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The development of hotcake products with reduced staling and reduction of microbiological growth

A thesis presented in partial fulfilment of the requirements for the degree of

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In

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At

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Abstract

Staling and microbiological spoilage are major issues in the market development of hotcake products. This project is aiming at reduce the staling rate of hotcake product during storage and review the methods that could be effective in reducing microbiological spoilage of hotcakes at ambient temperature.

The staling rate was reduced by incorporation of anti-staling ingredients into the formulation. A combination of anti-staling ingredients including Dimodan PH 320/B-M, a distilled monoglyceride; DATEM Palsgaard 3502, a Diacetyl Tartaric Acid Ester of Mono- and Diglycerides; and also Novamyl 10000 BG, a bake stable alpha amylase was effective to reduce the staling rate of hotcake when incorporate them into the hotcake formulation. The staling rate of hotcake products was reduced from 0.14N/day to 0.085N/day in commercial trial. In addition, the sensory results indicated the customers can not perceive a stale hotcake for the new formulation developed in this research and they also can not perceive the changes between original formulation and the new formulation.

Two applicable antimicrobial spoilage approaches were used; these were to increase the level of calcium propionate preservative and to reduce the oxygen content level to below 1% using O_2 absorber or 100% CO_2 in the packaging. The commercial trial showed decreasing the oxygen content level to less than 1% in the packaging and increasing the level of preservatives increased the shelf life by 1 or 2 days under the ambient storage condition used.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

AF: Amylofresh

BG: Novamyl 10000BG

BHA: Butylated hydroxyl anisole

BHT: Buytlated hydroxyl toluene

BP: Barrier pouch packaging

CA: Controlled atmosphere

Cont: Control

CFU: Colony forming unit

DATEM (DT): Diacetyl tartaric acid esters of mono and diglycerides

Dim: Dimodan PH 320/B-M, distilled monoglyceride

DP: Degree of polymerization

FB: Flour base

LDPE: Low density polyethylene

LSD: Least significant difference

MAP: Modified atmosphere packaging

NA: No Available data

Pro: Novamyl Pro BG

SAS: Sodium aluminium sulphate

SALP: Sodium aluminium phosphate

SSL: Grindsted ® SSL P 86 K, Sodium stearoyl lactylate

Std: Standard

TPA: Texture profile anaylsis

TPC: Total plate count

VDFF: Van Dyck Fine Foods Ltd.

Chapter 1 General introduction

Hotcakes are flat cakes prepared from a low viscosity batter and cooked for a short duration first one side and then on the other using a hot griddle. Most hotcakes are classified as quick breads; some use a yeast-raised or fermented batter others are chemically leavened. Hotcakes are widely consumed all over the world, depending on the region; they may be served at any time of the day and with a variety of toppings or fillings including jam, fruit, syrup or meat.

Due to their increasing popularity along with new automated processing technology, they are no longer only a homemade food, but also part of a steadily growing industry. The market for hotcakes has more than doubled over the last ten years. According to the '2006-2011 World Market Outlook' prepared by the ICON Group International, Inc. the product was worth $3183.95 Ml in 2001 and would reach approximately $8323.63 Ml by 2011(Parker, 2005).

Van Dyck Fine Foods Ltd is a company employing 14 staff based in New Plymouth which makes a range of chemically leavened, batter based foods that are produced using automated hot-plate cooking. These foods include hotcakes (pikelets), crepes and a range of corn and other vegetable fritters. Currently, their clients include a range of large fast food stores including Burger King and coffee shops along with several major airlines.

Market development is currently restricted by the approximate 10 days shelf life under refrigeration temperatures (4°C) of the products that is defined by staling to textures and flavours that are unacceptable to their customers; to avoid the staling to occur and store the product at ambient temperature (20°C), the products can only achieve a three days' shelf life due to the microorganisms becoming obvious as discoloured patches or the presence of mycelium or sporulation fungal bodies on the surface of the hotcakes. If the staling of the hotcake can be reduced and the shelf life of the product can be extended under the ambient storage condition, significant cost saving on product transportation and storage can occur. In addition, an expansion of current markets will become possible and product losses will be reduced.

Aim and Objectives

The aim of this research was to address these issues by develop the methods of retarding the staling and extending the shelf life of hotcakes.

The objectives were:

- Determine the effective method of retarding the staling process of the hotcake products
- Determine the best combination of anti-staling ingredients
- Review the methods that could be effective in reducing microbiological spoilage of hotcakes stored at ambient temperature

Project Constraints and mitigation of potential problems

Time constraints:

The project was to be completed within the time limit of one year Master of Food Technology course.

Ingredients and technical constraints:

Some of the potentially effective ingredients and technology are not available in New Zealand, or were not approved for use in New Zealand during the research period.

Product development constraints:

Customer's preference in the current packaging technique which is a loose packaging, therefore, the control atmosphere cannot apply into the real market situation.

The production procedure during this project had to carry out with the existing equipment in the lab or manufacture.

Experimental constraints:

Use lab scale equipment to mimic machinery production line in the manufacture.

Chapter 2 Literature review

2.1 Ingredients in bakery products

Bakery products which are predominantly based on wheat flour, have very diverse formulations they are usually cooked using dry heat applied directly by radiation or conduction from the walls and/or top and bottom of an oven, hotplate or other heating appliance. Leavening agents; water and oil are the basic ingredients mixed together with flour to form a batter or dough that is transformed by the cooking process into the final product. Other ingredients such as sugar, milk, eggs, shortening, flavours, spices, and functional ingredients such as emulsifiers, enzymes and stabilizers are optional. Bakery products such as breads, cakes, muffins and pies are all high moisture products with a water activity of around 0.95. The shelf lives of those products are three to five days at ambient storage condition due to the microbiological spoilage.

2.1.1 Flour

Wheat flour is commonly used in bakery goods and depending on the source, contains approximately 70% carbohydrates, 96% of which are starches; 14% moisture, 9 – 13% protein, small amounts of pentosans, lipids, fibre, minerals and vitamins (Pyler, 1988a).

When mixed with water, flour forms a viscous and elastic mixture because of the starches and proteins which develop as the dough is mixed to form an elastic continuum which binds the starch granules together (Pyler, 1988a). Two types of protein, gliadin and glutenin form gluten which is the component providing the viscosity and elasticity of the mixture (Edwards, 2007). The former is responsible for the extensibility and the latter is responsible for the elasticity (Conforti, 2006).

Starch is a combination of two types of polymers, typically 25% (wt/wt) of wheat starch is amylose and 75% (wt/wt) is amylopectin, both of polymers are formed from glucose. Amylose is a linear macromolecule consisting of α-D-glucopyranose residues linked together by (1, 4) bonds. Amylopectin is a highly branched polysaccharide which consists of α-(1,4)-linked glucopyranose residues (as in amylose) and a greater proportion of non-random α-(1,6)-linkages, which gives a highly branched structure and result in its huge molecular size

(Liu, 2005). Starch exists in a granule form in flour particles. The granule has an immense and highly organised semi-crystalline structure. It consists of the amorphous regions (the branching points of amylopectin) and crystalline regions (the clustered short branches of amylopectin molecules). Amylose and amylopectin molecules are associated by intermolecular hydrogen bonds and they are distributed throughout the granule (Liu, 2005).

Starch is insoluble in cold water, 10 - 20% of starch will swell at 20°C due to diffusion and absorption of water into the amorphous regions (Biliaderis, 1991) and this swelling is reversible upon drying; however, when a starch dispersion is heated to around 55 ~ 65°C in excess water, the starch granule swells dramatically and is accompanied with the increase in viscosity of the starch dispersion; the granules absorb water more than 20 times the weight of themselves; this process is termed gelatinization (Edwards, 2007). The swelling of starch granule upon gelatinization is irreversible and causes the ordered granule structure to become amorphous. Upon cooking, gluten protein undergoes heat coagulation (denaturation) and starch undergoes gelatinisation. In situations where the water availability is limited such as in many bakery goods, starches may absorb water from the gluten for its gelatinisation (Pyler, 1988a). This strengthens the baked item from a pourable batter to a solid and produces the typical textures of the final product (Conforti, 2006). The physical changes of starch and gluten during cooking and the moisture and fat content of the product are responsible for the softness, crumb structure and moisture retention.

2.1.2 Leavening agent

Leavening is the process by which gases are incorporated into the bakery product (Lai & Lin, 2006) to produce the typically light foamed structure of cakes, scones and breads. The gases retained in the baked item form the foam like structure that together with the texture of the bubble walls provides the softness of the product. Leavening gases are typically air, steam and carbon dioxide (CO_2) (Conforti, 2006). For CO_2 leavening there are two types of agents, biological, characterised by yeasts; and chemical, characterised by baking soda in combination with acidulants.

Yeast (*Saccharomyces cerevusae*) is a single cell fungus which is sensitive to temperatures with an optimal of 35°C (Lai & Lin, 2006). By fermentation, yeast acts on sugars and changes

them into CO_2 and alcohol (Lai & Lin, 2006). The released CO_2 gas produces leavening action and the alcohol evaporates during baking. Yeasts are not usually used in hotcakes and were not used during this project.

Chemical leavening agents including baking soda (sodium bicarbonate, $NaHCO_3$) and baking powder which contains around 1/3 of baking soda; one or more types of baking acids such as cream of tartar (potassium bitartrate), sodium aluminium sulphate (SAS), sodium aluminium phosphate (SALP) powder; and inert fillers which absorbs any excess moisture in the air, thus avoid caking and reducing its potency, usually starch (Conforti, 2006). When they are in contact with liquid and heat, a chemical reaction occurs which produces CO_2 and the product raises. Baking soda is used when there is an acid in the recipe such as sour milk, yogurt, or fruit juice. Sodium bicarbonate ($NaHCO_3$) reacts with the acid in the presence of water to yield CO_2 gas (equation 1). When there is no acid, $NaHCO_3$ react with itself upon heating and produce CO_2 gas and Na_2CO_3 which has slightly soapy flavour and a yellow colour (in equation 2).

1) $NaHCO_3 + HX \rightarrow H_2O + NaX + CO_2\uparrow$
2) $2NaHCO_3 \ \Delta \rightarrow Na_2CO_3 + H_2O + CO_2\uparrow$

There are two types of baking powder: fast (single acting powder made with baking soda and acidulants) and slow (double-acting baking powder made with baking soda and acidulants). A flour mixture made with fast acting baking powder should be handled quickly and placed in the oven as soon as possible, because it starts to produce CO_2 as soon as water is added (same as equation 1). Any delay can cause a decrease in its performance (Conforti, 2006).

Commercial bakers normally choose double acting baking powder that contains the acidulants such as potassium bitartrate, SALP or SAS; baking powder decomposes to form acids during the baking process: in the first stage, the reaction happens in the mixing bowl when potassium bitartirate, sodium aluminium sulphate or phosphate is solubilised in water at room temperature and produces tartaric, sulphuric or phosphoric acid. In the second stage, the reaction occurs when heat is applied, the acid reacts with the baking soda to release CO_2, normally the CO_2 start to release around 40°C and complete around 60°C (Conforti, 2006; Lai & Lin, 2006).

In the hotcake manufacturing industry, the company chose baking soda together with SALP. Firstly, it is easier to store a pure substance than a mixture of an alkali and an acid. Secondly, the leavening process needs to occur at cooking time in the hotcake manufacturing process. SALP is a temperature triggered acidulant that operates at 40 ~ 43°C (Lai & Lin, 2006).

The two steps of interactions of SALP ($NaAlPO_4$) with $NaHCO_3$ in the presence of water and heat are as follows:

1) $3NaAlPO_4 + 6H_2O \rightarrow 2H_3PO_4 + 2Na_3PO_4 + 3Al(OH)_2$
2) $3NaHCO_3 + H_3PO_4 \Delta \rightarrow Na_3PO_4 + 3H_2O + 3CO_2\uparrow$

2.1.3 Emulsifier

Emulsifiers function to disperse oil and water mixtures to form emulsions, typically they are amphiphlic materials with both lipophilic and hydrophilic properties (Stampfli & Nersten, 1995) that serve to improve the distribution of fats in baked goods. They also promote the formation and stabilization of emulsions (Hasenhuettl, 2008b). In bakery products they also stabilize bubbles in cake or bread structure by accumulating at the bubble and crumb interface.

Baking emulsifiers are normally divided into dough strengtheners and crumb softeners (Stampfli & Nersten, 1995). Dough strengtheners such as diacetyl tartaric acid esters of mono and diglycerides (DATEM) mostly act on proteins; while crumb softeners such as the monoglycerides or mixtures of mono and diglycerides mostly act on starches to form complex with starch helix (Orthoefer, 2008).

In the bakery system, emulsifiers also improve the texture and symmetry; improve product volume; strengthen the dough and by reducing the rate of staling rate and thereby increase the shelf life. Additional benefits are reducing mixing time and reducing egg and shortening usage (Orthoefer, 2008). Since commercial bakery goods require consistent quality and maximum shelf life, these properties of emulsifiers are often more important than its emulsification properties.

2.1.4 Other common ingredients

The functions of other common ingredients were summarized in Table 1:

Table 1 Optional ingredients used in baking products

Ingredients	Type	Function
Salt	-	Flavour Regulates leavening action Strengthens gluten May reduce bacterial growth Counteracts water softness
Sugar	Sucrose	Flavour Colour Regulates leavening action Influences the volume, moistness, tenderness Maillard reaction
Oil/fat	Liquid Solid	Imparting shortening, richness and tenderness to improve flavour and eating characteristics Increases softness Enhancing aeration for leavening action Providing lubrication to prevent the wheat gluten particles from adhering together to retard staling Increases moisture retention for shelf life improvement
Milk	Liquid Powder	Flavour Nutrients (complete protein, Vitamins and calcium) Help produce a velvety texture, a creamy crumb and a browner crust due to the role of lactose in Millard browning.
Egg	Egg white Egg yolk Whole egg	Moistening Aerating, due to the ability to form a foam when whisked, entangles large quantities of air Enriching, contains fat and protein Emulsifying, due to the presence of lecithin in the yolk Structural, due to the presence of the proteins in both the yolk and the white that coagulate upon heating

Summarized from (Assouad, 1996; Conforti, 2006; Lai & Lin, 2006)

2.2 Spoilage problems

Three types of spoilage are subject to bakery goods: microbial spoilage, chemical spoilage, and physical spoilage (staling).

2.2.1 Microbiological spoilage

The most common form of microbiological spoilage in bakery products is mould. Even through the baking process is generally sufficient to destroy microorganisms by thermal inactivation, recontamination by post-baking processes, such as adding toppings, the cooling process, human handling and the packaging processes are unavoidable (Magan & Aldred, 2006). This is because the products are exposed to the air in which are dispersed many mould spores which then contaminate the hotcakes prior to packaging. Thereafter, the conditions of high humidity, warm temperatures and the nutrients from the food product and during storage allow the spores to germinate and grow (Jay, Loessner, & Golden, 2005). The predominate fungal contaminants that have been isolated from intermediate and high moisture bakery products are of the *Penicillium*, *Aspergillus* and *Eurotium* fungal families (Suhr & Nielsen, 2004).

Techniques to control microbiological spoilage include the prevention of post baking contamination by manufacturing practices that either prevents contamination, kill existing contaminants or prevents points of infection from growing such as hurdle technology: a combination of barrier techniques to stop or slow down the growth of microorganisms (Gudsell, 2003).

2.2.2 Chemical spoilage

Rancidity is a type of chemical spoilage associated with bakery goods. It is caused by lipid degradation during storage which includes oxidative rancidity and lipolytic rancidity (Edwards, 2007). During this process, a large number of decomposition products, including short chain fatty acids, aldehydes and ketones are produced by an autolytic free radical mechanism. These free radicals have been shown to bleach pigments, breakdown proteins, destroy fat-soluble vitamins, cause darkening of fat, have an unpleasant taste and smell and are toxic in large amounts (Smith, Daifa, El-Khoury, Koukoutsis, & El-Khoury, 2004).

The prevention of chemical spoilage is usually by the addition of antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), by adding ascorbic acid or by replacing the atmosphere in the packaging by 100% N_2 or CO_2 or locate oxygen absorber sachet into the packaging which is also effective in managing microbiological spoilage (Smith, et al., 2004).

2.2.3 Physical spoilage

Physical spoilage is caused by moisture loss or gain or chemical changes that change the texture. Staling results in changes the texture of the food and includes increasing firmness (bakery goods), loss of crispiness (dry snacks) and softness (biscuits).

A loss of moisture in high moisture bakery products, such as breads and cakes, results in an increase in firmness. A gain of moisture in low moisture product turns the crispy product, such as biscuit and crackers, into a soggy texture. A packaging material with a very low moisture transfer rate for example, low density polyethylene (LDPE) can effectively eliminate moisture loss or gain between the surrounding air and the product (Assouad, 1996).

