milk protein, HPMC (K4M), psyllium husk, microbial transglutaminase and sugar as these were incorporated only in the gluten-free formulation.

The ingredients such as HPMC (K4M), maize starch, and potato starch increased the cost of gluten-free bread the most, yoghurt powder, psyllium husk moderately, and microbial transglutaminase, milk protein, canola oil, soy flour, and sugar contributed the least. Although the amount of water used in the gluten-free formulation was significantly higher compared to that in control wheat bread, its contribution towards the production cost of gluten-free bread was not substantial.

It is clear from the estimated cost of production of formulated gluten-free bread that Quality Bakers can sell the bread at lower price than that of existing commercial gluten-free breads.

Chapter 6 Conclusions and recommendations

Due to the increased awareness of the occurrence of coeliac disease and allergy to wheat – real and perceived – the demand for gluten-free carbohydrate product is increasing in the domestic and international food markets. In the context of bread, the gluten component of wheat has a crucial role in making dough, stabilising the gas-cell structure and maintaining the rheological properties of bread. Gluten's absence often results in a liquid batter rather than a pre-baking dough, thus making the mixture unsuitable for production on automatic or semi-automatic processing lines at bakeries. While such liquid batters can be manually processed for the production of gluten-free bread, the resulting textures are crumbly, with poor colour and other post-baking quality defects. Furthermore, manual production increases cost of the product.

Therefore, from the commercial perspective there was a need to develop a gluten-free bread with texture and flavour properties similar to those of conventional wheat flour bread. This research discovered an economic formulation for the production of a dough with suitable handling and processing properties for the production of high quality gluten-free loaf on existing processing lines at Quality Bakers.

In the initial experiments, various gluten-free flours (rice, maize, dahl flour, chickpea), protein concentrates (soy, rice, milk, egg), maize starch, hydrocolloids (carrageenan, guar gum, pectin, xanthan gum, locust bean gum, alginate, hydroxypropylmethylcellulose), hydrophilic psyllium husk, and enzymes microbial transglutaminase, glucose oxidase, alpha amylase were trialled in various concentrations and combinations for the production of gluten-free bread.

The results of these preliminary experiments, revealed four developmental issues that had to be solved for the production of gluten-free commercial bread. The first issue was to make a gluten-free non-sticky viscoelastic dough, the second was to entrap the carbon dioxide gas during proofing to achieve acceptable volume, the third was to avoid the collapse of cell structure by keeping the gas cells intact during baking, and the fourth was to prevent the collapse during cooling of the bread.

Subsequently, a 'shotgun' approach to formulations was adopted that pointed to the usefulness of rice flour, soy flour, maize starch, yoghurt powder, milk and rice proteins, hydrocolloids, microbial transglutaminase, fungal α-amylase, lipase, yeast, and whole eggs

for the production of gluten-free dough and, critically, in retaining the carbon dioxide gas. These ingredients were therefore chosen for more systematic work to develop a commercial gluten-free bread.

The development proceeded in roughly four phases. In Phase 1 a successful machine-processable dough was developed that was capable of holding the gas during proofing, but suffered from minor escape of gas during baking, which led to collapse of the bread to a minor extent. In Phase 2 the problem of baking collapse was solved by using tapioca starch, but the breads then tended to show minor side-and-surface collapse during cooling. This problem was solved in Phase 3.

Phase 3 was a deconstructive phase, in which ingredient with minor or contradictory functions in bread structure development were eliminated. Generally, it was observed that use of protein concentrates at high concentration in the formulation produced strong, cross-linked doughs with the best handling properties, but failed to rise due to increased dough firmness. Rice flour tended to increase dough stickiness and rice protein and egg added as the protein source adversely affected the crumb colour. Moreover, breads prepared using rice protein were too crumbly. Therefore, these ingredients were eliminated from the formulation.

By the end of Phase 3 the formulation was adjusted to create a gelation system of the lower-temperature -stable hydrocolloid psyllium husk, the heat-stable hydrocolloid hydroxypropylmethylcellulose, maize starch, and potato starch. This gelation system was stable at all stages, and thus temperatures, of the normal bread making process. In detail, psyllium has good water-holding and gel-forming capacities at lower temperatures to entrap carbon dioxide during proofing, and produce the required dough structure that can be processed on existing processing lines. The gel strength of hydroxypropylmethylcellulose increased, and starches tended to gelatinise as the temperature increased during baking. This stabilised the gas cells initially formed largely by the psyllium husk gel. Microbial transglutaminase increased the protein cross linking (protein in soy flour and supplemented milk protein) which further enhanced the dough-like structure and its gas entrapping abilities. The bread produced in this phase showed good oven spring, did not collapse while baking or cooling, and achieved a specific volume comparable to control wheat bread but became stale faster.

In Phase 4, the formulation was further refined to increase the moisture retention and to reduce the bread staling rate. Trial 124 (Bread 124) was the final recipe of Phase 4 in the scope of this development, and was objectively and subjectively compared with the control

wheat bread for its quality attributes. The evaluations on the day of baking were oven spring, moisture loss during baking, loaf volume and specific volume, crust and crumb colour. Crumb hardness, crumb water activity, and crumb and crust moisture contents, as well as preliminary sensory evaluation were conducted during storage.

