UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

# Effect of enzymes and emulsifiers on the shelf life of modified atmosphere packaged par-baked pizza

by

Yolandi Lemmer

This was submitted in partial fulfilment of the requirements for the degree

MSc Food Science

In the Faculty of Natural and Agricultural Sciences

Department of Food Science

University of Pretoria

Pretoria

Republic of South Africa

November 2009

## DEDICATION

This project is dedicated to my loving husband for all his patience and support. This project is also dedicated to my heavenly Father who has strengthened me to finish what I started.

## ACKNOWLEDGEMENTS

This project was supported by the National Research Foundation. The Winter Cereal Trust should be acknowledged for their financial student support. The supervisors of this project: Prof. EM Buys and Dr MN Emmanbux are acknowledged and greatly appreciated for their professional input, support, encouragement and patience. A special thank you goes to the following people: Mr. Luyanda Myhlanza who assisted during the microbiological analysis, Mrs. Helena de Wet at Ruto Mills for making their premises available for the preparation of the par-baked pizza bases and also for supporting this project with the basic raw ingredients needed and Dr Jane Murray and Mr Ed Murray for editing and formatting this document.

# ABSTRACT

**Effect of enzymes and emulsifiers on the shelf life of modified atmosphere packaged par-baked pizza**

By: Yolandi Lemmer
Supervisor: Prof. EM Buys
Co-supervisor: Dr MN Emmambux

Par-baked pizza was prepared with three different additives treatments and was packaged in three packaging treatments namely: air (A), 100% $CO_2$ modified atmosphere (MAP) and 100% $CO_2$ modified atmosphere with an oxygen absorber (MAP + OA). The additive treatments were diacetyl tartaric acid esters of mono- and diglycerides (DATEM), an enzyme combination treatment (EC) namely: lipase and maltogenic α-amylase and par-baked pizza without additives (C). The aim of the study was to determine the effects of the different additive and packaging treatments on the shelf life of par-baked pizza. Therefore the following physical measurements and microbiological analysis were conducted: water activity, crumb moisture content, thickness, firmness, stress, strain, springiness, aerobic plate count, lactic acid bacterial count and yeast and mould count.

The results showed that the water activity of par-baked pizza was between 0.95–0.98 . Thereafter the water activity remained stable at 0.96. As the storage time (d) progressed, the moisture content of the par-baked pizza crumb increased, from 36% on d0 to 41% on d16.

The par-baked pizza with added additives was thicker than par-baked pizza without additives. The thickness for par-baked pizza + DATEM, + EC and + C was 21.7 mm, 22.0 mm and 18.3 mm respectively.

The compression test and the three-point bend test showed that the firmness of par-baked pizza increased as the storage time increased until d8. The firmness then decreased from d12 to d16. The par-baked pizza packaged + MAP had a lower firmness than par-baked pizza + A. The firmness of par-baked pizza + EC was lower

than par-baked pizza + DATEM and par-baked pizza + C respectively. The results also showed that the re-baked pizza was even softer than originally.

The springiness of par-baked pizza decreased as the storage time progressed. The par-baked pizza + C had the highest springiness of 39% compared to the springiness of par-baked pizza + EC; 37%, and + DATEM; 35%, respectively. The springiness of the re-baked pizza showed trends similar to those of the par-baked pizza in terms of the main effects of the different additives. The springiness of the re-baked pizza was also higher than that of the par-baked pizza. Thus, the additive treatments, DATEM and EC, had beneficial effects on the texture and thickness of par-baked pizza.

The microbial analysis showed that the APC and the mould count were mostly affected by the different packaging treatments. The par-baked pizza + A showed visible mould growth from d12. Hence par-baked pizza + A reached the end of shelf life at d12. The par-baked pizza + MAP showed reduced APC levels, however, and as expected the yeast and lactic acid bacterial levels were unchanged. There were no signs of visible mould growth on any par-baked pizza + MAP. The microbial levels were the lowest with the par-baked pizza + MAP + OA packaging treatment.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# 1: INTRODUCTION

## 1.1 PROBLEM STATEMENT

Pizza has become very popular over the years as a convenient food product, which means it requires less preparation time or can be consumed as a ready-to-eat product (Pinho & Furlong, 2000). It can be sold freshly baked, pre- or par-baked, chilled or frozen (Pinho & Furlong, 2000). In comparison with other bakery products, the pizza remains under-researched (Larsen, Setser & Faubion, 1993).

It has been observed that during the industrial production of pizza, the quality may differ from day to day when undergoing the same processing method (Larsen *et al*., 1993). A par-baked pizza is a product with added benefit for it is convenient to use, fast to prepare and consistent in quality (Correll, 2002).

Par-baked refers to the method of manufacture. A par-baked product goes through two baking stages (Bárcenas & Rosell, 2007; Bent, 2007; Lainez *et al*., 2008). Firstly, the pizza is partially baked. The pizza crust remains uncoloured due to minimal maillard browning. The starch is gelatinised, the yeast killed and the protein in the dough is denatured. Hence, the pizza base structure is set during the first baking stage (Bárcenas & Rosell, 2007; Bent, 2007; Ribotta & Le Bail, 2007). When the consumer buys the par-baked pizza, the second baking stage takes place before consumption (Bárcenas & Rosell, 2007; Bent, 2007; Sluimer, 2005). This is when maillard browning of the pizza base is completed (Bent, 2007; Sluimer, 2005).

In addition, par-baked pizza has a limited shelf life of 5–8 days (d) stored at refrigeration temperatures of 7–10 ºC, packaged in air, with yeast and mould being the main shelf life limiting factors (Personal communication: Ina Screicher, dessert factory manager, Gull Foods, 2007). Rodriguez, Medina and Rafael (2003) show that the shelf life of par-baked pizza stored at 15–20 ºC is 7 d due to surface mould growth. There is limited information

on the shelf life of pizza. Most information is in relation to bread and other bakery products.

It is also mentioned by Bent (2007) that par-baked bread has a limited shelf life when stored at room temperature because of surface drying, rapid staling and surface mould (Bent, 2007). The shelf life of par-baked products can be extended by chilling, freezing and by modified atmosphere packaging (Bárcenas & Rosell, 2007; Bent, 2007). Rodriguez *et al*. (2003) show that the shelf life of par-baked pizza stored at 15–25 ºC could be extended to 13 d with modified atmosphere packaging. However, residual oxygen can cause a problem as <1% is needed to eliminate mould growth. Oxygen absorbers can be used to modify the atmosphere to prevent further mould growth by reducing the residual oxygen content to <1% (Smith *et al*., 2004).

Another factor that affects the shelf life of bakery products is known as staling, which is normally associated with the firming of the crumb (Smith *et al*., 2004). Avital, Mannheim and Miltz (1990) show that $CO_2$ might also delay the staling process of bread. Emulsifiers have been used in the bakery industry to delay the firming (staling) rate of breadcrumb (Selomulyo & Zhou, 2007; Stampfli & Nersten, 1995). However, the demand for natural products has driven the replacement of additives and emulsifiers with enzymes, for instance: α-amylase, xylanase and lipase (Si & Lustenberger, 2001).

The aim of the study is to determine the effect of modified atmosphere packaging and different additives such as DATEM or lipase and maltogenic α- amylase on the shelf life of par-baked pizza when it is stored at 10 ºC for 16 d.

## 1.2 LITERATURE REVIEW

### 1.2.1 Introduction to pizza

Pizza originated in Naples, Italy (Coppola, Pepe & Mauriello, 1998; Correll, 2002; Formato & Pepe, 2005). Pizza, a cereal-based product, is an oven-baked flat bread with a

spongy consistency (de Delahaye, Jimenez & Perez, 2005; Formato & Pepe, 2005). Pizza has a bread-like base and is topped with tomato sauce and cheese; other topping can also be used (Correll, 2002; Formato & Pepe, 2005; Sultan, 1990). Pizza can be classified according to its base thickness (Correll, 2002). In this case, the pizza base is the baked dough part of the pizza without a topping. It can be either thick-based or thin-based. A round thin pizza base is sometimes known as Neapolitan pizza (Correll, 2002; Sultan, 1990). Thick-based pizza is sometimes known as Sicilian pizza or deep-dish pizza (Correll, 2002; Sultan, 1990).

The appearance, taste and most importantly the texture of the pizza base (Figure 1) are important factors for product identification, differentiation and consumer acceptance (Larsen *et al*., 1993). The type of pizza dough, raw materials used, preparation and leavening process are the major influences on the end quality of the pizza (Formato & Pepe, 2005).

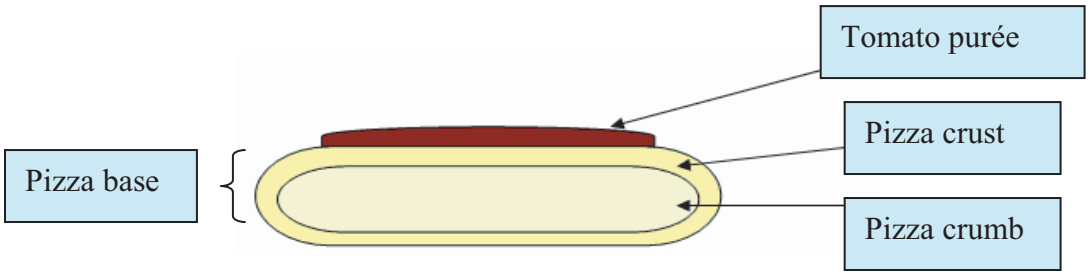



Figure 1: Illustration of the different parts of a pizza base

### 1.2.2 Basic ingredients of pizza

The basic ingredients include those essential for making the pizza base, for example, flour, water, salt and yeast (Sultan, 1990). Each ingredient has a specific role in the baking of a pizza base. The pizza base is usually made with bread dough (Sultan, 1990).

*1.2.2.1 Flour*

Flour consists of various components, namely protein, starch, fibre and minerals (Sluimer, 2005; Sultan, 1990). The flour used to bake pizza should have fine gluten-forming protein content of about 13–14% protein (Sultan, 1990). According to Sluimer (2005), wheat flour is normally used to bake bakery products such as bread and pizza. Larsen *et al*., (1993) have shown that hard spring wheat and hard winter wheat flour produce a thin pizza base with similar textural attributes. However, it was observed that the refrigeration or freezing period of dough before use known as retardation time significantly affected the textural attributes of pizza, suggesting that processing methods would have a great impact on the end quality of pizza (Larsen *et al*., 1993).

The starch and protein components of flour are important because they are essential for the transformation of a dough foam-type system to a bread-like sponge-type system (Hug-Iten *et al*., 1999). Baking causes the wet dough foam system with entrapped air in gas cells to solidify and form an open pore sponge system. These components also have an influential role in crumb firming upon cooling that will be discussed in later sections (Hug-Iten *et al*., 1999).

*1.2.2.2 Water*

Water affects the dough consistency, dough rheology and dough temperature (Correll, 2002; Wang, Choi & Kerr, 2004). It also serves as a dispersing medium bringing the ingredients into contact with each other and dissolving the water-soluble ingredients (Chieh, 2006; Correll, 2002; Mani, Trägårdh & Eliasson, 1992; Wang *et al*., 2004). When dough is formed while mixing water and flour, the water hydrates the protein (gluten) fragments know as gliadin and glutenin. During kneading, it forms a fragile network that entraps gases produced during fermentation (Chieh, 2006; Correll, 2002; Wang *et al*., 2004). This network forms the visco-elastic dough (Wang *et al*., 2004). Enzymes are also activated by the presence of water (Mani *et al*., 1992; Wang *et al*.,

2004). During baking, moisture plays a role in starch gelatinisation (Chieh, 2006; Correll, 2002; Wang *et al*., 2004).

*1.2.2.3 Salt*

Salt is added mainly to improve the taste (Brown, 1993; Chieh, 2006; Sluimer, 2005; Sultan, 1990). However, salt may also slow down gluten development. Hence, dough with a high salt content needs a longer mixing time to achieve its correct gluten development (Sluimer, 2005).

*1.2.2.4 Yeast*

*Saccharomyces cerevisiae* is normally used in the baking and brewing industry (Sluimer, 2005; Sultan, 1990; Williams & Pullen, 2007). It has been used in the baking industry for its ability to produce gas through the metabolism of glucose. In anaerobic conditions yeast ferments glucose to produce carbon dioxide and ethanol (Sluimer, 2005; Sultan, 1990; Williams & Pullen, 2007). The carbon dioxide goes into the dough/water phase, when it becomes saturated; the carbon dioxide is released into a gas cell that has been formed during mixing of the dough (Brown, 1993). Yeast also contributes to the flavour of baked products by the production of fermentation by-products (Brown, 1993). Yeast also produces reducing sugars that react with the amino groups of proteins during baking, forming part of the maillard reaction that causes browning of the outer crust. The maillard reaction also produces flavour compounds that are located in the crust (Brown, 1993).

### 1.2.3 Manufacture of pizza

The pizza industry can be separated into large-scale and small-scale production of pizza, based on the production process employed (Pizza crust production, 1996). Large-scale production companies usually produce frozen pizza dough, frozen par-baked pizza bases

and frozen topped par-baked pizzas. In general, these companies make use of automated lines (Liberopoulos & Tsarouhas, 2005).

The automated thick pizza base production processing steps are represented in Figure 2 (Pizza crust production, 1996).

Small-scale pizza production companies (pizzerias) generally use a more traditional manual process to produce pizzas (Correll, 2002). Wholesale companies sell in large quantities to be retailed by others like for instance; pizzerias use frozen dough balls or frozen par-baked pizza bases supplied by the wholesale chain to make fully-topped and baked pizzas (Correll, 2002).

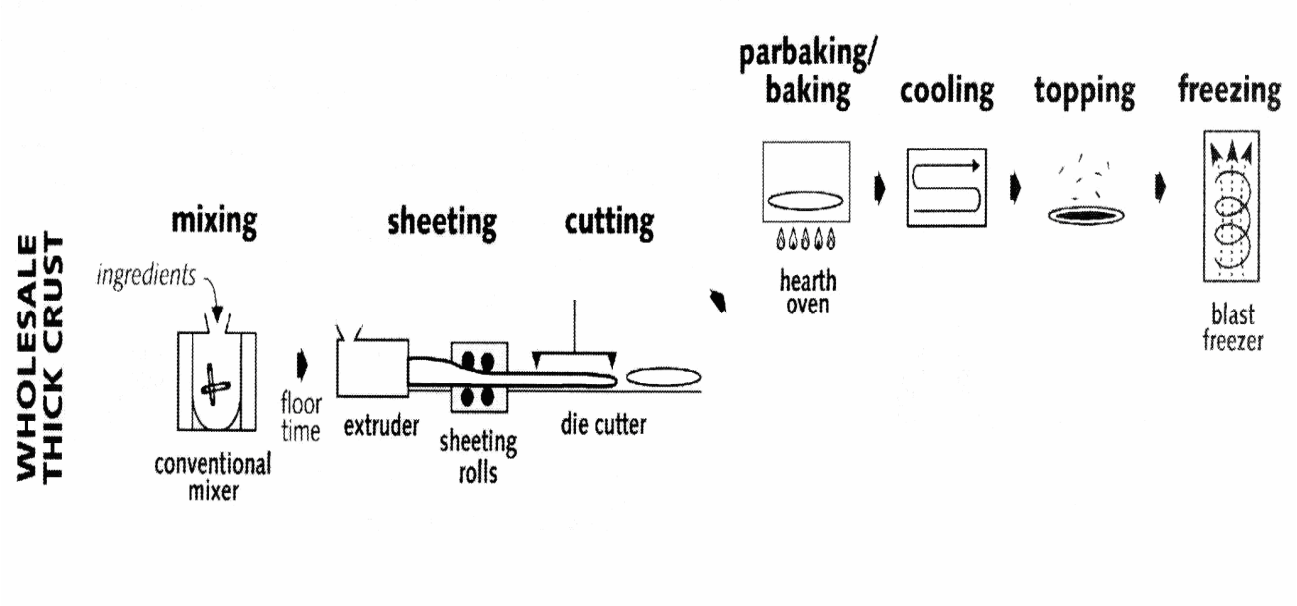



Figure 2: Wholesale production of thick par-baked pizza (Pizza crust production, 1996)

According to Correll (2002), there are advantages and disadvantages in preparing a pizza base from scratch on site. To eliminate dough making and pizza base formation processing steps, par- or pre-baked pizza bases can be used. However, this process also has its advantages and disadvantages.

The advantages to pizzerias of eliminating the dough-making procedure by using par-baked pizza bases are as follows:

- Less trained labour to make the pizza base;
- Less labour to clean up after dough making;
- Less equipment for mixing and shaping the dough;
- Less floor space and initial capital cost;
- No rolling, sheeting or handling of dough for the pizza base;
- Fewer ingredients;
- No proofing required, decreasing preparation time;
- Less final baking time as the base is partially baked; and
- Greater consistency in the quality of the base.

The disadvantages to pizzerias of eliminating the dough-making procedure by using par-baked pizza bases are as follows:

- A par-baked pizza base may cost more;
- Pizzerias are unable to create their own pizza-base recipe;
- Storage space is needed; and
- Because less baking time is needed the internal crumb may not be as warm as that of a freshly baked pizza.

### 1.2.4 Re-baking par-baked pizza

Re-baking the par-baked product occurs either just prior to its sale or prior to its consumption (Sluimer, 2005). The conditions for both par-and-re-baked pizzas may differ between manufactures because it will be prepared according to individual specifications. Par-baked pizzas are normally baked at 204 ºC for 3 to 4 minutes and re-baked pizzas are baked until crust is evenly browned (Pizza crust production, 1996).

The re-baked pizza may be of sub-optimal quality, which is one of the disadvantages of par-baking. The main reason why par-baked products are of sub-optimum quality is because the outer crust starts to wrinkle when re-baked and the crust may be thinner. The reason for a thinner crust is because during long storage periods, the moisture distribution between the outer crust and the inner crumb of the baked product start to reach equilibrium. When re-baked, the crispy crust has to be formed, thus requiring a longer baking time. The longer baking time may result in unfavourable final moisture content of the inner crumb; therefore, a shorter baking time is applied, however it will result in a thinner crust (Sluimer, 2005). Farahnaky and Majzoobi (2008) show that the re-baking process is mainly responsible for the development of flavour, crust and colour of the crust. They further state that the re-baking process is not responsible for the setting of the baked product structure and texture of the crumb.

Browning of the outer crust also occurs during the re-baking procedure. The maillard reaction is responsible for browning the crust and for the release of aromatic compounds (Chieh, 2006; Fik & Surówka, 2002; Sluimer, 2005). The maillard reaction is a condensation reaction of the reducing sugars and the amino groups in the proteins (Sluimer, 2005).

Fik and Surówka (2002) have shown that par-baked bread retains more of its freshness after the unfrozen bread is re-baked in comparison with full-baked bread. They explain that most of the flavour of the full-baked bread is lost after frozen storage indicating a loss of freshness, while the par-baked bread still has a sufficient formation of aromatic substances after re-baking due to the postponement of the maillard reaction (Fik & Surówka, 2000).

Fik and Surówka (2002) have concluded that re-baking par-baked bread levels out the textural changes that occur during storage. Staling is a result of many physico-chemical changes that occur during storage. Starch retrogradation and cross-linking between starch and gluten are some of the changes responsible for staling (Gray & Bemiller, 2003). When re-baked, the retrograded amylose is not reversed but the retrograded amylopectin does revert to its amorphous state by unfolding of its crystalline branches

(Bárcenas & Rosell, 2007; Hug-Iten *et al*., 2003; Escher & Conde-Petit, 2003; Stampfli & Nersten, 1995). During reheating, the cross-linkages between starch and gluten are also easily broken, thus the bread will return to its original softness (Bárcenas & Rosell, 2007; Gray & Bemiller, 2003).

The firmness of bread aged for 5 d, reheated to 80 ºC and stored again for another 2 d was compared with the firmness of bread stored for 7 d (Hoseney, 1986). After 7 d the re-heated bread's firmness was comparable to that of the non-reheated bread. Re-baked bread firmed faster than non-reheated bread. This may be because the amylopectin nuclei did not melt completely and therefore could have re-crystallized faster.

Bárcenas and Rosell (2007) have shown that the hardness of par-baked bread increased during storage at 2 ºC. They also observe that the re-baked bread retained its softness due to the melting of the amylopectin crystalline structure that formed during storage. It was further observed that amylopectin of the re-baked bread started to retrogradate again after a short period of storage. During storage of par-baked bread, amylopectin absorbs water from gluten that is required for the re-crystallization process that will lead to the firming of the par-baked bread. When re-baking the par-baked bread, the heat causes the re-crystallized amylopectin to unfold and release water and as a result the bread returns to its original softness. However, this released water in the re-baked bread is again available for the retrogradation of amylopectin, but to a lesser extent due to water evaporation (Bárcenas & Rosell, 2007).

### 1.2.5 Importance of moisture content and water activity of pizza dough

Water has a small molecular mass but causes great mobility (Chieh, 2006; Fessas & Schiraldi, 2005; Mani *et al*., 1992). Due to its high mobility, water acts as a plasticiser in the food system (Chirife & Buera, 1995; Fessas & Schiraldi, 2005). Water is therefore responsible for many physical properties of food as well as for microbial growth that can lead to spoilage and textural changes (Chieh, 2006; Fessas & Schiraldi, 2005). Water activity can be defined as the ratio of vapour pressure of water in equilibrium with a food

to the saturation vapour pressure of pure water at the same temperature (Chieh, 2006; Fontana, 1998; Lacey, 1989). It describes the degree to which water is bound and its availability to act as a solvent or to take part in biochemical/chemical reactions and to support the growth of microorganisms (Fontana, 1998).

Water activity can be used to indicate the stability of foods in terms of microbial growth, spoilage rates and physical/chemical properties (Chieh, 2006; Fontana, 1998). The ability of water to act as a solvent, medium and reactant, increases as the water activity increases. Water activity can be used to determine the potential occurrence of spoilage or growth of a pathogenic microorganism. Water activity also plays a role in the physical properties of food, such as texture and its shelf life (Chieh, 2006; Fontana, 1998).

The physical state of water in a food system can play an important role in the structure, physical, chemical and sensory properties (Wang *et al*., 2004). In a baked product, the proteins (gluten) form a continuous network of smooth thin strands with vacuoles (gas cells). The gelatinised starch is distributed as long fibrous strands, which are entangled in the protein network (Wang *et al*., 2004). There are two main partitions of water in wheat flour dough: the free-to-evaporate water from the core to the surface and the water bound within the gluten matrix which can only be released above high temperature thresholds (Chieh, 2006; Fessas & Schiraldi, 2005). The polymers in the flour, namely, starch, non-starch polysaccharides and gluten proteins, are incompatible and therefore compete for the available water forming separate aqueous phases (Fessas & Schiraldi, 2005; Piazza & Masi, 1995). Gluten has a higher affinity for water than for starch and starch has a higher water absorption capacity than gluten (Wang *et al*., 2004). During baking, moisture migrates from gluten to starch when starch gelatinises.

Dough normally contains 40–46% moisture and does not form when the water content is below 35% or when the moisture content is too high, namely above 50% moisture (Wang *et al.,* 2004). When dough moisture content is increased, the temperature for the release of water from the gluten matrix is reduced. Hence, the water binding strength within the structure is reduced (Fessas & Schiraldi, 2005). However, Fessas and Schiraldi (2005) have reported that higher dough moisture content resulted in better structural organisation

of water within the dough in the non-gluten phase, mainly as starch. The water in the discontinuous phases (starch) can still occupy intermediate sites through hydrogen bonding where the water remains relatively mobile. Therefore, the weak hydrogen bonding of the bridging water molecules can easily be broken through kneading, mixing or extruding (Fessas & Schiraldi, 2005).

Food with high water activities normally have a texture described as moist, juicy, tender and chewy (Fontana, 1998). When lowered to a low water activity, their texture changes and is normally described as hard, stale, dry and crunchy. Low moisture-content foods normally have a texture described as crispy and crunchy. When their water activities are increased, the texture changes to soggy (Fontana, 1998).

It has been reported that moisture content is inversely proportional to the rate of breadcrumb firming (Stampfli & Nersten, 1995). The higher the moisture content is, the slower the rate of firming (Stampfli & Nersten, 1995).

In food, water activity versus moisture content exists in a non-equilibrium system and can be plotted as an adsorption or desorption isotherm (Chieh, 2006). The sigmoidal relationship between moisture content and water activity in food is illustrated in Figure 3. It has also been observed that as the moisture content increased so did the water activity and followed the behaviour of a desorption isotherm. The water activity increased until 20% moisture content was reached. As the moisture content increased beyond 20%, the water activity started to reach equilibrium (Leuschner, O'Callaghan & Arendt, 1999).

Li, Kloeppel and Hsieh (1998) have shown that as the water activity increased to above 0.50 in corn cakes the hardness decreased. It was shown that hardness, glass transition temperature and crispiness are functions of moisture content. The hardness decreased as the moisture content increased from 9% and above with water activities higher than 0.60. The crispiness decreased within the glassy state with water activity between 0.5–0.6 when the corn cake was still at its greatest hardness. Li *et al*. (1998) explained that water plasticisation is responsible for the texture changes during the glassy state.

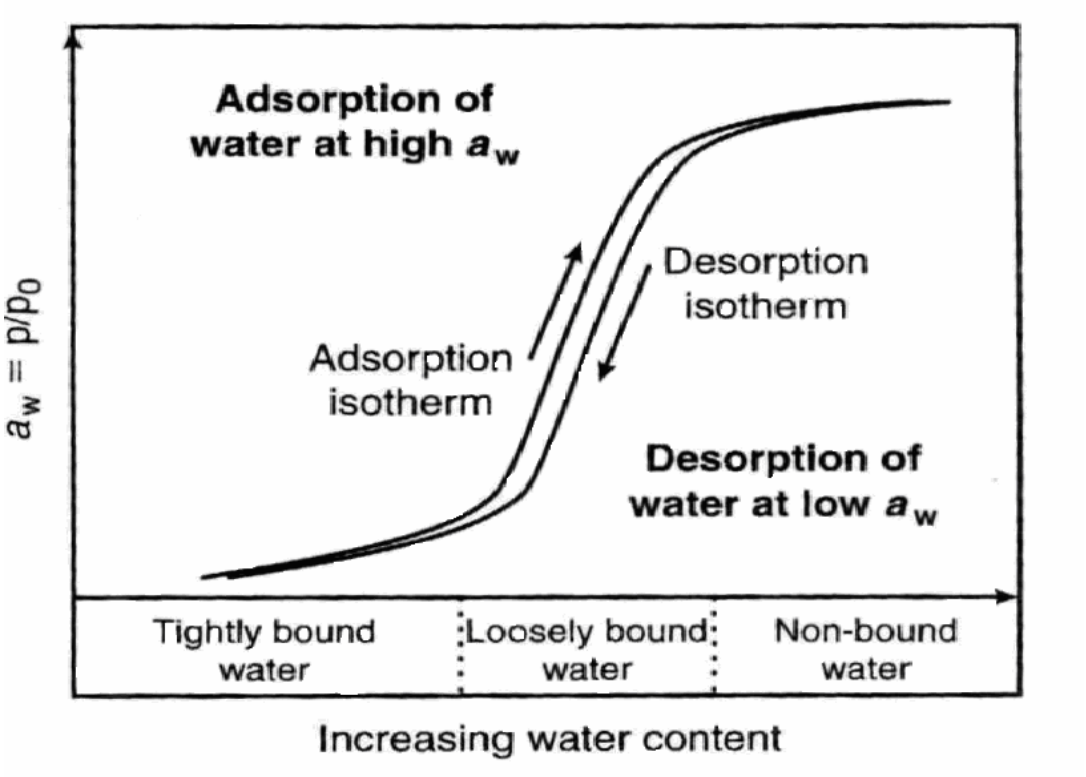



Figure 3: Sigmiodal relationship of moisture content and water activity in food (Chieh, 2006)

### 1.2.6 Spoilage patterns of pizza

Spoilage can be defined as any change of the food product that makes it less palatable at the time of consumption (Gram *et al*., 2002; Smith *et al*., 2004). Bakery products undergo deterioration during storage mainly because of high water activities (Piazza & Masi, 1995). Pizza is a product with high moisture content and with a water activity between 0.94–0.95 (Smith *et al*., 2004). During baking it undergoes physical, chemical and microbiological spoilage (Smith *et al.*, 2004).

Microbial growth or spoilage may change the sensory and nutritional quality of pizza (Pinho & Furlong, 2000). These spoilage microorganisms may also produce mycotoxins (Pinho & Furlong, 2000). Mycotoxins are produced by ubiquitous moulds namely, *Aspergillus* and *Penicillium* spp. (Abellana, Sanchis & Ramos, 2001; Pinho & Furlong, 2000).