Staling, caused by chemical and physical changes (Seyhun, Sumnu, & Sahin, 2003), is another type of deterioration of bakery goods. The softening of the crust; hardening of the crumb and the disappearance of the fresh aroma that is lost during staling makes the product less attractive to consumer and reduces the shelf life of the product (Arnaut, Verte, & Vekemans, 2005). In contrast to microbiological spoilage or the simple loss or gain of moisture, staling can also result from moisture migration among the components of the product during storage (Smith, et al., 2004). Several causes have been reported in the literature. These include moisture relocation and chemical rearrangement such as water redistribution, structural changes in protein and starch: starch retrogradation, cross-linking between partially solubilised starch and gluten and glassy-rubbery transition (Baik & Chinachoti, 2000). The details are reported in the staling mechanisms 2.3.

2.3 Staling mechanisms of moist baked goods

Crust staling and crumb staling are two well described staling processes among moist baked goods. The fresh crust is relatively dry and crisp in the freshly baked product but becomes soft

and leathery upon staling. The principal process is the transfer of moisture from either the interior of the product or the surrounding atmosphere to the crust. The gelatinised starch partially retrogrades and syneresis may occur which leads to increases in the water activity of the product (Pyler, 1988b).

Crumb staling is more important in hotcake products as there is no clearly defined crust in this type of product. Crumb staling is the reverse of crust staling and the end effect is a firming of the hotcake, the taste may become less sweet.

It was formerly believed that staling of bread crumb occurred mainly as a result of loss of moisture, however, for many products the original freshness can be restored by reheating. It also has been found that the amount of water-soluble starch that can be extracted from a stale crumb is less than that obtained from fresh crumb; it also observed that fresh crumb swells more with water than the staled one (Bechtel, Meisner, & Bradley, 1953). Therefore, in the later stage of staling research, in addition to the loss of moisture, scientists focused on other components in bakery products such as starch, protein and other minor ingredients such as pentosans and lipids (Kim & Appolonia, 1977; Kulp, Ponte-Jr, & Appolonia, 1981; Martin, Zeleznak, & Hoseney, 1991; Schoch & French, 1947). Staling is a phenomenon that describes the deterioration of product quality during storage; each of the components of staling and how it affects the properties of the crumb will be addressed specifically in the following session.

2.3.1 Moisture redistribution

Moisture redistribution among the bread constituents is one of the factors that contribute to the staling of baked goods because water is a plasticizer and its existence in either starch or the protein components has a very large influence on the mechanical properties of the crumb (Gray & BeMiller, 2003).

When hydrated, the tightly coiled wheat flour proteins swell, unravel and establish a complex mixture of chemical bonds, mostly disulfide (-S-S-) bonds (Edwards, 2007), eventually, forming gluten (a hydrated protein based elastic material in dough and batter); The gluten forms the elastic continuous phase of the dough and is a major contributor to the viscometric properties of the batter or dough (Patient & Ainsworth, 1994). In its simplest form of hotcake batter the hydrated network of gluten that comprises about 4% of the total batter weight. The

gluten binds the still uncooked and ungelatinised starch granules that comprise about 22% of the batter of which the remaining proportion is mostly water (50%). About 30% of the water is used to hydrate the starch granules and the remaining 70% is associated with the hydrated protein. In short, the 4% of protein holds 35% of the water present in the batter.

During cooking, gluten releases water as the starch granules swell and gelatinize. Together with the released gas from leavening agents, a soft, spongy alveolar crumb is created. In the crumb, gluten and starch formed interpenetrated gels which hold the aqueous interphases as reservoirs for water (Schiraldi & Fessas, 2001). Water has the tendency to move from the wet crumb to the dry crust and the walls of the crumb alveoli become more rigid (Gray & BeMiller, 2003).

During cooling and aging, gluten releases water as it undergoes a protein 1^{st}-order transformation (Kay & Willhoft, 1972). The water released from the gluten moves to the starch component of the crumb and thereafter to the crust although details of the mechanism are as yet uncertain. Some authors reported that starch takes up water from the gluten as the crumb ages (Gray & BeMiller, 2003). Other authors believed starch releases water during syneresis, driving force of which is starch retrogradation (Biliaderis, 2008). The released water moves to the surface causing crust staling and an increase in water activity (Schiraldi & Fessas, 2001). The more ordered structure of starch granule results in the increase in crumb firmness which is the crumb staling (Gray & BeMiller, 2003).

2.3.2 Starch

Starch is the main component of bakery products; native starch granules have ordered crystalline and semicrystalline structures; optical birefringence is observed under polarized light. Upon cooking the ordered structures disappear and become a disordered form and loss the birefringence. Starch crystallization and retrogradation are the most discussed and are considered as major factors that contribute to the staling process. Starch psdeudocrystallization is an ordering process through which starch molecules form into an ordered arrangement from a disordered melt or solution (Fisher & Thompson, 1997). Starch pseudocrystallization occurs during retrogradation which is commonly described as a process where the molecules of gelatinized starch start to associate in a more ordered structure (Fisher

& Thompson, 1997). It involves the formation of chain entanglement, short-range molecular order and crystallization of double helical aggregates (Biliaderis, 2008).

Before cooking, the starch granules are held together as a network of interlacing molecules (Kulp, et al., 1981). In the wet dough, the hydrated starch granules are coated by the hydrated continuous gluten phase (Hug-Iten, Handschin, Conde-Petit, & Escher, 1999). Gluten, with only around 10% wt/wt of the wheat flour composition and 5% wt/wt of the hydrated dough or batter, has high swelling capacity. It composes 40% of the batter volume and determines its rheological properties; while starch, with 70% wt/wt of flour is simply considered as filler (Bloksma, 1990).

During cooking, when the dough reaches about 53 to 62°C (Kulp, et al., 1981), starch granules absorb water and swell, this is accompanied by disordering of the molecular structure as the starch granules gelatinise and pseudocrystalline regions of the granule become amorphous (Biliaderis, 2008). In the fresh cooked item, the swollen and elongated starch granules may be partly fused with neighbouring granules. The hydrated gluten that surrounds the starch granules in the batter will denature as cooking proceeds. During cooking and gelatinisation of the starch amylose will tend to leach from the starch granules into the water associated with the gluten. The granules will still retain their granular identity (Hug-Iten, et al., 1999) so contributing to the strength of the cooked products. Gelatinization causes an irreversible transformation for starch granules and leads to a starch network (Hug-Iten, et al., 1999) in which the leached amylose extends as hair-like projections from the surface of the granule remnants (Toufeili, Sleiman, Salman, & Brockway, 1994) cited by (Assouad, 1996). The amylose also entangles with gluten fibrils to form a cross-linked network between starch and protein (Martin, et al., 1991). The fresh crumb can be described as a bicontinuous interwoven gluten network with gelatinized starch strands (Oates, 2001). No birefringence can be observed in the granules since the starch granules have lost the ordered structure during gelatinization (Biliaderis, 2008).

However, birefringence under polarised light was observed in aged crumb (Hug-Iten, et al., 1999) indicating that an ordered pseudocrystalline structure may form as the crumb ages. Assouad (1996) stated that after gelatinization, the amorphous, highly disorganized starch is

unstable and has a tendency to form an ordered structure. This leads to the retrogradation and syneresis of starch. Intra-granular and leached amylose, due to its linearity re-associates rapidly with amylose and more slowly with amylopectin after cooling to increase order within the structure and to make the structure more rigid, this occurs within a few hours after baking (Biliaderis, 2008). More slowly, the highly branched amylopectin begins to aggregate form a more ordered structure (Pyler, 1988a) again contributing to the rigidity of crumb.

The retrogradation processes are partially heat-reversible and it is well known that the freshness of bread can be restored by heat. When a paste of gelatinised starch is heated at 60°C or above, the recrystallized amylopectin melts (Gray & BeMiller, 2003); and structures formed by the weaker bonds between short linear branches of amylopectin are destroyed (Pyler, 1988b). When the bread is reheated, the softness and aroma reappears as moisture and flavour are released as the ordered amylopectin becomes amorphous (Assouad, 1996). However, when baked goods loose more than 30% of their moisture, the freshness cannot be restored. In addition, the crystallized linear long starch chain starch fractions, mostly amylose, are not able to melt. They require high temperatures of around 150°C to bring about their re-solution (Pyler, 1988b).

2.3.3 Protein

Protein transformation and its interaction with starch during storage of bakery product also contribute to the staling progresses. Hydrated flour protein forms gluten when the flour is mixed with water. During baking, heat causes the expansion of gas cells which produced by leavening agents and further stretching and thinning of the protein sheets; gelatinized softened starch granules are entirely enrobed by protein and distort to fit around air cells; hydrogen-bond-cross-links are produced between the starch and protein fibrils; the gluten system is reinforced and the final structure of baked item is developed as the protein denatures (Oates, 2001). During cooling, the crumb loses kinetic energy, the hydrogen cross-links between protein and swollen starch granules increase in number and strength and contributes to the firmness development (Martin, et al., 1991).

The quality and quantity of protein present in flour affects the crumb staling as it affects the interaction with starch granules. Poor quality protein has more hydrophilic properties and

hence forms more hydrogen bonds with starch therefore increasing the firming rate (Martin, et al., 1991). When the protein content is increased, the protein tends to link with other protein molecules by the disulphide bonds to form gluten (Patient & Ainsworth, 1994), this decreases the association with starch granules and serves as a moisture reservoir to buffer the hydration capacity of starch during storage thereby retarding the staling rate (Gray & BeMiller, 2003).

2.3.4 Pentosans

Wheat flour contains 2 ~ 3% pentosans, about 20 ~ 25% of which are water-soluble and the remainder water-insoluble (Pyler, 1988a). This small amount of pentosan cause increases in dough and batter viscosity. Pentosans are the polymers of pentoses (xylose and arabinose) the weight average molecular weight of wheat pentosans is 255,000 with a xylose to arabinose ratio of 1:16 (Girhammar & Nair, 1992). By interacting with amylose and amylopectin, pentosans also have the ability to retard staling by reducing the proportion of starch involved in crystallization, however they do not affect the mechanisms of staling discussed above (Gray & BeMiller, 2003).

When mixed with water, the small quantities of pentosans present in flour assist gluten to develop a highly viscous but less elastic batter thereby decreasing the initial crumb firmness (Gray & BeMiller, 2003). This is due to the high proportion of hydroxyl groups presenting on the highly branched pentosan polymers absorbing more water to produce a solution which has 15 to 20 times higher intrinsic viscosity than those of soluble flour protein extracts only (Pyler, 1988a). Furthermore, water-insoluble pentosans conjugate with gluten protein and possibly other polymers and contribute to the batter consistency and provide a uniform crumb structure (Pyler, 1988a).

2.4 Identification of staling

Staling is a complex process that involves macro changes in texture, flavour and appearance and micro changes in moisture content and distribution, and in the structure of starch and protein. Staling can be identified by a variety of techniques such as texture analysis, sensory organoleptic tests, thermal analysis (differential scanning calorimetry; DSC); molecular analysis (Nuclear magnetic resonance spectroscopy; NMR); x-ray crystallography; and

microscopic analysis. Other measurements such as Water activity (aw) and moisture content can also be used to help determine the staling process in bakery products.

Based on consumer perception of texture, the staled product becomes firmer, more crumbly and less spongy. Texture Profile Analysis (TPA) tests are designed to simulate the mechanical process the food undergoes during mastication. Different parameters that are related to sensory characteristics, such as hardness or firmness and springiness which is related to spongy textures can be measured.

The DSC has proven to be very useful in providing basic information on starch retrogradation which is directly related to bakery staling (Hug-Iten, Escher, & Conde-Petit, 2003; Karim, Norziah, & Seow, 2000). It measures the differential temperature or heat flow from a test sample compared to a reference material as a function of time and monitors the changes in phase transition (Karim, et al., 2000), such as from amorphous phase to crystal phase during the retrogradation of starch.

NMR techniques have been used to examine changes in the molecular mobility of water in bread (Karim, et al., 2000). At equilibrium bread contains mobile (liquid phase) and immobile (solid phase) protons associated with water. However, bread is always in a nonequilibrium state. During staling, the mobility of the less-mobile fraction of water decreases and the mobility of the more-mobile fraction of water increases (Gray & BeMiller, 2003).

2.5 Retardation of staling

Bakery products commence physical deterioration immediately after they are removed from the oven. Upon cooling, one of the first changes that begin is retrogradation of the gelatinised starch. To prevent or reduce the amount of starch that retrogrades is the main strategy used to retard staling. Several methods have been developed including freezing to reduce the redistribution of water and the addition of anti-staling agents such as emulsifiers, enzymes, humectants and gums can also reduce the staling through different mechanisms and this will be specifically discussed in the following session. In addition, avoiding storage at 4°C, the temperature at which staling is fastest (Pence & Standridge, 1957) will help bakery goods to maintain their fresh bake quality for longer.

2.5.1 Emulsifier

As it said in the section of ingredients in bakery products, emulsifiers are amphiphilic molecules usually composed of two components, the first a hydrophilic polar group and the second a long chain hydrophobic fatty acid.

Emulsifiers stabilize the starch-water suspension and retard staling by interacting with starch to form complexes. Amylose and the short side chains of amylopectin have a linear structure and form a left-handed helix with the hydrogen atoms oriented to the centre of the helix in aqueous systems. The centre of the molecule therefore tends to be hydrophobic and of sufficient diameter to accommodate fatty acids. As shown in Figure 1, this arrangement is suitable for an inclusion complex formation with the longer chain hydrophobic fatty acid molecules including those associated with the monoglyceride type emulsifiers (Hasenhuettl, 2008a). Emulsifiers can also form complexes with amylopectin, though, amylose complexes are more likely to occur. The highly branched amylopectin only forms a helical at the tertiary branches, so the monoglyceride emulsifiers can only interact with a small proportion of the molecule (Kulp, et al., 1981). The interaction of the monoglyceride with amylopectin may occur at higher dosage of the emulsifier. It is suggested by (Stampfli & Nersten, 1995) that for normal flour about 1% of added monoglyceride (wt/wt; monoglyceride: flour) is preferentially bound to the amylose component of the flour. They suggest that the monoglyceride is preferentially bound to unbranched regions of the starch molecule and may interact with the short terminal linear components of the amylopectin molecule when all the binding sites on the amylose present are occupied. For this reason, in excess of 1% of monoglyceride can ensure that the complexing would occur with the amylopecting component of the starch.

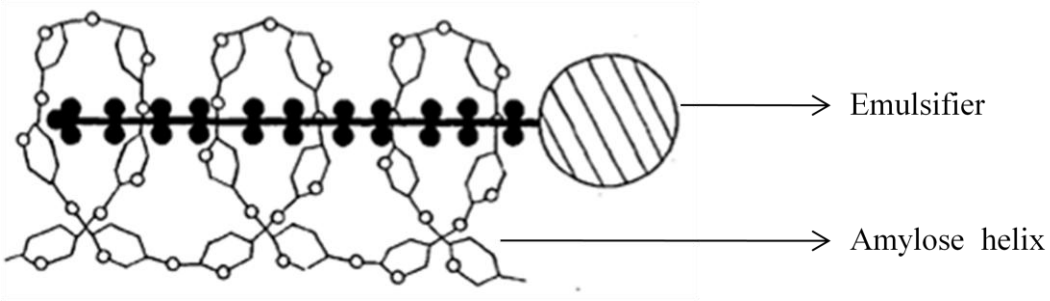


Figure 1 Emulsifier-amylose helical complex with the whole chain of fatty acid inside the helical space
Adopted from (Kulp, et al., 1981)

The complexed part of the starch molecules are not water-soluble and do not participate in the gelatinization process during baking and thus will not recrystallize and contribute to staling of the crumb (Leyn, 2006). The formation of complexes between starch and monoglyceride may occur inside the starch granule, thereby, reducing the leaching of amylose from the granule (Gray & BeMiller, 2003). The complexes may also inhibit amylose and amylopectin from forming bridges with other starch molecules and ultimately reducing the decrease in molecular volume and water exclusion that occurs during retrogradation (Schiraldi & Fessas, 2001).

In addition, some of the emulsifiers function to strengthen the gluten and prevent the firming between starch and gluten. The properties of emulsifiers also assist with the dispersion of a fat film between gluten and starch granules and increase the aeration in baked item (Orthoefer, 2008). Emulsifiers adsorbed onto the starch granule surface, prevent moisture uptake by the starch from gluten during aging, thus prevent contraction and firming of the gluten phase (Gray & BeMiller, 2003).

The common baking emulsifiers are monoglyceride, lecithin, sodium and calcium stearoyl lactylate and Datem. These emulsifiers all have the ability to form complex with starch; in addition, sodium and calcium stearoyl lactylate is able to form hydrophobic bonds with protein at its non-polar regions and ion pairing can be occur between the ionic carboxylic portion of the lacylate and the charged amino acid residues on protein (Boutte & Skogerson, 2004). Datem have the ability to form the hydrogen bridges with amidic groups of the gluten-protein (Gaupp & Adams, 2004).