Gluten-free bread had an oven spring comparable to that of the control wheat bread. The moisture loss during baking was less for gluten-free bread than for control wheat bread. The specific volume of the gluten-free bread (3.78 $cm^3.g^{-1}$) was a marked improvement on those of equivalent breads developed by Haque, Morris, & Richardson (1994) and Gujral & Rosell (2004a) (2.8 $cm^3.g^{-1}$ and 2.5 $cm^3.g^{-1}$, respectively), but still less than that of control wheat bread (3.85 $cm^3.g^{-1}$). For colour, the saturation and the hue angles of bread crust and crumb of gluten-free bread and control bread were closely similar. In contrast, the crust lightness of gluten-free was slightly higher than that of the control. In summary, the breads have a similar appearance.

The crumb hardness of gluten-free breads was higher than that of the control wheat bread, and it increased sharply during storage as calculated from the hardness index. Because the gluten-free bread was developed from amylose-rich maize starch, it tended to retrograde (stale) faster than the control wheat bread. The water activity, percent crumb moisture and percent crust moisture contents of gluten-free bread was higher and more stable than the control wheat bread, due to the higher moisture content used in the formulation, and subsequent retention by the hydrocolloids other than starch. Percent crust moisture contents increased with the storage time in both formulations. This increase was less marked in the gluten-free crust, which is an advantage because the crumb remains more moisture and thus may tend to mitigate the perception of staling.

In the sensory evaluations, there were no significant differences found in liking of crumb structure, taste, appearance, softness and flavour between formulated gluten-free bread and the control wheat bread. However, the texture of control wheat bread was liked more. In contrast, the formulated gluten-free bread was rated higher for appearance and crumb structure attribute than that of the control wheat bread. In general, formulated gluten-free bread is much more similar to control wheat bread than existing commercial gluten-free breads.

In conclusion, the formulation listed in Table 4 yielded high quality commercial gluten-free bread (Bread 124), that was 77 % more expensive to produce compared to control wheat bread, but had good crust and crumb characteristics, and high sensory acceptability scores.

The significance of this research is mainly commercial and the insights gained may extend to other bakery items that could be used by coeliacs.

In recommending the formulation for Bread 124 to Quality Bakers, there are several factors the company should be aware of.

Foods can be labelled 'gluten-free' if they contain no more than 20 mg of gluten.kg^{-1} food and are free from detectable gluten, oats and malt (Australia New Zealand Food Authority, 2003). Although coeliac disease and wheat allergy are two distinct phenomena, many people with wheat allergy may elect to eat Bread 124 simply because it is declared wheat-free. However, this will not guarantee safe consumption for wheat sensitive people unless cross-contamination in the bakery is thoroughly eliminated. The situation is not so serious for coeliacs, who require more than trace amount to elicit symptoms. But in general, strict food safety and quality systems should be adhered to prevent contamination. One must be able to track ingredients and show what is in the product, on the line, or in the plant. In the case of conventional bakery, it may still be necessary to have a dedicated plant for gluten-free production, since flour is easily dispersed in an industrial setting.

If and when Bread 124 becomes a commercial reality, the formula should be viewed as a starting point for further development. It is a well-recognised research and development principle that once the primary release of a product occurs, work should immediately start on improvements that will maintain market dominance.

Appendices

Appendix I Trial recipes

Phase 1										
Trial no →	25	26	27	28	29	31	32	33	34	35
Date →	15/2/06	20/2/06	21/2/06	21/2/06	22/2/06	23/02/06	24/02/06	24/02/06	27/02/06	27/02/06
Ingredients(g)↓										
Rice flour	170	170	160	160	160	150	130	130	110	110
Soy flour (full fat)	10	10	10	10	10	10	10	10	10	10
Potato starch										
Tapioca starch										
Maize starch	32	32	32	32	32	32	32	32	32	32
Yoghurt powder	5	5	5	5	5	10	10	10	10	10
Rice protein	10	10	10	20	20	30	40	44	50	50
Milk protein	2	2	3	3	4	5	5	6	6	8
Guar gum	2	2	2	2	2	2	2	2	2	3
Xanthan gum	2.5	2.5	2.5	2.5	2	2	1.5	1.2	1.2	
HPMC (K4M)	10	10	12	12	12	13	13	14	15	15
Psyllium husk	5	10	25	35	35	36	36	36	37	37
Microbial Transglutaminase	1	1	1	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	14.5	14.5	14.5	20	20	20	20	20	20	20
Vinegar	3	3	3	3	3	3	3	3	3	3
Canola oil	2.5	4.5	5	5	5	7	7	7	7	7
Salt	5.69	4.84	4.52	4.82	4.54	5.08	4.86	4.79	4.69	5.06
Sugar	5	7	7	10	7	7	10	10	10	10
Egg (g)	50	50	75	100	100	100	100	100	100	100
Total	330.29	338.44	371.62	425.42	422.64	433.18	425.46	431.09	418.99	421.16
Water	205	205	205	215	215	220	230	250	250	270
Sum	535.29	543.44	576.62	640.42	637.64	653.18	655.46	681.09	668.99	691.16