Another spoilage mechanism of bakery products is known as staling which is a process of chemical and physical changes that includes: moisture redistribution, drying, starch retrogradation, increased firmness and the loss of aroma and flavour (Goesaert *et al*., 2009; Kotsianis, Giannou & Tzai, 2002; Smith *et al*., 2004).

### 1.2.7 Staling

Staling is detected organoleptically by changes in the bread's texture as well as in its flavour and aroma (Hug-Iten *et al*., 2003; Pateras, 1998). Staling results in reduced consumer acceptance because of the physical-chemical alterations that take place during storage (Goesaert *et al*., 2009; Pateras, 1998). The organoleptic changes that occur during storage may result in hardening of the crumb and softening of the crust (Goesaert *et al*., 2009; Piazza & Masi, 1995). Staling has been extensively studied but is still not fully understood (Gray & Bemiller, 2003; Piazza & Masi, 1995; Ribotta & Le Bail, 2007; Sidhu, Al-Saqer & Al-Zeni, 1997).

There are two mechanisms of staling. The first is the moisture migration from the crumb to the crust during storage leaving the crust soft and leathery and the crumb hard and dry (Bhatt & Nagarju, 2009; Gray & Bemiller, 2003; Hug-Iten *et al*., 2003; Pateras, 1998; Sultan, 1990). The second mechanism of staling is due to intrinsic factors, for example, the re-crystallisation of starch that starts when the baked product is being cooled down after baking (Bhatt & Nagarju, 2009; Gray & Bemiller, 2003; Pateras, 1998; Sultan, 1990).

Factors that affect the staling rate are the following: storage temperature, moisture migration and processing factors (Gray & Bemiller, 2003; Halleberg & Chinachoti, 2002).

*1.2.7.1 Role of starch during staling*

It is believed that the first onset of staling is due to the rearrangement of the starch polymers followed by the moisture migration from the gluten protein to the crystalline starch (Gray & Bemiller, 2003; Hallberg & Chinachoti, 2002; Kamel & Ponte, 1993). Changes of the starch polymers during baking and storage determine the structure, texture and keeping quality of the baked product (Pateras, 1998).

Starch is located in spherical granules (Karim, Norziah & Seow, 2000; Pateras, 1998). Starch consists of two polymers, namely, amylose and amylopectin. Amylose is essentially a linear polymer whereas amylopectin is a highly branched polymer that is partially crystalline (Blanshard, 1986; Karim *et al*., 2000; Pateras, 1998). It is believed that amylopectin is responsible for the crystallinity and amylose is in a more amorphous state (Blanshard, 1986; Karim *et al*., 2000; Pateras, 1998).

During baking, as the temperature increases the starch granules start to absorb water and to swell (Chieh, 2006; Karim *et al*., 2000; Pateras, 1998). With increased temperature the starch polymers start to vibrate which leads to the breakage of intermolecular bonds thus allowing their hydrogen bonding sites to engage more water. The water penetration leads to a greater separation between the starch polymer chains that reduce the size and number of crystalline regions. When the temperature is further increased, starch will lead to a complete loss of crystallinity. This is known as starch gelatinisation, which occurs between 60–90 ºC depending on the starch (Chieh, 2006; Pateras, 1998).

Starch retrogradation is considered to be the main cause of staling (Avital *et al.*, 1990; Goesaert *et al*., 2009; Hallberg & Chinachoti, 2002; Hug-Iten *et al*., 2003; Karim *et al*., 2000; Ribotta & Le Bail, 2007). Starch retrogradation is a process in which gelatinised starch molecules re-associate to form double helical crystalline structures (Hug-Iten *et al*., 2003; Pateras, 1998).

The amylose starch retrogrades within a few hours after baking, which affects the initial firmness of the bread (Kamel & Ponte, 1993; Miyazaki, Maeda & Morita, 2004; Ribotta

& Le Bail, 2007; Stampfli & Nersten, 1995). Upon cooling, starch polymers start to lose mobility (Pateras, 1998). The amylose diffuses into the aqueous phase where through hydrogen bonding it forms a concentrated insoluble gel solution, thus contributing to the crumb structure (Pateras, 1998).

Firming during storage is due to the changes in the physical orientation of the branched amylopectin molecules within the swollen starch granule (Goesaert *et al*., 2009; Pateras, 1998). Amylopectin undergoes retrogradation after a longer storage period than amylose (Ribotta & Le Bail, 2007; Stampfli & Nersten, 1995). The re-crystallisation of amylopectin is thought to be the main cause of staling, but it is not solely responsible for the staling process (Durán *et al*., 2001; Goesaert *et al*., 2009; Gray & Bemiller, 2003; Hallberg & Chinachoti, 2002; Katina *et al*., 2006; Miyazaki *et al*., 2004; Ribotta & Le Bail, 2007; Smith *et al*., 2004). In a freshly baked product, the amylopectin chains are amorphous and unfolded (Pateras, 1998). These chains gradually start to aggregate, aligning with one another by various types of intra-molecular bonding which leads to the increased rigidity of the internal structure of the swollen starch granule causing crumb hardening (Pateras, 1998).

Amylopectin re-crystallisation requires moisture redistribution, so that moisture is available at the locus where crystallisation occurs (Bárcenas & Rosell, 2007; Goesaert *et al*., 2009; Gray & Bemiller, 2003). The moisture at the locus acts as a plasticiser and enables the polymer chains to be more mobile in order to crystallize (Gray & Bemiller, 2003). Hallberg and Chinachoti (2002) have shown that with excessive moisture loss the re-crystallisation of amylopectin could be retarded. However, this leads to the gluten network being less hydrated thus losing its flexibility (Goesaert *et al*., 2009).

It has been suggested that by keeping the amorphous starch phase plasticised the hardening of bread would be minimised (Hallberg & Chinachoti, 2002). Hallberg and Chinachoti (2002) have also suggested that as plasticising increases, the texture remains softer for longer although excessive amylopectin re-crystallisation was found to exist in such bread.

*1.2.7.2 Interaction between starch and protein*

If more protein is present in the formula, the rate of staling will be slower. This may be because the protein has a dilution effect on the starch (Gray & Bemiller, 2003; Pateras, 1998). It is also accepted that starch-gluten interaction is somehow responsible for the firming process (Gray & Bemiller, 2003; Keskin, Sumnu & Sahin, 2004). Starch exists in the discontinuous phase within the continuous gluten phase (gluten protein network). Therefore, it has been postulated that rheological properties are more likely to be governed by changes in the continuous phase (Hallberg & Chinachoti, 2002). It has also been suggested that the contribution of starch re-crystallisation to the changes in the rheological properties of bread becomes more significant when the crystal sizes become large enough to have an effect (Hallberg & Chinachoti, 2002). During baking, gluten and swollen starch start to cross-link (Kamel & Ponte, 1993; Keskin *et al*., 2004). These cross-links are the hydrogen bonding interaction between the hydroxyl groups of the protruding starch chains and the amide groups of the protein fibrils (Keskin *et al*., 2004; Pateras, 1998). However, during staling, the kinetic energy of the crumb decreases, which enable cross-linkages to increase both in number and in strength thus contributing to the firming of the crumb (Kamel & Ponte, 1993; Keskin *et al*., 2004) as illustrated below in Figure 4.

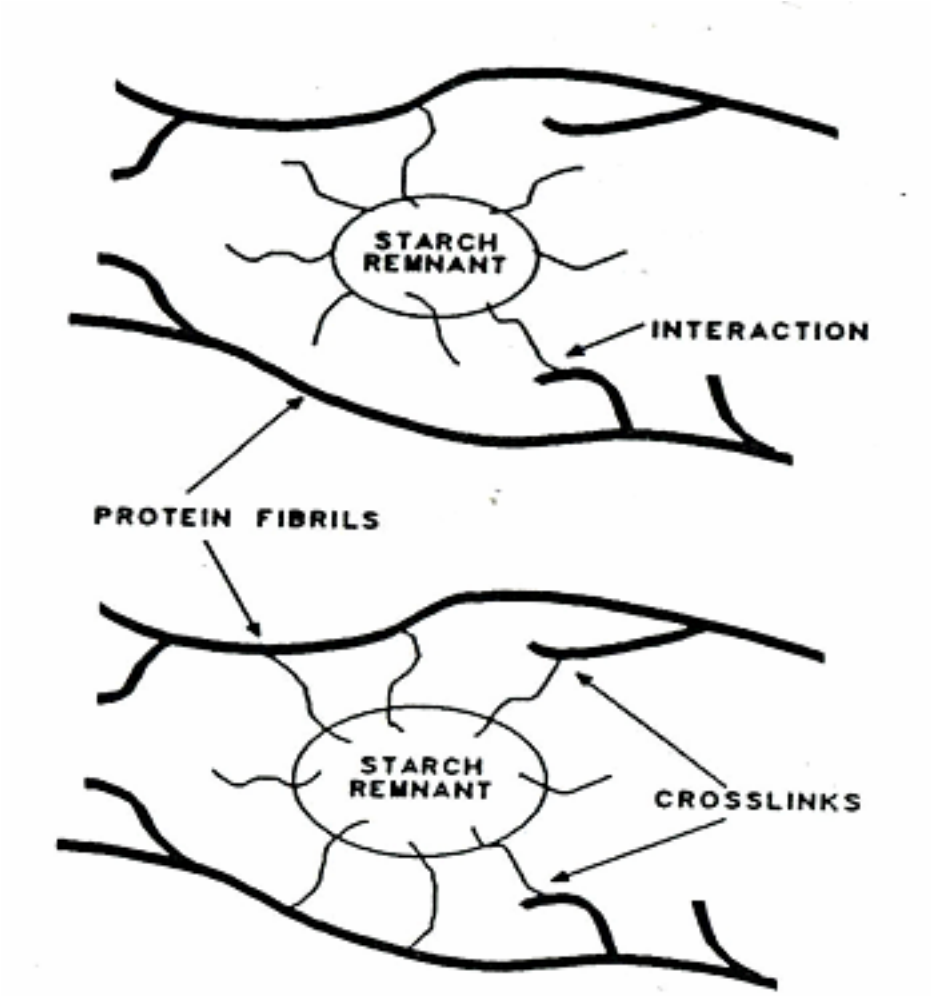



Figure 4: Starch-protein interaction during staling (Kamel & Ponte, 1993)

*1.2.7.3 Moisture and moisture migration*

Moisture migration from the crumb to the crust is due to a temperature gradient that forms during cooling (Piazza & Masi, 1995). The temperature of the crust and layers close to the crust is lower than the temperature in the centre of the crumb (Piazza & Masi, 1995). Therefore, vapour pressure varies between the crust and the centre crumb thereby promoting moisture migration (Gray & Bemiller, 2003; Piazza & Masi, 1995). This leads to a drier internal crumb and a moister crust (Gray & Bemiller, 2003). However, as the storage time increases, the moisture migration starts to reach equilibrium (Piazza & Masi, 1995). He and Hoseney (1990) show that the rate of bread firming increased during the

first 15 d and reached a maximum on d30. It was further observed that the moisture content of the crumb initially decreased fast and then slowed down as the moisture content difference between the crust and the crumb decreased (He & Hoseney, 1990). Therefore equilibrium of moisture content between crust and crumb was established. As moisture decreases, the effect of plasticising also decreases, leading to the formation of more cross-links between solubilised starch and the gluten proteins (Goesaert *et al*., 2009).

Water works as a plasticiser making bread components more flexible. If water were to be removed from the components it would lead to a more firm and rigid structure (Gray & Bemiller, 2003; Katina *et al*., 2006). The rigidity of bread increases as the moisture content decreases (Piazza & Masi, 1995).

The moisture also migrates from the gluten proteins to the crystallized starch (Avital *et al*., 1990; Gray & Bemiller, 2003). The water then becomes bound to the crystalline starch, leaving less water to act as a plasticiser (Avital *et al*., 1990; Goesaert *et al*., 2009; Gray & Bemiller, 2003).

Ricotta and Le Bail (2007) have shown that the moisture content and water activity of the crumb decreases while the crust moisture content and water activity increase during the staling of bread. It has also been observed that the freezable water decreased while the un-freezable water increased, suggesting that the water becomes more bound and immobilised during staling. In other words, the free water decreases while the bound water increases. Therefore, the decrease in freezable water is due to water migration to the crust, which will lead to significant firming of the bread and water being bound or trapped in the starch crystalline structure that develops because of staling (Hallberg & Chinachoti, 2002; Ricotta & Le Bail, 2007).

*1.2.7.4 Effect of storage temperature on the rate of staling*

There is a correlation between bread staling and starch crystallisation at the following storage temperatures: -1, 10 and 21 ºC (Mandala, Karabela & Kostaropoulos, 2007). At

refrigeration temperatures, the staling rate is greater than at room temperature (Leuschner *et al*., 1999). Lainez, Vergara & Bárcenas (2008) observe that storage temperature had a significant effect on the hardening of the par-baked bread. The par-baked bread hardened more quickly when stored at 1 ºC compared to the par-baked bread stored at 7 ºC. As mentioned earlier, starch retrogradation combined with other processes such as the re-crystallisation of amylopectin and the progressive aggregation of starch and gluten are responsible for the hardening of bread (Lainez *et al*., 2008). It has been explained that the crystallisation of semi-crystalline polymers, such as amylopectin, can only take place when the temperature is between the glass transition temperature (Tg) and the polymer melting point (Tm). Crystallisation occurs through three mechanisms, namely: nucleation, growth and annealing of crystals. Nucleation controls the crystallisation process. The nucleation rate is increased as the temperature is decreased until Tg is met, while the growth rate increases as the temperature increases until Tm is met (Lainez *et al*., 2008).

Lainez *et al*. (2008) explain that par-baked bread stored at 1 ºC has a higher rate of nucleation compared with bread stored at 7 ºC. Therefore, a higher rate of amylopectin re-crystallisation leads to a faster hardening of the crumb.

However, par-baked bread regains its original freshness when it is re-heated to 50 ºC and above (Gray & Bemiller, 2003; Leuschner *et al*., 1999; Stampfli & Nersten, 1995). The retrograded amylose is not reversed but the retrograded amylopectin does revert to its amorphous state (Stampfli & Nersten, 1995). Bread stored at low temperatures stales faster and undergoes firming faster, but after reheating the crumb regains its softness and the crust gets drier and becomes crispy. Therefore, the final product has the characteristics of freshly baked bread (Leuschner *et al*., 1999). During reheating, the cross-linkages between starch and gluten are also easily broken and the bread will return to its original freshness (Gray & Bemiller, 2003).

### 1.2.8 Methods to slow down staling

In the bakery industry, enzymes or emulsifiers have been used to slow down the rate of staling (Gray & Bemiller, 2003; Smith *et al*., 2004). Some of these enzymes include the following: α-amylase, lipase, xylanase. Emulsifiers normally used are: sodium stearoyl lactylate (SSL), diacetyl tartaric acid esters of mono- and diglycerides (DATEM) and monoglycerides. Modified atmosphere packaging especially high $CO_2$ packaging atmospheres have been shown to reduce the rate of staling (Gray & Bemiller, 2003; Smith *et al*., 2004).

#### *1.2.8.1 Enzymes*

The use of enzymes in the baking industry has increased in popularity due to the changes in demand for more natural products in this industry (Si, 1997). The following enzymes have gained a lot of interest in the industry: maltogenic α-amylase, lipase (Si, 1997).

##### 1.2.8.1.1 Maltogenic α-amylase

The bacterial *Bacillus stearothermophilus* maltogenic α-amylase has been found to have anti-staling properties by reducing the crumb-firming rate and the inhibition of amylopectin re-crystallisation (Cabellero, Gomez & Rosell, 2007; Durán *et al*., 2001; Goesaert *et al*., 2009; Gray & Bemiller, 2003; Jones *et al*., 2008; León, Durán & Benedito de Barber, 1997; Si, 1997). This enzyme belongs to the glycoside hydrolase GH 13 family (Goesaert *et al*., 2009; Jones *et al*., 2008). Novamyl® is a commercially available maltogenic α-amylase that has both endo- and exo-amylase functionality (Goesaert *et al*., 2009; Jones *et al*., 2008). Maltogenic α-amylase produces mainly maltose segments (Goesaert *et al*., 2009).

Alpha-amylase breaks down the amylopectin so that it has shorter branches; however, the amylopectin backbone remains intact (Cabellero *et al*., 2007; Keskin *et al*., 2004). These dextrins may interfere with the starch-gluten interaction and with the amylopectin re-

crystallisation, thereby reduce the rate of firming (Durán *et al*., 2001; Jones *et al*., 2008; Kestin *et al*., 2004; León *et al*., 1997; Miyazaki *et al*., 2004; Si & Lustenberger, 2001). Kestin *et al*. (2004) have shown that α-amylase reduces the firming of microwave bread by breaking down the starch and reducing the amount of leaking amylose. Hug-Iten *et al*. (2003) explain that the different starch degrading enzymes work through different mechanisms to reduce staling. Alpha-amylase degrades the starch molecules in the amorphous regions thus altering the link between the crystalline regions. In return, this leads to a reduced structural strength of the starch network (Hug-Iten *et al*., 2003; León *et al*., 1997). However, α-amylase is inactivated in the late stages of baking; it therefore does not break down the starch excessively (Si & Lustenberger, 2001). The enzymatic action of different amylolytic enzymes is shown in Figure 5.

Studies have shown that saccharides (glucose, maltose and fructose) can slow down starch retrogradation by modifying the hydrogen bonds of the starch chains (Durán *et al*., 2001). Durán *et al*. have shown (2001) that maltodextrins with a 3–5 degree of polymerisation (DP 3-5) reduce the rate of wheat starch retrogradation. They suggest that these oligosaccharides interact with the amorphous region (amylose) of the starch granule and act as stabilisers during the aging of the starch.

Maltogenic α-amylase reduces the retrogradation of starch and more so when storage time increases (Si, 1997). At gelatinisation temperature, it degrades both amylose and amylopectin to produce maltose segments (Hug-Iten *et al*., 2003; Jones *et al*., 2008; Si, 1997; Williams & Pullen, 2007). This leads to the enhancement of the mobility of these polymers (Hug-Iten *et al*., 2003). It has also been observed that bacterial maltogenic α-amylase softens the breadcrumb (Si, 1997). It has also been shown that maltogenic α-amylase produces a more elastic crumb during storage than fungal amylase and monoglycerides (emulsifier) combined (Si, 1997; Si & Lustenberger, 2001). Maltogenic α-amylase does not affect the volume. For this reason, it would be advantageous to use it in combination with other enzymes like lipase and/or xylanase that ensure bread quality parameters such as volume, dough stability and crumb structure (Si & Lustenberger, 2001).

However, Kestin *et al*. (2004) have noted that alpha-amylase has a positive effect on the specific volume of bread. They postulate that this might be because the amylase has an effect on starch. The amylase breaks down the starch into readily available sugars during dough preparation, so that yeast can ferment the available sugars into alcohol and carbon dioxide that cause the dough to rise.

The difference between fungal, bacterial and cereal α-amylase is their temperature optima (Sahlsröm & Bråthen, 1997). The temperature range of fungal α-amylase is 50–60 ºC and that of bacterial α-amylase 70–80 ºC (Sahlsröm & Bråthen, 1997; Williams & Pullen, 2007). Maltogenic α-amylase remains active during the gelatinisation of starch during baking (52–88 ºC). Therefore it can break down the starch into dextrins (Jones *et al*., 2008; Si & Lustenberger, 2001).

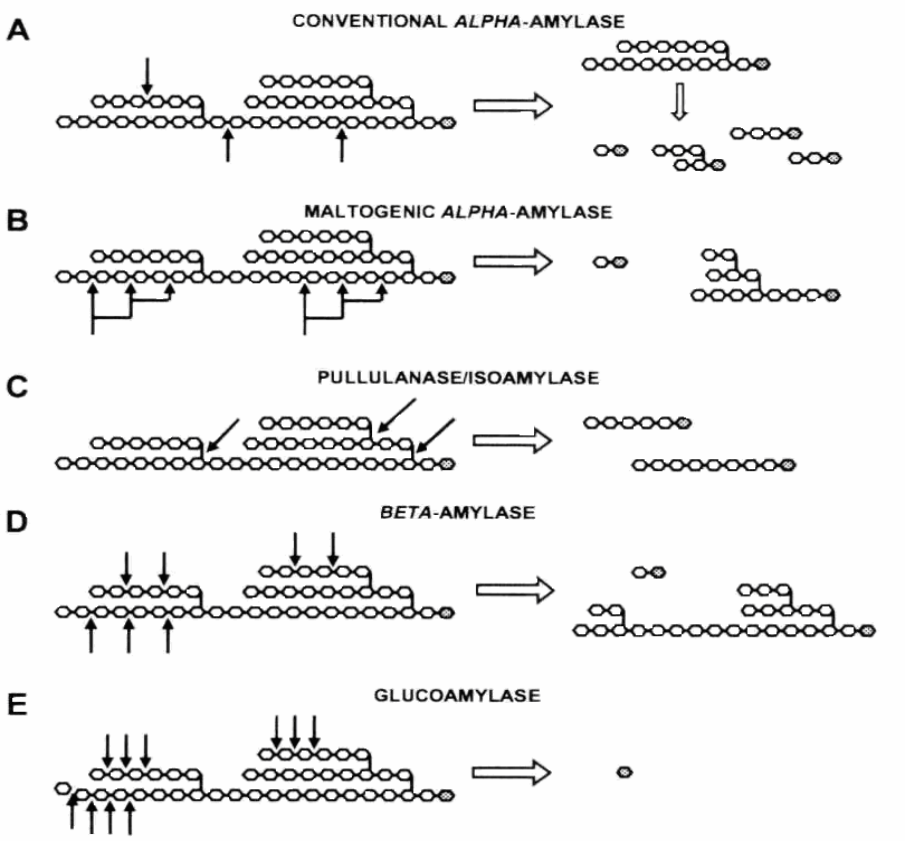



Figure 5: Action of different amylolytic enzymes on starch polymers. The darker ring structure represents a reducing sugar. (A) Alpha-amylase producing low molecular weight dextrins through and endo-type action; (B) maltogenic α-amylase producing mainly maltose through an exo-type action; (C) pullulanase producing linear dextrins through a debranching action; (D) beta-amylase producing maltose and beta-limit dextrins though and exo-type action; (E) glucoamylase producing glucose through an exo-type action (Goesaert *et al.*, 2009).

1.2.8.1.2 Lipase

Exogenous lipase affects the dough rheology and dough colour (Castello *et al*., 1999). Exogenous 1, 3- specific lipase improves dough elasticity and extensibility (Castello *et al*., 1999). Lipase is a strong dough-conditioning enzyme that can successfully replace dough-conditioning emulsifiers such as DATEM (Guy & Sahi, 2006; Keskin *et al*., 2004; Si & Lustenberger, 2001). Dough conditioners improve the dough strength, thus lipase also improves the gluten protein strength (Si, 1997). Its addition results in higher loaf volume and a uniform grain therefore improving crumb softness during storage (Castello *et al*., 1999; Gray & Bemiller, 2003; Keskin *et al*., 2004; Si, 1997).

During mixing and proofing, lipase increases the number of molecules with emulsifying properties by breaking down the triglycerides (TAG) into diglycerides (DAG), monoglycerides (MAG) and free fatty acids (FFA) (Castello *et al*., 1999). Polyunsaturated fatty acids (PUFA) and endogenous wheat lipooxygenase also form oxidised lipids, which can co-oxidise other molecules such as protein thiols (PSH) and carotenoid pigments (Fig 6). Therefore, exogenous lipases increase the co-oxidation reaction of lipids. This produces a strong gluten network with more double sulfide bridges (PSSP) and whiter dough by bleaching the carotenoid pigments in the flour. These pathways are the same as for endogenous wheat lipase. In this way exogenous and endogenous lipases increase the intensity of oxidation reactions by supplying more substrate to endogenous lipooxygenase (Castello *et al*., 1999).

Guy and Sahi (2006) have shown that Lipopan® F BG a commercial lipase enzyme, lowers the surface tension of a cake batter. This suggests that lipase produces surface-active material by its action on the native flour lipids or the added fat. The decrease in surface tension, lowering of the interfacial viscosity and increase of the bulk batter density may be attributed to the surfactants produced by lipase that help to hydrate the gluten. This leads to the increase of viscosity and stabilises the air bubbles by forming new interfacial membranes (Guy & Sahi, 2006).

The higher loaf volume may also be attributed to the production of more emulsifying molecules by lipase (Keskin *et al*., 2004). Guy and Sahi (2006) explain that lipase causes gas cells to increase in size rather than in number, thus leading to an increase of cake volume.

Lipase also reduces retrogradation in bread (Gray & Bemiller, 2003; Keskin *et al*., 2004; Si, 1997; Williams & Pullen, 2007). The exact mechanism is still unknown. It has been postulated that monoglycerides interact with amylose thus inhibiting it from forming double helices upon cooling. However, it has also been postulated that the amount of monoglycerides produced by the hydrolysis of triglycerides was not enough to retard staling (Keskin *et al*., 2004; Si, 1997).

Guy and Sahi (2006) have observed that lipase (Lipopan® F) reduces the firming of cake that includes this enzyme compared with cake that does not contain this enzyme. It was suggested that the reduced firming rate was because the cake with lipase had a higher specific volume than cake without it.

Lipase may be considered for use in conjunction with the other enzymes for it has shown synergistic effects on the quality of dough and bread, producing a reduced stickiness (Si, 1997). Siswoyo, Tanaka and Morita (1999) have shown that the use of an enzyme mixture containing lipase and α-amylase, produced synergistic positive effects on the specific volume and starch-lipid complex formations.

It was also observed that bread with lipase and α-amylase had reduced starch retrogradation compared with bread that contained these enzymes individually (Siswoyo *et al*., 1999).

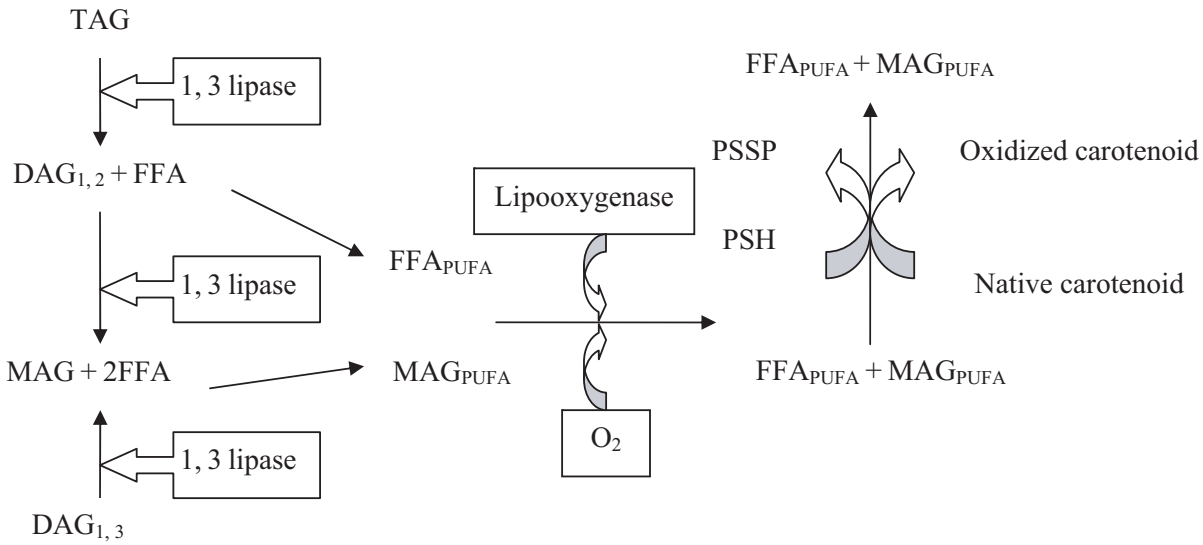



Figure 6: Biochemical changes induced by exogenous 1, 3- specific lipase during mixing of wheat dough, with triglycerides (TAG), diglycerides (DAG), monoglycerides (MAG), free fatty acids (FFA), polyunsaturated fatty acids (PUFA), protein thiols (PSH) and double sulfide bridges (PSSP) (Castello, 1999)

*1.2.8.2 DATEM emulsifier*

Emulsifiers are surface-active agents that are composed of lipophilic and hydrophilic properties (Stampfli & Nersten, 1995). Emulsifiers reduce the surface tension between two immiscible phases and so an emulsion is able to form (Stampfli & Nersten, 1995). Some of the characteristics expected of emulsifiers in the bakery industry are the following: improved dough handling and strength; increased rate of hydration and water absorption; improved crumb structure; creation of greater tolerance to resting time and fermentation; increased gas retention capacity; and increased shelf life (Stampfli & Nersten, 1995). An emulsifier can be classified as follows: its origin; solubility properties; presence of functional groups; hydrophilic/lipophilic ratio; and its potential for ionisation. Emulsifiers are normally used to increase dough strength or crumb softening, but some emulsifiers do possess the property of both functions. Dough in the bakery industry is expected to have great tolerance to vibration and mechanical shock (Stampfli & Nersten, 1995).