2.5.2 Enzyme

Amylases, one of the most commonly used enzymes, catalyse the hydrolysis of starch molecules, hence, decrease the structural strength of the starch phase and prevent the recrystallization of starch or reduce the connectivity between crystalline starch phase, and therefore, reduce the staling rate (Hug-Iten, et al., 2003). Amylases are divided into α-amylase, β-amylase, and maltogenic amylase. Among them, α-amylase (1, 4-α-D-glucan glucanohydrolase EC3.2.1.1) and maltogenic amylase (glucans, 1, 4-α-maltohydrolase, EC 3.2.1.33) are the most widely used amylases. α-amylase is endo-active enzyme that randomly

hydrolyzes the α-1, 4 glucosidic linkages in polysaccharides, resulting in short chain dextrin of Degree of Polymerization (DP) 2 ~ DP12; maltogenic amylase produces maltose and some longer maltodextrines in the α-configuration (Oort, 2010).

Degraded small chain dextrin reduce the batter viscosity during starch gelatinization, thus, increasing the leavening and providing the spongy crumb texture (Oort, 2010). Additionally, the small chain molecules are related to larger dextrin and amylose; they may diffuse away from the interface between starch and protein and inhibit the entanglements between starch and protein or protein and protein, as illustrated in Figure 2, resulting in the retardation of staling (Martin & Hoseney, 1991). Furthermore, the shorter chain amylose, degraded by enzyme, can interact with emulsifiers to form more amylose-complexes, hence, reducing the staling rate (Hasenhuettl, 2008a).

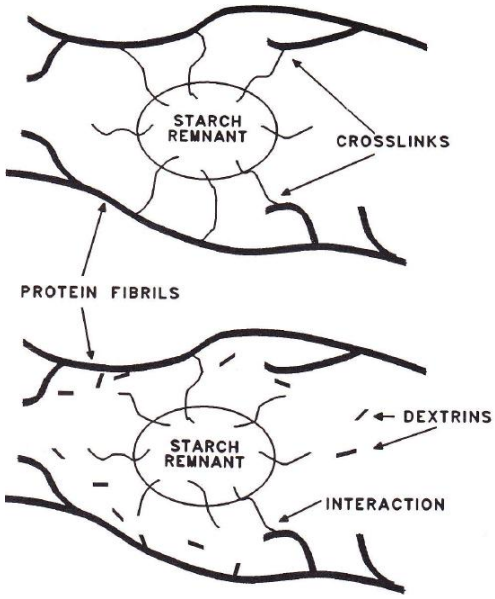


Figure 2 Mechanism of bread firming and the antifirming role of dextrins
Adopted from (Martin & Hoseney, 1991)

Ideally, the small chain molecules can be produced after the gelatinization (after the cooking procedure), when the amylose has leached from the granule and become accessible. Hence, heat stable enzymes are preferred in this situation. Bacterial enzymes are heat stable, and exhibit enzymatic activity after baking and continuously during the storage time, therefore,

overdosing will cause a collapse of the bakery texture, for this reason, bacterial amylases can be safely used only at very low dosage (Oort, 2010).

2.5.3 Other anti-staling agents

Shortening, humectants and gums are also reported to have anti-staling effects. Shortening is edible fat from various sources that is added to bakery products to make the dough less elastic and to improve the " bite tenderness" of the final product; it prevents adjacent starch granules binding tightly together during gelatinisation (Rogers, Zeleznack, Lai, & Hoseney, 1988) cited by (Assouad, 1996). Shortening also assists with the formation of hydrophobic films between gluten and starch ultimately increasing the aeration resulting in a softer baked product (Orthoefer, 2008). Humectants, such as glycerine and syrup, can reduce the staling rate by enhancing the water retention of baked goods. Moisture is prevented from moving to the crust and is retained in the crumb, therefore, keeping the product fresh (Assouad, 1996). Two proposed anti-staling mechanisms were reported for gums (Gray & BeMiller, 2003): gums can extend the freshness by prevention of moisture loss or they may inhibit the gluten-starch interaction in the same manner as small molecule dextrin may.

2.6 Extending the shelf life of bakery goods

Retardation of staling is the main objective of this project; suppressing the growth of micro-organisms is another challenge to extend the shelf life of hotcake products. Two basic strategies will be discussed to extend the shelf life of bakery products:

2.6.1 Prevention and destruction of post baking contaminants

Spoilage by fungal moulds is the predominate microbiological spoilage problem in bakery products. Most mould spores are destroyed during baking therefore contamination occurs largely due to the post baking contamination; such as from the air in the bakery or from the cooling and packaging environment. Air filtration/circulation systems are capable of removing all microorganisms from air and delivering this into the bakery environment, however it is impossible to remove all microorganisms from the bakery environment and the cost of aseptic packaging systems are a major disadvantage (Smith, et al., 2004).

Destroying contaminants before packaging is an approach that ensures a long shelf life for the products. Mould spores are sensitive to 70% ethanol, U.V.light, infrared radiation, and low dose irradiation. These methods may not kill the contaminants (mould or yeast), but they can inactivate their spores (Smith, et al., 2004). Due to the cost, processing and packaging constraints, these technologies are not often a commercially viable approach in extending the shelf life of bakery products. Therefore, controlling the growth of the post baking contaminants is a common approach in baking industry.

2.6.2 Controlling the growth of post baking contaminants (hurdle technology)

Hurdle technology is currently adopted among the food industry to assure a better shelf life. It is a combination of preservation techniques to control the microbiological spoilage and extend the shelf life such as lowering the storage temperature, water activity (a_w), redox potential (Eh), changing the acidity (pH), incorporation of preservatives and packaging (Leistner, 2000). In the application, a set of hurdles that is specific for a particular product can keep spoilage or pathogenic microorganisms under control because some of the microorganisms can overcome a number of hurdles, but none can overcome all the hurdles used together. For different microorganisms, the main hurdles can also be set at higher intensity than other hurdles to achieve an optimum performance.

Due to the nature of the hotcake product which is the subject of this work and the storage condition required by the company and supply chain, not all possible hurdles are applicable or can be used in this real product development situation. Storage temperature is fixed by the company and supply chain. pH, a_w and Eh are determined by the baking formulation. Therefore, incorporation of preservatives and modification of the packaging technique are the tangible factors.

2.6.2.1 Preservatives

Most factory practices making bakery products incorporate chemical preservatives as the easiest and cost efficient method to achieve a long shelf life. Three types of preservatives are available for bakery goods: propionates, sorbates and benzoate. Among those three, propionates inhibit mould spores; sorbates inhibit both moulds and yeast therefore are not preferred by breading making; while benzoate is mainly used for fruit preservation due to it is

active for low pH products (Suhr & Nielsen, 2004). The maximum dosage of sorbates and propionates is 0.12% and 0.4% (wt/wt) respectively (FSANZ, 2007). Within these allowable usage range the suitable dosage is also dependent on the pH value of the product (Suhr & Nielsen, 2004). Therefore, checking the pH value of hotcake products is needed when determining the type of preservatives and the usage of preservatives.

2.6.2.2 Modified atmosphere packing

Modified atmosphere packing (MAP) is another approach for hotcake product to control the mould growth prior to sale. The principle of MAP is to reduce the levels of O_2 and so prevent respiration in the strictly aerobic moulds. Oxygen levels below 1% are reported to be sufficient for the attainment of long shelf live (Guynot, Marin, Sanchis, & Ramos, 2003). With appropriate low gas permeable packaging materials, this can be achieved by vacuum packaging, flushing with CO_2 or N_2 and the use of oxygen absorbers. Vacuum packaging is the earliest and most basic MAP. By evacuating air, O_2 levels as low as 1% or less can be achieved. However, this process causes irreversible deformation of soft products by crushing due to air pressure and it is not used for most of the baking industry. The commonly used gases in MAP are CO_2, N_2 and combinations of these gasses. CO_2 is bacteriostatic, fungistatic and can kill insects in the package (Assouad, 1996). N_2 is an inert gas and usually used as a filler gas to prevent the packaging collapsing in products that could absorb some CO_2 during storage (Assouad, 1996). Oxygen absorbers are composed of chemical substances which react with oxygen to reduce the O_2 level and maintain this level in a sealed packaging film (Assouad, 1996).

Chapter 3 General materials and methodology

3.1 Project overview

Incorporation of generally known anti-staling ingredients was the approach identified from the literature to reduce the rate of staling. Although this process is well discussed for breads and cakes there was little literature on how to reduce the rate of staling in hotcakes. Bakery products begin to stale immediately after cooking and throughout the storage period at temperatures above freezing. The staling rate of hotcakes can be reduced by the use of appropriate anti-staling ingredients. A diverse variety of anti-staling ingredients including emulsifiers, enzymes, humectants and gums are available.

The shelf life of a final product, particularly if it contains high level of moisture, is usually determined by the presence of unacceptably large numbers of micro-organisms. The possible approaches of modified atmosphere packaging (MAP) and the incorporation of appropriate preservatives would be determined to extend the shelf life of hotcakes.

The first step in this project was to run preliminary and screening tests to select the most effective anti-staling ingredients. The second step was to combine the effective anti-staling ingredients together, based on their likely synergies to reach a best anti-staling combination. The third step was to test possible approaches such as MAP and incorporation of the appropriate preservatives to extend the shelf life by reducing the microbiological spoilage at ambient storage temperature. The forth step was to confirm the methods achieved from the laboratory work to reduce the staling rates and microbiological spoilage in a commercial scale trial at VDFF's manufacturing plant.

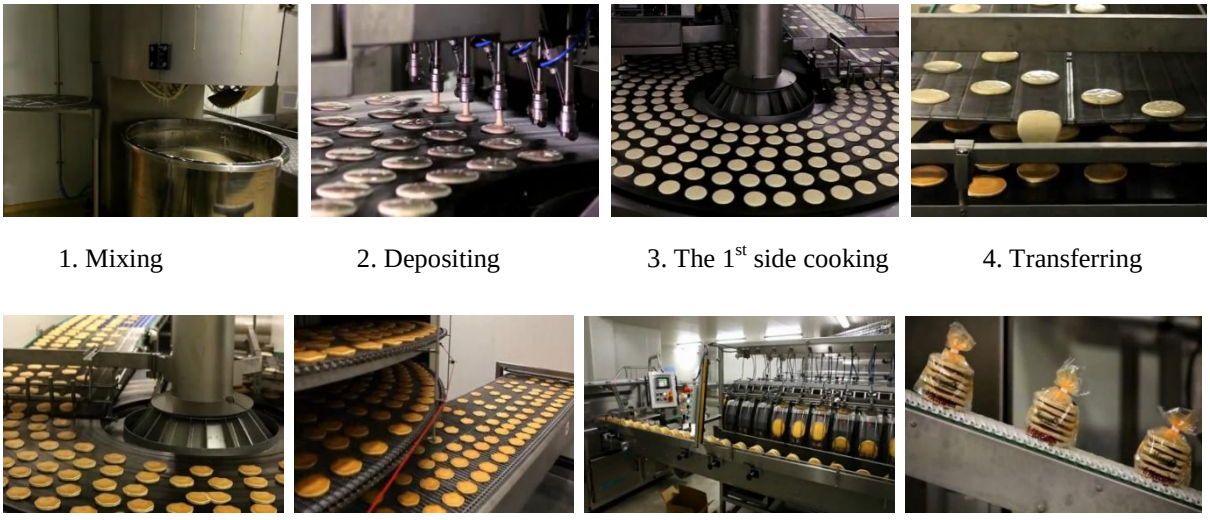
The general materials and methods used in this project are presented in this chapter.

3.2 Commercial hotcake ingredients and manufacturing procedure

Hotcakes for this work were made with flour, water, egg, butter milk powder, canola oil, starch, and salt; leavening ingredients including sodium bicarbonate and sodium aluminium phosphate; calcium propionate as a preservative; and vanilla essence for flavouring. Without anti-staling additives hotcakes stale within 24hours at temperatures above freezing and become firmer in texture and losing their fresh baked flavour. The anti-staling ingredients

Lecithin and the crude mono-diglyceride product (Grinstead®MONO-DI) are used in VDFF's current commercial formulation.

In the factory, dry ingredients and liquid ingredients are mixed at chilled temperature; the mixed batter is deposited on an automated rotating hotplate to cook one side of the hotcake. The partially cooked products are then transferred to a second hotplate to complete the cooking process. Cooking times are about 75 seconds for the 1st side and 50 seconds for the 2nd. Temperatures on the hotplate surfaces are around 110°C and 120°C respectively. After cooking, the hotcakes are cooled to -30°C in 30 minutes by blast freezing. In the end the frozen hotcakes are packed into bags with six in each by automatic packing machine in a positive air pressure clean room. The manufacturing flow chart is shown in Figure 3.



1. Mixing 2. Depositing 3. The 1st side cooking 4. Transferring

5. The 2nd side cooking 6. Cooling 7. Packing

Figure 3 Hotcake manufacturing flow chart

3.3 Laboratory materials

3.3.1 Basic hotcake formulation

Table 2 Van Dyck Fine Foods Standard hotcake formulation (Std) for one batch

Ingredient	Std formulation/g
water	900
flour	660
sugar	195
buttermilk powder	150
egg	75
canola oil	45
baking soda	13.5
SALP	13.5
maize starch	13.5
salt	5.1
calcium propionate	3.225
vanilla	0.405
Anti-stlaing ingredients	
Lecithin	6.99
Grindsted®MONO-DI	6.99

Flour was obtained from Bidvest Ltd. Butter milk powder was obtained from Fonterra co-operate Group Ltd. Canola oil was obtained from Sunnz Ltd. Maize starch was obtained from Penford Ltd. Sugar, egg, and salt were obtained from local super market. Anti-staling ingredients: Lecithin and Grindsted®MONO-DI; leavening ingredients: sodium bicarbonate and sodium aluminium phosphate acidulant, the preservative: calcium propionate; and vanilla essences were provided by VDFF throughout the project.

3.3.2 Anti-staling ingredients

Table 3 Anti-staling ingredients selected in this project

Ingredients	Category	Dosage (Flour Base, FB)	Supplier
Maltodextrin	Dextrin	7.5%	Salkat
Litesse Polydextrose	Humectants	7.5%	Danisco
Glucose syrup	Humectants	7.5%	Davis Trading
Glycerine	Humectants	1%	Davis Trading
Fruitrim	Humectants	1%	ANZCHEM
Xanthan gum	Gum	0.05%	ADM
Dimodan PH 320/B-M	Emulsifier	0.5%	Danisco
Grindsted ® SSL P 86 K	Emulsifier	0.5%	Danisco
DATEM	Emulsifier	1%	Palsgaard
Amylofresh	Enzyme	0.2%	ABF Ingredients
Novamyl 10000 BG	Enzyme	0.0075% (750MANU)	Novozymes
Novamyl Pro BG	Enzyme	0.0062% (62ppm)	Novozymes

The dosages of Maltodextrin, Litesse Polydextrose, Glucose syrup, Glycerine and Xanthan gum were recommended by New Zealand Sales manager of Danisco. The dosages of other ingredients were recommended by the product descriptions.

The Generic names or compositions for the emulsifiers:
Lecithin: phospholipids, a diglyceride and a phosphate group (Orthoefer, 2008).
Grindsted ® MONO-DI: a mixture of mono- and di- glyceride.
Dimodan PH 320/B-M (Dim): distilled monoglyceride.
Grindsted ® SSL P 86 K (SSL): sodium stearoyl lactylate.
DATEM (DT): a Diacetyl Tartaric Acid Ester of Mono- and Diglycerides.

Among those five emulsifiers distilled monoglyceride, mono-diglyceride and lecithin have the function of crumb softeners that only act on starch. SSL and DT have the functions of both crumb softeners and dough strengtheners. These two are not only able to interact with starch; they are also able to form liquid films of lamellar structure in the interphase between the

gluten strands and the starch and therefore improve the ability of gluten to form a film which retains the gas produced by the leavening agent (Stampfli & Nersten, 1995). However, during cooking when protein denaturation and starch gelatinization occurs, SSL transfers to the starch fraction while DT remains bound to the protein fraction (Boutte & Skogerson, 2004). Therefore, DT normally gives poor crumb softening compared to SSL.

The three enzymes trialled are all bacterial maltogenic amylases derived from *Bacillus subtilis* according to their product descriptions, the difference among them are the activity units in the liquids supplied at the recommended dosages. The function of enzymes is to catalyze the hydrolysis of starch molecules and decrease the structural strength of the starch phase and prevent the recrystallization of starch or reduce the connectivity between crystalline starch phase, and therefore, reduce the staling rate (Hug-Iten, et al., 2003). During experiments, the dosage of enzyme was carefully controlled because activity is a continuing process and started after cooking (Oort, 2010).

3.4 Methods

3.4.1 Mixing method and quality control

One anti-staling ingredient or an anti-staling combination was added into the control formulation (Cont, VDFF formulation from which the Lecithin and Grindsted®MONO-DI were omitted). Solid and liquid ingredients were weighed and mixed in a food mixer. Egg and sugar were mixed first; and then 50% of the total water at 9°C was added. The butter milk powder was then added and dissolved followed by the starch, leavening agents, anti-staling ingredients, salt, essences and oil. Flour was added at the end of the mixing process, half of this flour was chilled to 3°C and the other half was at ambient temperature. After mixing, the batter was kept below 9°C in a chilled water bath before cooking. Hotcake size was controlled using a measuring cup to deposit a uniform volume of batter.