Observations →	Dough like structure, bread raised up to 50 % of control wheat bread height	Dough like structure, bread raised up to 60 % of control wheat bread height, In next trial, increased HPMC, psyllium husk, egg protein and reduced rice flour	Bread raised up to 80 % of control wheat bread height, increased yeast , egg and psyllium in next exp.	Due to increased yeast and psyllium husk increased loaf volume, showed oven spring , dough was very sticky so reduced xanthan gum in next trial	Dough like structure but bit sticky, bread raised up to 60 % of control wheat bread height, in next trial increased psyllium husk, HPMC, milk and rice protein and reduced rice flour	Sticky dough, raised up to 80 % of control wheat bread height, in next trial reduced rice flour, xanthan gum, and increased rice protein	Sticky dough, reduced xanthan, in next trial, bread collapsed on baking, increased rice and milk protein, HPMC in next trials	Good dough, bread did not collapse, good crumb strength, but had less volume	Good dough but bit sticky ,bread raised equal to 85 % of control wheat bread height but collapsed, deleted xanthan gum and increased guar gum, milk protein and water in next trial	Dough like structure, raised up to 100 % of control wheat bread but collapsed on baking , in next trial increased guar gum and reduced rice flour

Phase 2							
Trial no →	36	37	38	39	40	41	42
Date →	28/02/06	28/02/06	28/02/06	1/3/06	1/3/06	2/3/06	6/3/06
Ingredients(g)↓							
Rice flour	100	100	90	90	90	80	80
Soy flour (full fat)	10	10	15	15	16	16	16
Potato starch							
Tapioca starch		5	5	8	10	12	13
Maize starch	44	45	45	50	50	50	87
Yoghurt powder	10	10	10	10	10	10	10
Rice protein	50	50	50	50	45	45	35
Milk protein	8	10	10	10	10	10	10
Guar gum	4	4	4	4	4	4	4
Xanthan gum							
HPMC (K4M)	7.5	7.5	13	14	16	16	10
Psyllium husk	37	37	40	40	42	42	42
Microbial Transglutaminase	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	20	20	20	20	20	20	20
Vinegar	3	3	3	3	3	3	3
Canola oil	8	8	7	7	7	6	14
Salt	5.29	5.33	5.38	5.19	5.08	5.26	5.89
Sugar	10	10	10	10	10	9	9
Egg (g)	100	100	100	100	100	100	100
Total	417.89	425.93	428.48	437.29	439.18	429.36	459.99
Water	280	280	300	300	300	330	365
Sum	697.89	705.93	728.48	737.29	739.18	759.36	824.99
Observations →	Bread raised up to control wheat bread, collapsed a little, crumb had weak structure, increased maize starch and milk protein in next trial	Dough like structure but did not rise even up to half of the height of control wheat bread, increased water, HPMC, psyllium husk, soy flour and decreased rice flour in next trial	Dough like structure but did not rise even up to half of the height of control wheat bread, increased tapioca starch, maize starch, HPMC in next trial	Dough like structure, bread raised equal to 90 % of control wheat bread height and did not collapse, increased soy flour, psyllium husk and decreased rice protein in next trial	Dough like structure, raised up to 100 % of control wheat bread, collapse on baking, in next trial increased tapioca starch, water decreased sugar and rice flour	Dough like structure, raised equal to the height of control wheat bread, decreased baking collapse	Dough raised equal to control wheat bread, less baking collapse than previous trial.

Phase 2							
Trial no →	43	44	45	46	47	48	49
Date →	13/03/06	14/03/06	22/03/06	22/03/06	28/03/06	28/03/06	29/03/06
Ingredients(g)↓							
Rice flour	80	70	70	70	65	65	65
Soy flour (full fat)	16	16	17	17	17	17	17
Potato starch							
Tapioca starch	13	13	13	13	14	14	14
Maize starch	90	95	105	105	105	110	110
Yoghurt powder	10	10	10	10	10	10	10
Rice protein	35	35	35	30	30	30	25
Milk protein	10	10	5	5	5	5	5
Guar gum	4	4	4	4	4	4	4
Xanthan gum							
HPMC (K4M)	5	10	10	10	7	8	5
Psyllium husk	42	42	44	44	44	46	46
Microbial Transglutaminase	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	20	20	20	20	20	20	15
Vinegar	3	3	3	3	3	3	3
Canola oil	17	14	18	20	16	17	15
Salt	5.67	5.80	5.82	6.06	4.90	5.07	5.07
Sugar	9	9	9	9	9	9	9
Egg (g)	100	100	100	100	100	100	100
Total	460.77	457.90	469.92	467.16	455.00	464.17	449.17
Water	365	370	360	354	320	300	325
Sum	825.77	827.90	829.92	821.16	775.00	764.17	774.17
Observations →	Bread raised equal to the height of control wheat bread, but collapsed on baking to more extent than previous experiment, increased HPMC and maize starch in next trial.	Dough like structure, raised equal to the height of control wheat bread, but collapsed a little on baking, increased psyllium husk and soy flour in next trial.	Dough like structure, raised equal to the height of control wheat bread, but collapsed a little on baking	Collapsed a bit, crumb less elastic and bread did not rise equal to the height of control wheat bread, decreased rice flour and increased tapioca starch in next trial	Bread raised equal to the height of control wheat bread and did not collapse on baking, but collapsed on cooling, decreased water in next trial to avoid cooling collapse	Bread raised up to tin surface, did not show oven spring and baking collapse but collapsed on cooling	Dough like structure, bread raised up to control wheat bread level but collapsed on baking and cooling