Diacetyl tartaric acid esters of mono and diglycerides (DATEM) are anionic oil-in-water emulsifiers (Ribotta *et al*., 2004; Selomulyo & Zhou, 2007; Stampfli & Nersten, 1995). DATEM is normally used for its dough-strengthening properties, especially during dough fermentation, dough handling, during proofing and during the first part of baking. The strengthening effect finally results in higher loaf volume, a resilient crumb texture and fine-grained crumb (Ribota *et al*., 2004; Selomulyo & Zhou, 2007; Stampfli & Nersten, 1995).

The mechanism is not yet fully understood but a possible theory is the following: the emulsifier forms liquid films of lamellar structure between the gluten network and starch (Stampfli & Nersten, 1995). It improves the ability of the gluten network to form films to retain gas (Stampfli & Nersten, 1995). The hydrophilic emulsifier can also form a lamellar liquid-crystalline phase in water that is associated with gliadin (Ribotta *et al*., 2004; Selomulyo & Zhou, 2007). These structures contribute to the elasticity of the

dough which enables the gas cells to expand that will lead to a higher loaf volume (Ribotta *et al*., 2004; Selomulyo & Zhou, 2007).

DATEM may also cause the aggregation of the gluten proteins by binding to the hydrophobic groups on the protein surface during dough formation (Köhler, 2001). This leads to the net charge of gluten being reduced, which will lead to the formation of a strong gluten network, thus producing bread with higher volume and better texture (Armero & Collar, 1998; Köhler, 2001; Ribotta *et al*., 2004; Selomulyo & Zhou, 2007; Williams & Pullen, 2007). The model for the dough-strengthening effect of DATEM is illustrated in Figure 7.

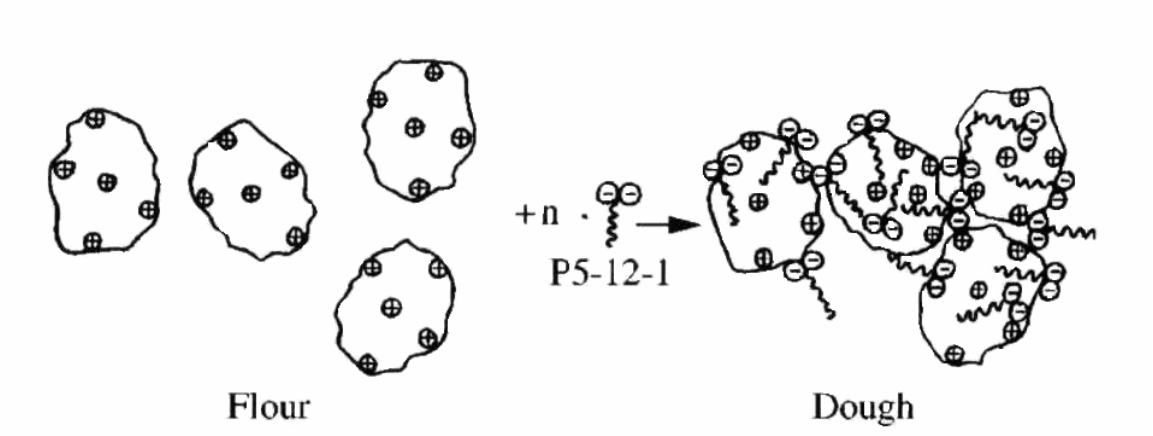



Figure 7: Interaction of gluten proteins and DATEM (P5-12-1) with two carboxyl groups forming a strong gluten network with gluten aggregates (Köhler, 2001)

It has been reported that DATEM has no effect on water absorption, but it does increase the dough stability and loaf volume, reduces proofing time and promotes gassing power (Stampfli & Nersten, 1995). DATEM has shown a positive correlation between dosage, and fermentation time and stability. It has been shown that with increasing dosage, the fermentation time and fermentation stability also increase (Stampfli & Nersten, 1995).

DATEM interacts with amylose and forms complexes (Selomulyo & Zhou, 2007). A crumb softener is an emulsifier or surfactant that interacts with starch thereby reducing the rate of staling (de Leyn, 2006; Stampfli & Nersten, 1995). The emulsifier may form complexes with amylose that will render it insoluble in water (de Leyn, 2006). The lipid-

amylose complex is unable to re-crystallize and hence cannot take part in the staling process (de Leyn, 2006; Gray & Bemiller, 2003; Ribotta *et al*., 2004; Stampfli & Nersten, 1995; Toufeili *et al*., 1995). The monoglycerides are normally used as softeners (Stampfli & Nersten, 1995).

Emulsifiers increase the amylose-lipid complexes and reduce amylopectin retrogradation, but DATEM is less efficient than distilled monoglyceride to act as a crumb softener (Gray & Bemiller, 2003; Ribotta *et al*., 2004; Stampfli & Nersten, 1995). It has been stated earlier that the re-crystallisation of amylopectin is the main reason for staling. Therefore, emulsifiers that form complexes with both amylose and amylopectin possess a higher efficiency to act as crumb softeners during storage, for instance sodium stearoyl lactylate (SSL) (Gray & Bemiller, 2003; Stampfli & Nersten, 1995; Toufeili *et al*., 1995). DATEM may also disrupt the water migration from the gluten to the starch, which will enable the amylopectin to retrogradate (Gray & Bemiller, 2003; Ribotta *et al*., 2004; Selomulyo & Zhou, 2007).

Armero and Collar (1998) have shown that DATEM is a good crumb softener when used in white and wholemeal breads, sourdough and straight-dough production processes. They found that it acted as a good softener for 4 d in sourdough bread and for 6 d in straight-dough bread. Higher specific volume and thinner gas cell walls correlated with softer crumbs. The effective resistance surface in a cross section decreases as the thickness of the gas cell walls decreases, leading to a reduced firming rate (Armero & Collar, 1998).

Ribotta *et al*. (2004) have shown that DATEM reduced the firming rate of bread, however, low storage temperatures reduced its efficiency. DATEM's association with starch is minimal compared with the other emulsifiers such as SSL and MG. It has been suggested that the anti-firming effects of DATEM are probably related to the changes in cell wall thickness and modification of elasticity (Gray & Bemiller, 2003; Toufeili *et al*., 1995).

*1.2.8.3 Modified Atmosphere Packaging (MAP)*

Modified atmosphere packaging has been applied in the food industry in order to increase shelf life (Kotsianis, Giannou & Tzia, 2002). This objective is met by changing the proportions of the atmospheric gases that normally consist of a $CO_2$, $O_2$, and $N_2$ combination (Kotsianis *et al*., 2002).

The firmness of bread is usually measured by using a compression test (Knorr & Tomlins, 1985). Knorr and Tomlins (1985) showed that the compressibility of white bread slices and whole wheat bread slices was lower when packaged in 100% $CO_2$ modified atmosphere compared with the bread slices packaged in air. The reason for the effect of $CO_2$ on the firming of the bread slices was not clear (Knorr & Tomlins, 1985).

Avital *et al*. (1990) have also shown that $CO_2$ delays the staling of bread when packaged in a carbon dioxide modified atmosphere. During its storage in a $CO_2$ atmosphere, bread loses its water-holding capacity. Gluten does not absorb water during its storage in baked products but starch does. Amylose is also retrograded after one day of storage therefore the amylopectin absorbs water during storage. The $CO_2$ may block the sites of hydrogen bonding between the water and amylopectin, thus reducing the water absorption capacity. Avital *et al*. (1990) explain that the formation of these hydrogen bonds is one of the basic reasons for staling. They also observe that the effect of $CO_2$ exists when the water is in a solute state and that carbon dioxide is soluble in water. This may enable $CO_2$ to bind with the amylopectin branches (Avital *et al*., 1990).

**1.2.9 Microbiological spoilage**

*1.2.9.1 Factors that affect microbiological spoilage*

One of the main predetermining factors of the microorganisms that may be present is water activity. Almost no microorganism will grow at and below the water activity of 0.6. Mould and osmophilic yeast can grow at the following water activity range of 0.6–

0.85. Almost all bacteria, yeast and mould grow and cause spoilage between 0.85–0.99 water activity range (Legan & Voysey, 1991; Leuschner *et al*., 1999; Smith *et al*., 2004).

The storage temperature also influences the rate of microbiological spoilage and the type of microorganism present. Lainez *et al*. (2008) noted that partially baked bread stored at 7 ºC, had surface mould growth after 9 d, whereas partially baked bread stored at 1 ºC had surface mould growth after 28 d. The aerobic plate count (APC) did increase from 3.4 log cfu/g on d1 to 5.5 log cfu/g on d7 when stored at 1 ºC. At 7 ºC it was even higher; on d1 the APC was 4.8 log cfu/g and on d7, 7.2 log cfu/g. The yeast count also increased from less than 1 log cfu/g on d1 to 3.8 log cfu/g on d28 when stored at 1 ºC. However, refrigeration reduced the APC and yeast count when compared to bread stored at 20 ºC with an APC of 1.04 x $10^8$ cfu/g and a yeast count of 2.8 x $10^5$ cfu/g on d7. When the partially baked bread was re-baked the microbial population reduced by 4 log cycles.

*1.2.9.2 Bacterial spoilage*

*Bacillus* species (spp) are the major contributor to spoilage of bakery products, in particular *B. subtilis,* which causes rope spoilage (Bailey & von Holy, 1993; Leuschner *et al*., 1999; Smith *et al*., 2004). Other *Bacillus* species of concern are *B licheniformis, B. cereus* and *B megaterium* (Bailey & von Holy, 1993).

The first indication of rope spoilage is a sweet fruity odour that can be noticed 12–24 hours after baking (Bailey & von Holy, 1993). At a later stage, the crumb becomes sticky and discoloured due to bacterial metabolism (Bailey & von Holy, 1993; Smith *et al*., 2004). The stickiness is caused by slime produced by the bacteria (Bailey & von Holy, 1993). When the crumb is broken open, web-like strands are visible at the stage when the crumb becomes discoloured and sticky (Bailey & von Holy, 1993).

It was shown by Bailey and von Holy (1993) that rope spoilage was evident at a spore level of 6.38 log cfu/g when brown bread was stored at 30 ºC for 3 d. It was also highlighted that under-baking can cause spores to survive the baking period. Once the

environmental conditions become favourable they start to grow (Bailey & von Holy, 1993). These bacteria require water activities of 0.9–0.99 to grow, so they will be limited to high moisture-content bakery products, like pizza (Smith *et al*., 2004).

In South Africa, rope spoilage may be more prevalent due to the warmer humid climate and poor sanitary practices followed in some bakeries (Bailey & von Holy, 1993). Bailey and von Holy (1993) have shown that flour, commercial yeast and bakery equipment are the main sources of *Bacillus* spp. spore introduction.

If toppings and fillings are added to pizza, different microorganisms may also contribute to spoilage of the product. For instance, if tomato paste is added the expected spoilage bacteria are lactic acid bacteria (Cabo *et al*., 2001). Lactic acid bacteria have also been isolated from pizza dough. It has been suggested that these bacteria were introduced by contaminated yeast or through the environment (Coppola *et al*., 1998). Lactic acid bacteria can cause spoilage through the production of lactic acid and carbon dioxide leading to a drop in pH and the formation of off-flavours (Huis in't Veld, 1996).

Leuschner *et al*. (1999) noted no rope spoilage in brown soda bread when it was packaged in a modified atmosphere with 40%:60% $CO_2$:$N_2$.

*1.2.9.3 Yeast spoilage*

Yeast normally spoils intermediate-moisture to high-moisture content bakery products (Smith *et al*., 2004). There are two types of yeast spoilage: visible yeast spoilage, which is yeast on the surface of the product that may appear as pink and/or white patches and fermentative spoilage that normally results in an alcoholic odour with or without visible gas bubbles (Legan & Voysey, 1991; Smith *et al*., 2004).

*Pichia burtonii*, *Candida guilliermondii*, *Hansenula anomala* and *Debaromyces hansenii* are normally associated with bakery products (Legan & Voysey, 1991; Smith *et al*., 2004). Contamination by yeast usually occurs during the post-baking period when unsanitary practices are followed and through flour and dust-air ventilation (Legan &

Voysey, 1991; Miller, 1979; Smith *et al*., 2004). Spoilage of bakery products is more often caused by mould rather than by yeast (Legan & Voysey, 1991).

The interaction between yeast and other groups of organisms on the same substrate is limited due to competition for available nutrients (Miller, 1979). Some microorganisms may make available the components of the substrate to yeast that otherwise would have been unavailable to the yeast. For example, mould and bacteria can hydrolyse cellulose and xylan with their extra cellular enzymes while yeast cannot do this. Breakdown products are then released and are readily available for the yeast metabolism to multiply (Miller, 1979).

Lactic acid bacteria may release inhibitory metabolic products such as lactic acid and acetic acid that are inhibitory to some microorganisms. However, there are yeasts that can utilise these acids in their disassociated form and gain competitive advantage over the inhibited microorganisms (Huis in't Veld, 1996; Miller, 1979).

*1.2.9.4 Mould spoilage*

Moulds are the main cause of spoilage of bakery products and may even be the shelf life determinant (Guynot *et al*., 2003a & b; Nielsen & Rios, 2000; Rodriguez, Medina & Jordano, 2000; Smith *et al*., 2004). They normally grow at a water activity of >0.80, however, a few can grow at very low water activity and are known as xerotolerant moulds (Lacey, 1989; Smith *et al*., 2004). Moulds grow more often in low water activity foods than high water activity foods because fewer competitive microorganisms are present (Leuschner *et al*., 1999). Lacey (1989) proposes that mould often forms in great numbers when the conditions are sub-optimal, for example, *Eurotuim* spp. predominate the grain at a water activity of 0.72, while their optimum is at 0.90. Abellana, Sanchis and Ramos (2001) have shown that none of the isolates tested grow below the water activity of 0.80.

Moulds are normally killed during the baking procedure but can re-contaminate the product after baking through airborne spores, unclean utensils and food handlers

(Abellana *et al*., 2001; Pateras, 1998; Guynot *et al*., 2003b; Smith *et al*., 2004). Therefore, the practice of good sanitation and hygienic practices may reduce the number of unwanted mould spores (Guynot *et al*., 2003a). Mould spoilage is more prolific during the summer months when temperatures are higher and the environment more humid than in other seasons of the year (Smith *et al*., 2004). The following species are mainly associated with the spoilage of food products: *Penicillium*, *Aspergillus*, *Eurotium* and *Cladosporium* spp. (Abellana *et al*., 2001; Nielsen & Rios, 2000; Smith *et al*., 2004). *Eurotium* spp. normally first colonise incorrectly dried and stored bakery products, elevating the level of available water and thereby making the environment more favourable for other moulds to grow (Abellana *et al*., 2001).

Pinho and Furlong (2000) showed that refrigeration did reduce the occurrence of moulds and yeast, due to reduced metabolism. However, refrigerated storage did not reduce the population count below the limit for marginal quality, which is 2 x $10^3$ cfu per gram. The level of moulds and yeast that is associated with unacceptable quality is 5 x $10^4$ cfu/g. However, the maximum tolerated level of moulds and yeasts in baked goods, according to the Brazilian guideline, is 5 x $10^3$ cfu/g. The following genera were most frequently isolated from the pre-baked pizza dough: *Penicillium* and *Aspergillus* spp (Pinho & Furlong, 2000).

Most mould can thrive at temperatures between 10–40 ºC, while their optima are at 25–35 ºC (Lacey, 1989). However, lowering the temperature may reduce the rate of mould metabolism and food deterioration. Mould has a selective advantage over bacteria at low temperatures. Although low temperatures of 1–2 ºC have been used to slow down spoilage of moist grain, *Penicillium* and *Fusarium* spp. were still able to grow and produce mycotoxins (Lacey, 1989).

Abellana *et al*. (2001) have shown that the growth rate of *Penicillium* and *Aspergillus* spp. are influenced by both minimum water activity required and storage temperature. At 15 ºC, the minimum water activity required for *Penicillium* spp. was 0.9 and for *Aspergillus flavus,* no growth occurred. At 20–25 ºC, the minimum water activity for *Penicillium* spp. was 0.85 and for *Aspergillus flavus,* 0.9. The highest growth rate

recorded for *Aspergillus flavus* was between 25–30 ºC at a water activity of 0.9. This information can predict the probability of the occurrence of mould spoilage (Abellana *et al*., 2001).

### 1.2.10 Inhibition of microbiological spoilage

According to Smith *et al*. (2004), there are three strategies to control microbiological spoilage of bakery products, as follows:

- Packaging the product before baking or immediately after baking under aseptic conditions that can be met with air filtration systems and thorough sanitary procedures;
- Destroying post-baking contaminants on the surface of the product after packaging by using UV light, infrared radiation, microwave heating, low dose irradiation and ultra high pressure; and
- Controlling the post-baking growth of microorganisms inside the packaged products by using preservatives and modified atmosphere packaging.

The control of microorganisms inside the packaged product after baking through the application of modified atmosphere packaging and the use of oxygen absorbers will be discussed.

### 1.2.11 Modified atmosphere packaging

Modified atmosphere packaging (MAP) is an alternative to chemical preservation to increase the shelf life of bakery products (Cabo *et al*., 2001; Church & Parsons, 1995; Kotsianis *et al*., 2002; Smith *et al*., 2004). It is the packaging of a food product in packaging materials with a gas-barrier and with a changed gaseous environment in order to prevent the growth of spoilage microorganisms, thereby maintaining a higher quality and an extension of shelf life (Church & Parsons, 1995; Smith *et al*., 2004). Gas flushing is the removal of air from the package and its replacement with a single gas or a

combination of gases (Church & Parsons, 1995). The gases generally used are carbon dioxide, nitrogen and oxygen (Church & Parsons, 1995; Guynot *et al*., 2003a; Kotsianis *et al*., 2002; Smith *et al*., 1986; Smith *et al*., 2004).

In order to extend a product's shelf life successfully, the correct gas combination is required. The product must be packaged with the correct packaging material or film, with the correct permeability properties and must be stored at the correct temperature (Kotsianis *et al*., 2002; Smith *et al*., 2004).

*1.2.11.1 Film type*

The film type also influences the effectiveness of modified atmosphere packaging to extend shelf life; it should have the right permeability to oxygen and carbon dioxide to maintain the correct gas mixture inside the package (Kotsianis *et al*., 2002; Smith *et al*., 2004). The film should have a low water transmission rate in order to prevent moisture loss or gain (Smith *et al*., 2004). Seal ability, ability to thermoform, film clarity and anti-fog properties should also be considered when choosing a film (Smith *et al*., 2004). Carbon dioxide is five times more permeable through food packaging material than oxygen (Labuza, Fu & Taoukis, 1992). Cooked foods have a low $CO_2$ consumption rate; therefore, $CO_2$ may slowly drop during the shelf life period (Labuza *et al*., 1992).

*1.2.11.2 Storage temperature*

The microbial shelf life of par-baked bread was extended when stored at low temperatures in combination with modified atmosphere packaging (Leuschner *et al*., 1999). However, it has been found that refrigeration temperatures accelerate the staling rate, but par-baked goods may regain their soft crumb after reheating. It is shown by Leuschner *et al*. (1999) that as storage temperature increases so does the *Bacillus* level, the lowest population level being obtained when par-baked bread is stored at 4 ºC in 40%:60% $CO_2$:$N_2$ atmosphere. When the $CO_2$ concentration increases, the minimum temperature required for growth may also rise (Labuza *et al*., 1992).

*1.2.11.3 Gases used in MAP*

1.2.11.3.1 Oxygen

Aerobic bacteria, mould and undesirable oxidative reactions require oxygen, therefore oxygen removal can inhibit spoilage and extend shelf life (Church & Parsons, 1995; Labuza *et al*., 1992). However, oxygen is required to prevent an anaerobic environment from developing when fresh commodities such as fruit and vegetables are packaged (Church & Parsons, 1995). Oxygen is also required in the meat industry to preserve the colour of red meat (Church & Parsons, 1995). In the bakery industry, no oxygen is needed to preserve or prevent deterioration reactions.

When oxygen is removed, the growth of gram-negative aerobes such as *Pseudomonas* spp. is restricted (Labuza *et al*., 1992). When the residual oxygen content is kept <1%, the growth of mould is restricted because mould is strictly aerobic (Guynot *et al*., 2003 a & b; Lacey, 1989). However, the removal of oxygen may enhance the growth of *Lactobacillus* spp. (Labuza *et al*., 1992).

1.2.11.3.2 Nitrogen

Nitrogen gas is a filler gas that prevents the package from collapsing and replaces oxygen (Church & Parsons, 1995; Kotsianis *et al*., 2002; Smith *et al*., 2004). Nitrogen is also tasteless and does not pass through the packaging material and food product as easily as the other gases discussed here (Church & Parsons, 1995; Guynot *et al*., 2003a).

1.2.11.3.3 Carbon dioxide

Carbon dioxide is used as a bacterial and fungal inhibitor (Church & Parsons, 1995; Kotsianis *et al*., 2002; Smith *et al*., 2004). The inhibitory effect of carbon dioxide is dependent on the following: amount of gas present, storage temperature, and growth

phase of the microorganism (Church & Parsons, 1995; Guynot *et al*., 2003a; Smith *et al*., 2004).

The inhibitory effect increases as the concentration of $CO_2$ increases. The inhibitory effect increases linearly as the concentration increases up to 50–60% but the effect changes little above this concentration (Church & Parsons, 1995; Smith *et al*., 2004).

When a 100% $CO_2$ is used, the product can become acidic and a vacuum may develop and cause the package to collapse. In order to prevent this, it is advisable to use low permeable packaging film and gas mixes (Church & Parsons, 1995; Smith *et al*., 2004).

Low $CO_2$ concentrations may lead to an increase in microbial metabolism, which may lead to product acidification as well as $CO_2$ production due to fermentative metabolism (Cabo *et al*., 2001). Fermentative metabolism is due to the growth of lactic acid bacteria and yeast that is the main micro-flora of tomato paste. However, the growth of yeast can be inhibited with high $CO_2$ atmospheres (Cabo *et al*., 2001).

The effect of $CO_2$ is also dependent on its solubility in the aqueous phases and fat phases on the surface of the food product. The solubility of $CO_2$ is higher at low temperatures than at high temperatures (Church & Parsons, 1995). It is thought that $CO_2$ dissolves to create carbonic acid on the food surface reducing the environmental pH, thus the microorganisms present must dissipate energy to maintain internal pH. Therefore, the reduction of the pH will result in a reduced growth rate of spoilage and potential pathogenic microorganisms (Labuza *et al*., 1992).

The lower the initial microbial load the more effective the $CO_2$ will be (Church & Parsons, 1995; Labuza *et al*., 1992; Smith *et al*., 2004). The inhibitory effect is greater during the lag growth phase than during the logarithmic growth phase (Church & Parsons, 1995; Labuza *et al*., 1992; Smith *et al*., 2004). Guynot *et al*. (2003a) have observed that $CO_2$ increased the lag phase of the mould growth. The lag phase of the mould growth was increased to more than 20 d when stored in a 100% $CO_2$ atmosphere.

These researchers also observed that the reduction of water activity to 0.85–0.90 increased the inhibitory effect of $CO_2$.

Carbon dioxide is most effective against aerobic bacteria and mould (Smith *et al*., 2004). Gram-negative bacteria are more sensitive to elevated $CO_2$ concentrations than gram-positive bacteria are (Smith *et al*., 2004). Bacteria like lactic acid bacteria and *Bacillus* spp. are more resistant to elevated $CO_2$ concentrations and anaerobic bacteria. For example, *Clostridium botulinum* is not affected by $CO_2$ (Smith *et al*., 2004). *Penicillium* spp. are more resistant to $CO_2$ than *Aspergillus* spp. (Smith *et al*., 2004). However, anaerobic growth may be reduced when high $CO_2$ levels are used in conjunction with low temperatures (Labuza *et al*., 1992).

Mould is one of the shelf life limiting factors in baked goods (Rodriguez *et al*., 2003). One way of extending the mould-free shelf life is by reducing their growth rate during post-baking and this can be achieved with modified atmosphere packaging (Knorr & Tomlins, 1985; Rodriguez *et al*., 2003). It has been shown that pizza's shelf life can be extended to 15–21 d when using a high barrier packaging film with a high $CO_2$ concentration of 50% (Rodriguez *et al*., 2003). Mould growth can be completely inhibited for 14 d at 25 ºC with a carbon dioxide concentration of 50–100% (Smith *et al*., 2004). However, Rodriguez *et al*. (2003) observed that the $CO_2$ concentration did not influence the growth patterns of the microorganisms. They explain it was due to $O_2$ draining through the packaging material during the storage period. Moulds like *Penicillium* and *Aspergillus* spp. require a minimum amount of 0.4% $O_2$. However, visible mould growth was evident after 13 d only on samples packaged in 100% $CO_2$, compared to samples packaged in lower concentrations of $CO_2$ where visible growth was evident within 7 d (Rodriguez *et al*., 2003). In pure $CO_2$ atmosphere, mould can be inhibited despite the residual oxygen (Guynot *et al*., 2003a). The inhibitory effect of $CO_2$ may be enhanced when low $O_2$ concentrations are achieved (Lacey, 1989).

The residual oxygen present may determine if aerobic spoilage could still occur (Guynot *et al*., 2003b). When mould growth still occurs, it may be due to the presence of residual oxygen that may have originated from trapped air by the product itself, oxygen

penetrating the oxygen permeable packaging material, inadequate sealing and/or gas flushing (Guynot *et al*., 2003b; Smith *et al*., 1986; Smith *et al*., 2004). Products may absorb headspace oxygen that may cause the package to cling to the product. Localised environments may then be created, possibly leading to increased oxygen levels and mould growth (Smith *et al*., 1986). Most bakery products are highly porous thereby making it difficult to remove all the oxygen from the packaging container (Guynot *et al*., 2003b).

### 1.2.12 Oxygen absorbers

A novel approach of controlling the oxygen concentration inside the package is to make use of oxygen absorbers (Guynot *et al*., 2003b; Kotsianis *et al*., 2002; Smith *et al*., 1986; Smith *et al*., 2004). Oxygen absorbers are packets that are included in the packaging to modify the atmosphere and reduce the residual oxygen in headspace through a chemical reaction (Guynot *et al*., 2003b; Kotsianis *et al*., 2002; Powers & Berkowitz, 1990; Smith *et al*., 1986). These oxygen absorbers typically contain ferrous compounds (Guynot *et al*., 2003b; Powers & Berkowitz, 1990; Smith *et al*., 1986). The iron absorbs the oxygen to form non-toxic iron oxide when the humidity condition is appropriate (Guynot *et al*., 2003b).

An oxygen absorber can reduce oxygen levels to below 0.05–0.1% and therefore reduce the growth of aerobic bacteria, mould and insects (Appendini & Hotchkiss, 2002; Guynot *et al*., 2003b). It may also prevent unwanted oxidative changes that may deteriorate the sensory quality of the bakery product (Appendini & Hotchkiss, 2002; Kotsianis *et al*., 2002).

Oxygen absorbers are reactive after the product has been packaged (Powers & Berkowitz, 1990). Oxygen absorbers can reduce the oxygen content inside the package to 0.01% within 15 hours after packaging (Guynot *et al*., 2003b). However, the packaging film permeability to $O_2$ is very important. A film with a medium permeability of less than 20 $cm^3/m^2d/atm$ is generally preferred, with the following barrier components: polyamide,

ethylene-alcohol vinyl or polyvinylidene chloride. If the packaging film has a high oxygen permeability, the oxygen absorber may become saturated and therefore lose its ability to absorb oxygen (Guynot *et al*., 2003b).

It has been shown that mould growth can be eliminated when oxygen levels are less than 0.4% (Powers & Berkowitz, 1990; Smith *et al*., 1986). Mould can grow at a high concentration of $CO_2$. For example, *Aspergillus* spp. can grow in an 85% $CO_2$ atmosphere with a 3% oxygen level and *Xeromyces bisporus* in a 95% $CO_2$ and 1% $O_2$ atmosphere (Smith *et al*., 1986). Powers and Berkowitz (1990) have shown that mould growth was inhibited for 13 months on meal ready-to-eat pouched bread, stored at 25 ºC. One of the reasons for shelf life extension by reducing the oxygen content is that it may lead to a reduced metabolic and chemical oxidation rate (Labuza *et al*., 1992). The pouched bread with no oxygen absorber present had excessive mould growth after 14 d stored at 25 ºC (Powers & Berkowitz, 1990).