3.4.2 Cooking method

In the laboratory, the hotcakes were cooked on a commercially made domestic hotcake grill (Sunbeam ReversaGrill HG3300) that had been modified by adding a proportional–integral–derivative (PID) temperature controller to improve temperature control of the cooking surface

to reducing the variation in cooking time and temperature between batches of hotcakes. The cooking temperature was set at 150 ± 3°C as the lower cooking temperature in the lab cannot produce an acceptable hotcake. The cooking time was around 1min for each side. Minor variations to adjust the degree of cook and the colour of the hotcakes occurred as production progressed during the day.

3.4.3 Post cooking treatment, packaging and storage condition

After cooking, the hotcakes were cooled to room temperature and sprayed with 2ml, 70% alcohol to destroy mould spores (Gudsell, 2003). Thereafter, the hotcakes were manually packed into bags with six hotcakes in each (the same as used in the commercial product) packaging bags were provided by VDFF and the material is low density polyethylene. The packed hotcakes were stored at 20°C in a controlled temperature room in Institute of Food, Nutrition and Human Health (IFNHH) for the staling study.

3.4.4 Sampling method

Two bags of hotcakes from each batch, a total of 12 hotcakes and two samples from the centre of each hotcake were selected for texture analysis. Therefore 24 measurements for each batch were analysed (2 sample points x 12 hotcakes). The firmness and springiness were measured at day 0 (within 3 hours of manufacturing the hotcakes) and thereafter at day 1, 3 and 6.

3.4.5 Texture measurement

In hotcake study, Texture Profile Analysis (TPA) was chosen to identify the staling rate throughout the project due to it is practical and imitative in daily texture examination.

Figure 4 shows the typical TPA graph would be obtained from Texture analyzer and Table 4 shows the relevant parameters would be obtained from the TPA graph. In this figure, A_1, A_{1W} and A_2, A_{2W} are areas under the compression and withdrawal portions of the first-bite and second-bite curve, respectively; A_3, d_3 are the negative work of force during the first withdrawal and the corresponding crosshead travel distance, respectively; P_1, P_2 and d_1, d_2 are the peaks of the first and second compressions and the corresponding crosshead travel distance; F_1 is the first significant break in the first compression curve.

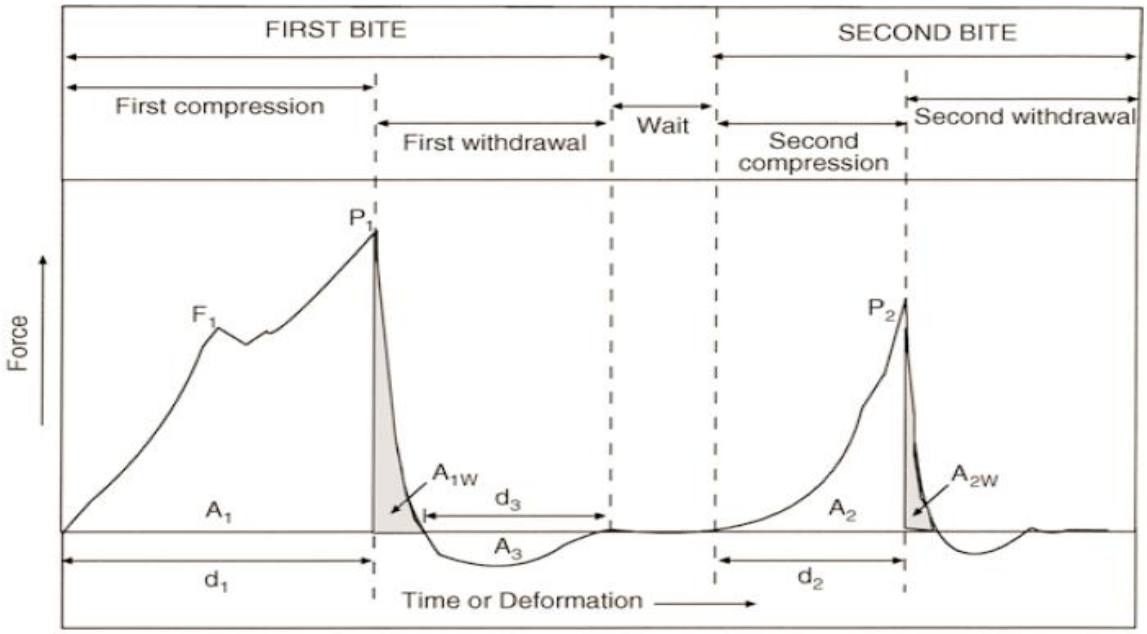


Figure 4 Typical two-bite Texture Profile Analysis force-time (deformation) curve

Table 4 Parameters obtained by TPA tests related to hotcake products

TPA Term (SI units) [dimensions]	Definition	How Measured Ref. Figure 1
Firmness (N) [MLT–2]	Force necessary to attain a given deformation	Force corresponding to P_1
Springiness (m/m) [L]	The distance of the detected height of the product on the second compression (d_2 on the graph above), as divided by the original compression distance (d_1).	d_2/d_1

Figure 4 is adopted from (Gunnasekaran, 2002) and Table 4 is adopted from TAXT2 Manuscript.

Firmness explains how hard the baked sample is; it increases with time, the higher the value, the harder the product and the staler the product. The springiness is a function of the elasticity of the products; it represents the sponginess and decreases with time, the higher the value, and the spongier the product, the fresher the product. The increase in firmness with time, such as per day, was calculated as the staling rate. It is the same as firming rate in some literature. In

addition, the hardness and springiness values are affected by the air cell structure of the hotcakes. For a solid material, the number of air cells and the size of air cells all affect the firmness and sponginess of the material. Large air cells provide softer and less spongy texture. Small evenly spread air cells provide a harder and spongier texture.

TAXT2 Texture Analyzer was used for TPA tests fitted with a 5kg Load Cell. A 50% compression strain was applied at a 5mm/second compression speed and the firmness was recorded as the force recorded on the load cell at a compression of 50%. The trigger force that initiated the measurement cycle was 0.05N.

3.4.6 Statistical methods

Firmness and Springiness was calculated according to the formula in Table 4 from the TPA test. Outliers of each formulation on each testing day were identified from Boxplot procedure of Minitab software. The outliers are defined as the values beyond the upper and lower whiskers as calculated by the Boxplot procedure.

After deleting the outliers, the data of each formulation was checked by normality test. If the data followed normal distribution; significant differences between each formulation on each testing day were identified by One-way ANONA, Minitab. If the data did not follow the normal distribution, general mean value comparison was used, and this was specifically illustrated in the next chapter 4.2.7.

One-way ANOVA was carried out on the normal distributed data using formulation as treatments and firmness or springiness as responds. The one-way ANOVA test was run separately for each testing day. If variation between treatments was significant at the 5% level probability of $F > 0.95$ then a least significant difference (LSD) analysis with fisher's individual error rate of 0.05% was carried out. The treatments were considered different if the differences between the means exceeded the LSD value.

Chapter 4 Anti-staling screening trial

4.1 Introduction

Staling is a major problem that reduces the fresh baked flavour and texture of the hotcakes and makes them less acceptable to the consumer. Rapid deterioration in these characteristics limits shelf life and increases product wastage and profitability as stale product is discarded and is fed to animals. The retention of fresh baked flavour and odour are difficult as volatile products are lost from the hotcakes. Many ingredients are available to the baking industry that assist in preserving fresh baked texture and the way in which these operate has been discussed in chapter 2 (literature review). The preliminary trial that lead to the work presented in this chapter also identified a number of possible bakery agents such as Dim, SSL and Datem that reduced the rate of staling. Humectants, dextrin and gum were rejected from the list in the preliminary trial because of the firmer products they made and less desirable texture imparted.

It is difficult to predict or choose the best combination of anti-staling ingredients without carrying out baking trials to determine the effectiveness of the potential anti-staling ingredients and in this chapter the firming rate of the hotcakes made with these ingredients will be reported. The tests to screen ingredients were performed using eight ingredients (including two ingredients used by VDFF) that are known from the literature and preliminary study to reduce staling in bakery goods.

The objectives of the screening trial were to determine how effective each anti-staling ingredient was in reducing the rate of firming of hotcakes. The most effective ingredients from this work would be selected for an ingredient combination study reported in later chapters. In all cases hotcakes made with the ingredients under test were compared with hotcakes made with the standard formulation used by VDFF (VDFF original formulation: Table 2).

4.2 Materials and Methods

4.2.1 Materials

The standard hotcake cooking ingredients were listed in Table 2. The anti-staling ingredients used in this chapter were Dimodan PH 320/B-M (Dim), Grindsted ® SSL P 86 K (SSL),

DATEM (DT), Amylofresh (AF), Novamyl 10000 BG (BG), and Novamyl Pro BG (Pro). The details of these ingredients were listed in Table 3. The dosages of enzyme used in the study were the same as listed in Table 3; and the dosages of emulsifiers were increased from the suggested level according to the literature and VDFF's original formulation, so that the qualities of the hotcakes are comparable with the Std formulation. Therefore, the dosages for Dim and SSL were 1.5% (FB) in each batch and the dosage for DT was 3% (FB) in each batch.

4.2.2 Mixing method and quality control

Mixing method and quality control was listed in 3.3.1.

4.2.3 Cooking method

Cooking method was listed in 3.3.2.

4.2.4 Post cooking treatment, packaging and storage condition

Post cooking treatment, packaging, and storage conditions were listed it 3.3.3.

4.2.5 Sampling method

Sampling method was listed in 3.3.4. In addition, the screening trial lasted for two months because one day can only produce one batch, and the experimental space and instruments were not available every day, considering the quality of general ingredients, and weather conditions changed to some extent over this long period; the effects of different cooking days might become obvious; duplicate treatment (two batches for each formulation) was performed. Therefore 48 measurements for each formulation were analysed (2 sample points x 12 hotcakes x 2 batches).

4.2.6 Texture measurement

Texture measurement was listed in 3.3.5.

4.2.7 Statistical methods

Statistical method was listed in 3.3.6. Due to the reason listed in 4.2.5, although each formulation had 48 measurements, the data of each formulation did not follow the normal

distribution while checking their normality, and hence, one-way ANOVA could not be used to test their significant difference between each formulation. In the end, the mean value of firmness, the proportional increase in firmness, staling rate and the mean value of springiness of the hotcakes made with the anti-staling formulation was compared with values of the hotcakes made with the control formulation and the standard VDFF's formulation (Std) for determine their effectiveness.

4.3 Results and Discussion

The average firmness values of hotcakes made from each formulation on each testing day was presented in Figure 5. The firmness of hotcakes made from all formulations increased with storage time. The control formulation and Std formulation showed similar firmness throughout the testing period. Most anti-staling ingredients, except the enzyme Pro, provide softer texture to the hotcakes. The enzyme did not and was not expected to affect the short term rate of staling of the hotcakes as it takes time for the effects of hydrolysing the starch component of the hotcakes to affect the firmness. The decrease in the rate of firming of the hotcakes containing Pro and AF enzymes slowed markedly after day 3 probably due to starch hydrolysis. The BG enzyme at the level used seen to be less effective than Pro and AF in preventing firming after day 3.

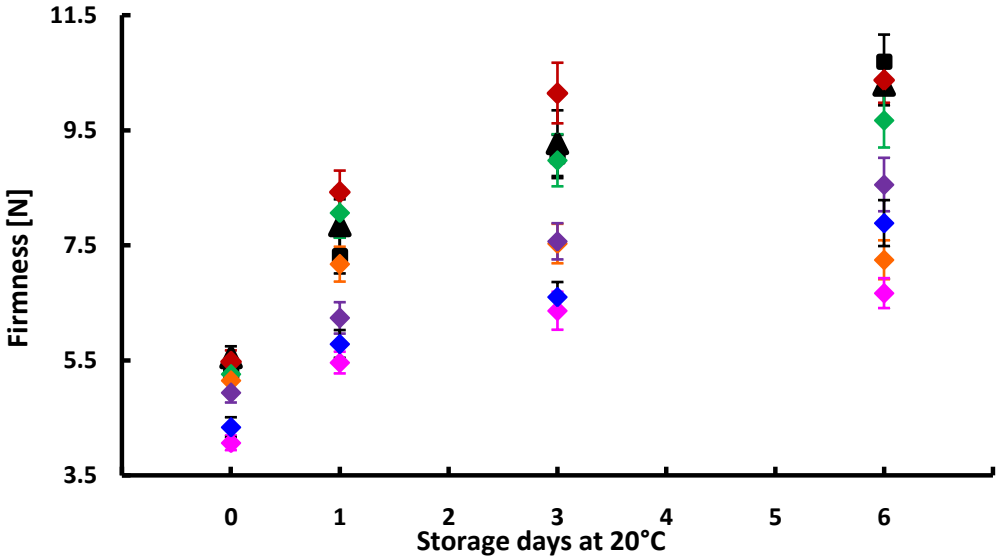


Figure 5 The changes in firmness of hotcakes made in screening trial

The firmness scale of this graph is from 3.5 to 11.5 to emphasis the difference between each formulation.

The hotcakes made with enzyme AF and emulsifiers of Dim, DT and SSL had the lowest firmness throughout the testing period suggesting that these products are among the most effective for reducing staling. It is notable that these ingredients produced hotcakes that were significantly less firm and had a slower firming rate than both the Cont and the Std product.

Two staling trends were evident, and were represented by the emulsifier and enzyme categories of anti-staling ingredients. The firmness of the Std and enzyme treatments increased at similar rates for the first 3 days before the effect of the starch hydrolysis became apparent. The firming rates of the hotcakes containing the emulsifiers were slower but the firming continued for the duration of the trial. This indicates that the emulsifiers were effective in giving the freshness of the hotcakes at the beginning. Based on this evidence, the hypothesis is to develop a combination of anti-staling ingredients including emulsifier and enzyme. The function of the emulsifier is to provide softer texture to the fresh baked hotcake, and the function of the enzyme is to maintain the softness during the storage time, especially from day 3 onwards.

This concept is supported by the anti-staling mechanisms involved. The emulsifiers interact with amylose and amylopectin to form complexes that stabilize the gelatinised starch-water association before the cooking process starts. As a result the total amount of amylose which will retrograde during storage is reduced, so providing softer texture and a slower staling rate than the hotcake made without emulsifier. The heat stable enzymes continue to hydrolyse starch throughout the 'life' of the hotcake, gelatinized amylose will be hydrolysed to short chain dextrins throughout the storage time. This process reduces the amount of amylose that will be involved in stabilizing and hence reduces the firming of bakery products.

The combination of monoglyceride emulsifier and an amylase enzyme was proven to be successful in reducing the staling rate of white pan bread. They found that the enzyme reduced the firming rate of bread while the emulsifier decreases both the initial firmness and the rate of bread firming. The combination of an enzyme and emulsifier resulted in bread that was less firm than that made with either enzyme or emulsifier alone (Valjakka, Ponte, JR, & Kulp, 1994). The mechanism of monoglycerides in reducing the staling rate is because the monoglyceride adhere to the starch granules and therefore decreases the amount of starch to

gelatinize (Lonkhuysen & Blankestijn, 1976). α-Amylase reduces the staling rate by degrading the long chain amylose into shorter chain dextrins and reduces the starch crystallization during storage after the gelatinization (Martin & Hoseney, 1991). However, there is no synergistic interaction between the two ingredients because the α-amylase cannot attack the clathrate formed between amylose and monoglyceride (Valjakka, et al., 1994).

Based on the function of each ingredient in the combination study, a specific analysis was carried out for each formulation in screening trial in Table 5. In this table, the initial hardness, initial staling rate, final staling rate and final proportional hardness increase were compared within each anti-staling category: emulsifier and enzyme.

Table 5 Specific analysis for each formulation in screening trial

Formulations	**Cont**	**Std**	**Enzymes**			**Emulsifiers**		
			Pro	BG	AF	Dim	SSL	DT
Initial firmness (N)	5.29	5.55	5.47	5.26	5.14	4.06	4.93	4.33
Initial staling rate (N/day)	2.02	2.29	2.95	2.80	2.02	1.39	1.30	1.45
Final staling rate (N/day)	0.55	0.34	0.08	0.23	-0.09	0.10	0.33	0.43
Final Prop. firmness increase	2.02	1.85	1.90	1.84	1.46	1.64	1.73	1.82

Initial firmness = firmness of fresh hotcakes tested on day 0
Initial staling rate = rate of firming (N/day; the slope) from fresh to day 1
Final staling rate = rate of firming (N/day; the slope) from day 3 to day 6
Proportional firmness increase = hardness on day6 / hardness on day0

Initial firmness and staling rate are important when selecting emulsifiers due to their anti-staling functions in the combination such as reducing the initial firmness and keeping the softness during the storage time. Compared with the Cont and Std formulations, all the tested emulsifiers gave lower values for initial firmness, initial staling rate and final proportional firmness increase. Among them, adding Dim gave lowest initial firmness (4.06 N), lowest final staling rate (0.10 N/day) and lowest final proportional hardness increase (1.64) and the second lowest initial staling rate (1.39N/day). Adding SSL resulted in the lowest initial staling rate (1.30), the other indicators of staling used were all much greater than for Dim. This phenomenon was more obvious in Figure 5 where the formulation containing Dim resulted in the lowest firmness throughout the whole storage period. Similar results were also found for a

wheat bread study where Dim also provided the lowest firmness compared with SSL and DT after short proofing periods (Gomez, et al., 2004).