Phase 2					
Trial no →	50	51	52	53	54
Date →	29/03/06	5/4/06	6/4/06	6/4/06	7/4/06
Ingredients(g)↓					
Rice flour	65	65	65	65	65
Soy flour (full fat)	17	25	25	25	25
Potato starch					
Tapioca starch	14	15	15	15	15
Maize starch	110	110	110	110	120
Yoghurt powder	10	10	10	10	10
Rice protein	25	20	20	20	20
Milk protein	5	5	5	5	5
Guar gum	4	4	4	4	4
Xanthan gum					
HPMC (K4M)	5	10	5	5	5
Psyllium husk	46	48	48	48	48
Microbial Transglutaminase	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	15	12	12	12	10
Vinegar	3	3	3	3	3
Canola oil	15	15	15	15	15
Salt	5.07	6.09	5.89	5.48	5.67
Sugar	8	8	8	8	8
Egg (g)	100	100	100	100	100
Total	448.17	457.19	451.99	451.58	459.77
Water	330	370	368	340	340
Sum	778.17	827.19	819.99	791.58	799.77
Observations →	Bread raised equal to the height of control wheat bread but collapsed on baking, increased soy flour, psyllium husk, tapioca starch, water and decreased rice protein and bakers' yeast.	Bread raised equal to the height of control wheat bread, collapsed to a minor extent on baking, but collapsed a bit more on cooling.	Had volume equal to control wheat bread, collapsed on baking to minor extent but more on cooling, decreased water in next trial.	Bread raised equal to control wheat bread and did not collapse on baking, but it collapsed from sides a little on cooling, increased maize starch in next trial.	Bread raised equal to control wheat bread and did not collapse on baking but collapsed on cooling

Phase 3

Trial no →	55	56	57	58	59	60	61
Date →	11/4/06	18/4/06	20/4/06	20/4/06	24/4/06	26/4/06	26/4/06
Ingredients(g)↓							
Rice flour	65	60	60	60	60	55	55
Soy flour (full fat)	25	20	20	20	17	17	15
Potato starch							
Tapioca starch	15	10	10	10	10	10	10
Maize starch	120	120	125	125	125	125	130
Yoghurt powder	10	10	10	10	10	10	10
Rice protein	20	20	20	20	20	20	20
Milk protein	5	5	5	5	5	5	5
Guar gum	4	4	4	4	4	4	4
Xanthan gum							
HPMC (K4M)	5	6	6	6	6	6	6
Psyllium husk	46	46	46	46	46	44	44
Microbial Transglutaminase	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	10	11	11	11	11	11	11
Vinegar	3	3	3	3	3	3	3
Canola oil	10	16	11	10	5	14	14
Salt	4.96	5.88	5.88	5.24	6.10	6.08	6.08
Sugar	8	8	8	8	8	8	8
Egg (g)	100	90	90	90	90	90	90
Total	452.06	435.98	435.98	434.34	427.20	429.18	432.18
Water	330	364	370	360	350	365	370
Sum	782.06	799.98	805.98	794.34	777.20	794.18	802.18
Observations →	Dough like structure, bread raised equal to the height of control wheat bread, and did not collapse on baking but collapsed on cooling, decreased rice flour, soy flour, tapioca starch, egg and increased HPMC and water in next trial	Dough like structure, bread raised equal to height of control wheat bread but collapsed on cooling, increased maize starch in next trial.	Bread raised equal to the height of control wheat bread, but collapsed on cooling to a large extent, therefore reduced water in next trial	Dough like structure, bread raised equal to control wheat bread, but collapsed on cooling	Dough like structure, bread raised equal to the height of control wheat bread but collapsed on cooling, decreased rice flour, psyllium husk in next trial.	Dough like structure, bread raised equal to height of control wheat bread but collapsed on cooling to minor extent, decreased soy flour, increased water, maize starch in next trial	Dough like structure, bread raised equal to height of control wheat bread but collapsed on cooling to lesser extent than previous trial