Reducing the oxygen level may reduce the growth of gram-negative, aerobic spoilage microorganisms, for example *Pseudomonas* spp. (Labuza *et al*., 1992). However, it may enhance the growth of gram-positive microaerophillic spp., for example *Lactobacillus* spp. Facultative anaerobic microorganisms are usually unaffected by a reduction of the oxygen level. Yeast may grow at low levels of oxygen, however, its growth is reduced (Legan & Voysey, 1991).

## 1.3 HYPOTHESES

### 1.3.1 Hypothesis one

The shelf life of the par-baked pizza will be increased by MAP and by the addition of an oxygen absorber. Mould is one of the main shelf life determining factors of pizza and needs oxygen to grow. Hence, a reduced oxygen level <1% in the atmosphere will reduce mould growth (Smith *et al*., 1986).

### 1.3.2 Hypothesis two

The firming rate of par-baked and re-baked pizza will be reduced in the MAP with DATEM or an enzyme combination treatment (lipase & maltogenic α-amylase). High $CO_2$ atmosphere packaging has been shown to reduce the force needed to compress bread compared to bread stored in air (Knorr & Tomlins, 1985).

DATEM interacts with amylose and amylopectin and forms complexes, thereby limiting the retrogradation of starch, which is the main cause of staling, but the anti-firming effect of DATEM is probably related to changes in cell-wall thickness and modification of elasticity (Armero & Collar, 1998; Gray & Bemiller, 2003; Toufeili *et al*., 1995).

Maltogenic α-amylase, partially degrades amylopectin and amylose which will reduce their ability to retrogradate (Gray & Bemiller, 2003; Si, 1997). In addition, the production of low weight dextrins will limit the cross-linking between starch and gluten (Gray & Bemiller, 2003; Si, 1997).

Lipase also reduces retrogradation in bread (Gray & Bemiller, 2003). Its addition results in a higher loaf volume, a uniform grain, and thereby improves crumb softness during storage (Castello *et al*., 1999; Gray & Bemiller, 2003; Si, 1997). Lipase produces molecules with emulsifying properties like monoglycerides (Keskin *et al*., 2004; Si, 1997). It has been postulated that the monoglycerides interacts with amylose thus inhibiting the formation of double helices upon cooling, which is associated with staling (Keskin *et al*., 2004; Si, 1997).

## 1.4 OBJECTIVES

### 1.4.1 Objective one

To determine the effect of 100% $CO_2$ modified atmosphere stored at 10 ºC on the shelf life of par-baked and re-baked pizza with and without DATEM or lipase and maltogenic α-amylase in terms of staling (textural changes).

### 1.4.2 Objective two

To determine the effect of 100% $CO_2$ modified atmosphere with and without an oxygen absorber stored at 10 ºC on the shelf life of par-baked pizza in terms of microbiological spoilage.

## 2: RESEARCH ABSTRACT

Par-baked pizzas prepared with different additives were stored in air (A), 100% $CO_2$ modified atmosphere (MAP) and 100% $CO_2$ modified atmosphere with an oxygen absorber (MAP + OA). The additive treatments were diacetyl tartaric acid esters of mono- and diglycerides (DATEM), enzyme combination treatment (EC) (lipase and maltogenic α-amylase) and par-baked pizza without additives (C). The pizza was stored at 10 ºC for 16 d.

The results showed that the moisture content of the par-baked pizza crumb increased, from 36% on d0 to 41% on d16. The additive treatments, DATEM and EC had beneficial effects on the firmness and thickness of par-baked pizza. The par-baked pizza with additives was thicker than par-baked pizza + C and had a reduced rate of firmness. The par-baked pizza + MAP had a lower firmness than par-baked pizza + A. The firmness of par-baked pizza + EC was lower than par-baked pizza + DATEM and par-baked pizza + C. The results also showed that the re-baked pizzas were even softer than the original par-baked pizzas.

The microbial analysis showed that the different packaging atmospheres had the greatest effect on the APC and the mould count. The microbial levels were the lowest when the par-baked pizza + MAP + OA were used. However, the LAB and YC were not affected by MAP and MAP + OA.

The shelf life of the par-baked pizza + MAP and par-baked pizza + MAP + OA increased. The par-baked pizza + A reached the end of its shelf life at d12 stored at 10 ºC due to visible mould growth.

### 2.1 INTRODUCTION

Par-baked pizza is a convenient food product because it can be stored frozen or refrigerated. However, it has been shown that refrigeration storage increases the staling process of baked products (Correll, 2002). There is limited information on the

shelf life of pizza as most scientific information is in relation to bread and other bakery products. It has been observed that the shelf life of par-baked pizza is limited to 7 d with mould appearing to be the shelf life limiting factor when stored at 15–20 ºC (Rodriguez *et al*., 2003).

The bakery industry has used emulsifiers, for example DATEM, to produce a product with better quality in terms of volume and texture (Ribotta *et al*., 2004; Selomulyo & Zhou, 2007; Stampfli & Nersten, 1995). It has been proposed that DATEM interacts with amylose and forms complexes and that this may also contribute to a reduced staling rate (Selomulyo & Zhou, 2007). However, the demand for natural products has driven the replacement of additives and emulsifiers with enzymes, for instance, α-amylase, xylanase and lipase (Si & Lustenberger, 2001). León *et al*. (2002) observed that lipase and α-amylase in bread reduced the rate of firming (staling).

The rate of staling and mould growth has been reduced using modified atmosphere packaging. White bread slices and whole wheat bread slices showed delayed staling in 100% $CO_2$ modified atmosphere (Avital *et al*., 1990; Knorr & Thomsin, 1985). Rodriguez *et al*. (2003) also found that 50% of the pre-baked pizza showed visible signs of mould growth after 13 d packaged in 100% $CO_2$ atmosphere. Even better results can be obtained using an oxygen absorber that reduces the oxygen content in the headspace to <1%, which is required to eliminate mould growth (Smith *et al*., 2004).

The aim of the study is to determine the effects of different additives like DATEM and enzyme combination treatment (lipase and maltogenic α-amylase) and a 100% $CO_2$ modified atmosphere packaging with and without an oxygen absorber on the shelf life of pizza in terms of staling (firming) and microbiological spoilage.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Experimental design

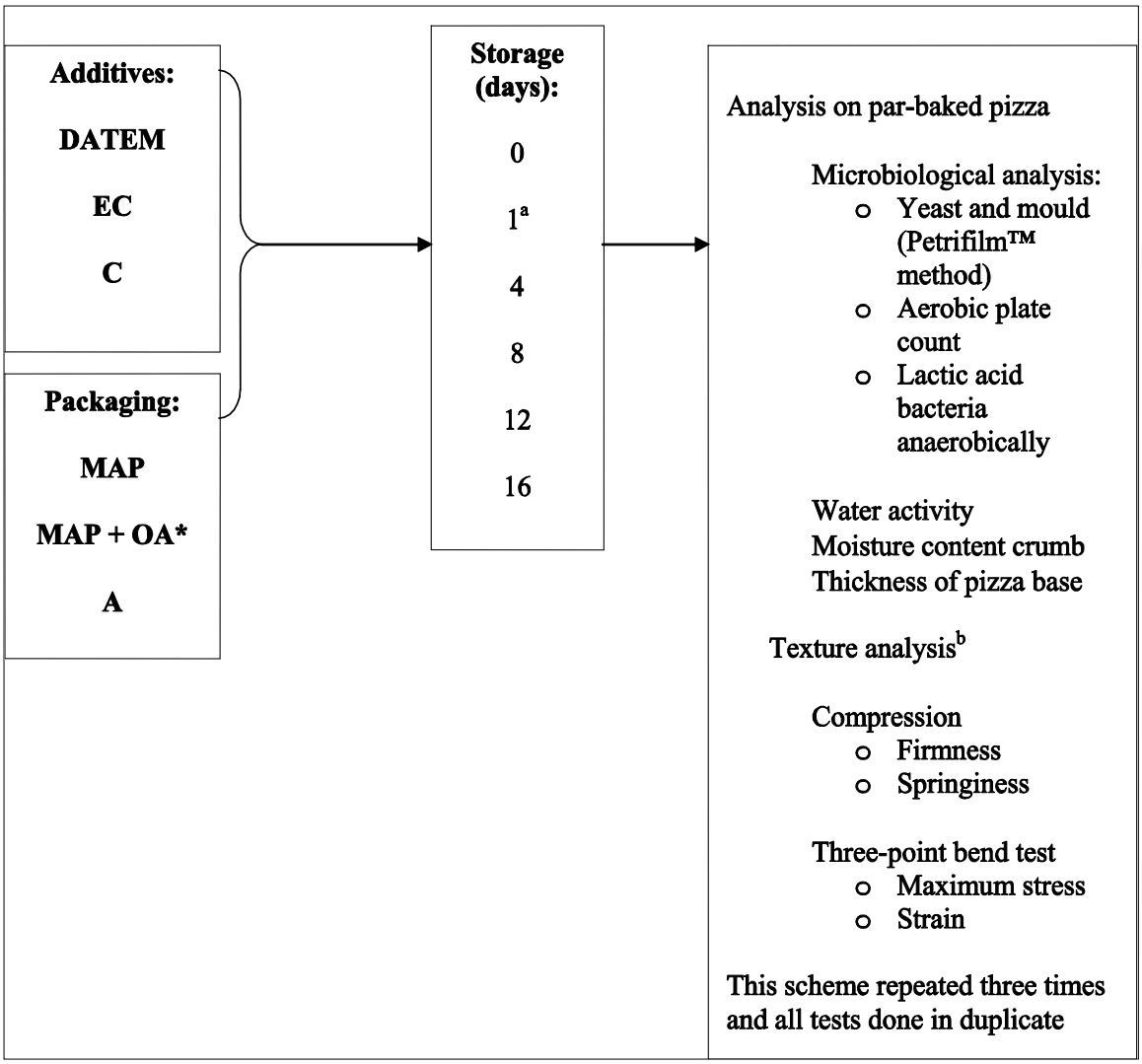
The experimental design was a multi-factorial experiment with three factors, as follows:

- The first factor was par-baked pizza with different additives: no additives that served as the control (C), diacetyl tartaric acid esters of mono- and diglycerides (DATEM) and enzyme combination treatment (EC) (lipase and maltogenic α-amylase).
- The second factor was the packaging systems: air (A), 100% $CO_2$ modified atmosphere (MAP) and MAP with an oxygen absorber (MAP + OA).
- The third factor was time of storage: 0, 1, 4, 8, 12 and 16 d.

Microbiological analyses were done: yeast and mould count, aerobic plate count and the lactic acid bacterial count. For these analyses the third factor (storage time) only included 5 levels of d: 0, 4, 8, 12 and 16 (Figure 1).

The textural changes measured were the firmness, springiness, and maximum stress and strain. For these analyses the second factor packaging systems included only 2 levels, namely, par-baked pizza packaged in air and MAP. Par-baked pizzas were re-baked and the following textural changes were also measured: firmness, springiness, stress, and strain.

Other analyses done were water activity, % moisture of par-baked pizza crumb and the thickness of the par-baked pizza base.




* The MAP + OA was not included when the following was measured: water activity, moisture-content crumb, thickness of pizza base and texture analysis
[a] Day 1 was not included in the microbiological analysis
[b] Textural analysis was also done on re-baked pizza

Figure 1: Schematic representation of the experimental design with the following factors: additives (diacetyl tartaric acids of mono- and diglyceride (DATEM), enzyme combination treatment (EC) and no additives (C)), packaging systems (air (A), 100% $CO_2$ modified atmosphere (MAP), (MAP) with an oxygen absorber (MAP + OA) and storage time (0, 1, 4, 8, 12, 16 d)

### 2.2.2 Preparation of par-baked pizza base

Par-baked pizza bases were prepared at the product development laboratory of Ruto Mills (Tshwane, South Africa). There were pizzas with three different additive

treatments and three different packaging systems as shown in Figure 1. The basic ingredients for the par-baked pizza bases are shown in Table 1.

Table 1: Ingredients used to prepare par-baked pizza bases

| Ingredient | Mass per batch (2 kg) |
|---|---|
| Flour (12.3% proteinwb) | 1128 g |
| Chilled water (16 ºC) | 640 g |
| White sugar | 120 g |
| Palm fat super shortening | 60 g |
| Salt | 22.5 g |
| Compressed Anchor yeast | 20 g |
| DATEM[*] (Panodan® A2020) | 22 g |
| Lipase[a] (Lipopan® Xtra BG) | 0.06 g |
| Maltogenic α-amylase[a] (Novamyl®1500MG) | 0.15 g |

[*]: For treatment 1 only; [a]: for treatment 2 only

There were three par-baked recipes and all three contained the basic ingredients (Table 1). The par-baked pizza + C was prepared with only the basic ingredients. DATEM (Panodan® A2020) was used in the par-baked pizza that contained an emulsifier. The par-baked pizza + EC contained the following enzymes: lipase (Lipopan® Xtra BG) and maltogenic α-amylase (Novamyl® 1500MG).

The dough of par-baked pizza was formed in a spiral mixer, mixing slowly for 2 minutes and fast for 6 minutes. After the dough was properly formed it was covered with a plastic cover and laid to rest for 10–12 minutes. The relaxed dough was then sheeted in four steps, first to the thickness of 15 mm, second to 10 mm, third to 7mm and finally to a uniform thickness of 0.5 cm. The dough sheet was pierced using a docking tool to prevent the dough to sweat during baking. The individual pizza bases were formed using a 9 cm in diameter, circular moulder. The pizza bases were proofed in a humidity-controlled proofer for 20 minutes at 46 ºC and 89 RH (relative humidity). The proofed pizza bases were par-baked in a rotary oven at 190 ºC for 8–9 minutes. After baking, they were removed from the oven to cool down to room

temperature on wire racks. The par-baked pizza bases were smeared with 11–12 g of tomato purée (All Gold, South Africa) 1 cm from the rind of the pizza base. The par-baked pizzas were then packaged.

### 2.2.3 Packaging, gas flushing and gas analysis

Par-baked pizza samples were packaged in Cryovac® bags (BB4L Cryovac® barrier bag, OTR – 20 cc/$m^2$24h/atm at 22 ºC & 75% RH). There were three packaging systems. The control packaging system contained par-baked pizza that was packaged in Cryovac® bags with sealed-in air. The bags of the MAP and MAP + OA packaged par-baked pizza were vacuum sealed with a Multivac A300 vacuum packer (Gesprüfte Sicherheit, sepp Haggenmüller KG, 8941 Wolfertschwenden, Germany). The MAP and MAP + OA packaged par-baked pizzas were gas flushed manually with 100% $CO_2$ gas. The MAP + OA packaging system included an oxygen absorber. Gas analysis was conducted during the shelf life study with gas headspace analyser (Gaspace 2, Systech Instruments, Thame, UK).

### 2.2.4 Water activity determination

Water activity ($a_w$) was measured using a water activity meter (Pawkit, Decagon Devices, England). The sample was placed in a small sampling cup and spread out evenly. The sampling cup was placed under the water activity meter to obtain a reading.

### 2.2.5 Moisture content

The moisture content was determined according to the American Association of Cereal Chemists (AACC), method number 44–01 (AACC, 2000). This method is an oven drying method. A 5 ± 1 g sample of the crumb was placed in a pre-dried metal tin. The metal tins with samples were placed in an oven at 103 ºC for 3 hours. The moisture content was determined as the weight lost after drying.

### 2.2.6 Sampling method of pizza bases for microbiological analysis

Random 2 x 5 g pieces were cut from different quarters of the par-baked pizza bases (Figure 2).

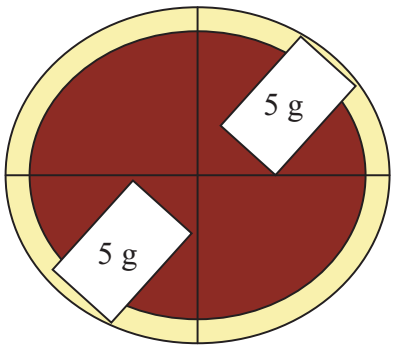



Figure 2: Method applied for random sampling of pizza bases for microbiological analysis

### 2.2.7 Microbiological analysis

A pizza sample of 10 g was added to 90 ml of peptone water in a stomacher bag and homogenised for 1 minute in stomacher (Lab Blender 400, Art Medical Equipment, Johannesburg: South Africa). Colonies were counted in the range of 30–300 and reported as cfu/g for all microbiological analysis.

Appropriate dilutions were prepared and plated on yeasts and mould petri film (3M™, Petriflim™, YM, 3M Company, South Africa; Beuchat *et al.*, 1991; Vlaemynck, 1994). The petri films were incubated for 5 d at 25 ºC ± 1 ºC. Yeast colonies were counted as the blue-green colonies and mould was counted as the larger black, yellow, green and other pigmented colonies.

The aerobic plate count was done according to the Association of Official Analytical Chemists (AOAC) (2000), method number 990.12. An aerobic plate count petri film (3M™, Petriflim™, AC, 3M™ Company, South Africa) was placed flat on a surface. Then a 1ml sample was transferred onto the centre of the petri film and spread out

over the growth area. Petri films were incubated for 48 hours ± 3 hours at 35 ºC ± 1 ºC, with the clear side facing upwards. Aerobic bacteria formed red colonies.

The pour plate technique was used, using MRS agar (Merck) to determine the lactic acid bacterial count. The plates were incubated inversely for 48–72 hours at 30 ºC anaerobically (de Man, Rogosa & Sharp, 1960).

### 2.2.8 Textural changes analysis

#### *2.2.8.1 Compression test*

The compression test was done in order to determine the firmness of the crumb. It was done according to AACC method number 74-09 (AACC, 2000) using a texture analyser (TA.TX2, Stable Micro Systems Ltd., England). The sample preparation was as follows: the pizza base was cut in a circle of 50 mm in diameter. The top crust was removed because of the tomato sauce.

The 10 mm thick sample was placed on a heavy-duty platform HDP/90 and compressed with a 50 mm (p50) aluminium cylinder probe up to 25% at 1.67 mm/s speed. The force (N) at 25% compression was the target parameter, indicating the firmness of the crumb. The springiness was also determined with this method. The sample was compressed as mentioned above and then relaxed for 60 s. The springiness was determined using the following formula:

$$\% \text{ Springiness} = \frac{F2 \text{ (force after relaxation at 60 s)}}{F1 \text{ (force at peak)}} \times 100$$

#### *2.2.8.2 Three-point bend test*

The pizza base was cut to a rectangular shape (80 mm x 30 mm). The crust was not removed. The sample was placed on two supporting vertical wedges that were 60 mm apart from one another. The sample was bent with a three-point bend rig (HDP/3PB)

compressing until it broke at a crosshead speed of (1 mm/s) using a texture analyser TA.TX2 (Stable Micro Systems Ltd, England; Figure 3).

Maximum stress (MPa) was obtained using the following formula:

$$\sigma_{max} = \frac{\pm 3 F L}{2 b h^2}$$

In the above formula, $\sigma$ indicates maximum stress (MPa), $F$ the peak force (N), $L$ the distance (mm) between supporting bars, $b$ the width (mm) of the test piece, and $h$ the height (mm) of the test piece.

The corresponding strain (unit less):

$$\varepsilon = \frac{6 h \Delta y}{L^2}$$

In the above formula ε indicates strain, $\Delta y$ the maximum deflection (mm) at the centre of the beam, $h$ the height (mm) of the test piece and $L$ the distance (mm) between supporting bars.

### 2.2.9 Statistical analysis

A multi-factor Analysis of Variance (ANOVA) was conducted on the data with Statistica software Windows version 7 (Tulsa, Oklahoma: USA, 2003).

The independent variables were:

- Factor one: additives (C/DATEM or EC)
- Factor two: packaging (A, MAP, MAP + OA[*])
- Factor three: storage time (0, 1[a], 4, 8, 12, 16 d).

*: Only included during the microbiological analysis
a: Only included during the textural changes analysis

The dependent variables were: water activity,% moisture of par-baked pizza crumb and thickness of par-baked pizza base, microbiological levels (APC, LAB, YC and MC) and textural changes (firmness [compression test and three-point bend test] and springiness).

The interactions between factors were also determined. When insignificant interaction was found, only the main effects of each factor were reported. The experiment was repeated three times.

## 2.3 RESULTS

### 2.3.1 Gas composition

Gas analysis was used as a control point for the experiment, therefore no statistical analysis was done and only approximate values were reported. It was observed that the oxygen content of the par-baked pizza + A was unchanged throughout the storage period of 16 d at 10 ºC at 16% $O_2$ (Appendix D). The oxygen content for the par-baked pizza + MAP changed throughout the storage period. The initial $O_2$ content was 0.9% and increased to a maximum of 4.6% on d12. The carbon dioxide content of the par-baked pizza + MAP was initially 77% and decreased to 58% on d12. The $O_2$ content for par-baked pizza + MAP + OA was 0.8% and increased to a maximum of 2.8% on d12. The $CO_2$ content was also 77% initially and decreased to 69% on d12. The analysis showed that the oxygen content of MAP + OA remained the lowest throughout the storage period of 16 d.

### 2.3.2 Water activity of par-baked pizza

According to the Analysis of Variance (ANOVA), the main effects of packaging, additives and time of storage did not significantly ($p \geq 0.05$) affect the water activity of

par-baked pizza (Table 2). The $a_w$ of par-baked pizza was between 0.95–0.98 irrespective of the treatments (Table 3).

### 2.3.3 Moisture content of the par-baked pizza crumb

The moisture content of par-baked pizza was not significantly ($p \geq 0.05$) affected by the different additive treatments and packaging systems (Table 2). Only storage time significantly ($p < 0.05$) affected the moisture content of par-baked pizza crumb, therefore only the main effect of storage time was reported. The main effect of storage showed that there was increase of moisture content with storage time (Table 4).

### 2.3.4 Thickness of par-baked pizza

According to the ANOVA, the only the factor, namely, additives significantly ($p < 0.05$) affected the thickness of par-baked pizza bases (Table 2). Thus only the main effect of additives is reported. Par-baked pizza + EC had the thickest pizza base of 22 mm thick and was statistically similar to par-baked pizza + DATEM with a base thickness of 21.7 mm. The par-baked pizza + C was the least thick with a thickness of 18.3 mm (Figure 4).

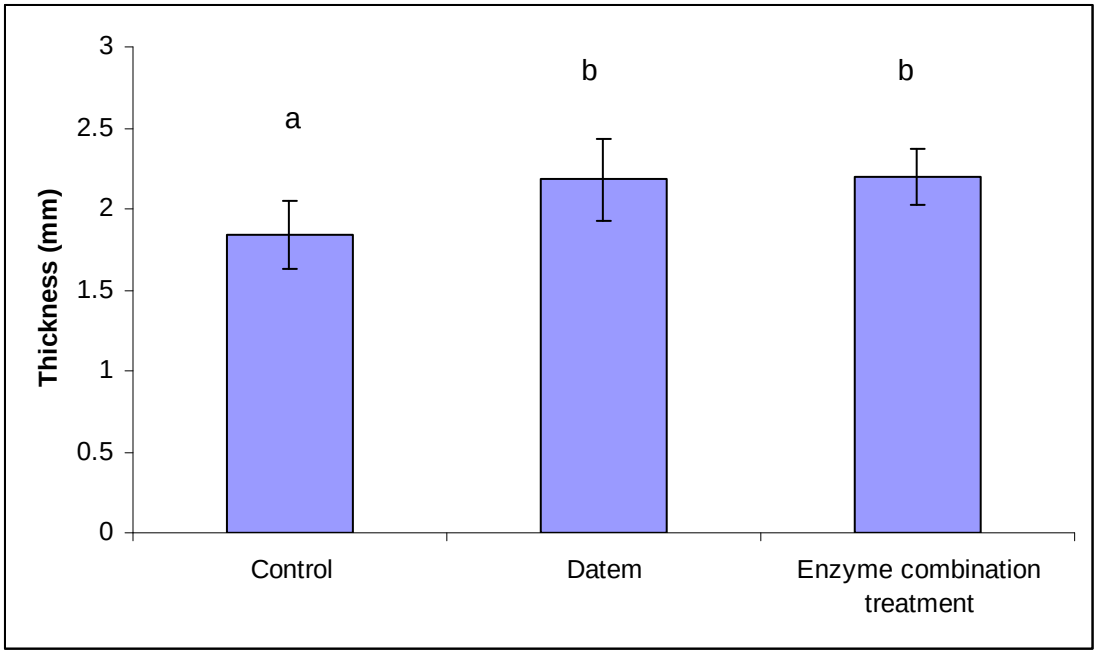



Figure 3: Thickness (mm) of par-baked pizza with different additives

a, b: Mean values with different letters differ significantly from each other ($p<0.05$)

Table 2: Analysis of Variance (ANOVA) for various physical parameters of par- and re-baked pizza stored at 10 ºC for 16 d

| Source of variance | $a_w$ | % Moisture of crumb | Firmness | Springiness | Max Stress | Max Strain | Thickness |
|---|---|---|---|---|---|---|---|
| **Par-baked pizza** | | | | | | | |
| Additive (A) | ns | ns | * | * | * | * | * |
| Packaging (P) | ns | ns | * | ns | ns | ns | ns |
| Days (D) | ns | * | * | * | ns | * | ns |
| A x P | ns | ns | * | ns | ns | ns | ns |
| A x D | ns | ns | * | ns | ns | ns | ns |
| P x D | ns | ns | * | ns | ns | ns | ns |
| P x A x D | ns | ns | * | ns | ns | ns | ns |
| **Re-baked pizza** | | | | | | | |
| Additive (A) | ND | ND | ns | * | * | * | ND |
| Packaging (P) | ND | ND | ns | ns | ns | ns | ND |
| Days (D) | ND | ND | * | * | * | * | ND |
| A x P | ND | ND | ns | ns | ns | ns | ND |
| A x D | ND | ND | ns | ns | * | ns | ND |
| P x D | ND | ND | ns | ns | ns | ns | ND |
| P x A x D | ND | ND | ns | ns | ns | ns | ND |

* Significant difference when $p<0.05$; ns Not significant when $p\geq0.05$, ND Not determined

Table 3: Effect of different additives and packaging systems on the water activity of par-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging Systems | | Main effect of additive[d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 0.95 (± 0.01) | 0.95 (± 0.01) | 0.96[a] |
| | 1 | 0.95 (± 0.02) | 0.96 (± 0.01) | |
| | 4 | 0.96 (± 0.02) | 0.97 (± 0.02) | |
| | 8 | 0.95 (± 0.01) | 0.96 (± 0.01) | |
| | 12 | 0.95 (± 0.01) | 0.96 (± 0.01) | |
| | 16 | 0.95 (± 0.01) | 0.96 (± 0.00) | |
| DATEM | 0 | 0.96 (± 0.01) | 0.96 (± 0.01) | 0.96[a] |
| | 1 | 0.96 (± 0.02) | 0.95 (± 0.01) | |
| | 4 | 0.97 (± 0.01) | 0.97 (± 0.01) | |
| | 8 | 0.95 (± 0.02) | 0.96 (± 0.02) | |
| | 12 | 0.96 (± 0.01) | 0.96 (± 0.01) | |
| | 16 | 0.96 (± 0.00) | 0.97 (± 0.01) | |
| Enzyme combination (EC) | 0 | 0.96 (± 0.01) | 0.96 (± 0.01) | 0.96[a] |
| | 1 | 0.96 (± 0.01) | 0.95 (± 0.01) | |
| | 4 | 0.96 (± 0.01) | 0.97 (± 0.01) | |
| | 8 | 0.95 (± 0.01) | 0.96 (± 0.01) | |
| | 12 | 0.96 (± 0.01) | 0.96 (± 0.01) | |
| | 16 | 0.97 (± 0.01) | 0.98 (± 0.01) | |
| Main effect of packaging [e] | | 0.96a | 0.96a | |

| Main effect time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 0.95[a] | 0.95[a] | 0.97[a] | 0.96[a] | 0.96[a] | 0.96[a] |

[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[c] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

Table 4: Effect of different additives and packaging systems on the moisture content (%) of par-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging systems | | Main effect of additive [d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 38.24 (± 3.75) | 38.24 (± 3.75) | 39.60[a] |
| | 1 | 35.74 (± 2.74) | 35.85 (± 4.65) | |
| | 4 | 40.14 (± 1.63) | 39.88 (± 0.44) | |
| | 8 | 43.87 (± 5.74) | 36.62 (± 5.84) | |
| | 12 | 41.35 (± 0.87) | 40.77 (± 2.49) | |
| | 16 | 42.63 (± 1.29) | 41.92 (± 0.97) | |
| DATEM | 0 | 37.05 (± 1.02) | 37.05(± 1.02) | 38.48[a] |
| | 1 | 34.83 (± 2.87) | 37.13 (± 2.08) | |
| | 4 | 38.93 (± 1.09) | 34.87 (± 7.37) | |
| | 8 | 39.68 (± 0.41) | 39.88 (± 0.40) | |
| | 12 | 40.27 (± 0.37) | 40.24 (± 0.57) | |
| | 16 | 40.17 (± 1.70) | 41.70 (± 1.17) | |
| Enzyme combination (EC) | 0 | 34.92 (± 2.10) | 34.92 (± 2.10) | 38.88[a] |
| | 1 | 36.80(± 2.01) | 36.67 (± 2.25) | |
| | 4 | 39.26 (± 0.48) | 39.06 (± 0.46) | |
| | 8 | 40.61 (± 0.71) | 39.56 (± 0.49) | |
| | 12 | 40.51 (± 0.47) | 41.33 (± 1.04) | |
| | 16 | 41.88 (± 0.34) | 41.03 (± 0.89) | |
| Main effect of packaging [e] | | 39.27[a] | 38.71[a] | |

| Main effect of time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 36.74[a] | 36.17[a] | 38.69[b] | 40.04[bc] | 40.75[c] | 41.56[c] |

[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

### 2.3.5 Textural changes

*2.3.5.1 Firmness of par- and re-baked pizza*

According to the ANOVA, the packaging, additives and time of storage significantly ($p<0.05$) affected the firmness of par-baked pizza (Table 2). There were also two-way and three-way interactions between the main factors. However, the firmness of re-baked pizza was significantly ($p<0.05$) affected only by the time of storage (Table 2). Although there were some interactions between the different factors, only the main effects of the following factors were reported: additives, packaging and storage time. The effects of packaging, additives and storage time on the firmness of par- and re-baked pizza are shown in Table 5 and Table 6 respectively. The main effect of time of storage showed that the firmness of par-baked pizza significantly ($p<0.05$) increased with storage time until d8 (Table 5). The main effect of storage time further showed that the firmness of par-baked pizza significantly ($p<0.05$) decreased from d12 to d16. The main effect of storage time on the firmness of re-baked pizza showed that the firmness increased significantly from d0 to d1. The firmness of re-baked pizza stayed similar from d1 onwards to d16 (Table 6).