Among those glycerides, the effects of lecithin and mono-diglyceride are small and occurred only at high dosages (Stampfli & Nersten, 1995). This because lecithin is mainly a mixture of phospholipids which is a diglyceride and a phosphate group (Orthoefer, 2008). Distilled monoglyceride contains more monoglyceride compared with mono-diglyceride (Hasenhuettl, 2008c). The research of interaction between starch and lipid additives has found that monoglyceride has the ability to form more starch complexes than diglyceride or triglyceride because monoglyceride has more hydroxyl groups than the other two types of glycerides (Eliasson & Ljunger, 1988). Therefore, at similar dosage level, the least firmness provided by Dim agreed with its character of distilled monoglyceride and Dim is the most effective emulsifier (Eliasson & Ljunger, 1988) to interact with starch and form starch complex to give the softness to hotcake at the beginning, and would be used in the combination study.

The hotcakes made containing DT had lower initial firmness than the hotcakes made with SSL. However, the values of staling rate and final proportional firmness increase were higher than those of SSL formulation. This proves the findings of (Boutte & Skogerson, 2004) that DT normally gives poorer crumb softening than SSL. They explained, before cooking, SSL and DT functioning the same to improve the ability of gluten to form a film which retains the gas produced by the leavening agent (Stampfli & Nersten, 1995), and during cooking, SSL moves to starch fraction and reduce the staling rate, while DT always binds with gluten protein fraction. Due to the interaction of DT and flour components which is different from monoglyceride, DT was also chosen for the combination study.

Three types of enzymes were tested in the screening trial. Their final performances are essential in the following combination study. Among them, AF seemed to be the best ingredient as it provided the lowest values on all the compared parameters. However, a sticky and doughy crumb, which reduces the springiness of the crumb and results in a stale product, was observed for the hotcakes containing AF at later stage of storage time. The stickiness was expressed by the springiness in Figure 6.

Enzyme Pro provided lower final staling rate than enzyme BG (0.08N/day and 0.23N/day respectively), but all the other parameters, especially the firmness values (Figure 5) are much higher than those of BG and Std formulations. Therefore, BG would be used for the combination study. The different performances between these three enzymes are caused by their dosages, since they all are bacterial maltogenic amylases derived from *Bacillus subtilis*, which was said in 3.3. The only difference between them was the activity units and the recommended dosages according to their suppliers. Bacterial amylases are heat stable; their enzymatic activities exhibited after baking and continued during the storage period. Lack of dosage will not be effective in reducing the firmness (probably in the case of Pro enzyme). Over dosage will cause a sticky texture and result in too much small chain dextrins degraded from the enzymatic activities (probably in the case of AF enzyme).

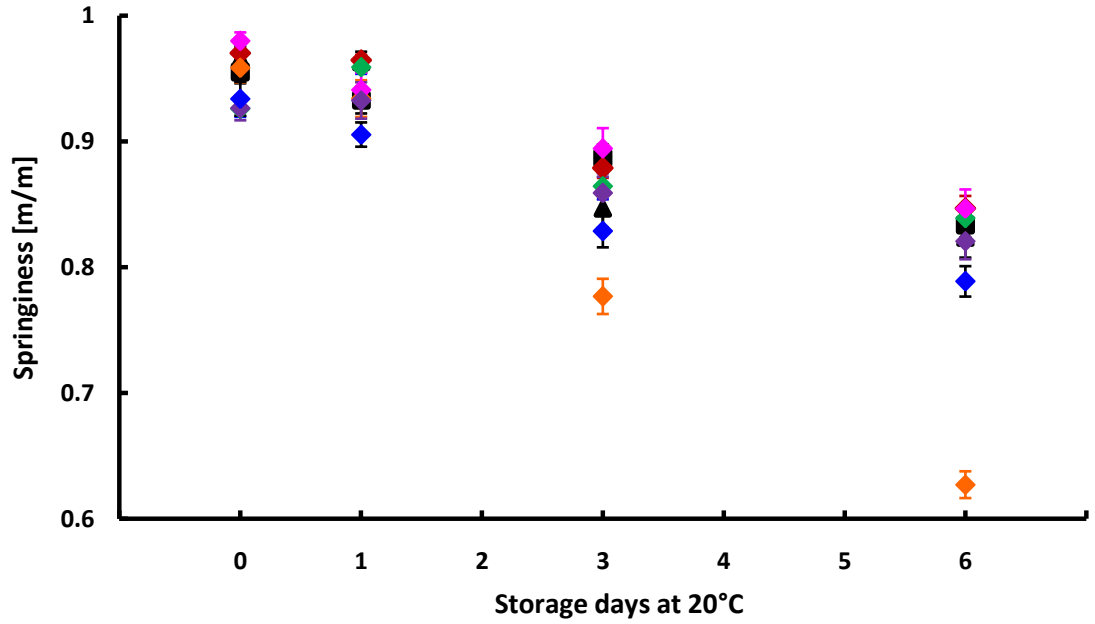


Figure 6 The changes in springiness of hotcakes made in screening trial

The springiness scale of this graph is from 0.6 to 1 to emphasis the difference between each formulation.

Springiness represents the sponginess and was considered when selecting the anti-staling ingredients. It was expected that the hotcakes should not lose their springiness and become rigid as they aged nor should they become too soft and sticky, perhaps through the action of the enzymes added. Overall, the springiness of hotcakes made from all the formulations

decreased during storage. Among them, the formulation containing AF showed the most rapid decline in springiness among all formulations. The springiness value of the hotcakes containing AF was the lowest of all formulations from day 3 onwards. Therefore, AF was judged unsuitable for the combination study. Of the enzyme treatments BG showed greater springiness than other ingredients, and along with Dim and DT were chosen for the following combination study.

4.4 Conclusions

This work shows that emulsifiers function to provide a soft texture to hotcakes immediately after baking. The most effective emulsifier was Dim (distilled monoglyceride). Enzymes have the function of reducing the staling rate at later stage of storage time by hydrolysing starch throughout the 'life' of the hotcake. The most effective enzyme in this study was BG. As the modes of action of these two materials are different it is likely that they can be used in combination to decrease the staling of hotcakes. The reduced rate of staling conferred by Dim compared to the Std treatment that contains the cheaper mixed mono-diglyceride combination is likely to be due to an increased level of monoglyceride, the most effective agent in reducing staling (Eliasson & Ljunger, 1988). Monoglyceride and enzyme are functioning on starch while DT had a different mode to continue functioning on protein; for this reason it may be possible to develop an anti-staling system for the hotcakes using all three ingredients.

Chapter 5 Anti-staling combination trial

5.1 Introduction

Evidence was shown in Chapter four that a reduction in the rate of staling could be affected by increasing the level of monoglyceride added to the hotcakes; later, hydrolysis of the starch component of the hotcakes was shown to soften the product, reversing the effects of staling. As these two mechanisms affected different components of the staling process it seemed possible to combine them to retard staling more effectively.

It was not known if an interaction between Dim and DT occurs. In the published information it is suggested that Dim primarily acted to reduce bonds forming between the starch components (amylose and amylopectin) thus reducing the tendency of the gelatinised molecules to retrograde to a more ridged state. DT on the other hand is thought to prevent water redistribution within and between the protein components after cooking (Boutte & Skogerson, 2004); however, there is no information on the effect of combining the Dim and DT together.

In this part of the thesis, combinations of the ingredients shown in Chapter 4 to have anti-staling properties were evaluated with the aim of determining if Dim and DT could act together to reduce staling in hotcakes or to determine if either in combination with the BG could decrease of prevent firming due to staling in hotcakes.

5.2 Materials and Methods

5.2.1 Materials

Basic hotcake ingredients used in this chapter were listed in Table 2. Three anti-staling ingredients from previous work (Chapter 4) were chosen for this combination study. They were the emulsifiers Dim and DT and enzyme BG.

The dosage of enzyme was kept the same as suggested. The dosages of emulsifiers were studied at two levels in this chapter. They are the dosage modified by the Company's formulation (dosage 1: 1.5% for Dim and 3% for DT, the dosage used in screening trial,) and

the lower level suggested by the ingredients suppliers (dosage 2: 0.5% for Dim and 1% for DT).

The anti-staling combinations used in the chapter were listed below:
Dimodan + enzyme BG @ dosage1 = DE1
Dimodan + enzyme BG @ dosage2 = DE2
Dimodan + enzyme BG + Datem @ dosage1 = DET1
Dimodan + enzyme BG + Datem @ dosage2 = DET2

5.2.2 Mixing method and quality control

Four types of the anti-staling ingredient combinations were added into the control formulation (Cont, Std formulation without Lecithin and Grindsted®MONO-DI). Mixing method and quality control was listed in 3.3.1.

5.2.3 Cooking method

Cooking method was listed in 3.3.2.

5.2.4 Posting cooking treatment, packaging and storage condition

Post cooking treatment, packaging, and storage conditions were listed in 3.3.3.

5.2.5 Sampling method

Sampling method was listed in 3.3.4. The combination trial was finished within two weeks; one batch of experiment for each formulation was carried out. The total measurements for each formulation in this chapter were 24 (2 samples x 12 hotcakes).

5.5.6 Texture measurement

Texture measurement was listed in 3.3.5.

5.2.7 Statistical methods

The statistical method was listed in 3.3.6.

5.3 Results and discussion

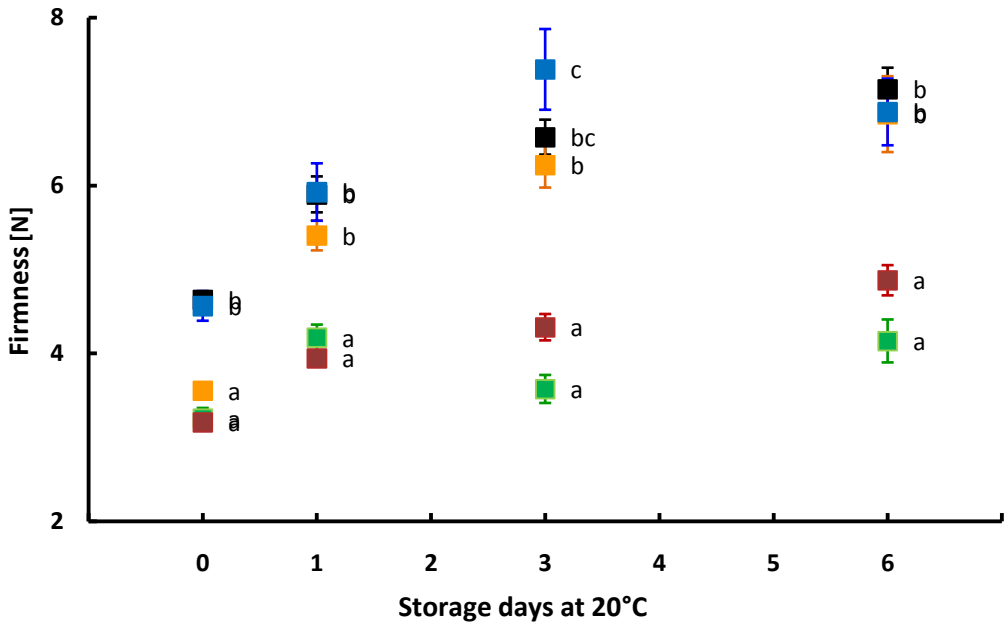


Figure 7 The changes in firmness of hotcakes made in combination trial

■Std ■DE1 ■DE2 ■DET1 ■DET2

The solid squares in different colours represent the average firmness of the hotcakes made with different formulations on each testing day. Values in a particular block not showing the same letter (a-c) are significantly different ($P<0.05$). The firmness scale of this graph is from 2 to 8 to emphasis the difference between each formulation.

Firmness values from the combination trial were presented in Figure 7. Two groups of treatments are presented. The first group includes the Std formulation, and the combinations of DE which had the ingredients of Dim and the enzyme BG at low and high dosage. The second group is the combinations of DET which had the ingredients of Dim, DT and BG at both dosages. The firmness values of the hotcakes in first group made without DT are significantly higher compared to the second group with DT added at all testing days except for the firmness of DE1 on day zero. This treatment was a little firmer than the corresponding treatment containing DT but the difference was not significant.

This shows that adding the monoglyceride (Dim) together with enzyme BG did not significantly reduce the rate of firming or the absolute firmness of the hotcakes compared with the standard treatment. The absence of a significant effect of DE formulations may be

due to insufficient time for the enzyme to act in this trial. Or alternatively, the monoglyceride may have blocked some of the effects of the enzyme. In the DE1 and DE2 formulations, Dim and the enzyme BG are combined together, the thermostable α-amylase hydrolyse the complexed amylose only occur at high temperature such as during cooking of hotcake or at very slow rate during storage (Putseys, Lamberts, & Delcour, 2010). Therefore, by adding emulsifiers in combination with the enzyme treatment only a small proportion of starch susceptible to enzyme digestion may have been present and as a result the reduction in staling may have been small.

Compared with the firmness values of hotcakes in Figure 5, this result did show that the combination of monoglyceride and enzyme produced hotcakes that were less firm than those made when these additives were used separately, this result was also found by (Valjakka, et al., 1994).

Adding Datem to the combination formulation produced significantly less firm hotcakes. Stampfli and Nersten (1995) also found similar results and they considered that the softer texture provided by Datem (DT) was the result of more air cells in the crumb which are retained by the gluten films, the formation and stability of which is assisted by the DT. It therefore appears that the firmness of DET formulation probably had more air cells and therefore had lower firmness than the formulation that only contains monoglyceride (DE combination).

Higher dosages of Dim (DE1) produced softer (less firm) hotcakes than lower dosages of Dim (DE2). This indicated when higher dosage of monoglycerides were added to the batter system, more monoglyceride is available to interact with amylose, and more starch complexes were formed, and provided lower firmness.

No statistical differences were found between the dosages of DET combination. Although higher dosage of DT produced hotcakes with higher firmness for the fresh hotcakes measured on day 0 and day 1, the firmness of lower dosage of DT hotcakes had higher firmness when measured on day 3 and day 6, this inconsistent result may be due to the inconsistent testing condition.

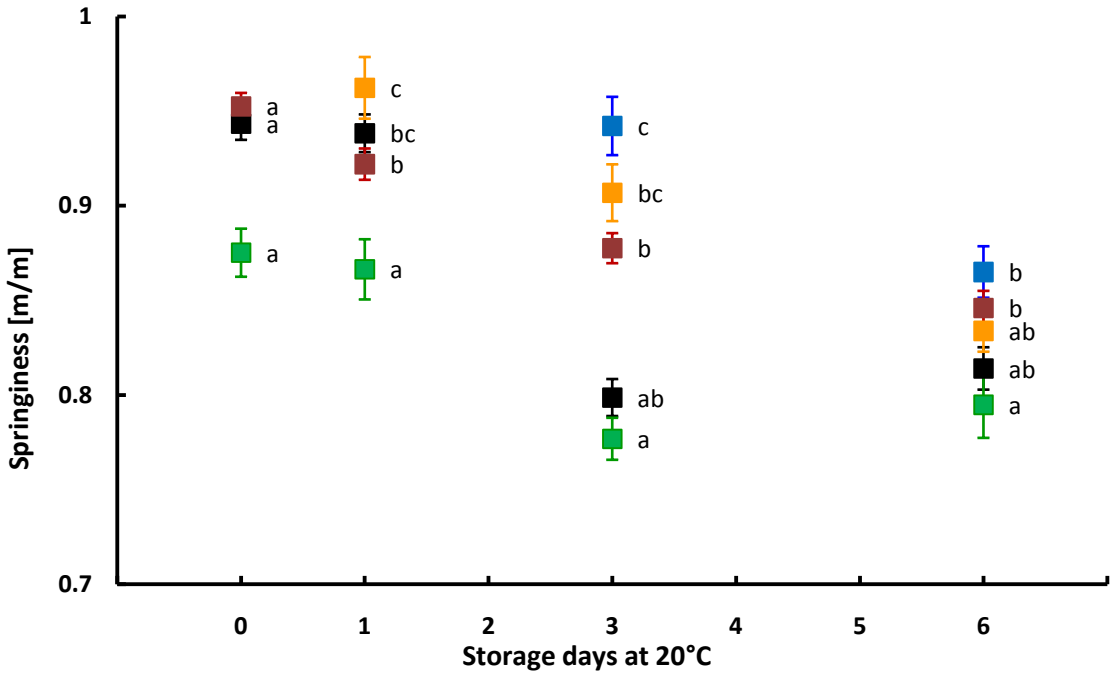


Figure 8 The changes in springiness of hotcakes made in combination trial

Std DE1 DE2 DET1 DET2

The solid squares in different colour represent the average springiness of the hotcakes made with different formulations on each testing day. Values in particular block not showing the same letter (a-d) are significantly different ($P<0.05$). The springiness scale of this graph is from 0.7 to 1 to emphasis the difference between each formulation.