Phase 3							
Trial no →	62	63	64	65	66	67	68
Date →	26/4/06	27/4/06	27/4/06	4/5/06	4/5/06	9/5/06	10/5/06
Ingredients(g)↓							
Rice flour	55	55	55	50	50	50	50
Soy flour (full fat)	15	15	15	15	15	15	15
Potato starch						1	1
Tapioca starch	10	10	10	10	10	10	10
Maize starch	130	130	130	135	135	135	135
Yoghurt powder	10	10	10	10	10	10	10
Rice protein	20	20	20	20	20	20	20
Milk protein	5	5	5	5	5	5	5
Guar gum	4	4	4	4	4	4	4
Xanthan gum							
HPMC (K4M)	6	6	6	6	6	6	6
Psyllium husk	44	44	42	42	42	42	42
Microbial Transglutaminase	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	11	11	11	11	11	10	10
Vinegar	3	3	3	3	3	3	3
Canola oil	14	14	12	0	0	10	10
Salt	6.08	6.01	6.08	6.34	6.34	5.08	5.07
Sugar	8	8	8	8	8	8	8
Egg (g)	80	80	80	70	70	70	60
Total	422.18	422.11	418.18	396.44	396.44	405.18	395.17
Water	358	358	350	360	380	365	365
Sum	780.18	780.11	768.18	756.44	776.44	770.18	760.17
Observations →	Bread raised equal to the height of control wheat bread, but collapsed to very minor extent, developed big holes in crumb	Bread raised up to 90 % of height of control wheat bread and collapsed to a minor extent on cooling, very good crumb structure and strength, decreased psyllium husk and water in next trial	Raised equal to the height of control wheat bread, but collapsed to very minor extent on cooling, decreased rice flour, egg and increased maize starch and water in next trial.	Bread did not rise equal to the height of control wheat bread, did not collapse on baking or cooling, increased water in next trial	Bread raised equal to the height of control wheat bread, added potato starch in next trial	Bread raised equal to the height of control wheat bread, and collapsed a bit on baking.	Dough like structure, bread raised equal to the height of control wheat bread, and collapsed a bit on baking

Phase 3							
Trial no →	69	70	71	72	73	74	75
Date →	18/05/06	23/05/06	30/05/06	1/6/06	1/6/06	6/6/06	6/6/06
Ingredients(g)↓							
Rice flour	50	50	45	45	45	45	40
Soy flour (full fat)	15	15	15	15	15	15	15
Potato starch	1	1	2	2	2	2	5
Tapioca starch	10	8	8	8	8	8	5
Maize starch	140	140	140	150	150	150	160
Yoghurt powder	10	10	10	10	10	10	10
Rice protein	20	20	20	15	15	15	15
Milk protein	5	5	5	5	5	5	5
Guar gum	4	4	4	4	4	4	4
Xanthan gum							
HPMC (K4M)	6	6	6	6	6	6	6
Psyllium husk	42	40	40	40	40	40	40
Microbial Transglutaminase	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	10	10	10	10	12	10	10
Vinegar	3	3	3	3	3	3	3
Canola oil	10	10	12	10	10	10	10
Salt	4.92	5.08	5.09	5.09	5.09	5.08	5.09
Sugar	9	9	9	9	9	9	9
Egg (g)	60	50	50	50	50	40	40
Total	401.02	387.18	385.19	388.19	390.19	378.18	383.19
Water	355	355	345	350	355	355	358
Sum	756.02	742.18	730.19	738.19	745.19	733.18	741.19
Observations →	Dough like structure, bread raised equal to the height of control wheat bread, and collapsed a bit on baking	Bread raised equal to the height of control wheat bread, collapsed a bit and attained final volume up to 90 % of control wheat bread	Raised equal to the height of control wheat bread, collapsed to minor extent but developed holes in the crumb	Non sticky dough, collapsed to very minor extent, crumb was soft and had good strength	Non sticky, dough raised equal to the height of control wheat bread, and collapsed to very minor extent	Dough like structure, bread raised u equal to the height of control wheat bread, and collapsed on baking	Bread did not rise equal to the height of control wheat bread and also did not collapse, increased water and decreased psyllium husk

Phase 3							
Trial no →	76	77	78	79	80	81	82
Date →	13/6/06	15/6/06	16/06/06	16/06/06	16/06/06	19/06/06	19/06/06
Ingredients(g)↓							
Rice flour	40	40	30	30	30	20	20
Soy flour (full fat)	15	15	15	15	15	15	15
Potato starch	5	5	10	10	15	15	20
Tapioca starch	5	5	5	3	2		
Maize starch	160	176	176	185	190	190	200
Yoghurt powder	10	10	10	10	10	10	10
Rice protein	15	15	10	10	10	10	10
Milk protein	5	5	5	5	5	5	5
Guar gum	4	4	4	4	4	4	3
Xanthan gum							
HPMC (K4M)	6	6	6	6	6	6	6
Psyllium husk	38	38	38	38	38	36	36
Microbial Transglutaminase	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	10	10	10	8	8	8	8
Vinegar	3	3	3	6	6	6	6
Canola oil	10	10	10	10	10	10	10
Salt	5.08	5.04	5.06	5.05	5.06	5.05	5.04
Sugar	9	9	9	9	9	9	9
Egg (g)	40	40	40	30	30	30	30
Total	381.18	397.14	387.16	385.15	394.16	380.15	394.14
Water	370	344	345	340	340	330	330
Sum	751.18	741.14	732.16	725.15	734.16	710.15	724.14
Observations →	Bread raised equal to the height of control wheat bread, collapsed a little from sides on cooling , but uniform cell structure and good crumb strength	Bread raised equal to the height of control wheat bread and did not collapse on baking collapsed to very minor extent on cooling	Bread collapsed on cooling may be due to more moisture, in next experimen t reduced water	Dough like structure, bread raised up to control wheat bread level and collapsed a bit from sides and top on cooling	Bread showed oven spring and did not collapse, good volume but collapsed from sides to a bit on cooling and developed holes in crumb.	Nice bread with good texture and volume and showed oven spring, but developed holes in crumb but less than previous trial	Very good bread no collapse on baking but collapse to minor extent on cooling and showed oven spring