The main effect of packaging showed that par-baked pizza + MAP had a significantly ($p<0.05$) lower firmness than par-baked pizza + A. The main effect of packaging did not significantly ($p\geq0.05$) affect the firmness of re-baked pizza (Table 6).

The main effect of the different additives showed that the firmness of par-baked pizza + EC was significantly ($p<0.05$) lower than that of par-baked pizza + DATEM and of par-baked pizza + C respectively (Table 6). The different additive treatments did not significantly ($p\geq0.05$) affect the firmness of re-baked pizza (Table 6). It could be observed that the firmness of par-baked pizza was always higher compared with the firmness of re-baked pizza (Table 5 and Table 6).

Table 5: Effect of different additives and packaging systems on the firmness (N)[a] of par-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging systems | | Main effect of additive[d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 38.43 (± 1.69) | 38.43 (± 1.69) | 44.17[a] |
| | 1 | 51.40 (± 6.74) | 36.06 (± 3.15) | |
| | 4 | 50.10 (±3.34) | 40.54 (± 5.49) | |
| | 8 | 57.07 (±7.24) | 38.22 (± 4.59) | |
| | 12 | 42.74 (± 1.06) | 55.73 (± 7.95) | |
| | 16 | 42.17 (± 10.27) | 39.20 (± 7.68) | |
| DATEM | 0 | 30.36 (± 0.90) | 30.36 (± 0.90) | 32.0[b] |
| | 1 | 35.97 (± 8.20) | 32.81 (± 3.36) | |
| | 4 | 31.01 (± 2.11) | 32.48 (± 6.65) | |
| | 8 | 33.01 (± 2.13) | 32.05 (± 4.60) | |
| | 12 | 34.62 (± 0.13) | 28.21 (± 4.54) | |
| | 16 | 40.31 (± 9.39) | 22.51 (± 7.74) | |
| Enzyme combination (EC) | 0 | 11.52 (± 4.76) | 11.52 (± 4.76) | 30.79[c] |
| | 1 | 21.11 (± 0.53) | 32.11 (± 2.45) | |
| | 4 | 26.72 (± 7.42) | 37.37 (± 6.25) | |
| | 8 | 52.44 (± 12.10) | 34.95 (± 1.52) | |
| | 12 | 34.16 (± 2.52) | 41.34 (± 4.10) | |
| | 16 | 28.23 (± 3.98) | 31.80 (± 2.87) | |
| Main effect of packaging [e] | | 36.67[a] | 34.08[a] | |

| Main effect of time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 26.77[a] | 34.70[b] | 36.16[bc] | 40.58[d] | 39.06[cd] | 33.86[b] |

[a] Firmness in Newton at 25% compression by compression test
[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

Table 6: Effect of different additives and packaging systems on the firmness (N) a of re-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging systems | | Main effect of additive [d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 10.22 (± 1.21) | 10.22 (± 1.21) | 12.22[a] |
| | 1 | 15.99 (± 3.01) | 11.54 (± 1.99) | |
| | 4 | 13.88 (± 3.07) | 13.99 (± 4.09) | |
| | 8 | 13.28 (± 4.18) | 12.48 (± 3.19) | |
| | 12 | 11.16 (± 0.77) | 13.03 (± 1.53) | |
| | 16 | 10.99 (± 0.62) | 9.17 (± 1.51) | |
| DATEM | 0 | 9.41 (± 1.05) | 9.41 (± 1.05) | 11.19[a] |
| | 1 | 11.42 (± 2.28) | 13.23 (± 2.29) | |
| | 4 | 9.57 (± 1.11) | 13.19 (± 4.61) | |
| | 8 | 10.52 (± 3.67) | 11.26 (± 2.03) | |
| | 12 | 10.20 (± 1.96) | 10.83 (± 0.90) | |
| | 16 | 12.69 (± 1.56) | 12.48 (± 1.02) | |
| Enzyme combination (EC) | 0 | 9.67 (± 2.07) | 9.67 (± 2.07) | 11.30[a] |
| | 1 | 11.35 (± 2.50) | 12.15 (± 0.63) | |
| | 4 | 12.97 (± 4.26) | 10.97 (± 1.58) | |
| | 8 | 10.60 (± 2.55) | 11.82 (± 3.35) | |
| | 12 | 10.78 (± 2.92) | 11.19 (± 2.28) | |
| | 16 | 13.09 (± 4.44) | 11.31 (± 1.90) | |
| Main effect of packaging [e] | | 11.55[a] | 11.56 | |

| Main effect of time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 9.77[a] | 12.68[b] | 12.43[b] | 11.66[b] | 11.22[ab] | 11.66[b] |

[a] Firmness in Newton at 25% compression by compression test
[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

*2.3.5.2 Springiness of par- and re-baked pizza*

There were no interactions between the different factors for the dependent variable springiness, therefore only the main effects were reported (Table 2). According to the ANOVA, only the factors time of storage and additives significantly ($p<0.05$) affected the springiness of par-and re-baked pizza (Table 2). The main effect of time showed that as the storage time increased the springiness decreased (Table 7). The main effect of time showed that the springiness of re-baked pizza declined initially from d0 to d1. There were no significant changes in the springiness of re-baked pizza after d1 until d16 (Table 8).

The main effect of additives on the springiness showed that the par-baked pizza + C and par-baked pizza + EC had the highest springiness of 39% and 37% respectively (Table 7). The par-baked pizza + DATEM had the lowest springiness at 35%. As for the par-baked pizza, the additives significantly ($p<0.05$) affected the springiness of re-baked pizza (Table 2). The main effect showed that the re-baked pizza + C and re-baked pizza + EC had the highest springiness, 48% and 45% respectively (Table 8). The springiness of the re-baked pizza + DATEM was the lowest at 42%.

Table 7: Effect of different additives and packaging systems on the springiness of par-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging Systems | | Main effect of additive [d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 44.78 (± 5.83) | 44.78 (± 5.83) | 39.33[c] |
| | 1 | 39.26 (± 3.21) | 39.51(± 2.23) | |
| | 4 | 37.32 (± 0.77) | 41.50 (± 0.56) | |
| | 8 | 37.47 (± 1.62) | 37.33 (± 1.18) | |
| | 12 | 37.65 (± 2.03) | 37.77 (± 1.31) | |
| | 16 | 36.42 (± 0.92) | 37.70 (± 3.90) | |
| DATEM | 0 | 36.68 (± 3.29) | 36.68 (± 3.29) | 35.51[a] |
| | 1 | 35.09 (± 1.32) | 38.46 (± 1.67) | |
| | 4 | 34.53 (± 1.31) | 36.62 (± 1.86) | |
| | 8 | 35.30 (± 1.17) | 33.84 (± 3.53) | |
| | 12 | 34.89 (± 1.38) | 33.25 (± 3.93) | |
| | 16 | 35.16 (± 0.63) | 36.27 (± 2.38) | |
| Enzyme combination (EC) | 0 | 40.46 (± 5.01) | 40.46 (± 5.01) | 37.59[b] |
| | 1 | 38.10 (± 3.11) | 38.80 (± 3.49) | |
| | 4 | 35.67 (± 0.57) | 35.40 (± 0.35) | |
| | 8 | 36.10 (± 0.95) | 39.33 (± 2.75) | |
| | 12 | 34.97 (± 1.89) | 35.89 (± 0.96) | |
| | 16 | 36.51 (± 2.92) | 38.69 (± 2.86) | |
| Main effect of packaging [e] | | 36.98[a] | 37.95[a] | |

Main effect of time

| Days | 0 | 1 | 4 | 8 | 12 | 16 |
|---|---|---|---|---|---|---|
| Main effect [e] | 40.64[a] | 38.20[b] | 36.92[bc] | 36.55[bc] | 35.49[c] | 36.79[bc] |

[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean valueFs with different letters in same row differ significantly from each other ($p<0.05$)

Table 8: Effect of different additives and packaging systems on the springiness of re-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging systems | | Main effect of additive [d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 48.91 (± 1.68) | 48.91 (± 1.68) | 48.28[c] |
| | 1 | 45.51 (± 1.32) | 45.53 (± 3.02) | |
| | 4 | 48.21 (± 0.46) | 49.61 (± 1.99) | |
| | 8 | 48.94 (± 0.56) | 48.83 (± 2.14) | |
| | 12 | 49.23 (± 1.69) | 48.63 (± 2.07) | |
| | 16 | 47.41 (± 2.94) | 49.68 (± 2.27) | |
| DATEM | 0 | 41.03 (± 2.59) | 41.03 (± 2.59) | 41.33[a] |
| | 1 | 39.17 (± 2.05) | 39.42 (± 1.73) | |
| | 4 | 41.25 (±0.58) | 41.57 (± 2.65) | |
| | 8 | 41.41 (± 1.12) | 41.78 (± 0.90) | |
| | 12 | 42.46 (± 0.61) | 42.32 (± 1.31) | |
| | 16 | 42.46 (± 1.87) | 42.36 (± 1.70) | |
| Enzyme combination (EC) | 0 | 44.28 (± 1.22) | 44.28 (± 1.22) | 44.95[b] |
| | 1 | 42.68 (± 1.80) | 44.00 (± 1.90) | |
| | 4 | 45.11 (± 0.71) | 44.04 (± 1.91) | |
| | 8 | 47.08 (± 0.74) | 45.27 (± 1.29) | |
| | 12 | 45.01 (± 0.24) | 45.92 (± 1.43) | |
| | 16 | 45.92 (± 2.18) | 45.81 (± 1.20) | |
| Main effect of packaging [e] | | 44.78[a] | 44.99[a] | |

| Main effect of time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 44.74[b] | 42.72[a] | 44.96[b] | 45.55[b] | 45.78[b] | 45.61[b] |

[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

*2.3.5.3 Texture measurements with the three-point bend test: maximum stress of par- and re-baked pizza*

There were no interactions between the factors for the dependent variable maximum stress, therefore only the main effects were reported (Table 2). According to the ANOVA, the main effect of packaging did not significantly ($p \geq 0.05$) affect the maximum stress of par- and re-baked pizza (Table 2). However, the storage time and additives did significantly ($p < 0.05$) affect the maximum stress of par- and re-baked pizza (Table 2). The main effect of storage time showed that the maximum stress of par-baked pizza increased from 13.84 MPa on d0 to 14.69 MPa on d1 (Table 9). The maximum stress decreased from 14.69 on d1 to 12.39 MPa on d4 where it stabilised until d16. The main effect of storage time showed that the maximum stress of re-baked pizza increased from 12.47 MPa to 14.03 MPa on d1. The maximum stress decreased to 10.34 MPa on d4 and did not significantly ($p \geq 0.05$) change until d16 (Table 10).

The main effect of additives showed that the par-baked pizza + C had the highest maximum stress throughout the storage period. The main effect of additives also showed that the par-baked pizza + DATEM and par-baked pizza + EC did not differ significantly ($p \geq 0.05$), however, it could be noted that the par-baked pizza + EC had the lowest maximum stress (Table 10). The main effect of additives on the maximum stress of re-baked pizza showed that the re-baked pizza + C had a higher overall maximum stress than the re-baked pizza + DATEM and re-baked pizza + EC, 15.82 MPa, 9.85 MPa and 9.51 MPa respectively (Table 10).

Table 9: Effect of different additives and packaging systems on the maximum stress (MPA)* of par-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging systems | | Main effect of additive[d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 18.16 (± 7.62) | 18.16 (± 7.62) | 15.13[b] |
| | 1 | 16.82 (± 2.80) | 19.42 (± 5.82) | |
| | 4 | 13.09 (± 4.92) | 13.26 (± 2.91) | |
| | 8 | 10.98 (± 2.92) | 13.71 (± 1.68) | |
| | 12 | 12.97 (± 3.90) | 15.38 (± 4.41) | |
| | 16 | 15.92 (± 2.01) | 13.99 (± 2.70) | |
| DATEM | 0 | 12.79 (± 3.89) | 12.79 (± 3.89) | 12.22[a] |
| | 1 | 14.15 (± 2.00) | 12.81 (± 2.81) | |
| | 4 | 12.30 (± 3.42) | 13.43 (± 4.93) | |
| | 8 | 11.98 (± 2.09) | 12.18 (± 3.40) | |
| | 12 | 11.03 (± 3.20) | 11.79 (± 2.89) | |
| | 16 | 9.42 (± 2.68) | 11.94 (± 2.56) | |
| Enzyme combination (EC) | 0 | 10.56 (± 2.21) | 10.56 (± 2.21) | 11.30[a] |
| | 1 | 12.03 (± 2.76) | 12.92 (± 5.40) | |
| | 4 | 11.63 (± 2.72) | 10.65 (± 0.94) | |
| | 8 | 12.65 (± 3.14) | 11.76 (± 3.07) | |
| | 12 | 13.35 (± 5.85) | 9.83 (± 2.73) | |
| | 16 | 8.43 (± 1.7) | 11.28 (± 2.23) | |
| Main effect of packaging [e] | | 12.62[a] | 13.10[a] | |

| Main effect on time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 13.84[ab] | 14.69[b] | 12.39[ab] | 12.21[a] | 12.39[ab] | 11.59[a] |

* The maximum stress in MPa, measured using the three-point bend test
[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

Table 10: Effect of different additives and packaging systems on the maximum stress (MPa)* of re-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging Systems | | Main effect of additive[d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 18.40 (± 2.45) | 18.40 (± 2.45) | 15.82[b] |
| | 1 | 15.60 (± 3.80) | 19.04 (± 5.80) | |
| | 4 | 12.37 (± 2.51) | 14.92 (± 8.20) | |
| | 8 | 22.08 (± 8.88) | 16.70 (± 8.82) | |
| | 12 | 16.04 (± 3.23) | 11.69 (± 3.94) | |
| | 16 | 13.67 (± 1.84) | 10.98 (± 1.11) | |
| DATEM | 0 | 10.54 (± 1.78) | 10.54 (± 1.78) | 9.85[a] |
| | 1 | 12.43 (± 4.34) | 12.66 (± 2.13) | |
| | 4 | 8.15 (± 2.76) | 10.38 (± 1.41) | |
| | 8 | 9.67 (± 2.66) | 10.23 (± 3.90) | |
| | 12 | 7.99 (± 0.18) | 8.68 (± 0.83) | |
| | 16 | 7.90 (± 1.51) | 9.01 (± 0.93) | |
| Enzyme combination (EC) | 0 | 8.49 (± 2.36) | 8.49 (± 2.36) | 9.51[a] |
| | 1 | 12.12 (± 1.67) | 12.34 (± 2.57) | |
| | 4 | 7.72 (± 1.50) | 8.48 (± 0.87) | |
| | 8 | 5.12 (± 7.78) | 9.19 (± 2.86) | |
| | 12 | 10.74 (± 1.25) | 10.16 (± 2.14) | |
| | 16 | 9.07 (± 1.83) | 12.03(± 4.06) | |
| Main effect of packaging [e] | | 11.61[a] | 11.88[a] | |

| Main effect of time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 12.47[ab] | 14.03[b] | 10.34[a] | 12.16[ab] | 10.89[a] | 10.52[a] |

* The maximum stress in MPa, measured using the three-point bend test
[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

*2.3.5.4 Strain of par- and re-baked pizza*

Only the main effects were reported due to no interactions between factors for the dependent variable strain (Table 2). According to the ANOVA, the strain of par- and re-baked pizza was not significantly ($p \geq 0.05$) affected by the main effect of packaging (Table 2). However, the strain of par- and re-baked pizza was significantly ($p < 0.05$) affected by the storage time and by the different additives.

The main effect of time showed that the strain of par- and re-baked pizza decreased as the storage time increased (Table 11 and Table 12). The strain of par-baked pizza decreased until d4 where no further significant ($p \geq 0.05$) changes occurred until d16 (Table 11). However, the strain of re-baked pizza decreased until d16 (Table 12).

The main effect of additives on the strain of par-baked pizza showed that the strain of the par-baked pizza + C was the lowest; 0.35, and the par-baked pizza + EC was the highest with a strain of 0.47 (Table 11). The main effect of additives showed that the strain of re-baked pizza + C was the lowest at 0.60, and that of the re-baked pizza + EC was the highest with a strain of 0.73 (Table 12).

It was also noted that the strain of re-baked pizza was higher than the strain for par-baked pizza.

Table 11: Effect of different additives and packaging systems on the strain of par-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging Systems | | Main effect of additive [d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 0.48 (± 0.10) | 0.48 (± 0.10) | 0.35[b] |
| | 1 | 0.34 (± 0.04) | 0.35 (± 0.08) | |
| | 4 | 0.34 (± 0.07) | 0.33 (± 0.03) | |
| | 8 | 0.38 (± 0.12) | 0.31 (± 0.03) | |
| | 12 | 0.34 (± 0.12) | 0.27 (± 0.04) | |
| | 16 | 0.30 (± 0.02) | 0.27 (± 0.08) | |
| DATEM | 0 | 0.49 (± 0.18) | 0.49 (± 0.18) | 0.46 |
| | 1 | 0.45 (± 0.05) | 0.62 (± 0.08) | |
| | 4 | 0.50 (± 0.19) | 0.44 (± 0.13) | |
| | 8 | 0.46 (± 0.22) | 0.47 (± 0.04) | |
| | 12 | 0.40 (± 0.01) | 0.35 (± 0.05) | |
| | 16 | 0.48 (± 0.08) | 0.36 (± 0.03) | |
| Enzyme combination (EC) | 0 | 0.50 (± 0.12) | 0.50 (± 0.12) | 0.47 |
| | 1 | 0.54 (± 0.06) | 0.47 (± 0.10) | |
| | 4 | 0.49 (± 0.06) | 0.42 (± 0.08) | |
| | 8 | 0.47 (± 0.19) | 0.50 (± 0.18) | |
| | 12 | 0.51 (± 0.05) | 0.39 (± 0.10) | |
| | 16 | 0.46 (± 0.12) | 0.40 (± 0.07) | |
| Main effect of packaging [e] | | 0.44 | 0.41[a] | |

| Main effect of time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 0.49[b] | 0.46[ab] | 0.42[ab] | 0.43[ab] | 0.38[a] | 0.38[a] |

[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

Table 12: Effect of different additives and packaging systems on the strain of re-baked pizza stored at 10 ºC for 16 d

| Additives | Days | Packaging Systems | | Main effect of additive [d] |
|---|---|---|---|---|
| | | Air (A) | Modified atmosphere (MAP) | |
| No additives (C) | 0 | 0.62 (± 0.11) | 0.62 (± 0.11) | 0.60[a] |
| | 1 | 0.61 (± 0.12) | 0.67 (± 0.10) | |
| | 4 | 0.66 (± 0.10) | 0.68 (± 0.11) | |
| | 8 | 0.48 (± 0.28) | 0.67 (± 0.21) | |
| | 12 | 0.46 (± 0.31) | 0.61 (± 0.25) | |
| | 16 | 0.54 (± 0.17) | 0.53 (± 0.14) | |
| DATEM | 0 | 0.78 (± 0.24) | 0.78 (± 0.24) | 0.67[ab] |
| | 1 | 0.71 (± 0.18) | 0.84 (± 0.07) | |
| | 4 | 0.61 (± 0.21) | 0.79 (± 0.12) | |
| | 8 | 0.62 (± 0.09) | 0.57 (± 0.11) | |
| | 12 | 0.65 (± 0.22) | 0.64 (± 0.22) | |
| | 16 | 0.48 (± 0.20) | 0.54 (± 0.01) | |
| Enzyme combination (EC) | 0 | 0.77 (± 0.08) | 0.77 (± 0.08) | 0.73[b] |
| | 1 | 0.82 (± 0.13) | 0.82 (± 0.12) | |
| | 4 | 0.73 (± 0.28) | 0.70 (± 0.18) | |
| | 8 | 0.69 (± 0.31) | 0.65 (± 0.04) | |
| | 12 | 0.77 (± 0.14) | 0.70 (± 0.16) | |
| | 16 | 0.63 (± 0.18) | 0.63 (± 0.24) | |
| Main effect of packaging [e] | | 0.65[a] | 0.68[a] | |

| Main effect of time | | | | | | |
|---|---|---|---|---|---|---|
| Days | 0 | 1 | 4 | 8 | 12 | 16 |
| Main effect [e] | 0.73[ab] | 0.75[b] | 0.70[ab] | 0.61[ac] | 0.64[abc] | 0.56[c] |

[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

### 2.3.6 Microbiological analysis of par-baked pizza in different packaging treatments

*2.3.6.1 Effect of air on the shelf life of par-baked pizza*

The aerobic plate count (APC), lactic acid bacterial count (LAB) and mould count (MC) of par-baked pizza packaged + A were not significantly ($p \geq 0.05$) affected by the different additive treatments (Table 13). Although there were no significant ($p \geq 0.05$) differences between the different additive treatments for APC, LAB and YC, a clear trend was observed and reported. The main effects of time on the microbial levels tested were also reported.

The highest APC and LAB counts were recorded for the par-baked pizza + C throughout the storage period with 3.52 and 4.17 log cfu/g on d16 respectively. The APC and LAB counts for par-baked pizza + EC were the lowest, 2.1 and 2.94 log cfu/g respectively on d16. The APC and LAB maximum counts for par-baked pizza + DATEM were 2.91 and 3.96 log cfu/g respectively on d16. The par-baked pizza + EC had significantly lower ($p<0.05$) YC than the par-baked pizza + C and par-baked pizza + DATEM throughout the storage period except on d16.

As storage time progressed ($p<0.05$) the APC and LAB increased for all the treatments. However, this was not observed for the yeast (YC).

The par-baked pizza + C reached a maximum YC on d12, 3.8 log cfu/g; from there it decreased to 2.58 log cfu/g on d16. The same trend was observed for the par-baked pizza + DATEM, with a YC of 3.2 log cfu/g on d12 and then decreased to 2.79 log cfu/g on d16 although the par-baked pizza + EC reached a maximum YC on d16.

The interactions of the different groups of microorganisms tested for on par-baked pizza + A were also reported. With regard to the par-baked pizza + C, the APC remained the lowest throughout the storage period of 16 d at 10 ºC, compared to the LAB and the YC (

Figure 5, Appendix A, B, C). The LAB count of par-baked pizza + C remained the highest as the storage period progressed compared to the APC and YC. The same was observed for the APC, LAB and YC of the par-baked pizza + DATEM and par-baked pizza + EC (Figure 4).

All the air packaged par-baked pizza showed visible mould growth from d12 onwards, indicating the end of shelf life (Table 14).

Table 13: Analysis of Variance (ANOVA) for various microbiological parameters of par-baked pizza stored at 10 ºC for 16 d

| Source of variance | Aerobic Plate count | Lactic acid bacteria | Yeast | Mould |
|---|---|---|---|---|
| Packaging treatment (P) | * | ns | ns | * |
| Additive (A) | ns | ns | * | ns |
| Days (D) | * | * | * | * |
| P x A | ns | ns | ns | ns |
| P x D | ns | * | ns | * |
| A x D | ns | ns | * | ns |
| P x A x D | ns | ns | ns | ns |

*, Significant at $p < 0.05$, ns, not significant at $p \geq 0.05$

Table 14: Visible mould growth (Log cfu/g) of par-baked pizza stored in A, MAP and MAP + OA, stored at 10 ºC for 16 d

| Packaging | Additive | Storage time | | | | | | Shelf life (d) |
|---|---|---|---|---|---|---|---|---|
| | | 0–8 d | | 12 d | | 16 d | | |
| | | Log cfu/g | No. of samples with visible mould growth (n=4) | Log cfu/g | No. of samples with visible mould growth (n=4) | Log cfu/g | No. of samples with visible mould growth (n=4) | |
| A | C | ND | 0 out of 4 | ND | 4 out of 4 | 3.70 | 4 out of 4 | 12M |
| | DATEM | ND | 0 out of 4 | ND | 4 out of 4 | 3.26 | 4 out of 4 | 12M |
| | EC | ND | 0 out of 4 | ND | 4 out of 4 | 3.10 | 4 out of 4 | 12M |
| MAP | C | ND | 0 out of 4 | ND | 0 out of 4 | ND | 0 out of 4 | > 16 |
| | DATEM | ND | 0 out of 4 | ND | 0 out of 4 | ND | 0 out of 4 | > 16 |
| | EC | ND | 0 out of 4 | ND | 0 out of 4 | ND | 0 out of 4 | > 16 |
| MAP + OA | C | ND | 0 OUT OF 4 | ND | 0 OUT OF 4 | ND | 0 OUT OF 4 | > 16 |
| | DATEM | ND | 0 OUT OF 4 | ND | 0 OUT OF 4 | ND | 0 OUT OF 4 | > 16 |
| | EC | ND | 0 OUT OF 4 | ND | 0 OUT OF 4 | ND | 0 OUT OF 4 | > 16 |

ND: not detected below $10^1$ cfu/g; M: Visible mould growth indicating cut-off point for shelf life

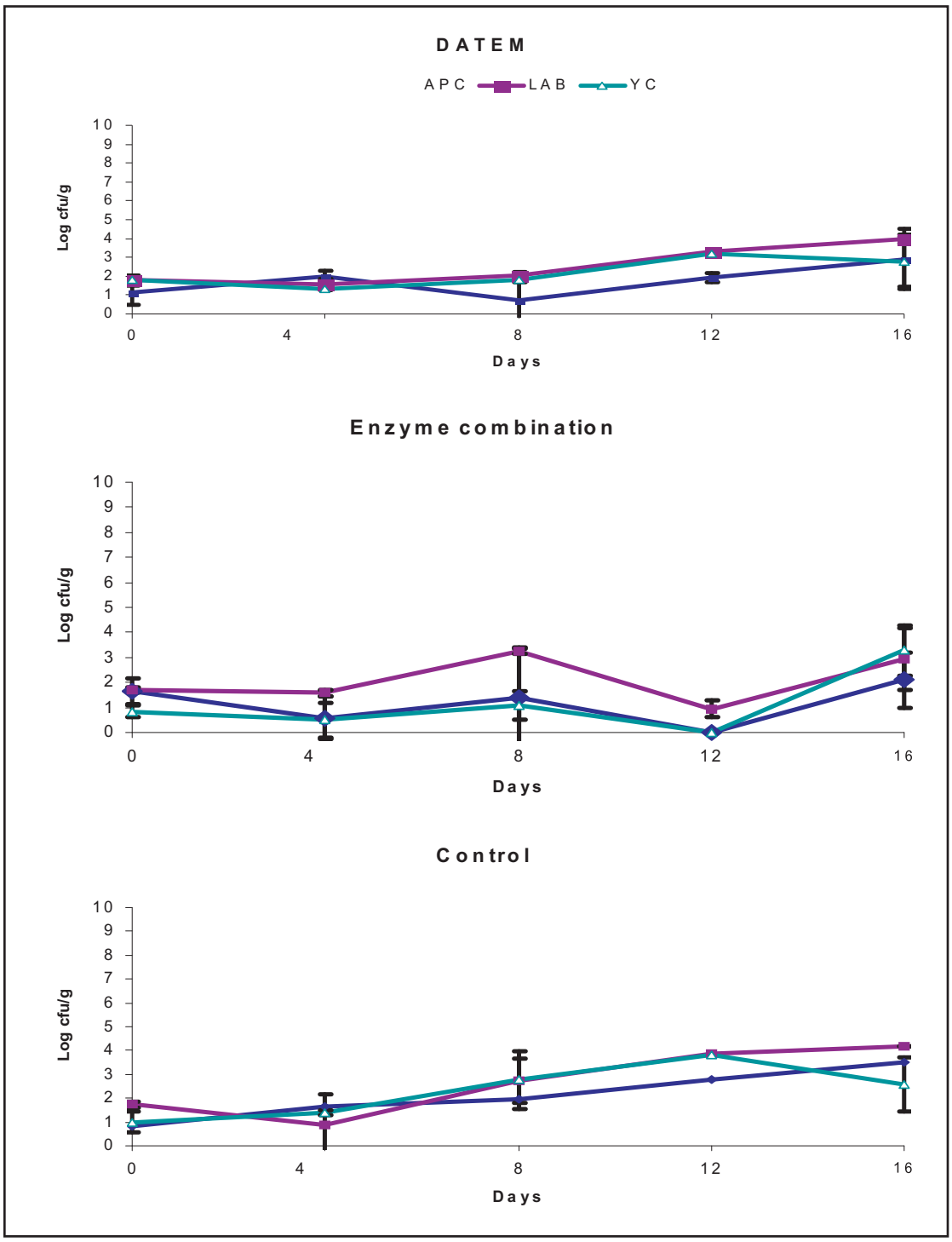



Figure 4: Main effect of aerobic plate (APC), lactic acid bacteria (LAB) and yeast (YC) counts of par-baked pizza with either: DATEM or enzyme combination (EC): lipase & maltogenic α-amylase or the control (C), packaged in air (A) for 16 d at 10 ºC

*2.3.6.2 Effect of 100% $CO_2$ on the microbiological shelf life of par-baked pizza*

The packaging's main effect significantly ($p<0.05$) affected the APC and the MC (Table 13). There was no visible mould growth for any of the par-baked pizza + MAP throughout the storage period of 16 d at 10 ºC. Therefore, the shelf life of par-baked pizza + MAP was >16 d (Table 14).