The springiness values of the hotcakes made from each anti-staling combination formulation are shown in Figure 8. Combinations of DE1 and DE2 provided extremely high springiness (1.70 and 1.87 respectively, data points were out of scale in Figure 8) on day zero. The springiness of these two formulations decreased by day 1 but the values were still greater than other formulations.

According to the Figure 7 and Figure 8, DE combinations provide higher firmness and higher springiness than the DET combination. Since the amount of Enzyme in all the formulation is the same apart from the Std, the difference between DE and DET combinations is caused by Dim (monoglyceride) and Datem. As it was said in 3.4.5, the differences of hardness and springiness are greatly affected by the air cell structure of the hotcakes. In DET combination, Datem had the ability to interact with gluten strands and form more air cells in the crumb.

According to this theory, the hotcakes made with DET combination had more air cells and therefore were softer and less spongy than the hotcakes made with DE combination.

After 6 days of storage a proportion of the amylose is likely to have retrograded or become bound to the amylopectin firming and reducing the elasticity of the food structure and as a consequence, reducing the springiness values of the DE formulations to the similar value as DET and Std formulations. So, by day six, no statistically significant difference was evident between the DET2, DE1, DE2 and the Std formulations.

The DET1 formulation provided the lowest springiness throughout the testing period. This low springiness may be due to the over dosing of the emulsifiers, especially DT. This may have been due to the formation of too many large bubbles and the reduction in solid material to support the crumb or the formation of a squashy and less elastic product when the batter was cooked.

Staling rate and springiness decreasing rate were calculated from the data of Figure 7 and Figure 8 and the corresponding results were listed in Table 6 and Table 7 respectively.

Table 6 Staling rate analysis for each formulation in combination trial

Formulation	Linear equation of firmness VS testing day	Staling rate (N/day)	R^2
Std	y = 0.37x + 4.8	0.37	0.84
DE1	y = 0.48x + 3.8	0.48	0.78
DE2	y = 0.36x + 4.9	0.36	0.58*
DET1	y = 0.09x + 3.5	0.09	0.28*
DET2	y = 0.25x + 3.2	0.25	0.90

"y" represents the firmness of the corresponding formulations; "x" represents the testing day. "R^2" is the coefficient of determination. "*" indicates the equation of the treatment is poorly represents the data because of the low R^2 value.

The slope of the linear equation of firmness versus testing day represents the staling rate of each formulation. In table 6, values for the coefficient of determination (R^2) were high and above 0.75 except the DE2 treatment (0.58) and DET1 treatment (0.28); firmness values of

Std, DE1 and DET2 treatment are therefore closely related to the age of the hotcakes. Among the closely related to the age of hotcakes, DET2 combination showed lowest staling rate (0.25 N/day).

Table 7 Springiness decreasing rate for each formulation in combination trial

Formulation	Linear equation of springiness VS testing day	Springiness reducing rate (day^{-1})	R^2
Std	y = -0.024x + 0.96	-0.024	0.70
DE1	y = -0.110x + 1.49	-0.110	0.53
DE2	y = -0.127x + 1.62	-0.127	0.52
DET1	y = -0.015x + 0.88	-0.015	0.65
DET2	y = -0.017x + 0.96	-0.017	0.95

"y" represents the springiness of the corresponding formulations; "x" represents the testing day. "R^2" is the coefficient of determination.

Firmness increased with time whereas springiness decreased, however the relationship with hotcake age and the decrease in springiness is less well defined as is seen from the lower R^2 values with springiness compared to firmness. Therefore, although springiness and firmness seem to be aspects of textural changes that can be associated with staling that occurs as the hotcakes age, firmness would appear to be the better predictor of staling.

5.4 Conclusions

In this chapter it has been shown that combinations of Datem and a starch hydrolysing enzyme can interact to provide an effective system to reduce the rate of staling of hotcakes. The combination of Dim at a concentration of 0.5% (FB); Datem at a concentration of 1% (FB) and the enzyme at a concentration of 0.0075% (FB) resulted in hotcakes that firmed at about 1/3 the rate of the standard formulation (0.25N/day for the DET2 combination formulations compared with the Std staling rate of 0.37N/day) and the combination formulation also had much lower firmness when freshly made. The much lower firmness and greater springiness of the hotcakes incorporating DT and Enzyme suggests that the combination of Datem, Dimodan and enzyme (DET2) gave the best performance of all the combination treatments. Although the difference between DET1 and DET2 for firmness was

not significant, at later storage days the DET1 treatment had the lowest firmness, probably a consequence of being softer, and the lowest rate of hardening with storage; however, the lowest firmness and springiness are not very desirable, if cost is a consideration the small difference in firmness between the DET1 and DET2 treatments can probably be ignored to effect a small saving in the cost of Dimodan and Datem's usage. Overall, the project has identified a combination of food grade ingredients that approximately extend the shelf life of VDFF hotcakes to 1.5 times than the original shelf life which caused by staling with only a bare minimum of reformulation.

The major barrier to the shelf life of hotcakes is now microbiological contamination and this will be briefly addressed in the next chapter.

Chapter 6 Mould control study

6.1 Introduction

Hotcakes are a high moisture product that typically have a water activity of greater than 0.95 (Sutton & Rout, 2010). For this reason they are highly susceptible to microbial spoilage when held at room temperature and on the supermarket shelf typically have a useable life of less than 5 days before microorganism growth becomes unacceptable for human consumption. Based on hurdle technology as discussed in the literature review, three possible approaches to extend the shelf life of hotcake products at ambient temperature are: 1) to incorporate the right preservatives at right dosage into the formulation; 2) to modify of packaging techniques; and 3) to manufacture and pack a sterile product in a sterile environment.

In the Std VDFF formulation, calcium propionate is used to assist with the control of microorganisms, however, it was clear from initial tests carried out in a 20°C room mould colonies were apparent by day 6 or so and within 3 days at 30°C. This means that the reduced rate of staling developed in this work cannot be effectively used to increase the shelf life of the product. For this reason, works to investigate methods of reducing or inhibiting mould growth were investigated.

The appropriate preservative and its dosage level used in hotcake product was checked in this chapter to make sure all the tangible factors were considered to extend the shelf life. The suitable preservative and its dosage depend on the pH value of the product; therefore, the pH value of hotcake was evaluated.

Shelf life can also be extended by controlled atmosphere (CA) packaging technique, because by CA packaging, with the appropriate permeable packaging material and the techniques of vacuum packaging, CO_2 or N_2 gas flush or oxygen absorbers, the levels of O_2 residual can be reduced to less than 1%, and aerobic moulds are not able to grow without enough O_2. Therefore the shelf live can be extent (Guynot, et al., 2003).

Although controlled atmosphere packaging was investigated in this work for comparison, it was unlikely to be used for the final marketed product due to the public perception that the

shift from a light weight polythene pack to a controlled atmosphere pack was associated with a more artificial product. When VDFF marketed identical products with identical labels, product packed in a flexible polythene bag outsold that packed in a rigid barrier pouch by 3 to 1 with comments from the public that the barrier pouch pack represented a less desirable "artificial" product. Oxygen absorber sachets to temporarily reduce oxygen levels in the standard VDFF packs was considered as a possible solution to the problem that fitted within the constraints of the work.

This work was constrained by the desire to retain the original hotcake formulation and packaging. A range of methods, some of which would not meet the company criteria discussed above were compared. On one hand to determine the duration of an acceptable product when mould growth was inhibited and on the other to determine how much extra shelf life could be obtained using methods acceptable to VDFF.

6.2 Materials and methods

6.2.1 pH

The pH value of a product determines the choice of the preservative and the dosage of the preservative (Suhr & Nielsen, 2004). The pH value of fresh hotcakes was determined by the 14.022 Potentiometric Method (AOAC, 1980). To measure the pH value, 10g of fresh hotcake samples were weighed into clean, dry Duran bottles and 100mL of Reverse Osmosis hyperfiltration (RO) water at room temperature (20°C) was added. The slurry was shaken for 30min on a Chiltern flask shaker at speed 6 until the hotcake particles were evenly suspended and mixture was free of lumps. The mixture was allowed to stand for 10min before decanting the supernatant and immediately measuring its pH.

6.2.2 Packaging

The standard VDFF packaging for their hotcakes was a Low Density Polythene (LDPE) bag with loose closure clip. This material was highly permeable to oxygen ($150 cm^3 \cdot mm/m^2 \cdot day \cdot atm$) and CO_2 and is generally unsuitable for controlled atmosphere packaging. Four types of packaging products and atmosphere control techniques were compared with the standard VDFF packaging. They included 1) the VDFF's packaging to

which an O2 absorber sachet (Environmental Control Ltd.) was added (VDFF packaging + O2 absorber); 2) a gas barrier pouch (Contour Packaging Ltd.) with an oxygen permeability of 50cm^3·mm/m^2·day·atm that was flushed with a controlled atmosphere of 100% CO_2 (BP + CA); 3) BP + O_2 absorber sachet; and 4) BP + O_2 absorber sachet + CA treatments (Table 8).

The hotcakes were made according to VDFF's Std formulation at its manufacture site according to the procedure which was introduced at 3.2 and sent to Massey University in frozen condition and repacked after defrosting according to the above packaging treatments. After the hotcakes were packed into the bags they were manually sealed using an impulse heat sealer. All the CA packages were gassed and sealed using a Cryovac Grace Packaging machine (Type Multivac A300/42, Sepp Haggenmuller KG, Wolfertschewenden, Germany).

6.2.3 Gas analysis

O_2 level in the treatment packs was analysed at the end of the trial period to test the quality of the packaging technique. If the packaging technique can keep the O_2 level under 1% until the end of the trial period, the packaging technique is capable to extent the shelf life.

The concentration of O_2 in the 1 ml of gas sample which was removed from each package was measured using a miniature infrared CO_2 transducer (Analytical Development Co, Hoddesdon, UK), in series with a paramagnetic O_2 sensor (servomex) using N_2 as carrier gas at 35ml.min^{-1}. The equipment was calibrated with commercially produced β-standard 0.49 ± 0.01% CO_2 (BOC, New Zealand). Output signals were linear over the range analysed and recorded using an HP 3396A (Hewlett Packard, USA) integrator.

6.2.4 Mould colony observation

During storage, visual observations of the mould colony development at 20°C storage condition were conducted daily at IFNHH temperature control storage room. Two bags of packed hotcake of each treatment were stored at 20°C storage room, the storage time of the first mould colony appeared at hotcake surface was recorded for each treatment.

6.2.5 Second trial of packaging techniques

The second trial of packaging methods was identical to the first trial with the exception that no combination of VDFF packaging + O_2 absorber and combination of CA + O_2 absorber were tested in this trial and by adding single BP packaging technique to be a Control to compare with other techniques used in the laboratory condition. The analysis of O_2 content for each type of packaging treatment was conducted using sterile conditions (flaming in ethanol) before microbiology Total Plate Count (TPC) test.

6.2.6 Microbiology TPC test

After the gas analysis, the same packs of hotcakes in the second trial of packaging techniques were delivered to the microbiology department at IFNHH for the TPC test for colony forming of aerobic and anaerobic organisms.

6.2.6.1 Preparation of samples

Outside of the sample bag was sprayed down with 70% ethanol; left for 30seconds or so, and then wiped down with a tissue. Scissors and tweezers were sterilised by flaming in ethanol and used to cut the package. The sample from top and middle hotcake in a pack was selected by sterilised equipment; weighed 25g into a sterile stomacher bag; added with 225ml of peptone diluents (5g peptone per litre); and stomached the sample for 2 minutes.

6.2.6.2 Testing

The stomached samples were diluted to appropriate dilutions from their respective shelf life days. Dilutions were carried out also using peptone water in 9ml volumes and transferred 1ml of sample to the 9ml then vortexing the bottle. 0.1ml amounts of the appropriate dilutions were pipetted onto prepoured and dried Standard Plate Count Agar plates and spread with a sterilised spreader. Duplicate plates were done for each dilution for aerobic and anaerobic test. The plates were then inverted and incubated at 30°C for 48hours under aerobic and anaerobic condition. The anaerobic plates were inverted in sealed anaerobic jars with an anaerobic gas pack to reduce oxygen and maintain anaerobic conditions.

6.2.6.3 Counting

Plates that had colonies between 30 ~ 300 were counted and counts averaged from duplicate plates. The average counts were then multiplied by 1/dilution factor on the plate counted then multiplied another factor of 10 to take into account that only 0.1ml of the sample was plated. Results reported as colony forming unit per gram sample (CFU/g).

The tests were conducted on day 0 (the day in which they were repackaged), on day1 (1day after repackaging) and on days 3 and 5 after repackaging. A limit of $1x10^5$ CFU/g is considered the safe upper limit for foods (Jay, et al., 2005); therefore, once the TPCs of the hotcakes reached or passed this limit, no further TPC was conducted.

6.3 Results and discussion

6.3.1 pH value of hotcake and the choice of preservative

pH values of the fresh hotcakes made from different anti-staling formulations were determined during screening trial. Control products had the highest pH value of 7.72. The hotcakes made with DT had the lowest pH value around 7.4, the pH drop in DT formulation due to the minor acidity of the Diacetyl Tartaric acid. The hotcake made with emulsifiers of Dim and SSL had similar values of 7.5 and the hotcake made with enzyme (BG, Pro and AF) had similar values around 7.6. Generally the anti-staling ingredients did not have a significant effect on pH value. Hotcakes are a low alkaline product with the pH value about 7.5. This alkaline value is contributed to by the chemical leavening agents (sodium bicarbonate and sodium aluminium phosphate) added to the formulation.

The low alkalinity of the hotcakes suggests that sorbates and propionates should be good preservatives for hotcakes (Suhr & Nielsen, 2004). Sorbates have been shown to be twice as effective as propionates to control mould growth (Suhr & Nielsen, 2004). However the food legislation of New Zealand allows a maximum dosage of sorbates of 0.12%, less than 1/3 that of propionates (0.4%) (FSANZ, 2007). At the maximum dose rates, sorbate is not as effective as propionate in hotcake products. It is suggested by the company Mycoban (Inc., 2007), that the dosage of calcium propionate in low alkaline baking products sufficient to retard microbial growth is 0.375% of the batter weight and this is within the maximum dosage

allowed by New Zealand food legislation. Due to time constraints this treatment was not examined in this chapter but was added to the commercial trial and described in Chapter seven.

6.3.2 First trial of packaging treatments

The results of O_2 content and the appearance of mould colonies on the surface of the hotcakes at days till the appearance of visible colonies are shown in Table 8. The O_2 content of VDFF original packaging was found to be 20 ~ 21% and was close to the normal atmospheric O_2 composition, this because the pack was not tightly closed and the gas exchange rate was high. The gas atmosphere in the VDFF packaging + O2 absorber had an O_2 content of 1.2 ~ 4.3%. This is about 10% of the loose closure, however, it is still higher than the critical 1% limit to inhibit mould growth and as a result the shelf life was similar to the control treatment of the standard VDFF packaging. This high O_2 content level is due to the high permeability of LDPE material to O_2.

The O_2 contents for the treatments of BP bags with either CA or the O2 absorber or the combined CA+O2 absorber were all lower than the critical value of 1%, therefore, these three treatments were expected to result in a longer shelf life than the standard VDFF polythene bags. In these three treatments no visible growth of mould colony was observed until day 10.

Table 8 Effects of packaging techniques on O_2 content and mould spoilage of hotcakes from the 1st packaging trial

Packaging techniques	O_2 content (%)	Days to visible mould growth
VDFF packaging	20 ~ 21	5
VDFF packaging + O_2 absorber	1.2 ~ 4.3	5
BP + CA	0.33 ~ 0.74	NVG until day 10
BP + O_2 absorber	0.15 ~ 0.24	NVG until day 10
BP + CA +O_2 absorber	0 ~ 0.1	NVG until day 10

NVG = No visible growth of mould colony

6.3.3 Second trial of packaging treatments

To determine the levels of contamination, total plate counts (TPC) of colony forming of both aerobic and anaerobic organisms were conducted on the second packaging trial. The TPCs of

aerobic and anaerobic for each treatment are the same on each testing day, therefore, only a combined TPC were presented in the Table 9.

Table 9 Effects of packaging treatments on O_2 content and mould spoilage of hotcakes from the 2nd packaging trial

Packaging Techniques	O_2 Content (%)	TPC on day 0 (CFU/g)	TPC on day 1 (CFU/g)	TPC on day 3 (CFU/g)	TPC on day 5 (CFU/g)
VDFF Packaging	NA	<10	NA	680	$1.1x10^5$
BP	18 ~ 20	<10	400	$1.5x10^4$	$6X10^7$
BP + CA	0.33 ~ 0.74	<10	<10	$1.7x10^3$	$6x10^5$
BP + O_2 absorber	0 ~ 0.1	<10	150	$1.3x10^4$	$3x10^6$

The data of VDFF packaging were obtained from VDFF shelf life evaluation report from Formula Foods Corporation Limited (Sutton & Rout, 2010). "NA" = no available data .cfu=colony forming unit

The TPCs of all the packaging techniques are the same on day 0. This indicates the initial detectable microorganisms are the same for each packaging technique. On day1, BP technique had the highest TPC then followed by BP + O_2 absorber technique. BP + CA technique had the lowest TPC. This ranking continued until the last testing day (day 5). The BP bags without an oxygen depleted atmosphere had an O_2 content between 18 and 20%. This is slightly lower than the normal atmosphere composition (21%) and may represent microorganism respiration. The O_2 content of BP + CA treatment and BP + O_2 absorber treatment were less than 1%, indicating that the packages were well sealed.