Phase 3							
Trial no →	83	84	85	86	87	88	89
Date →	19/06/06	21/06/06	21/06/06	23/06/06	29/06/06	30/06/06	30/06/06
Ingredients(g)↓							
Rice flour	20	10	10				
Soy flour (full fat)	15	15	15	15	15	12	12
Potato starch	20	25	25	25	30	30	30
Tapioca starch							
Maize starch	200	210	220	220	230	240	260
Yoghurt powder	10	10	12	12	12	12	12
Rice protein	5	5	5	5	5	5	
Milk protein	5	5	5	5	5	5	6
Guar gum	3	3	3	3	2	1	
Xanthan gum							
HPMC (K4M)	6	6	6	6	6	6	6
Psyllium husk	36	34	34	34	34	32	32
Microbial Transglutaminase	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	8	8	8	8	8	8	8
Vinegar	6	6	6	6	6	6	6
Canola oil	10	10	10	10	10	10	10
Salt	5.04	5.05	5.05	5.06	5.04	5.02	5.02
Sugar	9	9	9	9	10	10	10
Egg (g)	20	20	20	10	10	10	
Total	379.14	382.15	394.15	374.16	389.14	393.12	398.12
Water	320	310	300	320	320	320	320
Sum	699.14	692.15	694.15	694.16	709.14	713.12	718.12
Observations →	Bread had height equal to control wheat bread and showed oven spring, loaf volume was equal to the previous loaf	Dough achieved good proofing volume and showed oven spring, no collapse on baking but a little collapse on sides during cooling	Bread did not collapse on baking or cooling showed oven spring during baking	Dough like structure, raised up to control wheat bread height and had minor side and surface collapse on cooling	Good bread with a very minor side collapse and open texture	Very good bread without any side or surface collapse, crumb was slightly gummy and of dark yellowish colour, eliminated rice protein, egg and guar gum in next trial	Bread did not rise equal to the height of control wheat bread, increased water in next trials

Phase 3							
Trial no →	90	91	92	93	94	95	96
Date →	3/7/06	3/7/06	4/7/06	5/7/06	7/7/06	11/7/06	13/7/06
Ingredients(g)↓							
Rice flour							
Soy flour (full fat)	12	12	12	10	10	10	10
Potato starch	35	35	35	40	40	40	45
Tapioca starch							
Maize starch	260	265	265	270	270	273	275
Yoghurt powder	12	12	12	12	14	14	14
Rice protein							
Milk protein	6	6	6	6	6	6	6
Guar gum							
Xanthan gum							
HPMC (K4M)	6	6	6	6	6	6	6
Psyllium husk	32	32	25	25	20	20	18
Microbial Transglutaminase	1	1	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	8	8	8	8	8	8	8
Vinegar	6	6	6	6	6	6	6
Canola oil	10	10	10	10	10	10	10
Salt	5.00	5.02	5.12	6.92	7.52	7.66	8.00
Sugar	10	11	11	11	12	12	12
Egg (g)							
Total	403.10	409.12	402.22	412.02	410.62	413.76	419.10
Water	335	335	340	340	320	320	320
Sum	738.10	744.12	742.22	752.02	730.62	733.76	739.10
Observations →	Bread did not collapse on baking or cooling, had yellow crumb	Bread had height equal to the height of control wheat bread and did not collapse on cooling and baking, increased water in next trial and reduced psyllium husk to reduce yellowness of bread	Bread had height equal to the height of control wheat bread and did not collapse on cooling and baking, reduced soy flour in next trial to reduce yellowness of bread	Bread raised up to control wheat bread level, did not collapse on baking and cooling, bread was more yellowish than control wheat bread, so reduced psyllium husk in next trial and adjusted water contents	Bread raised equal to the height of control wheat bread, had less yellowish crumb than previous trial	Good bread with slightly more yellow crumb colour than control wheat bread, so reduced psyllium husk	Good bread raised equal to the height of control wheat bread, no side collapse, nice uniform cell structure but slightly more yellowish than control wheat bread