The interactions of the different groups of microorganisms tested on par-baked pizza + MAP were reported. With regard to the par-baked pizza + C, the APC remained the lowest throughout the storage period of 16 d at 10 ºC, compared to the LAB and the YC (

Figure 5; Appendix A, B, C). The LAB count remained the highest as the storage period progressed with a maximum count of 3.59 log cfu/g on d16, compared to APC (2.25 log cfu/g) and YC (2.92 log cfu/g). The YC increased until d8 to 2.39 log cfu/g, decreasing to 1.56 log cfu/g on d12, reaching levels of 2.92 log cfu/g on d16.

The same trend was observed for par-baked pizza + DATEM and par-baked pizza + EC. However, for par-baked pizza + EC, the APC, LAB and YC remained low; the counts did not exceed 3 log cfu/g throughout the storage period of 16 d at 10 ºC.

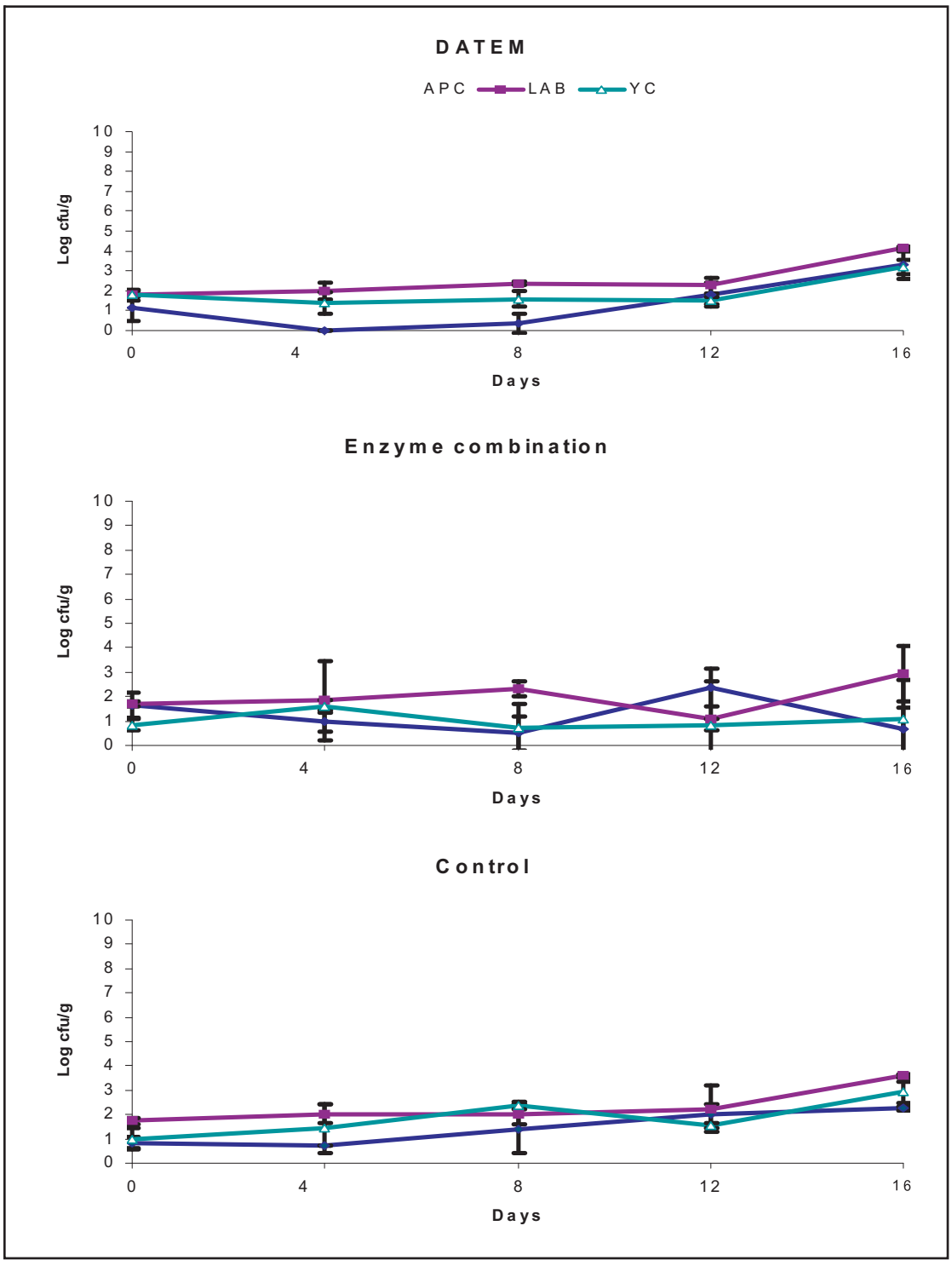



Figure 5: Main effect of aerobic plate count, lactic acid bacterial and the yeast counts of par-baked pizza with either: DATEM or enzyme combination (EC): lipase and maltogenic α-amylase or control (C), packaged in 100% $CO_2$ modified atmosphere (MAP) for 16d at 10 ºC.

*2.3.6.3 Effect of 100% $CO_2$ with an oxygen absorber on the microbiological shelf life of par-baked pizza*

The packaging main effect significantly ($p<0.05$) affected the APC and the MC, but not ($p\geq0.05$) LAB and YC (Table 13).

The interaction of the different microbial groups tested for were reported. The APC for the par-baked pizza + C, remained the lowest throughout the storage period when stored for 16d at 10 ºC, with 1.35 log cfu/g on d16 compared to LAB and YC with 2.96 and 3.5 log cfu/g respectively (Figure 6; Appendix A, B, C).

The APC for the par-baked pizza + DATEM increased throughout the storage period of 16 d at 10 ºC to 1.88 log cfu/g. The LAB count increased to 2.91 log cfu/g on d16 and the levels were higher than that of the APC and YC, 1.88 and 2.47 log cfu/g respectively.

The APC for the par-baked pizza + EC remained the lowest throughout the storage period when stored for 16 d at 10 ºC, with 1.29 log cfu/g on d16 compared with the LAB and YC with 3.00 and 2.10 log cfu/g respectively.

There was no visible mould growth during the shelf life of 16 d at 10 ºC; therefore, the shelf life of par-baked pizza + MAP + OA was > 16 d (Table 14).

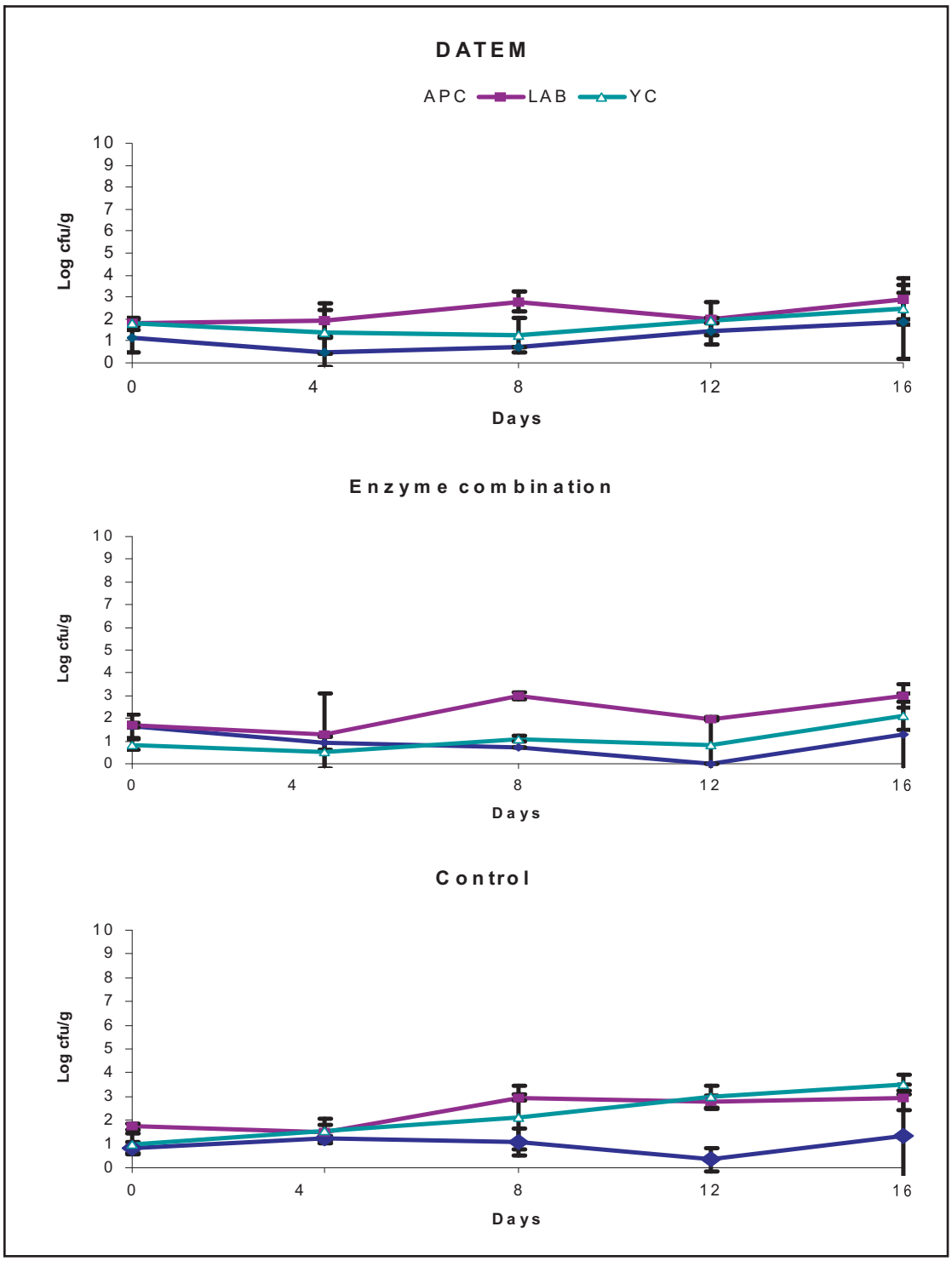



Figure 6: Main effect of aerobic plate count, lactic acid bacteria and the yeast count of par-baked pizza with either: DATEM or enzyme combination (EC): lipase and maltogenic α-amylase or control (C), packaged in 100% $CO_2$ modified atmosphere with an oxygen absorber

## 2.4 DISCUSSION

### 2.4.1 Water activity and moisture content of par-baked pizza crumb

The water activity of par-baked pizza crumb was between 0.95 and 0.98. Smith *et al*. (2004) state that the average water activity of pizza is between 0.94 and 0.95. The reason for the higher water activity of par-baked pizza may be that the par-baked pizza is a partially baked product and therefore would have a higher water activity than a fully baked product. It was also observed that the moisture content of par-baked pizza crumb increased from 36.74% on d0 to 41.56% on d16. Similar moisture content was found by Leuschner *et al*. (1999) for yeast rolls with moisture content ranging from 36–46%. The increase of moisture content of the crumb may be attributed to moisture migration from the tomato purée on the top crust to the internal crumb. Karathanos *et al*. (1995) showed that the moisture of the dough with higher moisture content diffused to the dried raisins with lower moisture content. The tomato purée had moisture content of 87.7%, thus the purée had higher moisture content than the inner par-baked pizza crumb, and this may have promoted moisture migration from the sauce to the crumb. This would in turn lead to the increase of water activity. Leuschner *et al*. (1999) also found small increases of water activity when the moisture content increased. This moisture redistribution may also lead to textural changes that will be discussed in later sections (Barrett *et al*., 2005).

Although the main effect of time showed that the water activity started to reach equilibrium and stabilise at 0.96, for the remaining storage period the moisture content continued to increase until d16. A similar trend was observed by Leuschner *et al*. (1999), namely, as the moisture content of re-baked soda bread and yeast rolls increased so did the water activity up to a certain point, then as the moisture content increased the water activity started to reach equilibrium. The reason for levelling off of the water activity may be that the moisture content and water activity relationship may have followed a desorption isotherm (Leuschner *et al*. 1999). Hence, equilibrium may start to form between the moisture migrating from the tomato purée topping to the internal crumb and moisture being lost from the crumb through evaporation.

### 2.4.2 Thickness of par-baked pizza

As expected, the par-baked pizza + DATEM and par-baked pizza + EC had similar thickness and were thicker than par-baked pizza + C. Primo-Martin, Hamer and De Jongh (2006) showed that both DATEM and lipase increased the volume of the bread, but DATEM produced 28% increase of the bread volume while the lipase enzyme produced a 16% increase of bread volume. Ying *et al*. (2008) also showed that the addition of DATEM increased the specific volume of Chinese steam bread. Guy and Sahi (2006) showed similar trends when lipase was used in cake formula. Keskin *et al*. (2004) also found that fungal α-amylase as well as lipase increased the specific volume of breads baked in a microwave oven.

DATEM is normally used for its dough strengthening properties; the strengthening effect finally results in higher loaf volume, a resilient crumb texture and fine-grained crumb (Ribota *et al*., 2004; Selomulyo & Zhou, 2007; Stampfli & Nersten, 1995). The mechanism is not yet fully understood but a possible theory is the following: the emulsifier DATEM improves the ability of the gluten network to form films to retain gas (Stampfli & Nersten, 1995). It causes the aggregation of the gluten proteins in the dough by binding to the hydrophobic sites. The hydrophilic emulsifier can also form a lamellar liquid-crystalline phase in water that is associated with gliadin (Ribotta *et al*., 2004; Selomulyo & Zhou, 2007). These structures contribute to the elasticity of the dough, which enables the gas cells to expand, and that will lead to higher loaf volume. Ying *et al*. (2008) showed that DATEM increased gluten strength that resulted in better gas retention and increased specific volumes.

Another mechanism of increasing volume by DATEM may be by stiffening the surface around the gas bubble (Kokelaar, Garritsen & Prins, 1995). Thus, this may prevent the collapse of small bubbles to form a larger bubble; therefore the disproportionation problem is retarded. The growth of larger gas bubbles at the expense of the surrounding smaller ones is due to a difference in Laplace pressure because of different size in gas cell radius. The gas solubility is higher around the smaller bubbles than around the large

bubbles, thereby promoting gas flow from the small bubbles into the large bubbles. DATEM can also lower the surface tension, thus allowing incorporation of more small bubbles during mixing.

It has also been reported that lipase is a strong dough-conditioning enzyme, which can successfully replace dough-conditioning emulsifiers like DATEM (Guy & Sahi, 2006; Keskin *et al*., 2004; Si & Lustenberger, 2001). Maltogenic α-amylase does not affect the volume of bread thus it has been suggested that this enzyme be used in conjunction with lipase (Si & Lustenberger, 2001). The increase in thickness of the par-baked pizza + EC is attributed more often to the function of the lipase enzyme. Lipase also improves the gluten protein strength (Si, 1997). With its addition, it results in higher loaf volume, a uniform grain, and therefore improves crumb softness during storage (Castello *et al*., 1999; Gray & Bemiller, 2003; Keskin *et al*., 2004; Si, 1997). During mixing and proofing, lipase increases the number of molecules with emulsifying properties by breaking down the triglycerides (TAG) into diglycerides (DAG), monoglycerides (MAG) and free fatty acids (FFA) (Castello *et al*., 1999). In addition, polyunsaturated fatty acids and endogenous wheat lipoxygenase form oxidised lipids. These oxidised lipids can co-oxidise others with molecules like protein thiols to form a strong gluten network by increasing the disulphide bridges (Castello *et al*., 1999).

### 2.4.3 Firmness of par- and re-baked pizza

Firmness can be defined as the force necessary to attain a given deformation (Carr & Tadini, 2003; Carson & Sun, 2001). Firmness was measured using a compression test and the three- point bend test.

It was observed that the firmness of par-baked pizza increased until d8, using the compression test. The strain values of par-baked pizza decreased until d4 and stabilised until d16, using the three-point bend test. This would indicate that the structure became more resistant and more firm and resistant to deformation. Thus, this correlated with the findings of the compression test. The stress values also initially increased which shows

that the par-baked pizza increased in firmness from d1. Lainez *et al*. (2008) also observed breadcrumb firming as storage time increased until d7 stored at 7 ºC. Firming of the par-baked pizza may be associated with the spoilage process known as staling. Staling is detected organoleptically by changes in texture as well as in the flavour and aroma (Pateras, 1998). The organoleptic changes that occur during storage may result in hardening of the crumb and softening of the crust (León, Durán & Benedito de Barber, 2002; Piazza & Masi, 1995). There are many theories of the mechanism of staling that are complex and not yet fully understood. The theories include the following: the migration of moisture from the crumb to the crust during storage, the recrystallisation of amylose during cooling of the baked product, followed by the recrystallisation of amylopectin and the association of the swollen starch granules with the gluten fibrils creating a more rigid structure (Gray & Bemiller, 2003; Halleberg & Chinachoti, 2002; Kamel & Ponte, 1993; Pateras, 1998; Piazza & Masi, 1995; Ribotta & Le Bail, 2007; Sidhu *et al*., 1997). The firmness of bread depends on the extent of plasticisation (Hug-Iten *et al*., 2003).

It was also observed that the firmness of par-baked pizza started to decrease from d12 until d16. This may indicate the degradation of the structure components of the par-baked pizza. Visible mould growth was evident on the air-packaged samples from d12. Extra-cellular enzymes of mould can be amylolytic and proteolytic and therefore are able to degrade starch and gluten. Yeast and lactic acid bacterial growth was also evident in samples packaged in MAP. These microorganisms have also been shown to be amylolytic and proteolytic. Gram *et al*. (2002) explain that microbial spoilage may result in textural changes due to the degradation of polymers. Fleet (2007) explains that some yeast produce extra-cellular proteases, lipases, amylases and pectinases that would affect the flavour and texture of the food product.

Another explanation for the softening of the par-baked pizza from d12 can be attributed to the moisture migration from the tomato purée to the crumb. It has been observed that the moisture content of the par-baked pizza crumb did increase as storage time increased. Water works as a plasticiser making bread biopolymer components more flexible. If

water were to be removed from the components it would lead to a firmer and more rigid structure (Gray & Bemiller, 2003; Katina *et al.,* 2006). The rigidity of bread increases as the moisture content decreases (Piazza & Masi, 1995). Thus, the moistening of the par-baked pizza will leave it less rigid in structure and less firm. Hallberg and Chinachoti (2002) showed that the meal ready-to-eat bread remained soft due to the retained moisture through effective packaging and by the humectants acting as plasticisers. Barrett *et al*. (2005) also showed that increasing strain resulted from moisture migration from the cheese to the bread, which reflected its plasticising effect and thus reduced the bread's firmness.

It was also observed that the par-baked pizza + EC was less firm than the par-baked pizza + DATEM and par-baked pizza + C respectively. Jones *et al*. (2008) and Si & Lustenberger (2001) showed that maltogenic α-amylase reduced the rate of bread firming. Kestin *et al*. (2004) showed that α-amylase and lipase reduced the rate of firming of microwave bread. Guy and Sahi (2006) also showed that lipase reduced the rate of firming of cake products. Si (1997) suggested that using α-amylase and lipase in conjunction would produce synergistic effects. Thus, the lower firming rate of par-baked pizza with lipase and maltogenic α-amylase may be due to the synergistic effect of these two enzymes.

Lipase and maltogenic α-amylase have shown anti-staling properties through different proposed mechanisms. Alpha-amylase breaks down the amylopectin so that it has shorter branches, however, the amylopectin backbone still stays intact (Cabellero *et al*., 2007; Keskin *et al.,* 2004; León *et al*., 2002). These dextrins may interfere with the starch-gluten interaction and with the amylopectin recrystallisation, thereby reducing the rate of firming (Durán *et al*., 2001; Jones *et al*., 2008; Kestin *et al*., 2004; León *et al.,* 1997; Miyazaki *et al*., 2004; Rojas, Rosell & Benedito de Barber, 2001; Si & Lustenberger, 2001). Rojas *et al*. (2001) showed that maltodextrins reduced the initial firmness of starch gels during the storage period, with maltose being the most effective in the reduction of firmness. León *et al*. (2002) also made this observation.

As stated earlier, during mixing and proofing, lipase increases the number of molecules with emulsifying properties. The exact mechanism by which lipase inhibits staling is not yet fully understood. It has been postulated that the monoglycerides interacts with amylose thus inhibiting the formation of double helical structures upon cooling (Gray & Bemiller, 2003; Keskin *et al*., 2004; León *et al*., 2002; Si, 1997; Williams & Pullen, 2007). These double helices that form during the re-crystallisation of amylose have been thought to be responsible for the initial firming of the crumb after baking (Pateras, 1998). León *et al*. (2002) observed similar effects of lipase and α-amylase. León *et al*. (2002) found that the initial firmness of the enzyme treated bread was lower compared to the control. They explained that amylose-lipid complexes might have been formed as a consequence of the lipase action producing emulsifying compounds like monoglycerides (MAG) that forms complexes with the amylose. This may contribute to the retardation of the crystallisation of amylose.

The par-baked pizza + DATEM was less firm than the par-baked pizza + C throughout the storage period. Ribotta *et al.* (2004) showed that DATEM reduced the rate of firming of bread compared to the rate of firming of bread without additives. DATEM interacts with amylose and forms complexes (Selomulyo & Zhou, 2007). Because the complex amylose is unable to re-crystallize it cannot take part in the staling process (de Leyn, 2006; Gray & Bemiller, 2003; Ribotta *et al*., 2004; Stampfli & Nersten, 1995; Toufeili *et al*., 1995). Armero and Collar (1998) showed that bread with DATEM had a higher specific volume and thinner gas cell walls correlated with softer crumbs compared to bread without DATEM. They explained that the effective resistance surface in a cross-section decreases as the thickness of the gas cell walls decreases thereby leading to a reduced firming rate (Armero & Collar, 1998).

Par-baked pizza + A was firmer than par-baked pizza + MAP. Similar trends have been observed by Knorr and Tomlins (1985) using white bread slices and whole wheat bread slices. Avital *et al*. (1990) also showed that $CO_2$ delayed the staling of bread when packaged in a carbon dioxide modified atmosphere. Avital *et al*. (1990) postulated $CO_2$ might block the sites of hydrogen bonding between the water and amylopectin, thus

reducing the water absorption capacity. It has been reported that these hydrogen bonds are involved in the staling process (Avital *et al*., 1990). They also observed that the effect of $CO_2$ existed when the water was in a solute state and that carbon dioxide is soluble in water. This may enable the $CO_2$ to bind to the amylopectin branches, thus reducing the rate of amylose recrystallisation.

The re-baked pizza remained less firm than the par-baked pizza throughout the storage period. Leuschner *et al*. (1999) recorded similar results; the soda bread also retained its freshness after re-baking. Bárcenas and Rosell (2007) explained that the softening effect of re-baking was due to the melting of the amylopectin crystals that formed during storage.

The re-baked pizza only showed significant increase in firmness from d0 to d1. Bárcenas and Rosell (2007) showed similar trends with re-baked bread. After one day of storage there would have been more amylopectin re-crystallisation, thus more crystalline amylopectin to be unfolded during the reheating process. Therefore, the re-baked pizza of d1 would be firmer than the re-baked pizza of the initial day.

### 2.4.4 Springiness of par- and re-baked pizza

Springiness can be defined as the rate a deformed material goes back to its un-deformed condition following removal of the deformed force (Carr & Tadini, 2003; Carson & Sun, 2001). Carons and Sun (2001) explained that as the deformation force was removed the bread recovered instantaneously due to its elastic components. The viscous components were responsible for the permanent deformation of the bread. Singh *et al*. (2006) explained that the food became more elastic when stress relaxation (SR) became close to 100% and when SR is <100%, the food became more viscous. The SR for baked goods is normally in the 40–50% SR range, which can be correlated to a 50:50 ratio of gliadin (viscous component) and glutenin (elastic component) fractions being present (Singh *et al*., 2006). The visco-elastic properties of the baked product will depend on the level of moisture and cross-linking of the gluten proteins during baking.

The springiness of par-baked pizza decreased as the storage time increased. Vulicevic *et al*. (2004) found similar trends, namely that the springiness of frozen par-baked bread decreased as storage time increased. Hug-Iten *et al*. (2003) also showed similar results. Fik and Surówka (2002) showed that the springiness of par-baked bread decreased when re-baked and it became exceedingly moist and gummy. They observed that high moisture crumbs produced increased stickiness and adhesiveness. Moisture may have migrated from the tomato purée to the internal crumb of the par-baked pizza. Thus, the par-baked pizza crumb could have become exceedingly moist and gummy thus reducing the springiness.

The par-baked pizza + EC and par-baked pizza + DATEM, springiness were lower than the par-baked pizza + C; it is not clear why the DATEM par-baked pizza was the least springy. It was shown in the previous section that the EC treatment and DATEM reduced the rate of firming thereby producing par-baked pizza with a softer crumb. Carson and Sun (2001) showed that soft bread had offered little resistance from the elastic components to the deformation force, thus is less springy but more viscous and sticky. Hug-Iten *et al*. (2003) showed that amylase treated bread also had a reduced springiness in comparison with the control. Hug-Iten *et al*. (2003) explained that reduced springiness is normally associated with sticky and chewy bread determined by sensory evaluation.

Siswoyo *et al*. (1999) explained that α-amylase produced shorter starch chains that made the dough more viscous. Carson and Sun (2001) also explained that more springy bread would have a larger recovery than less springy bread due to complex elasticity being larger or the complex viscosity being smaller. Should the bread be sticky or cohesive the viscous components would be larger than the elastic components. This would inhibit the bread recovery and mean that the bread is less springy and would leave permanent deformations. Hug-Iten *et al*. (2003) showed that bread treated with α-amylase had reduced springiness and increased stickiness. The α-amylase weakened the structure to a point where it collapsed during deformation. This may explain the reduce springiness of the par-baked pizza + EC.