Compared with VDFF Packaging, none of the treatments provide longer shelf life and the VDFF packaging had the lowest TPC from day 3 onwards. The much greater CFU counts for the BP packs on a given test day, compared to the standard packaging, probably represents the effect of inadvertent contamination by microorganisms as the hotcakes were transferred from the standard VDFF packs to the BP's for the packaging test. It is also clear that the in most cases the original contamination was quite low at <10 organisms per gram sample but they multiplied very rapidly over the test period. The reason for BP + O_2 absorber treatment having a higher TPC than the BP + CA treatment may be due to inadvertent contamination in the surface of the O^2 absorber sachet.

The BP bags can retain a controlled atmosphere for at least 10 days and when the oxygen level in the bag is controlled can also increase the shelf life estimated as the number of days before the TPC reaches 1 x 10^5 by approximately 1 day compared to the BP treatment without an oxygen depleted atmosphere (Table 9).

6.4 Conclusions

This section of the work has shown small decreases in the rate of microorganism growth can be achieved by controlling the atmosphere in the packaging. Due to the recontamination which occurred during repacking the hotcakes in the laboratory environment, the effectiveness of reducing the O_2 rate in the packaging to extend the shelf life in terms of microbiological spoilage would be tested in the manufacture commercial packaging environment in commercial trial.

In addition, the pH value of hotcake products were determined at about 7.5, although not tested in this series of experiments, it may also be useful to increase the level of calcium propionate to the maximum allowable level (0.375% batter weight) to maximize this barrier to micro-organism growth in commercial trial chapter seven.

Chapter 7 Commercial trial

7.1 Introduction

In chapter five, it was shown that the rate of staling of hotcakes was reduced in comparison with the VDFF's standard formulation by increasing the proportions of the emulsifier Dim and DT; and adding an enzyme BG to the hotcake formulation. This new formulation including Dim, DT, BG and together with VDFF's original dosage of lecithin (Soy lecithin, Ultralec P) which assisted the release of the hotcakes from the platters after cooking was used in a trial conducted at the VDFF manufacturing site on 5th December 2011 using a commercial batch size of 140kg and commercial processing equipment. Together with this new formulation, two other modified formulations based on the New formulation suggested by in-house expert were conducted, one using a high dosage (level compared with level) of lecithin (L) that may cause a finer and perhaps more desirable texture in the cooked hotcakes; the other treatment used a high dosage of Dim (D) (level compared with level) was conducted to determine if increasing the level of monoglyceride in the hotcakes had the same effect as adding the lecithin and would also result in further decreases in the rate of staling. Dim has a greater proportion of the monoglyceride component compared to the mixture of mono and diglycerides in the Grindsted®MONO-DI currently used by VDFF. The monoglyceride component is the most important in reducing the rate of staling in these products.

Furthermore, as suggested by antimicrobial studies discussed in chapter six, two treatments, one using a high dosage of calcium propionate preservative in the VDFF standard formulation and the other incorporating O_2 absorbers with barrier pouch packaging were used.

The products using 3 formulations and two preservation techniques were analysed at Massey University for the textural attributes of firmness and springiness, the sensory qualities and the shelf life in terms of microbial contamination (TPC test).

The objectives of this trial were to determine if the anti-staling properties of the new formulation developed in the laboratory trials or the proposed effects of the increased proportions of lecithin and Dimodan were translated into improved hotcake quality in commercial production. Secondly, to determine if the growth of microorganisms could be

markedly reduced during storage at ambient conditions by increasing the level of preservatives or decreasing oxygen levels in the packaging.

7.2 Materials and methods

7.2.1 Materials

The formulations used in commercial trial were listed in Table 10.

Table 10 Commercial trial formulations

Ingredient/Kg	Std	HP	N	L	D
water	60	60	60	60	60
flour	44	44	44	44	44
sugar	13	13	13	13	13
buttermilk powder	10	10	10	10	10
egg	5	5	5	5	5
canola oil	3	3	3	3	3
baking soda	0.9	0.9	0.9	0.9	0.9
SALP	0.9	0.9	0.9	0.9	0.9
maize starch	0.9	0.9	0.9	0.9	0.9
Grindsted®MONO-DI	0.466	0.466	None	None	None
lecithin	0.466	0.466	0.466	0.699	0.466
salt	0.34	0.34	0.34	0.34	0.34
calcium propionate	0.215	0.535	0.215	0.215	0.215
vanilla	0.027	0.027	0.027	0.027	0.027
Dim	None	None	0.22	0.22	0.33
Datem	None	None	0.44	0.44	0.44
Enzyme /g	None	None	3.3	3.3	3.3

The listed formulations were VDFF original formulation (Std); Std formulation with higher dosage of preservative Calcium Propionate (HP); the best combination from laboratory trials (N): low dosage of DET combination (0.5% of Dimodan PH 320/B-M; 1% of Palsgaard Datem 3502; and 0.0075% of Novamyl 10000 BG); 1.59% of lecithin (L) based on the best

laboratory combination of N; and 0.75% of Dimodan (D) based on the best laboratory combination of N. The percentages are all based on flour weight.

7.2.2 Production procedure

Hotcake manufacture production procedure was listed in 3.2.

7.2.3 Packaging techniques

After manufacture, two types of packaging methods were applied to the hotcakes: the first method was the VDFF's original packaging technique (LDPE bag with a loose clip closure). In the second method, the second best packaging technique (BP + O2 absorber) identified in laboratory scale trials (Table 9) was chosen as the modified technique for the extension of shelf life study. Addition of the controlled atmosphere CA (100% CO_2 flush) would be preferred but this equipment was not available at the VDFF plant. A bag of hotcakes in VDFF's original packaging were put into a barrier pouch bag with oxygen absorber sachet inside and heat sealed to prevent gas exchange with the atmosphere. Retaining the original packaging of the hotcakes prevented contamination occurring through product transfer between packaging while the relatively high CO_2 and O_2 permeability of the original polythene bags rapidly allowed the depletion of oxygen due to the scavenging sachet.

7.2.4 Commercial trial product evaluations

After packaging, all the products were stored at -18°C for about 10 hours before being transferred to Massey University the next day for further tests. The products were defrosted overnight before determining differences in texture and staling rate at a room temperature of close to 20°C. The texture and microbiology tests were conducted on day 0, immediately after thawing, on day1, 3 and day 5.

7.2.4.1 Texture analysis

The texture analysis methods including sampling method, TA texture analysis, and statistical methods were listed in 3.4.4; 3.4.5; and 3.4.6 respectively.

7.2.4.2 Gas analysis for packaging study

Gas analysis method was listed in 6.2.3.

7.2.4.3 Microbiology TPC test

The microbiology TPC test methods including preparation of sample, testing and counting were listed in 6.2.6.

7.2.4.4 Sensory test

The sensory tests were conducted at IFNHH Sensory Lab. In sensory tests, two types of question were asked of the panellists. The first question was to determine if the panellists could differentiate between fresh hotcakes and hotcakes that had been stored for 3 days (maximum safety human consumption condition after the microbiology TPC test); the second question was to determine if the panellists could differentiate between formulations; these two questions are Sensory Difference and Similarity Tests; triangle test was chosen as a testing method (Maximo C. Gacula, Singh, Bi, & Altan, 2009). In the triangle test, the properly coded samples are presented simultaneously to the panellists. Two of the samples are identical, and the remaining one is different. The order of sample presentation was balanced throughout the entire experiment.

In the first question, four sets of samples (formulation Std, N, D & L) were presented to the panellists, each set containing one fresh hotcake sample and two samples of the same formulation that had been stored for 3 days or one sample of 3 days' old and two fresh samples from same formulation. The samples were coded with 3 random numbers; the fresh (thawed overnight) and the 3 day old samples were presented in different orders to the panellists.

In the second question, four formulations (Std, N, D &L) were combined with each other in six different pairs: Std & N, Std & D, Std & L, N & D, N & L, and D & L. Six sets of samples were presented to the panellists, in each set, two of the samples are from one formulation, and the remaining sample was from a different formulation. In order to know in what way and by how much the odd sample is different from the others, the indication of the degrees of difference of hardness and sponginess which are the firmness and springiness in texture

analysis were asked after the triangle test which is to pick the odd sample. All the samples tested in the second question were presented to the panellists one day after thawing. This testing time is within the normal consuming period for hotcakes, the results were intended to determine whether the customer can perceive differences between formulations.

The questionnaire used is shown in the Appendix, 32 panellists for each question were recruited for this work.

7.3 Results and discussion

7.3.1 Texture

7.3.1.1 Firmness

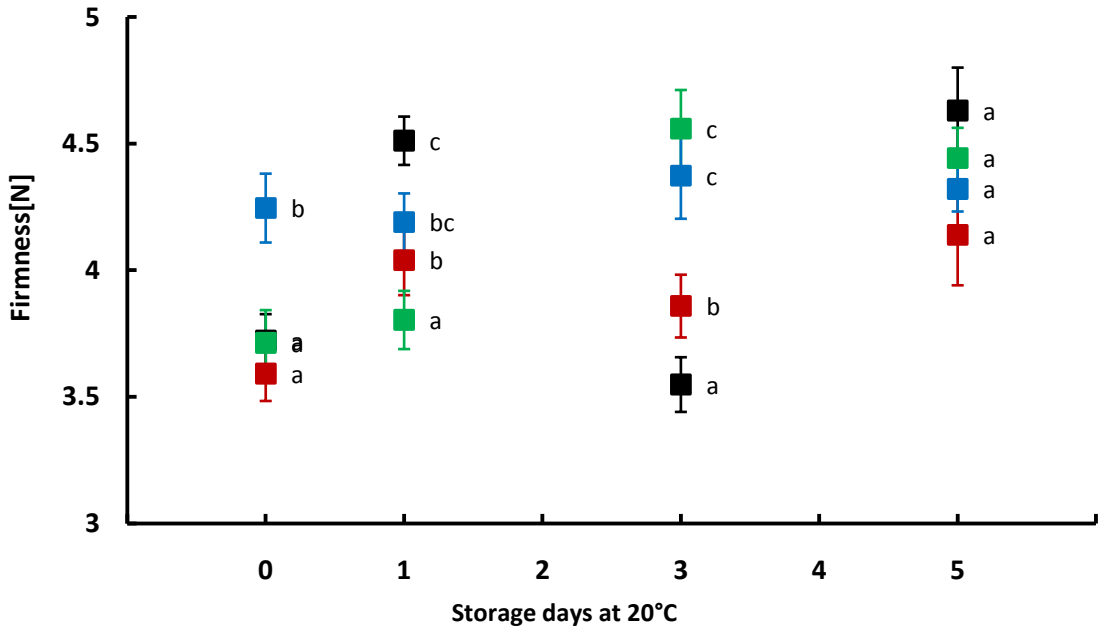


Figure 9 The changes in firmness of the hotcakes made in commercial trial

Std N L D

The solid squares represent the average firmness of the hotcakes on each testing day and the various colours represent the different formulations. Values in a particular block not showing the same letter (a-c) are significantly different ($P<0.05$). The firmness scale of this graph is from 3 to 5 to emphasis the difference between each formulation.

The firmness values for the hotcakes made from each formulation were presented in Figure 9. Generally, the firmness values of hotcakes made at VDFF were between 3.5 and 5 Newton. They were less firm and show less change over time than those made at lab scale which

ranged from about 4 to 11 Newton as they staled in chapter 4, Figure 5 and about 3.5 to 7.5 Newton as they staled in chapter 5, Figure 7.

For the product made in the trial at VDFF, there was no evidence of the marked changes in firmness with time that occurred for hotcakes made in the laboratory, differences between the formulations for this work were also small. The lower final firmness values in this commercial trial may be due to the commercial cooking conditions: the lower cooking temperature and longer cooking time may create larger air cells in the product than the hotcake cooked in the lab. It is thought that the large gas bubble that appeared in the commercially produced hotcakes resulted or contributed to the reduced firmness of that product.

In Figure 9, although not significantly different from the other formulations, the hotcakes made using the new (N) formulation were among the least firm when measured on day 0 and day 5. The hotcakes made with the increased level of Dim had an initial firmness of about 4.2N which is statistically higher than other formulations but firmness showed little change during storage. Increasing the level of Lecithin results in a softer texture for the fresh sample; but the final firmness is higher than for the N and D formulations. The analysis for staling rate of the commercial trial was stated in Table 11.

Table 11 Staling rate analysis for each formulation in commercial trail

Formulation	Linear equation of firmness VS testing day	Staling rate (N/day)	R^2
Std	y = 0.14x + 3.87	0.14	0.55
N	y = 0.085x + 3.67	0.085	0.60
L	y = 0.15x + 3.54	0.15	0.99
D	y = 0.02x + 4.19	0.02	0.65

The firmness values for the hotcakes tested on day 3 were considered as outliers because the values did not follow staling trend and did not fit into linear equations. In the linear equation, "y" represents the firmness of the corresponding formulations; "x" represents the testing day. "R^2" is the coefficient of determination.

The slope of the linear equation of firmness versus testing day represents the staling rate of each formulation. However, except for high lecithin dose formulation (L) which had and R^2 value of 0.99, the relationship between firmness and time was less clear due to their lower R^2 values.

All the formulations containing amylase (N, L, and D) had slower firming rates than Std formulation which did not contain amylase. This is not surprising as the amylase used in the formulation was intended to slowly digest the starch as the product aged, counteracting the usual firming that occurs as the product stales.

Among these three modified formulations, high dosage of Dim (D) had the slowest staling rate (0.02N/day) during storage. The New formulation (N) staled at the rate of 0.09N/day and high dosage of lecithin (L) had the fastest rate of staling (0.15 N/day) that was similar to that for the Std formulation which is 0.14N/day. Compared with the New formulation, the high dosage of Dim treatment may have formed more amylose - monoglyceride complex, resulting in less amylose participating in retrogradation during storage and therefore resulting in a slower staling rate.

The formulation containing a high dosage of lecithin showed a faster rate of staling than the formulations that contained a lower dosage of lecithin. This indicates that lecithin is not very effective in reducing the rate of staling in hotcakes. Similar results were also found in the wheat bread study at 1.5hrs proofing time, in which the firmness of the bread containing lecithin staled faster than the bread containing monoglyceride (Gomez, et al., 2004). Both lecithin and monoglycerides are regarded as emulsifiers but at similar dosage the monoglyceride forms more complex with amylose and possibly amylopectin.

7.3.1.2 Springiness

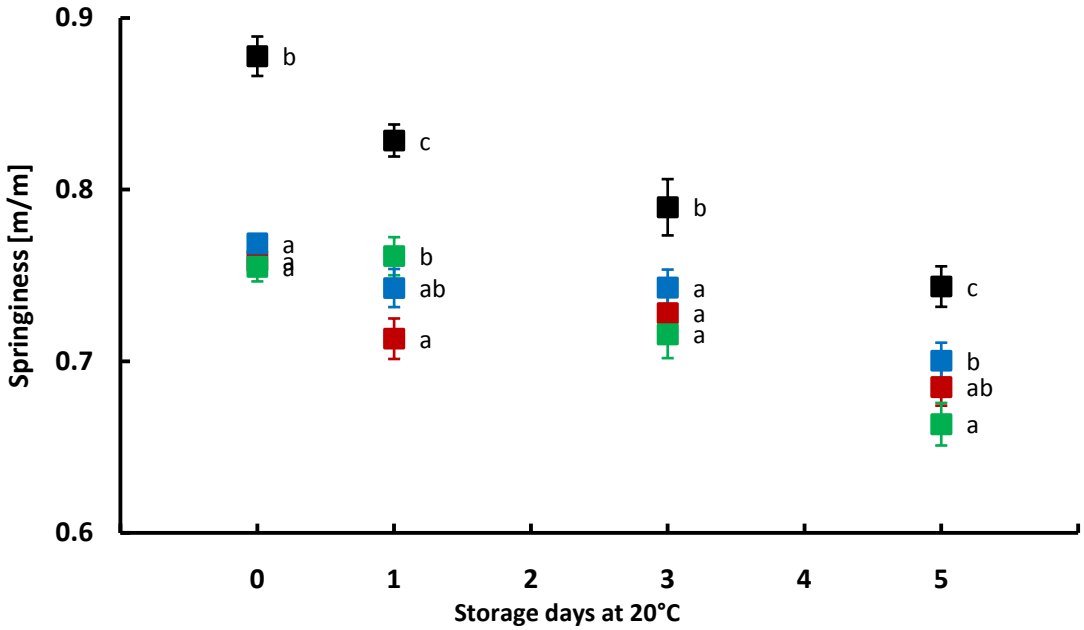


Figure 10 The changes in springiness of hotcakes made in commercial trial

Std N L D

The solid squares represent the average springiness of the hotcakes on each testing day and various colours represent the different formulations. Values in particular block not showing the same letter (a-c) are significantly different ($P<0.05$). The springiness scale of this graph is from 0.6 to 0.9 to emphasis the difference between each formulation.