Phase 3					
Trial no →	97	98	99	100	101
Date →	13/7/06	2/8/06	2/8/06	3/8/06	4/8/06
Ingredients(g)↓					
Rice flour					
Soy flour (full fat)	10	8	8	7.35	7.35
Potato starch	45	45	50	50	50
Tapioca starch					
Maize starch	277	279.8	279.8	288	288
Yoghurt powder	14	17.17	17.17	17.67	17.67
Rice protein					
Milk protein	6	7	7	7	7
Guar gum					
Xanthan gum					
HPMC (K4M)	6	6.5	6.5	6.5	7
Psyllium husk	18	15	15	14	14
Microbial Transglutaminase	1	1	1	1	1
Alpha amylase	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	8	8	8	8	8
Vinegar	6	6	6	6	6
Canola oil	10	12	12	12	12
Salt	8.74	9.00	9.00	9.50	9.50
Sugar	12	12	12	12	12
Egg (g)					
Total	421.84	426.57	431.57	439.12	439.62
Water	320	300	300	300	300
Sum	741.84	726.57	731.57	739.12	739.62
Observations →	Good bread raised up to control wheat bread height, but slightly more yellowish crumb than control wheat bread, so increased HPMC and decreased psyllium husk and soy flour and adjusted water addition in next trial	Good dough and bread, no baking or cooling collapse	Good bread raised up to control wheat bread level, no side collapse, nice uniform cell structure, slightly more yellowish crumb than control wheat bread, so reduced soy flour and psyllium husk in next trial	Very good dough and very good bread, but stalled quickly, so increased HPMC in next trial	very good dough and bread, good crumb strength, crumb and crust colour comparable to control wheat bread , but bread stalled on fifth day of storage, so increased psyllium husk, sugar and water in next trial

Phase 4						
Trial no →	102	103	104	105	106	107
Date →	9/8/2006	14/8/2006	14/8/2006	14/8/2006	21/8/2006	25/8/2006
Ingredients(g)↓						
Rice flour						
Soy flour (full fat)	7.35	7.35	7.35	7.35	7.35	7.35
Potato starch	50	50	50	50	50	50
Tapioca starch						
Maize starch	288	288	288	288	288	288
Yoghurt powder	17.67	17.67	17.67	17.67	17.67	17.67
Rice protein						
Milk protein	7	7	7	7	7	7
Guar gum						
Xanthan gum						
HPMC (K4M)	7	7	7	7	7	7.25
Psyllium husk	14.5	14.5	14.5	14.5	15	15
Microbial Transglutaminase	1	1	1	1	0.5	0.5
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	8	8	8	8	8	8
Vinegar	6	6	6	6	6	6
Canola oil	12	12	12	12	12.5	12.5
Salt	9.50	9.50	9.50	9.50	9.50	9.50
Sugar	15	15	15	15	15	15
Egg (g)						
Total	443.12	443.12	443.12	443.12	443.62	443.87
Water	305	305	308	308	310	310
Sum	748.12	748.12	751.12	751.12	753.62	753.87
Observations →	Good dough and volume, staled in similar way as in previous trial	Good dough and volume, staled in similar way as in previous trial	Increased water, good dough and volume, staled in similar way as in previous trial	Good dough and volume, staled in similar way as in previous trial, increased water, canola oil and psyllium husk in next trial	Good dough and bread , stalling rate decreased, increased HPMC in next trial to further decrease the stalling rate, but by increasing psyllium husk, crumb yellowness increased	Good dough and bread , stalling rate slightly decreased by HPMC addition, but volume slightly decreased, hence increased yeast in next trial. Increasing HPMC reduced the crumb yellowness

Phase 4						
Trial no →	108	109	110	111	112	113
Date →	30/8/2006	30/8/2006	4/9/2006	4/9/2006	8/9/2006	8/9/2006
Ingredients(g)↓						
Rice flour						
Soy flour (full fat)	7.35	7.35	7.35	7.35	7.35	7.35
Potato starch	50	50	50	50	50	50
Tapioca starch						
Maize starch	288	288	288	288	288	288
Yoghurt powder	17.67	17.67	17.67	17.67	17.67	17.67
Rice protein						
Milk protein	7	7	7	7	7	7
Guar gum						
Xanthan gum						
HPMC (K4M)	7.25	7.25	7.5	7.5	7.5	7.5
Psyllium husk	15	15	15.5	15.5	15.5	16
Microbial Transglutaminase	0.5	0.5	0.5	0.5	0.5	0.5
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	9	9	9	10	10	11
Vinegar	6	6	6	6	6	6
Canola oil	12.5	12.5	12.5	12.5	12.5	12.5
Salt	9.50	9.50	9.50	9.50	9.50	9.50
Sugar	15	15	15	15	15	15
Egg (g)						
Total	444.87	444.87	445.62	446.62	446.62	448.12
Water	310	310	315	315	315	315
Sum	754.87	754.87	760.62	761.62	761.62	763.12
Observations →	Same results as in previous trial	Same results as in previous trial, so increased HPMC, psyllium husk and water in next trial	Nice non sticky dough and bread stalling rate decreased and was less than previous trials, but volume slightly decreased, so increased yeast in next trial	Same results as of previous trial	Same results as of previous trial, increased psyllium husk in next trail to further reduce stalling rate, increased yeast to maintain volume as increased hydrocolloids increased dough viscosity and reduced volume	Good non sticky dough, nice bread with good oven spring, bread was firm on fifth day, so increased canola oil in next trial