The springiness of the re-baked pizza also decreased from the initial day to d1. Fik and Surówka (2002) found similar results with re-baked bread. As mentioned above, the re-baked bread became exceedingly moist and gummy thus reducing its springiness. Bárcenas and Rosell (2007) explained that during the second baking, heat causes the amylopectin to unfold and release the water. This moisture may produce a more sticky and adhesive crumb than a springy crumb. After d1 the springiness increased until d4. Bárcenas and Rosell (2007) further explained that the free water, released after the second bake, is again available for the second retrogradation of amylopectin, but to a lesser extent due to water evaporation. This would lead to the firming of the bread where it becomes more elastic than viscous (less sticky and adhesive). Hence, the re-baked pizza would have increased springiness.

The re-baked pizza was springier than the par-baked pizza. The par-baked pizza was also firmer than the re-baked pizza. As moisture is released during the re-baking process and the crystallized amylopectin unfolds, the re-baked pizza returns to its original softness. The free water is again available to plasticise the structure components (gluten and starch), thus the structure components would be less rigid and more elastic compared to the par-baked pizza crumb structure components.

### 2.4.5 Effect of air on the microbiological shelf life of par-baked pizza

Shelf life of par-baked pizza samples stored in air was restricted to 12 d at 10 ºC due to visible mould growth. Rodriguez *et al*. (2000) also found signs of visible mould growth on wheat bread stored in air at 15–20 ºC after 8 d. Huis in't Veld (1996) explained that yeast and mould spoilage resulted in sensorial changes: visible pigmentation, the production of slime, acids, gas and alcohol that lead to off-flavour and odour development.

During the 16 d storage period of par-baked pizza the APC and the LAB counts did not reach the level at which spoilage has been indicated to occur between $10^7$–$10^8$ cfu/g (Gram *et al*., 2002). This may be because the initial microbial load for APC and LAB

was low. Rodriguez *et al*. (2003) also found similar results concerning the APC and LAB levels of pre-baked pizza when stored at 15–20 ºC for 17 d.

The LAB levels and the yeast levels were similar with LAB having a slight competitive advantage over the yeast. Visible mould growth was evident before the LAB and YC could reach maximum levels. Thus, both lactic acid bacteria and the yeast were outgrown by mould. This may explain why the yeast levels declined from d12. The predominant species may be influenced by the water activity, temperature and gas composition of the environment (Lacey 1989). The water activity of par-baked pizza remained high (0.96) throughout the storage period. Thus all microbial growth was possible in the air-packaged samples. Although LAB, yeast and mould are able to grow at low storage temperatures, it has also been found that low storage temperatures may increase the lag phase by reducing the metabolism thus slowing down deterioration. Lacey (1989) has stated that most mould can grow in a temperature range of 10–40 ºC. Low temperatures have shown to give mould selective advantage over bacteria in meat, possibly explaining why the mould became the predominant group in par-baked pizza. Another possible contribution to the competitive advantage of mould over LAB may be that LAB grows slower in aerobic environments than in anaerobic environments (Huis in't Veld, 1996).

Wai Lee *et al*. (1999) reported that DATEM showed bactericidal and anti-viral action. DATEM has been effective against gram-positive and gram-negative bacteria. The native wheat lipase may have introduced monoglycerides as a result of its enzymatic activity. The antibacterial lipids (monoglycerides) together with DATEM may act on the organism's lipid envelope or membrane and affect its permeability thus reducing bacterial growth (Wai Lee *et al*., 1999). This may explain why the LAB levels for par-baked pizza + DATEM were lower than those for the par-baked pizza + C.

Thormar and Hilmarsson (2007) also explained that through the action of lipase in breast milk, antibacterial and antiviral free fatty acids and monoglycerides are released. This may explain why par-baked pizza + EC also showed reduced LAB levels compared to the par-baked pizza + C. As mentioned earlier, TAG is broken down by lipase to DAG,

MAG and FFA. The antibacterial activity of these lipids MAG and FFA has been attributed to the disruption of the cell membrane (Thormar & Hilarsson, 2007). Gram-positive bacteria are more susceptible to the anti-microbial lipids than gram-negative bacteria.

### 2.4.6 Effect of 100% $CO_2$ on the microbiological shelf life of par-baked pizza

Unlike, the air samples, the par-baked pizza + MAP showed no visible mould growth after 16 d of storage. The reason may be because of the effect of $CO_2$ on mould. Rodriguez *et al*. (2003) found 50% of the pre-baked pizza showed visible signs of mould growth after 13 d packaged in 100% $CO_2$ atmosphere at 15–20 ºC. Leuschner *et al.* (1999) showed that mould growth was inhibited for 13 weeks when par-baked bread was packaged in 40% $CO_2$/60% $N_2$ and stored at 4 ºC.

Fernandez *et al.* (2006) said that $CO_2$ is most effective against aerobic bacteria and moulds. It is well known that $CO_2$ is bacteriostatic and fungistatic, by penetrating the microbial cell and lowering the internal pH thereby rendering metabolism and enzymatic activity (Labuza *et al*., 1992). Fernandez *et al*. (2006) showed that the APC of soy bread was reduced by MAP (20% $CO_2$/ 80% $N_2$ & 50% $CO_2$/ 50% $N_2$). Rodriguez *et al*. (2003) also found lower APC growth in the 100% $CO_2$ packaged pre-baked pizza. However, the aerobic growth was still supported due to $O_2$ migrating through the packaging film (Rodriguez *et al*., 2003). This may explain why no visible mould growth occurred and the low APC levels of par-baked pizza + MAP.

The LAB favours anaerobic environments because the reactive oxygen species like superoxide anion, hydrogen peroxide and hydroxyl radical build-up in aerobic environments are toxic to LAB (Higuchi, Yamamoto & Kamio, 2000). These bacteria lack the enzymes catalase and citochrome oxidase that is required for oxygen metabolism. The build up of toxic oxygen species could be because of incomplete reduction of oxygen to water during respiration, radiation and light (Higuchi *et al*., 2000). This would explain why the LAB levels are unaffected by MAP due to the anaerobic

environment. Rodriguez *et al*. (2003) also found that the LAB of pre-baked pizza was unaffected by MAP. Cabo *et al*. (2001) even found enhanced LAB activity under MAP conditions. It was thought that the LAB that has been previously isolated from pizza dough was introduced by contaminated yeast and the environment (Coppola *et al*., 1998).

Yeast levels were also unaffected by MAP. However, they did not exceed the level regarded as unacceptable in terms of quality. The limit between acceptable quality and marginal quality for yeast and moulds is 2 x $10^3$ cfu/g and the limit between marginal quality and unacceptable quality is 5 x $10^4$ cfu/g (Pinho & Furlong, 2000). When yeast causes spoilage, it is usually when it is initially present as a contaminant and/or environmental conditions enhanced yeast growth more than bacteria and mould growth.

Once again the par-baked pizza + EC had the lowest microbial levels; the reason may be similar to that given in the previous section.

### 2.4.7 Effect of 100% $CO_2$ with an oxygen absorber on the microbiological shelf life of par-baked pizza

As expected the LAB and YC were unaffected by MAP + OA packaging treatment, for these microorganisms are facultative anaerobic and therefore can grow in both aerobic and anaerobic conditions. However, LAB grows more favourably in anaerobic environments.

The reason for no visible sign of mould growth and the very low APC is the inhibitive effect of $CO_2$ and the reduced oxygen content inside the packaged produced by the oxygen absorber. Guynot *et al*. (2003b) found similar results on cake analogues and Smith *et al*. (1986) found these results on sponge cake. It has been shown that mould growth can be eliminated when oxygen levels are less than 0.4% (Powers & Berkowitz, 1990; Smith *et al*., 1986). Oxygen absorbers can reduce residual oxygen within the package to 0.01% in 15 h after packaging (Guynot *et al*., 2003).

Guynot *et al*. (2003a) explain that the reduced oxygen content increases the lag phase of the mould. However, the packaging film permeability to $O_2$ is very important. Guynot *et al.* (2003b) recommend using a packaging film that has permeability less than 20 $cm^3/m^2$24h/atm, which was used in this study. Another problem that may affect the efficiency of an oxygen absorber is that par-baked pizza is a porous product that would hinder the complete elimination of oxygen. Thus, if the film has high oxygen permeability and the residual oxygen level is too high, the oxygen absorber would reach saturation point quicker and would lose its oxygen-absorbing ability (Guynot *et al*., 2003b). This would lead to increased oxygen concentration inside the package and would allow aerobic bacterial and mould growth. Smith *et al*. (1986) have explained that even though there is some residual oxygen present, it would be used by other microorganisms like LAB and yeast and would prevent the occurrence of mould growth.

## 2.5 CONCLUSIONS

It can be concluded that the par-baked pizza + A reaches the end of its shelf life at d12 due to visible mould growth. Thus, mould growth is the main shelf life limiting factor. The par-baked pizza packaged in MAP and MAP + OA shelf life exceeds the storage period of 16 d in terms of microbiological limits. Therefore it can be concluded that the shelf life of the par-baked pizza is increased by modifying the packaging atmosphere (100% $CO_2$) and with the addition of an oxygen absorber. To increase the effectiveness of MAP and MAP + OA to increase the shelf life of par-baked pizza, preservatives such as sodium pyrophosphate and sodium bicarbonate can also be added.

Not only does MAP increase the shelf life microbiologically but it also reduces the rate of firming, therefore MAP increases the shelf life of par-baked pizza. However, staling is not the shelf life determinant of par-baked pizza, for the re-baked pizza was even softer than it had been originally. The additive treatments, DATEM and the enzyme combination treatment (lipase and maltogenic α-amylase), reduces the rate of firming and produce thicker par-baked pizza bases than par-baked pizza without additives. Hence, the addition of these additives increase the shelf life of par-baked pizza by reducing the

staling rate. With the physical parameters tested in this study it can be concluded that the demand for more natural additives can be met by replacing DATEM with an enzyme combination like lipase and maltogenic α-amylase. However, further research is required in order to determine whether par-baked pizza with an enzyme combination treatment would be sensorial the same as par-baked pizza with DATEM, in terms of crumb cell structures, mouthfeel and colour.

## 2.6 RESEARCH CHAPTER REFERENCES

AACC see American Association of Cereal Chemists.

American Association of Cereal Chemists (AACC). 2000a. Approved Methods of the American Association of Cereal Chemists, Method:74–09, AACC, St Paul, MN.

AACC. 2000b. Approved Methods of the American Association of Cereal Chemists, Method:44-01, AACC, St Paul, MN.

AOAC see Association of Official Analytical Chemists.

AOAC Official Methods of Analysis. 2000. Microbiological Methods, Association of Official Analytical Chemists (AOAC) International, 17th Ed, Gaithersburg, MD, Method:990.12.

ARMERO, E. & COLLAR, C. 1998. Crumb Firming Kinetics of Wheat Breads with Anti–staling Additives, *Journal of Cereal Science,* (28):165–174.

AVITAL, Y., MANNHEIM, C.H., & MILTZ, J. 1990. Effect of Carbon Dioxide Atmosphere on Staling and Water Relation in Bread, *Journal of Food Science*, (55), 2:413–416.

BÁRCENAS, M.E. & ROSELL, C.M. 2007. Different Approaches for Increasing the Shelf Life of Partially Baked Bread: Low Temperatures and Hydrocolloid Addition, *Food Chemistry*, (100):1594–1601.

BARRETT, A., CARDELLO, A., MAGUIRE, P., & PELEG, M. 2005. Moisture Redistribution and Textural Changes Stored Model Sandwiches, *Journal of Texture Studies*, (36):569–588.

BEUCHAT, L.R., NAIL, B.V., BRACKETT, R.E. & FOX, T.L. 1991. Comparison of the Petrifilm™ Yeast and Mould Culture Film Method to Conventional Methods for Enumerating Yeast and Molds in Foods, *Journal of Food Protection*, (54), 6:443–447.

CABALLERO, P.A., GOMEZ, M. & ROSELL, C.M. 2007. Improvement of Dough Rheology, Bread Quality and Bread Shelf–life by Enzymes, *Journal of Food Engineering*, (81):42–53.

CABO, M.L., PASTORIZA, L., BERNÁRDEZ, M. & HERRERA, J.J. 2001. Effectiveness of $CO_2$ and Nisaplin on Increasing Shelf–life of Fresh Pizza, *Food Microbiology*, (18):489–498.

CARR, L.G. & TADINI, C.C. 2003. Influence of Yeast and Vegetable Shortening on Physical and Textural Parameters of Frozen Part Baked French Bread, *Swiss Society of Food Science & Technology,* (36):609–614.

CARSON, L. & SUN, X. 2001. Creep-Recovery of Bread and Correlation to Sensory Measurements of Textural Attributes, *American Association of Cereal Chemists*, (78), 1:101–104.

CASTELLO, P., BARET, J.L., POTUS, J. & NICOLAS, J. 1999. Technological and Biochemical Effects of Exogenous Lipase in Breadmaking, *2nd European Symposium on Enzymes in Grain Processing,* ESEPC 2, Helsinki, Finland, 193–199.

COPPOLA, S., PEPE, O. & MAURIELLO, G. 1998. Effect of Leavening Microflora on Pizza Dough Properties, *Journal of Applied Microbiology*, (85):891–897.

CORRELL, J. 2002. Encyclopizza, Correll Consulting. Available on the Internet at:http://www.correllconcepts.com (accessed on 30 October 2008).

DE LEYN, I. 2006. Functional Additives, In: Hui, Y.H., Corke, H., De Leyn, I., Nip, W., Cross, N., (Eds), *Bakery Products Science & Technology*, Blackwell Publishing, U.S.A., 233–243.

DE MAN, J.C., ROGOSA, M. & SHARP, M.E. 1960. A Medium for the Cultivation of *Lactobacilli*, *Journal of Applied Bacteriology*, (23):130–135.

DURÁN, E., LEÓN, A.E., BARBER, B. & BENEDITO DE BARBER, C.B. 2001. Effect of Low Molecular Weight Dextrins on Gelatinization and Retrogradation of Starch, *European Food Research & Technology*, (212):203–207.

FARAHNAKY, A. & MAJZOOBI, M. 2008. Physicochemical Properties of Partbaked Breads, *International Journal of Food Properties*, (11):186–195.

FERNANDEZ, U., VODOVOTZ, Y., COURTNEY, P. & PASCALL, M.A. 2006. Extended Shelf life of Soy Bread Using Modified Atmosphere Packaging, *Journal of Food Protection*, (69), 3:693–698.

FIK, M. & SURŎWKA, K. 2002. Effect of Prebaking and Frozen Storage on the Sensory Quality and Instrumental Texture of Bread, *Journal of Food Science & Agriculture,* (82):1268–1275.

FLEET, G.H. 2007 Yeast in Foods and Beverages: Impact on Product Quality and Safety, *Current Opinion in Biotechnology*, (18):170–175.

GRAM, L., RAVN, L., RASCH, M., BRUHN, J.B., CHRISTENSEN, A.B. & GIVSKOV, M. 2002. Food Spoilage–Interactions between Food Spoilage Bacteria, *International Journal of Food Microbiology*, (78):79–97.

GRAY, J.A. & BEMILLER, J.N. 2003. Bread Staling: Molecular Basis and Control, *Comprehensive Reviews in Food Science & Food Safety*, (2):1–21.

GUY, R.C.E. & SAHI, S. 2006. Application of a Lipase in Cake Manufacture, *Journal of the Science of Food & Agriculture*, (86):1679–1687.

GUYNOT, M.E., SANCHIS, V., RAMOS, A.J. & MARIN, S. 2003a. Mould-free Shelf Life Extension of Bakery Products by Active Packaging, *Journal of Food Science*, (68), 8:2547–2552.

GUYNOT, M.E., MARÍN, S., SANCHIS, V. & RAMOS, A.J. 2003b. Modified Atmosphere Packaging for Prevention of Mould Spoilage of Bakery Products with Different pH and Water Activity Levels, *Journal of Food Protection*, (66), 10:1864–1872.

HALLEBERG, L.M. & CHINACHOTI, P. 2002. A Fresh Perspective on Staling: The Significance of Starch Recrystallization on the Firming of Bread, *Journal of Food Science,* (67), 2:1092–1096.

HIGUCHI, M., YAMAMOTO, Y. & KAMIO, Y. 2000. Molecular Biology of Oxygen Tolerance in Lactic Acid Bacteria: Functions of NADH Oxidase and Dpr in Oxidative Stress, *Journal of Bioscience & Bioengineering*, (90), 5:481–493.

HUG-ITEN, S., ESCHER, F. & CONDE-PETIT, B. 2003. Staling of Bread: Role of Amylose and Amylopectin and Influence of Starch-Degrading Enzymes, *Cereal Chemistry*, (80), 6:654–661.

HUIS IN'T VELD, J.H.J. 1996. Microbial and Biochemical Spoilage of Foods: an Overview, *International Journal of Food Microbiology*, (33):1–18.

JONES, A., LAMSA, M., FRANDSEN, T.P., SPENDLER, T., HARRIS, P., SLOMA, A., XU, F., NIELSEN, J.B. & CHERRY, J.R. 2008. Directed Evolution of a Maltogenic α-Amylase from *Bacillus* spp. TS-25, *Journal of Biotechnology*, (134):325–333.

KAMEL, B.S. & PONTE, J.G. 1993. Emulsifiers in Baking. In: Kamel B.S., Stauffer, C.E., (Eds.), *Advances in Baking Technology*, Blackie Academic & Professional, London, 180–215.

KARATHANOS, V.T., KOSTAROPOULOS, A.E. & SARAVACOS, G.D. 1995. Diffusion and Equilibrium of Water in Dough/Raisin Mixtures, *Journal of Food Engineering*, (25):113–121.

KATINA, K., SALMENKALLIO–MARTTILA, M., PARTANEN, R., FORSSELL, P. & AUTIO, K. 2006. Effects of Sourdough and Enzymes on Staling of High-fiber Wheat Bread, *Swiss Society of Food Science & Technology*, (39):479–491.

KESTIN, S.O., SUMNU, G. & SAHIN, S. 2004. Usage of Enzymes in a Novel Baking Process, *Die Nahrung*, (48), 2:156–160.

KOKELAAR J.J., GARRITSEN, J.A. & PRINS, A. 1995. Surface Rheological Properties of Sodium Stearoyl-2-lactylate (SSL) and Diacetyl Tartaric Esters of Mono (and di) Glyceride (DATEM) Surfactants after a Mechanical Surface Treatment in Relation to Their Bread Improving Abilities, *Colloids & Surfaces A: Physicochemical & Engineering Aspects*, (95):69–77.

KNORR, D. & TOMLINS, R.I. 1985. Effect of Carbon Dioxide Modified Atmosphere on the Compressibility of Stored Baked Goods, *Journal of Food Science*, (50):1172–1173.

LABUZA, T.P., FU, B. & TAOUKIS, P.S. 1992. Prediction for the Shelf Life and Safety of Minimally Processed CAP/MAP Chilled Foods: A Review, *Journal of Food Protection*, (55), 9:741–750.

LACEY, J. 1989. Pre- and Post-harvest Ecology of Fungi Causing Spoilage of Foods and Other Stored Products, *Journal of Applied Bacteriology Symposium Supplement*, (18):11S–25S.

LEÓN, A.E., DURÁN, E. & BENEDITO DE BARBER, C. 1997. Firming of Starch Gels and Amylopectin Retrogradation as Related to Dextrin Production by α-Amylase, Z *Lebensmittel Unters Forsch* A, (205):131–134.

LEÓN, A.E, DURÁN, E. & BENEDITO DE BARBER, C. 2002. Utilization of Enzyme Mixtures to Retard Bread Crumb Firming, *Journal of Agriculture & Food Chemistry*, (50):1416–1419.

LEUSCHNER, R.G.K., O'CALLAGHAN, M.J.A. & ARENDT, E.K. 1999. Moisture Distribution and Microbial Quality of Part Baked Bread as Related to Storage and Rebaking Conditions, *Journal of Food Science*, (64), 3:543–546.

LAINEZ, E., VERGARA, F. & BÁRCENAS, M.E. 2008. Quality and Microbial Safety of Partially Baked Bread during Refrigerated Storage, *Journal of Food Engineering*, (89):414–418.

MIYAZAKI, M., MAEDA, T. & MORITA, N. 2004. Effect of Various Dextrin Substitutions for Wheat Flour on Dough Properties and Bread Qualities, *Food Research International*, (37), 59–65.

PATERAS, I.M.C. 1998. Bread Spoilage and Staling, In: Cauvain, S.P., Young, L.S., (Eds), *Technology of Breadmaking*, Springer, New York, 240–254.

PIAZZA, L. & MASI, P. 1995. Moisture Redistribution Throughout the Bread Loaf During Staling and Its Effect on Mechanical Properties, *Cereal Chemistry,* (72), 3:320–325.

POWERS, E.M. & BERKOWITZ, D. 1990. Efficacy of an Oxygen Scavenger to Modify the Atmosphere and Prevent Mould Growth on Meal, Ready-to-Eat Pouched Bread, *Journal of Food Protection*, (53), 9:767–771.

PRIMO–MARTÍN, C., HAMER, R.J. & DE JONGH, H.H.J. 2006. Surface Layer Properties of Dough Liquor Components: Are Key Parameters in Gas Retention in Bread Dough? *Food Biophysics*, (1), 2:1–22.

PINHO, B.H. & FURLONG, E.B. 2000. The Occurrence of Mould, Yeasts and Mycotoxins in Pre–cooked Pizza Dough Sold in Southern Rio Grande Do Sul, *Brazilian Journal of Microbiology*, (31):99–102.

RIBOTTA, P.D. & LE BAIL, A. 2007. Thermo–physical Assessment of Bread during Staling, *Swiss Society of Food Science & Technology*, (40):879–884.

RIBOTTA, P.D., PÉREZ, G.T., LEÓN, A.E. & AÑÓN, M.C. 2004. Effect of Emulsifier and Guar Gum on Micro Structural, Rheological and Baking Performance of Frozen Bread Dough, *Food Hydrocolloids,* (18):305–313.

RODRIGUEZ, V., MEDINA, L. & JORDANO, R. 2000. Effect of Modified Atmosphere Packaging on the Shelf Life of Sliced Wheat Bread, *Die Nahrung*, (44), 4:247–252.

RODRIGUEZ, V., MEDINA, L. & RAFAEL, J. 2003. Influence Modified Atmosphere Packaging on the Shelf Life of Pre–baked Pizza Dough with and without Preservatives Added, *Die Nahrung*, (47), 2:122–125.

ROJAS, J.A., ROSELL, C.M. & BENEDITO DE BARBER, C. 2001. Role of Maltodextrins in the Staling of Starch Gels, *European Food Research & Technology*, (212):364–368.

SELOMULYO, V.O. & ZHOU, W. 2007. Frozen Bread Dough: Effects of Freezing Storage and Dough Improvers, *Journal of Cereal Science,* (45), 1–17.

SI, J. 1997. Synergistic Effect of Enzymes for Breadbaking, *Cereal Food World*, (42), 10: 802–807.

SI, J.Q. & LUSTENBERGER, Q. 2001. Novamyl® – A True Anti-staling Enzyme, *Cereal Food*, Novozymes, 1699-01.pdf, 1–20.

SIDHU, J.S., AL-SAQER, J. & AL-ZENKI, S. 1997. Comparison Methods for the Assessment of the Extent of Staling in Bread, *Food Chemistry*, (58), 1–2:161–167.

SINGH, H., ROCKALL, A., MARTIN, C.R., CHUNG, O.K. & LOOKHART, G.L. 2006. The Analysis of Stress Relaxation Data of Some Viscoelastic Foods using a Texture Analyzer, *Journal of Texture Studies*, (37):383–392.

SISWOYO, T.A., TANAKA, N. & MORITA, N. 1999. Effect of Lipase Combined with α–Amylase on Retrogradation of Bread, *Food Science Technology Research*, (5), 4:356–361.

SMITH, J.P., OORALKUL, B., KOERSEN, W.J., JACKSON, E.D. & LAWRENCE, R.A. 1986. Novel Approach to Oxygen Control in Modified Atmosphere Packaging of Bakery Products, *Food Microbiology*, (3):315–320.

SMITH, J.P., DAIFAS, D.P., EL-KHOURY, W., KOUKOUTSIS, J. & EL-KHOURY, A. 2004. Shelf Life and Safety Concerns of Bakery Products – A Review, *Critical Review in Food Science & Nutrition*, (44):4419–4455.

STAMPFLI, L. & NERSTEN, B. 1995. Emulsifiers in Bread Making, *Food Chemistry,* (52):353–360.

THORMAR, H. & HILMARSSON, H. 2007. The Role of Microbicidal Lipids in Host Defence Against Pathogens and Their Potential as Therapeutic Agents, *Chemistry & Physics of Lipids*, (150):1–11.

TOUFEILI, I., SHADAREVIAN, S., MISKI., A.M.A. & HANI, I. 1995. Effect of Shortening and Surfactants on Selected Chemical/Physicochemical Parameters and Sensory Quality of Arabic Bread, *Food Chemistry,* (53):253–258.

VLAEMUNCK, G.M. 1994. Comparison of Petrifilm™ and Plate Count Methods for Enumerating Molds and Yeasts in Cheese and Yoghurt, *Journal of Food Protection*, (57), 10:913–914.

VULICEVIC, I.R., ABADEL-AAL, E-S.M., MITTAL, G.S. & LU, X. 2004. Quality and Storage Life of Par-baked Frozen Breads, *Swiss Society of Foods Science & Technology*, (37):205–213.

WAI LEE, T.S, SCHALLER, J., ANDERSON, S.N., MAZER, T.B., HILTY, M.D., GUZMAN-HARTY, M., MEULBROEK, J.A., EWING, P.J. & LUI, J. 1999. Water-miscible Esters of Mono-and-Diglycerides Having Antibacterial Activity and Their Use in Inhibiting Infection, *United States Patent*, 5,908,862.

WILLIAMS, T., PULLEN, G. 2007. Functional Ingredients In: Cauvain, S.P., Young, L.S., (Eds), *Technology of Breadmaking*, 2nd ed, Springer, New York, 51–92.

YING, B., JUN, L., YUNZI, F., ZHUO, C. & ZAIGUI, L. 2008. Application of DATEM for Chinese Steamed Bread Made from Flours of Different Gluten Qualities, *Journal of Science Food & Agriculture*, (89):227–231.

# 3: GENERAL DISCUSSION

## 3.1 DISCUSSION AND RECOMMENDATIONS ON METHODOLOGY AND FURTHER RESEARCH

### 3.1.1 Packaging

It was difficult to remove the air prior to gas flushing, because pizza is a baked product and a complete vacuum would deform the par-baked pizza base. Thus a complete vacuum could not be achieved prior to gas flushing. This may explain why the initial $CO_2$ content of the gas headspace of the MAP and MAP + OA was lower than 100% (section 2.3.1; Appendix D). The absorption of $CO_2$ by the par-baked pizza base is another reason for lower headspace $CO_2$ content. The reduced vacuum contributed to residual oxygen being present inside the MAP and MAP + OA sample packages. It has been shown by Smith *et al.* (1986) that oxygen absorbers absorb the residual oxygen so that the headspace contains less than 1% $O_2$. Therefore, the oxygen headspace composition of the MAP samples was higher than the MAP + OA samples throughout the storage period of 16 d at 10 ºC.

However, it has also been stated that when packaging material has a high permeability, oxygen might migrate through the packaging material into the gas headspace (Guynot *et al.*, 2003b). This would cause the oxygen absorber to reach saturation point and ingressing oxygen would no longer be absorbed (Guynot *et al.*, 2003b). Therefore, the packaging material may have been too permeable and would have allowed the $CO_2$ composition of the gas headspace of the MAP and the MAP + OA to decline while the $O_2$ started to increase from d12. The film used had the following permeability properties: 20 $cm^3/m^2$24h/atm at 22 ºC and 75% RH. To improve the gas packaging of par-baked pizza, the following recommendations are made:

- Use of a film with a medium permeability of less than 20 cm3/m2d/atm, (Guynot et al., 2003b);

- Use of a gas mixture (60% CO2 / 40% N2) instead of a single gas to minimise the CO2 absorption into the par-baked pizza base; and
- Use of a gas-flushing and package-sealing method designed for pizza, for example, a thermoforming packaging system.

Thermoforming packaging involves the use of flexible or semi-rigid packaging material. This material is heated and transformed into a mould to the required shape and size to fit the food product (Kotsianis *et al*., 2002). A thermoforming packaging machine for pizza is currently supplied by Ulma packaging. This packaging machine allows flexible and rigid packaging and the option of creating a vacuum or gas injection (MAP) inside a package (Fresh pizza packaging in thermoforming in modified atmosphere (MAP) in rigid film, 2009).