In Figure 10, at all measurement times the hotcakes made from Std formulation were springier and springiness decreases faster with age than the hotcakes made using the modified formulations (N, L and D). The springier texture of Std formulation may be due to the absence of softening of the hotcake matrix that may occurs in the formulations to which enzyme was added. The springiness values of the N, L and D formulations throughout the trial show no consistent differences and are probably similar although some small significant differences are present.

The rate of reduction of springiness with time calculated as the slope of the linear regression of springiness with time for the different formulations is shown in Table 12.

Table 12 Springiness reducing rate analysis for each formulation made in commercial trial

Formulation	Linear equation of springiness VS testing day	Springiness reducing rate (day^{-1})	R^2
Std	y = -0.025x + 0.89	-0.025	0.97
N	y = -0.012x + 0.76	-0.012	0.80
L	y = -0.020x + 0.79	-0.020	0.94
D	y = -0.012x + 0.78	-0.012	0.96

The springiness values for the hotcakes tested on day 3 were considered as outliers because the corresponding firmness values did not follow corresponding trend and were not fit into linear equations. In the linear equation, "y" represents the springiness of the corresponding formulations; "x" represents the testing day. "$R^{2"}$ is the coefficient of determination.

The rate of reduction in springiness with time is fastest for the Std formulation and slowest for the N, D and L formulations for which the rates of change were similar. The R^2 values for springiness were all greater than for firmness suggesting that in the commercial trial, the springiness characteristic may be a less variable estimate of changes in the hotcakes with age.

The texture results from firmness and springiness in the commercial trial showed the formulations containing the bake stable α-amylase enzyme showed less firming and less reduction in springiness than the VDFF formulation. Among the formulations containing enzyme, that with the high dosage of Dim were marginally better than those formulated with the high dosage of lecithin.

7.3.2 Appearance

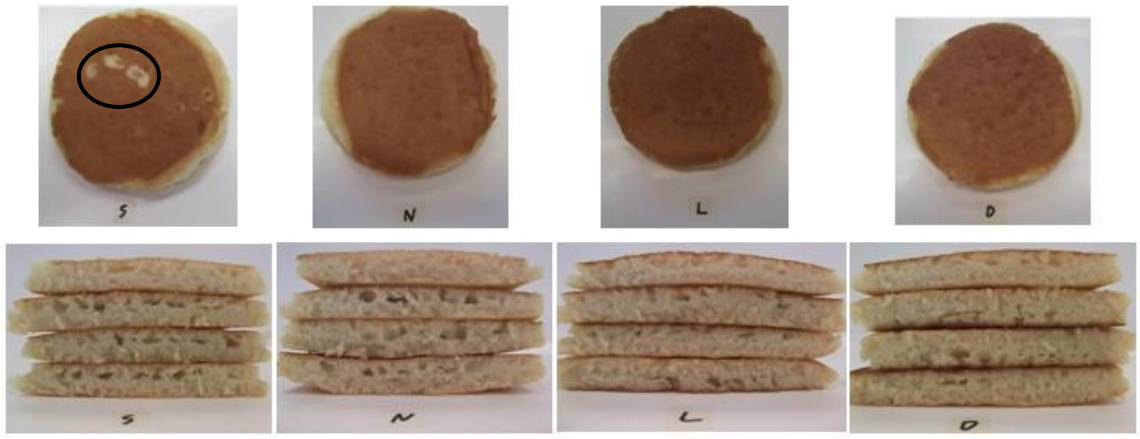


Figure 11 The appearances and air cell structures of hotcakes made from different formulations

The appearances of the hotcakes made using the modified formulations were better than the VDFF standard formulation. In Figure 11, the samples made from the N, L and D formulations had smoother external appearance compared with the Std (S) sample which often had large bubbles or voids on the surface (black circle in Figure 11). In the images of sectioned hotcakes, the bubble structures of the hotcakes made using formulations S, N and L are larger than the hotcakes made with formulation D. The bubble structures could explain the differences in initial firmness of day 0. The formulation D had more small air cells than the other formulations and had a slightly firmer texture than the hotcakes with larger air cells.

7.3.3 Microbiology of the commercially prepared formulations

The TPCs of aerobic and anaerobic for each treatment are the same on each testing day, therefore, only the combined TPCs were presented in the Figure 12.

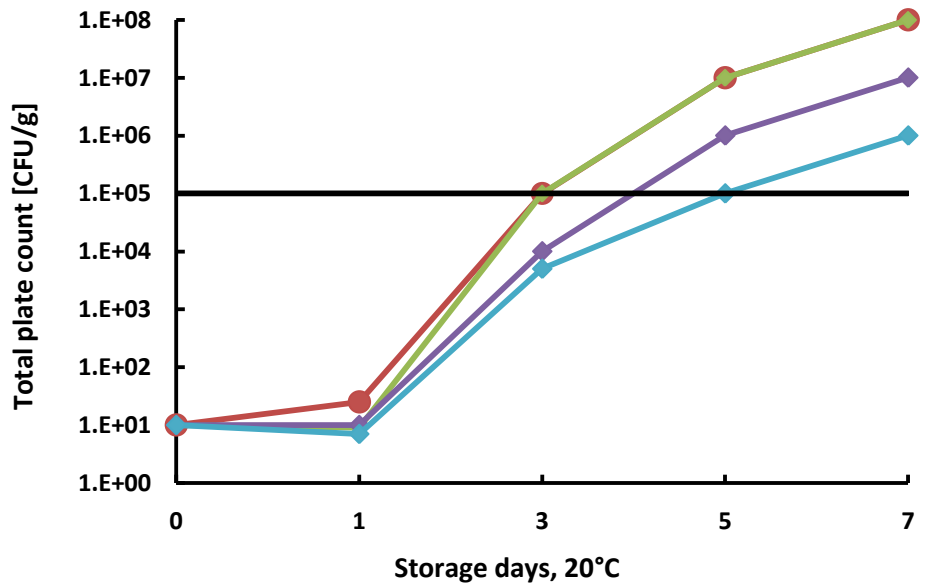


Figure 12 Shelf life tests of different preservation techniques

Std= VDFF original LDPE packaging; HP= higher dosage of preservatives; O2= barrier pouch bag with oxygen absorber; HP & O2= higher dosage of preservatives and barrier pouch bag with oxygen absorber. The black horizontal line is the limit of 1.E+05 CFU/g, the safe upper limit of microorganism contamination in foods. CFU=colony forming units

The Std treatment had reached the maximum allowable colony count by day 3, this result is two days less than the VDFF Shelf life evaluation made by Formula Foods (Sutton & Rout,

2010). The shorter shelf life from this trial may be due to the condensation during thawing which wets the surface of the hotcakes, and creating a better environment for microorganism growth. The formulation containing the increased level of calcium propionate had the same shelf life as the Std formulation, clearly calcium propionate at the level used in the Std treatment was already achieved maximum microbiological control. Reducing the level of oxygen in the packaging using O_2 absorption sachets increased shelf life by one day compared to the original packaging. Combining the increased levels of preservative with the reduced levels of O_2 further increased shelf life by two days compared to the standard VDFF formulation in original packaging.

From this result, reductions of the level of oxygen in the packaging and increasing the level of preservative did not result in commercially useful increases the in shelf life of the hotcakes at ambient conditions. Possible explanations for this are: 1) Post baking contamination may have left large numbers of spores on the hotcake surface; 2) Controlling oxygen levels in the pack below 1% along with the slightly higher dose of preservative are insufficient to control microbiological spoilage on hotcake product due to its nutrient character which is highly vulnerable to spoilage by microorganisms.

7.3.4 Sensory

Table 13 Sensory result to differentiate the fresh and stale hotcakes

	Std	N	L	D
Correct responds	16*	13	9	16*
Total countable comparisons	32	32	32	32

When comparing fresh and 3 day old hotcakes, panellists were unable to consistently differentiate between the two age groups and exactly half the panellists incorrectly identified the staleness of the Std and D formulation hotcakes (Table 13). To determine the difference between fresh and stale hotcakes at the 5% significance level, the minimum number of correct responses for triangle testing using the forced choice method for the 32 comparisons used in this work is 16 (Maximo C. Gacula, et al., 2009). The Std formulation and D formulation had the correct response of 16, which indicated customers can barely perceive a stale hotcake

when it was made using these two formulations. The hotcake made from formulations of N and L had the correct response lower than 16; thus, the customer can not perceive the staling of hotcakes when made from the New formulation or the modified formulation containing a high dosage of lecithin (L).

This result is slightly different from the firmness result from Texture analysis, especially for the high dosage of lecithin and high dosage of Dim formulations. From the TPA results it was evident that hotcakes made using a high dosage of lecithin stale faster than those made using other formulations.

To interpret the difference between texture and the sensory analyses, the definition for staling was considered again. In the sensory test, stale hotcakes were defined as being firmer, doughier and to have less fresh baked aroma. Therefore, the aroma may cause the different perception by human sensory and machinery tests of texture. In addition it is hard for humans to notify the difference within 1 Newton.

Table 14 Sensory result to differentiate the different formulations

	Std-N	Std-L	Std-D	N-L	N-D	L-D
Number of correct response	9	7	7	8	10	10
Total countable comparisons	28	27	28	28	29	26
Minimum correct response for difference @ α=0.05	14	14	14	14	15	14

The results to differentiate each formulation were presented in Table 14. In this test, some of the panellists did not follow the instruction, hence, not all the finished questionnaires were considered as countable comparisons. The total countable comparisons were shown at the second row of Table 14 and the corresponding number of correct responses required to show a difference significant at the 5% level were listed at the third row. None of the correct responds for each paired comparison had equal or greater value than the critical significantly different value in row three; therefore, the conclusion is the panellists could not differentiate between formulations. And the difference in firmness and springiness among the formulations did not correspond to the differences between the formulations.

Since the panellists could not differentiate the difference between formulations, the following questions of, in what way, and how much was the difference between them were not considered any more.

According to sensory evaluations, the panellists could not detect the differences between the fresh hotcake and the hotcake stored for three days or the differences between the modified formulation and VDFF's original formulation.

7.4 Conclusions

Both texture results and sensory evaluation indicated the anti-staling properties of the new formulation developed in the laboratory trials were successfully translated into in commercial production which improved hotcake quality.

The increased proportions of lecithin and Dim showed different results in texture analysis and sensory evaluation. According to texture results, high dosage of lecithin caused a faster staling rate to the hotcake product than other formulations, while the high dosage of Dim provided higher initial firmness to the fresh hotcakes but they showed little change during storage. According to the sensory evaluation, panellists could not differentiate between fresh and 3 day old samples for the hotcake made with New formulation and high dosage of lecithin but fresh, and 3 day old hotcakes could be differentiated when the hotcakes were made with high dosages of Dim.

Decreasing the oxygen content level to less than 1% in the packaging extended the shelf life by one day compared to the original packaging; combining the oxygen absorber with the increased levels of preservative further increased shelf life by two days. Unfortunately, increasing the level of preservatives did not fully control microorganisms at ambient storage conditions.

Chapter 8 General Conclusions and recommendations

8.1 General conclusions

Staling has been a major problem limiting the shelf life of hotcake product for Van Dyck Fine Foods Ltd. Their market development is restricted due to the short shelf life of the product. Therefore, methods to control the staling and extend shelf life of hotcakes at ambient temperature were studied in this research.

Incorporation of anti-staling agents into the formulation was the initial approach to reduce the staling rate of the hotcakes. Eleven anti-staling agents including emulsifiers, enzymes, gums, and humectants were evaluated during the preliminary and screening trials at the early stage of the study. Dimodan PH 320/B-M, a distilled monoglyceride; DATEM Palsgaard 3502, a Diacetyl Tartaric Acid Ester of Mono- and Diglycerides; and also Novamyl 10000 BG, a bake stable alpha amylase showed better anti-staling properties than other ingredients and were selected for the combination trial to determine if the effects of the major classes of anti-staling agents were additive.

The assessment for combination trial determined the best anti-staling combination that includes Dimodan, DATEM and Novamyl 10000 BG. In the commercial trial, when the best combination of ingredients were incorporated into the hotcake formulation, the sensory panellists were unable to detect the difference between a fresh product and a product stored for 3 days at ambient temperature. In addition, the panellists could not detect the changes made to the hotcake formulations. This is useful as the modification to the formulation developed in this work can be used without noticeably altering taste or texture of the product.

It is expected that if the hotcakes could be made microbiologically safe for storage periods greater than 6 days at ambient temperature, the new formulation will have an advantage in maintaining the fresh baked texture of the product. Two antimicrobial spoilage approaches were assessed during this project. The first approach was to increase the level of preservative and the second approach was to reduce the oxygen content level in the packaging. Other approaches such as reducing the water activity; changes in the pH level of the product and altering storage conditions are not applicable due to the natural character of this product and

the available commercial storage conditions. The commercial trial showed that decreasing the oxygen content to less than 1% in the packaging and increasing the level of preservatives increased the shelf life by only 1 or 2 days at ambient storage condition.

This unpromising result may be due to post baking contamination that left too many spores on the hotcake surface. Also, the hotcakes are a high moisture product containing high levels of sugar and starch which are highly vulnerable to spoilage by microorganisms, and low oxygen concentrations and increases in the level of preservative are not sufficient to keep the microbiological spoilage under control at ambient storage condition.

8.2 Recommendations

From the work done during this research program it is recommended that VDFF should focus on reducing microbiological contamination of the hotcakes at production by using sterile packaging methods. Although the new anti-staling formulation appears to offer reduced rates of staling when the hotcakes are held at 20°C this is of little importance as microorganism counts have already exceeded saleable levels before staling becomes important.

Since the maximum legal level of preservative and controlled atmosphere packaging are not sufficient to extend the shelf life at ambient temperature. The only strategy remaining to extend the shelf life is to reduce post baking contamination of the hotcakes such as packing the hotcakes in a near sterile environment. A possible approach is to install positive pressure air filtration and circulation systems which are capable of removing all microorganisms from the air. Installing UV lighting and perhaps hydrogen peroxide sprays are also possible approaches to sterilize the cooked hotcakes before packing into bags. Good Manufacturing Practice (GMP) to eliminate points of infection may help with maintaining existing standards. These practices include the hygiene control of ceilings, windows, doors, floors, pest control and personnel.

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Appendix

Sensory questionnaire

Name:_____

Date:_____

Stale hotcakes are firmer, doughier and contain fewer aromas.

You are going to assess 4 sets of hotcakes. Each set contains 3 samples.

Rinse your mouth with water before beginning. Expectorate the water into the container provided. In one set of three coded samples. Two of these samples are the same and one is different. Please taste the samples in the order listed below, from left to right. Circle the number of the sample that is **Staler** than the other two samples. Rinse your mouth with water between samples and expectorate all samples.

Set 1:	859	684	271
Set 2:	182	318	295
Set 3:	503	174	638
Set 4:	351	832	462

Thank you for participating in this project!

Name:_____

Date:_____

You are going to assess 6 sets of hotcakes, each set contains 3 samples.

Rinse your mouth with water before beginning. Expectorate the water into the container provided. In one set of three coded samples. Two of these samples are the same and one is different. Please tasted the samples in the order listed, from left to right. Circle the number of the sample that is **different (odd),** and in **what way** & by **how much** it is odd from others. Rinse your mouth with water between samples and expectorate all samples.

Set 1: **472** **815** **526**

Indicate the degree of difference between the duplicate samples and the odd sample:

1. the odd sample is **harder** or **softer** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

2. the odd sample is **Spongier** or **doughier** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

Set 2: **370** **907** **805**

Indicate the degree of difference between the duplicate samples and the odd sample:

1. the odd sample is **harder** or **softer** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

2. the odd sample is **Spongier** or **doughier** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

Set 3: **658** **823** **371**

Indicate the degree of difference between the duplicate samples and the odd sample:

1. the odd sample is **harder** or **softer** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

2. the odd sample is **Spongier** or **doughier** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

Please return the sample tray and wait for the second tray

Set 4: **584** **815** **264**

Indicate the degree of difference between the duplicate samples and the odd sample:

1. the odd sample is **harder** or **softer** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

2. the odd sample is **Spongier** or **doughier** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

Set 5: **947** **591** **153**

Indicate the degree of difference between the duplicate samples and the odd sample:

1. the odd sample is **harder** or **softer** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

2. the odd sample is **Spongier** or **doughier** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

Set 6: **379** **826** **283**

Indicate the degree of difference between the duplicate samples and the odd sample:

1. the odd sample is **harder** or **softer** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

2. the odd sample is **Spongier** or **doughier** than the other two
   By how much: **Very slight** **slight** **moderate** **large** **extreme**

Thank you for participating in this project!