Phase 4						
Trial no →	114	115	116	117	118	119
Date →	13/9/2006	18/9/2006	25/9/2006	2/10/2006	9/10/2006	16/10/2006
Ingredients(g)↓						
Rice flour						
Soy flour (full fat)	7.35	7.35	7.35	7.35	7.35	7.35
Potato starch	50	50	50	50	50	50
Tapioca starch						
Maize starch	288	288	288	288	288	288
Yoghurt powder	17.67	17.67	17.67	17.67	17.67	17.67
Rice protein						
Milk protein	7	7	7	7	7	7
Guar gum						
Xanthan gum						
HPMC (K4M)	7.5	7.5	7.5	8	8	8
Psyllium husk	16	17	17	18	18	22
Microbial Transglutaminase	0.5	0.5	0.5	0.5	0.5	0.5
Alpha amylase	0.05	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	11	12	12	13	13	14
Vinegar	6	7	7	7	8	8
Canola oil	13	13	13	13	13	14
Salt	9.50	9.50	9.50	9.50	9.50	9.50
Sugar	15	15	15	15	15	15
Egg (g)						
Total	448.62	451.62	451.62	454.12	455.12	461.12
Water	315	315	320	320	320	325
Sum	763.62	766.62	771.62	774.12	775.12	786.12
Observations →	Nice dough and bread, firm crumb, increased, psyllium husk, vinegar and yeast in next trial	Bread had firm crumb on fifth day, increased water in next trial	Good dough, good oven spring, less firm crumb on fifth day than previous trial, increased psyllium husk and HPMC in next trial	Good dough, good oven spring, less firm crumb on fifth day, increased vinegar in next trail to inhibit any microbial growth during storage	Very nice dough and bread, softer crumb than previous trials on fifth day, increased bakers yeast, water canola oil , and psyllium husk in next trial	Nice dough and bread, softer crumb than previous trials on fifth day

Phase 4					
Trial no →	120	121	122	123	124
Date →	23/10/2006	1/11/2006	6/11/2006	13/11/2006	20/11/2006
Ingredients(g)↓					
Rice flour					
Soy flour (full fat)	7.35	7.35	7.35	7.35	7.35
Potato starch	50	50	50	50	50
Tapioca starch					
Maize starch	288	288	288	288	288
Yoghurt powder	17.67	17.67	17.67	17.67	17.67
Rice protein					
Milk protein	7	7	7	7	7
Guar gum					
Xanthan gum					
HPMC (K4M)	8	8.5	8.5	9.12	9.12
Psyllium husk	22	22	24	24	24
Microbial Transglutaminase	0.5	0.5	0.5	0.5	0.5
Alpha amylase	0.05	0.05	0.05	0.05	0.05
Lipase	0.05	0.05	0.05	0.05	0.05
Bakers' yeast	14	16	16	18	18
Vinegar	8	10	10	10	10
Canola oil	14	14	14	14	14
Salt	9.50	9.50	9.50	9.50	9.50
Sugar	15	15	15	15	15
Egg (g)					
Total	461.12	465.62	467.62	470.24	470.24
Water	335	348	348	355	355
Sum	796.12	813.62	815.62	825.24	825.24
Observations →	Good dough and bread, good oven spring, softer and moist crumb, increased HPMC, yeast, water and vinegar in next trial	Very nice dough and bread remained soft on storage due to increase in water and hydrocolloids, further increased psyllium husk	Very nice dough and bread remained soft on storage, increased HPMC, yeast and water in next trial	Nice dough and bread, bread had good volume, showed oven spring, relatively softer crumb than previous trials	Similar results to that of trial 123. Produced nice dough, good volume and showed oven spring. Bread had softer crumb on storage compared to the trials prior to 123. This was the final recipe in the scope of this development (final recipe)

Appendix II Forms used for sensory analysis of breads

Gender: M ☐ F ☐

Age range: 18- 30 ☐ 31- 45 ☐ 46 and older ☐

Sample Code ☐

How much do you like this bread for following attributes?

For each attribute tick the box that best describes your liking/disliking

	Taste	Texture	Appearance	Softness	Flavour
 Like extremely	☐	☐	☐	☐	☐
Like a lot	☐	☐	☐	☐	☐
Like moderately	☐	☐	☐	☐	☐
Like slightly	☐	☐	☐	☐	☐
 Neither like nor dislike	☐	☐	☐	☐	☐
Dislike slightly	☐	☐	☐	☐	☐
Dislike moderately	☐	☐	☐	☐	☐
Dislike a lot	☐	☐	☐	☐	☐
 Dislike extremely	☐	☐	☐	☐	☐

Any comments?

Gender: M ☐ F ☐

Age range: 18- 30 ☐ 31- 45 ☐ 46 and older ☐

Key : Satisfactory = crumb with small holes and thin cell walls
Unsatisfactory = crumb with large holes and thick cell

What do you think about the crumb structure of this bread?

Observe visually from left to right
For each type of bread tick the box that best describes your liking/disliking

	Wheat Bread Control	Formulated gluten-free	Gluten-free A (from market)	Gluten-free B (from market)
 **Satisfactory (S)**	☐	☐	☐	☐
Questionable to satisfactory (QS)	☐	☐	☐	☐
 **Questionable (Q)**	☐	☐	☐	☐
Questionable to unsatisfactory (Q-U)	☐	☐	☐	☐
 **Unsatisfactory (U)**	☐	☐	☐	☐

Any comments?

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