### 3.1.2 Determining the crumb moisture content

An oven-drying method was used to determine the moisture content of the par-baked pizza crumb. Sampling was very important in order to minimise the variations within samples. The top and bottom crust was removed ± 2 mm from the edge and a sample piece was cut from the area with the most uniform pore formation. The morphology (porosity, gas cell sizes) of the baked product has also been shown to influence the moisture migration from a high concentration to a lower concentration (Primo-Martín *et al*., 2008). Hence, this would influence the moisture content and may explain the high standard deviation within treatments. However, notwithstanding these limitations, a trend could be observed in terms of crumb moisture content during storage (section 2.3.3, **Error! Reference source not found.**).

The internal crumb moisture content of par-baked pizza did increase over time, thus moisture migration was possible from the tomato purée topping. It has been shown that moisture migration plays a vital role in the textural changes that occur during storage (Hallberg & Chinachoti, 2002; Ricotta & Le Bail, 2007). Farahnaky and Majzoobi (2008) have shown that the different parts of par-baked bread vary in their moisture

content due to moisture gradients that form during baking. The factors that play a critical role in the firming of bakery products are influenced by the distribution of moisture (Lodi, Abduljalil & Vodovotz, 2007). An example is the following: amylopectin retrogradation has been shown to play a significant role in the firming of bread during storage; however, it requires moisture in order to be able to crystallize (Lodi *et al*., 2007). A topping may change the moisture migration process that was normally to be expected. A proposed model (Figure 1) describes how moisture may have migrated between the different parts of par-baked pizza

In order to better understand the effect of moisture migration on the texture of par-baked pizza base during storage, it is recommended that the moisture migration of par-baked pizza with a topping be mapped by determining the complete moisture distribution profile and the textural change from glassy to rubbery state.

The moisture distribution can be illustrated by using a magnetic resonance imaging (MRI) method. Lodi *et al*. (2007) have shown that MRI can be used to determine the moisture distribution profile of bread during storage. The advantage of using this method is that non-invasive and non-destructive sample preparation is used and a larger sample portion can be tested (Lodi *et al*., 2007). Therefore, this method can be used to study the moisture distribution in inhomogeneous food products such as pizza base.

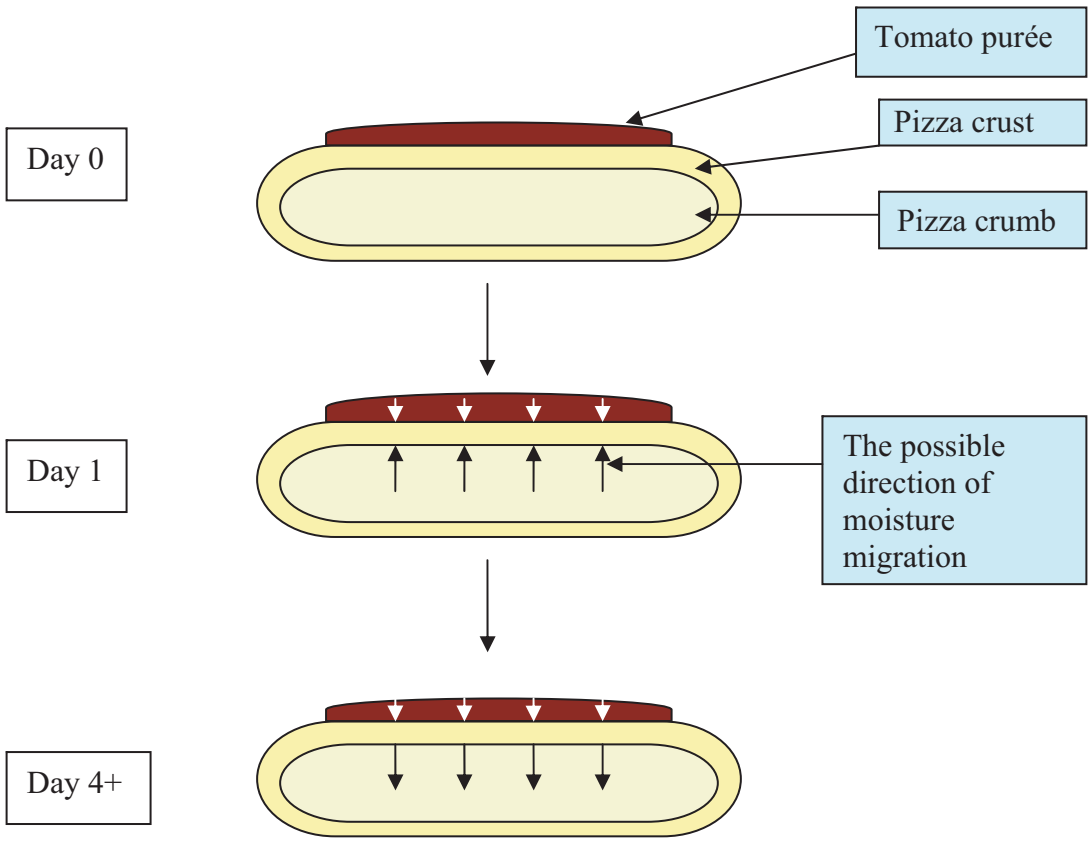



Figure 1: Illustration of moisture migration between the different parts of a pizza

As mentioned in previous sections, moisture plays an important role in the texture of foods. Bhatt and Nagaraju (2009) have shown that electrical impedance spectroscopy could be used to study the change of bread crust from a glassy to a rubbery state due to moisture migration from crumb to crust during storage. This method can be used to simultaneously determine the changes in resistance of the breadcrumb during storage due to staling. The moisture content of par-baked pizza increases over time. Thus this method can also be used to determine whether the par-baked pizza changed its state from rubbery to glassy and returned to rubbery state due to moisture migration that took place between the different parts of the pizza base.

Therefore, it can be hypothesised that par-baked pizza with a tomato purée topping changes its state from rubbery to glassy to rubbery.

The first change of state from rubbery to glassy may be due to starch retrogradation while the second change of state from glassy to rubbery may be due to moisture redistribution from the topping to the crumb.

### 3.1.3 Textural changes analysis

Firmness was measured using the compression test and the three-point bend test. The compression test used peak force at 25% compression to indicate the firmness of the par-baked pizza crumb (section 2.3.5.1, **Error! Reference source not found.**). The three-point bend test used stress and strain at breaking point to describe the changes of firmness during the storage period (sections 2.3.5.3 and 2.3.5.4, **Error! Reference source not found.** and **Error! Reference source not found.**).

The three-point bend test correlated well with the results obtained by the compression test. However, the differences in firmness of par-baked pizza with different additive treatments and storage time obtained by the compression test were clearer than the differences obtained by the three-point bend test. The samples for the three-point bend test differed in thickness because the crusts were not removed. The top crust was also irregular. This may also explain the standard deviations obtained using the three-point bend test. Bourne (2002) stated that it is difficult to map stress when the samples are not uniform in composition, structure or surface. Therefore, it was difficult to map stress in par- and re-baked pizza. The fracture behaviour of pizza is influenced by changes in temperature and moisture content (Bourne, 2002). There may have been moisture migration from the topping to the internal crumb, thus the firmness could have decreased because the crumb moved from a glassy state to a rubbery state. This transformation, from more brittle to more ductile texture, could also have made it difficult to map the stress and strain values, because moisture migration could have been different for every sample due to different pore structures. Therefore the pore-structured pizza base influenced the results obtained during the three-point bend test. When force is applied, the pores collapses and buckle and when further force is applied the sample tears. A

thick-based pizza does not bend. Therefore, the three-point bend test might not be suitable for thick-based pizza but rather for thin, crisp pizza.

The springiness measurements were also obtained using the compression test (section 2.3.5.2, **Error! Reference source not found.**) thereby giving further insight into the textural changes that occurred during storage of par-baked pizza. This is also another reason why the compression test is preferred above the three-point bend test.

The firmness and springiness measurements provided useful information on the textural changes of par-baked pizza that occurred during storage. However, the variability was greatly dependent on the experimental conditions and procedures used. Karim *et al*. (2000) have explained that it is difficult to compare texture profile analysis results from different authors due to non-standardisation of test procedures. In addition, the end of shelf life cannot be determined by instrumental methods alone.

Therefore, it is recommended that in order to determine the end of shelf life as an effect of the textural changes that occur during storage, the instrumental methods should be combined with sensory evaluation methods.

Angioloni and Collar (2009) have shown that a plural physical approach measuring the physical and mechanical properties of white and whole wheat bread samples give a better insight into consumer awareness than using only the instrumental method (compression test) to assess breadcrumb quality. This may also be applicable to the assessment of par-baked pizza quality. The plural physical approach consisted of various physical measurements. It included the following: static technique (texture profile analysis: firmness and springiness); dynamic deformation technique (innovative oscillatory test); image analysis; and sensory analysis and colour measurements. The results obtained by these analyses were correlated; it was observed that the different instrumental methods determining stress, firmness, hardness and chewiness were complementary and could be related to sensory attributes (Angioloni & Collar, 2009).

Another suggestion to improve on the textural study of par-baked pizza is to determine the crumb pore structure through image analysis.

It was observed that some sensory attributes of bakery products could also be influenced by the size and structure of the crumb cells (Angioloni & Collar, 2009; Lassoued *et al*., 2008). How the crumb feels by hand or in the mouth is mostly influenced by the size and structure of the crumb cells (Lassoued *et al*., 2008). Finer, thin-walled uniform-sized cells yielded a softer, more elastic texture than a coarse and thick-walled cell structure (Angioloni & Collar, 2009; Lassoued *et al*., 2008). These observations were made possible with image analysis. The following crumb cell structure can be derived from this test: mean cell area; cells/cm$^2$; cells to total area ratio; wall to total area ration; and crumb area to total cells ratio. The additives used in this study (DATEM and EC) may also produce par-baked pizza crumb with different cell structures. These additives have been shown to modify breadcrumb structure (Primo-Marín *et al*., 2008). Image analysis has been correlated with sensory attributes such as the fineness of the crumb cell structures (Lassoued *et al*., 2008). Lassoued *et al*. (2008) showed that image analysis gave an indication of the homogeneity of the cell distribution on the crumb texture evaluated by the panellist. Image analysis may also explain the differences in results on firmness and springiness obtained from the instrumental analysis in this study.

Starch retrogradation is thought to be the main reason for staling, thus differential scanning colourimetry (DSC) may indicate the onset of staling. However, starch retrogradation is not the only explanation for staling (Durán *et al*., 2001; Gray & Bemiller, 2003; Hallberg & Chinachoti, 2002; Katina *et al*., 2006; Miyazaki *et al*. 2004; Ribotta & Le Bail, 2007; Smith *et al*., 2004). There are two categorical methods used to study starch retrogradation (Karim *et al*., 2000). Macroscopic methods monitor the physical alterations that are manifested due to starch retrogradation, for example, textural and mechanical changes. Molecular methods monitor the changes in starch polymer conformation or water mobility in starch gels at molecular level (Karim *et al*, 2000). The following are examples of macroscopic methods applied: rheological techniques; sensory evaluation and differential scanning calorimetry (DSC); X-ray diffractometry, nuclear

magnetic resonance (NMR) spectroscopy and Fourier transform infrared spectroscopy are examples of molecular techniques (Karim *et al*., 2000). Hug-Iten *et al*. (2003) showed that the re-baked bread was softer than the aged bread but did not return to its original softness. Using DSC and X-ray diffraction methods, they found that re-crystallized amylose does not melt during the second baking stage due to the temperature being <150 ºC. However, the amylopectin did melt. In this study, the re-baked pizza was softer than the par-baked pizza in its original form.

It can be hypothesised that either the amylopectin content of the par-baked pizza was higher than the amylose content or the internal temperature was above 150 ºC in order to have produced re-baked pizza that was softer than par-baked pizza in its original form. This hypothesis could be determined by further analysis using DSC, X-ray diffraction and NMR methods.

The anti-staling effect of the additives (DATEM/EC) used in this study work on different mechanisms. It has been postulated by many authors that the anti-staling effect of DATEM is due to its ability to forming complexes with amylose. Lipase is responsible for the release of monoglycerides that is also able to form complexes with the helices of amylose. The ability of amylose to form double helices is retarded thereby reducing the initial firming of the baked product (Kamel & Ponte, 1993; Miyazaki *et al*., 2004; Ribotta & Le Bail, 2007; Stampfli & Nersten, 1995). Should this be the case, par-baked pizza with DATEM or lipase would have more amylose-lipid complexes than par-baked pizza without these additives. These formations have been studied using differential scanning calorimetry (DSC) that gave an endothermic peak at 90–120 ºC (Farahnaky & Majzoobi, 2008). It not only gives information on the amylose-lipid complexes that form, but is also able to indicate when starch retrogradation takes place with an endothermic peak at 45–65 ºC (Farahnaky & Majzoobi, 2008; Gray & Bemiller, 2003).

### 3.1.4 Microbiological analysis

MRS agar plates were incubated anaerobically and used to determine LAB. Bell, Penney and Moorhead (1997) determined that aerobic and anaerobic routine methods were compatible when analysing vacuum and carbon dioxide packed meat. It was difficult at times to count the colony-forming units because there were also spreading colonies. The pour plate technique also reduced plate over growth that can occur with spread plate techniques due to limited surface to grow on. Vanos and Cox explained (1986) that the commercial methods (MRS agar media) used were not selective enough for lactic acid bacteria because these bacteria are diverse with highly complex nutritional requirements. It was further observed that MRS allowed other gram-positive microbial growth (yeast and mould). Vanos and Cox (1986) suggested additional confirmation or the use of a more selective medium. However, this method was adequate to investigate the objectives of this study.

The petrifilm™ methods used to determine APC and yeast and mould count (Y&M) were easy and quick. De Sousa *et al*. (2005) found the conventional method for determining the APC and the petrifilm™ method compatible. However, under- estimation has been reported when lactic acid bacteria is part of the main microflora. It was postulated that it might be because the contents of the different media vary. Vlaemynck (1994) also compared a conventional method for determining Y&M with the petrifilm™ method and found it compatible. It was easy to distinguish the yeast colonies from the mould colonies on the petrifilm™. However, the mould would overgrow the yeast colonies making it difficult to count the yeast colonies beneath. On d12 visible mould growth was detected. No log cfu/g was determined for the air- packaged par-baked pizza because mould growth on the samples was not evenly distributed (**Error! Reference source not found.**). Yeast colonies also grew faster than mould colonies; by the end of the incubation period of 5 d yeast colonies were sometimes overgrown. Therefore, the yeast colonies were counted on d3 and d5. These methods served the purpose of determining the shelf life of par-baked pizza. These methods also showed that none of the

microorganisms tested for reached the level regarded as that indicating the end of shelf life within the testing period of 16 d (section 2.3.6, Figures 11, 12 and 13).

### 3.1.5 Determining the shelf life of par-baked pizza

Textural analysis showed the textural changes that occurred during the storage period of 16 d. It was not possible to tell when par-baked pizza became undesirable to the consumer in terms of its textural attributes. Although the re-baked pizza was even softer in texture than the initial par-baked pizza, it was also not possible to tell if the consumer preferred it to the par-baked pizza.

Finally, to complete the shelf-life study, it is hypothesised that sensory evaluation is required in order to determine when par-baked pizza would become undesirable to the consumer in terms of texture.

A consumer sensory test can be used to determine the sensory shelf life. The sensory shelf life of a food product is dependent on the interaction between a food product and the consumer. Giménez *et al*. (2007) showed that survival analysis gave good results for the cut-off point of shelf life. Survival analysis is based on consumer rejection.

This study showed that the microbial growth, in this instance mould growth, is the main factor determining the end of shelf life of par-baked pizza. It showed that visible mould growth limited the shelf life of par-baked pizza + A to 12 d. Although the MAP samples did not reach the end of shelf life in 16 d due to visible mould growth, it is not clear what the limiting factor would be beyond 16 d. Therefore an extended storage period is required to determine what the shelf-life limiting factor would be.

Therefore, it can be hypothesised that the shelf-life limiting factor of the MAP par-baked pizza would be lactic acid bacterial growth and/or yeast growth because these microorganisms are not affected by MAP and reduced oxygen levels inside the packaging headspace.

Lactic acid bacteria and yeast produce metabolic products that would render the par-baked pizza unacceptable to the consumer. For example, yeast may cause spoilage of par-baked pizza packaged in MAP by visible pigmentation or through producing fermentation by-products such as alcohol which leads to the formation of an alcoholic odour (Legan & Voysey, 1991; Smith *et al*., 2004). Par-baked pizza shelf life may also be reduced by product acidification caused by lactic acid bacteria (Cabo *et al*., 2001).

# 4: GENERAL CONCLUSIONS

It can be concluded that modified atmosphere packaging (100% $CO_2$) increase the shelf life of par-baked pizza microbiologically as well as physically by reducing the staling rate. It is assumed that with the addition of an oxygen absorber the shelf life of par-baked pizza is even further increased due to the low microbial levels.

The shelf life determining factor of par-baked pizza + A is visible mould growth, thus the shelf life of air packaged par-baked pizza is limited to 12 d.

The additive treatments, DATEM and EC, had beneficial effects on the firmness and thickness of par-baked pizza. The shelf life of par-baked pizza is increased with the addition of DATEM or the EC treatment, because these additives reduce the rate of firming. However staling is not the shelf life limiting factor of par-baked pizza because the re-baked pizza was even softer than the original form. Thus, the effects of these additives on the textural properties of par-baked pizza would be more evident if further analysis were done, for example, by image analysis or sensory evaluation.

Therefore, based on the parameters issued in this study, it can be concluded that DATEM can be effectively replaced with an enzyme-combination treatment (lipase and maltogenic α-amylase). However, further research is needed to determine if par-baked pizza with an enzyme-combination treatment would produce similar sensorial attributes, like mouthfeel and crumb cell structures, as par-baked pizza with DATEM.

# 5: REFERENCES

(AACC) see American Association of Cereal Chemists.

American Association of Cereal Chemists (AACC). 2000a. Approved Methods of the American Association of Cereal Chemists, Method:74–09, AACC, St Paul, MN.

AACC. 2000b. Approved Methods of the American Association of Cereal Chemists, Method:44-01, AACC, St Paul, MN.

ABELLANA, M., SANCHIS, V. & RAMOS, A.J. 2001. Effect of Water Activity and Temperature on Growth of Three *Penicillium* Species and *Aspergillus flavus* on a Sponge Cake Analogue, *International Journal of Food Microbiology*, (71):151-157.

ANGIOLONI, A. & COLLAR, C. 2009. Bread Crumb Quality Assessment: a Plural Physical Approach, *European Food Research & Technology*, (229):21-30.

AOAC see Association of Official Analytical Chemists.

AOAC Official Methods of Analysis. 2000. Microbiological Methods, Association of Official Analytical Chemists (AOAC) International, 17th Ed, Gaithersburg, MD, Method:990.12.

APPENDINI, P. & HOTCHKISS, J.H. 2002. Review of Antimicrobial Food Packaging, *Innovative Food Science & Emerging Technologies*, (3):113-126.

ARMERO, E. & COLLAR, C. 1998. Crumb Firming Kinetics of Wheat Breads with Anti-staling Additives, *Journal of Cereal Science*, (28):165-174.

AVITAL, Y., MANNHEIM, C.H. & MILTZ, J. 1990. Effect of Carbon Dioxide Atmosphere on Staling and Water Relation in Bread, *Journal of Food Science*, (55), 2:413-416.

BAILEY, C.P. & VON HOLY, A. 1993. *Bacillus* Spore Contamination Associated with Commercial Bread Manufacture, *Food Microbiology*, (10):287-294.

BÁRCENAS, M.E. & ROSELL, C.M. 2007. Different Approaches for Increasing the Shelf Life of Partially Baked Bread: Low Temperatures and Hydrocolloid Addition, *Food Chemistry*, (100):1594-1601.

BARRETT, A., CARDELLO, A., MAGUIRE, P. & PELEG, M. 2005. Moisture Redistribution and Textural Changes Stored Model Sandwiches, *Journal of Texture Studies*, (36), 569-588.

BELL, R.G., PENNEY, N. & MOORHEAD, S.M. 1997. Comparison of Aerobic and Anaerobic Methods for the Microbiological Monitoring of Chilled Packaged Meat During Storage, *Letters in Applied Microbiology*, (24):265-268.

BENT, A.J. 2007. Part Baked Breads In: Cauvain, S.P., Young, L.S., (Eds), *Technology of Breadmaking*, 2nd ed, Springer, New York, 255-261.

BEUCHAT, L.R., NAIL, B.V., BRACKETT, R.E. & FOX, T.L. 1991. Comparison of the Petrifilm™ Yeast and Mould Culture Film Method to Conventional Methods for Enumerating Yeast and Molds in Foods, *Journal of Food Protection*, (54), 6:443-447.

BHATT, C.M. & NAGARAJU, J. 2009. Studies on Glass Transition and Starch Re-crystallization in Wheat Bread During Staling Using Electrical Impedance Spectroscopy, *Innovative Food Science & Emerging Technologies*, (10), 241-245.

BLANSHARD, J.M.V. 1986. The Significance of the Structure and Function of the Starch Granule in Baked products In: Blanshard, J.M.V., Frazier, P.J., Galliard, T., (Eds), *Chemistry & Physics of Baking*, The Royal Society of Chemistry, London, 1-13.

BOURNE, M.C. 2002. Physics and Texture, In: Bourne M.C, *Food Texture & Viscosity: Concept & Measurements*, 2nd Ed, 62-65, 101-102.

BROWN, J. 1993. Advances in Breadmaking Technology In: Kamel, B.S., Strauffer, C.E., (Eds), *Advances in Baking Technology* , Blackie Academic & Professional, New York, 38-87.

CABALLERO, P.A., GOMEZ, M. & ROSELL, C.M. 2007. Improvement of Dough Rheology, Bread Quality and Bread Shelf-life by Enzymes, *Journal of Food Engineering*, (81):42-53.

CABO, M.L., PASTORIZA, L., BERNÁRDEZ, M. & HERRERA, J.J. 2001. Effectiveness of $CO_2$ and Nisaplin on Increasing Shelf-life of Fresh Pizza, *Food Microbiology*, (18):489-498.

CARR, L.G. & TADINI, C.C. 2003. Influence of Yeast and Vegetable Shortening on Physical and Textural Parameters of Frozen Part Baked French Bread, *Swiss Society of Food Science & Technology,* (36):609-614.

CARSON, L. & SUN, X. 2001. Creep-Recovery of Bread and Correlation to Sensory Measurements of Textural Attributes, *American Association of Cereal Chemists*, (78):1, 101-104.

CASTELLO, P., BARET, J.L., POTUS, J. & NICOLAS, J. 1999. Technological and Biochemical effects of Exogenous Lipase in Breadmaking, *2nd European Symposium on Enzymes in Grain Processing,* ESEPC 2, Helsinki, Finland, 193-199.

CHIEH, C. 2006. Water In: Hui, Y.H., Corke, H., De Leyn, I., Nip, W., Cross, N., (Eds), *Bakery Products Science & Technology*, Blackwell Publishing, U.S.A., 211-231.

CHIRIFE, J. & BUERA, M.P. 1995. A Critical Review of Some Non-equilibrium Situations and Glass Transitions on Water Activity Values of Foods in the Microbiological Growth Range, *Journal of Food Engineering,* (25):531-552.

CHURCH, I.J. & PARSONS, A.L. 1995. Modified Atmosphere Packaging Technology: A Review, *Journal of the Science of Food and Agriculture*, (67), 2:143-152.

COPPOLA, S., PEPE, O. & MAURIELLO, G. 1998. Effect of Leavening Microflora on Pizza Dough Properties, *Journal of Applied Microbiology*, (85):891-897.

CORRELL, J. 2002. Encyclopizza, Correll Consulting. Available on the Internet at:http://www.correlllconcepts.com (accessed on 30 October 2008).

DE DELAHAYE, E.P., JIMENEZ, P. & PEREZ, E. 2005. Effect of Enrichment with High Content Dietary Fiber Stabilized Rice Bran Flour on Chemical and Functional Properties of Storage Frozen Pizzas, *Journal of Food Engineering*, (68):1-7.

DE LEYN, I. 2006. Functional Additives, In: Hui, Y.H., Corke, H., De Leyn, I., Nip, W., Cross, N., (Eds), *Bakery Products Science & Technology*, Blackwell Publishing, U.S.A., 233-243.

DE MAN, J.C., ROGOSA, M. & SHARP, M.E. 1960. A Medium for the Cultivation of *Lactobacilli*, *Journal of Applied Bacteriology*, (23):130-135.

DE SOUSA, G.B., TAMAGNINI, L.M., GONZÁLEZ, R.D. & BUDDE, C.E. 2005. Evaluation of Petrifilm™ Method for Enumerating Aerobic Bacteria in Crottin Goat's Cheese, *Revista Argentina de Microbiologia*, (37):214-216.

DURÁN, E., LEÓN, A.E., BARBER, B. & BENEDITO DE BARBER, C. 2001. Effect of Low Molecular Weight Dextrins on Gelatinization and Retrogradation of Starch, *European Food Research & Technology*, (212):203-207.

FARAHNAKY, A. & MAJZOOBI, M., 2008. Physicochemical Properties of Partbaked Breads, *International Journal of Food Properties*, (11):186-195.

FESSAS, D. & SCHIRALDI, A. 2005. Water properties in Wheat Flour Dough II: Classical and Knudsen Thermogravimetry Approach, *Food Chemistry*, (90):61-68.

FERNANDEZ, U., VODOVOTZ, Y. & COURTNEY, P., PASCALL. 2006. Extended Shelf life of Soy Bread Using Modified Atmosphere Packaging, *Journal of Food Protection*, (69), 3:693-698.

FIK, M. & SURÓWKA, K. 2002. Effect of Prebaking and Frozen Storage on the Sensory Quality and Instrumental Texture of Bread, *Journal of Food Science & Agriculture,* (82):1268-1275.

FLEET, G.H. 2007. Yeast in Foods and Beverages: Impact on Product Quality and Safety, *Current Opinion in Biotechnology*, (18):170-175.

FONTANA, A.J. 1998. Water Activity: Why it is Important for Food Safety, *NSF International Conference on Food Safety*, 177-185.

FORMATO, A. & PEPE, O. 2005. Pizza Dough Differentiation by Principal Component Analysis of Alveographic, Microbiological and Chemical Parameters, *Cereal Chemistry*, (82), 4:356-360.

GIMÉNEZ, A., VARELA, P., SALVADOR, A., ARES, G., FISHZMAN, S. & GARITTA, L. 2007. Shelf Life Estimation of Brown Pan Bread: A Consumer Approach, *Food Quality & Preference*, (18):196-204.

GOESAERT, H., SLADE, L., LEVINE, H. & DELCOUR, J.A. 2009. Amylase and Bread Firming – An Integrated View, *Journal of Cereal Science*, in press, 1-8.

GRAM, L., RAVN, L., RASCH, M., BRUHN, J.B., CHRISTENSEN, A.B. & GIVSKOV, M. 2002. Food Spoilage-Interactions between Food Spoilage Bacteria, *International Journal of Food Microbiology*, (78):79-97.

GRAY, J.A. & BEMILLER, J.N. 2003. Bread Staling: Molecular Basis and Control, *Comprehensive Reviews in Food Science & Food Safety*, (2):1-21.

GUY, R.C.E. & SAHI, S. 2006. Application of a Lipase in Cake Manufacture, *Journal of the Science of Food & Agriculture*, (86):1679-1687.

GUYNOT, M.E., MARÍN, S., SANCHIS, V. & RAMOS, A.J. 2003a. Modified Atmosphere Packaging for Prevention of Mould Spoilage of Bakery Products with Different pH and Water Activity Levels, *Journal of Food Protection*, (66), 10:1864-1872.

GUYNOT, M.E., SANCHIS, V., RAMOS, A.J. & MARIN, S. 2003b. Mould-free Shelf Life Extension of Bakery Products by Active Packaging, *Journal of Food Science*, (68), 8:2547-2552.

HALLEBERG, L.M. & CHINACHOTI, P. 2002. A Fresh Perspective on Staling: The Significance of Starch Recrystallization on the Firming of Bread, *Journal of Food Science,* (67), 2:1092-1096.

HE, H. & HOSENEY, R.C. 1990. Changes in Bread Firmness and Moisture during Long-Term Storage, *American Association of Cereal Chemist*, (67), 6:603-605.

HIGUCHI, M., YAMAMOTO, Y. & KAMIO, Y. 2000. Molecular Biology of Oxygen Tolerance in Lactic Acid Bacteria: Functions of NADH Oxidase and Dpr in Oxidative Stress, *Journal of Bioscience & Bioengineering*, (90), 5:481-493.

HOSENEY, R.C. 1986. Component Interaction during Heating and Storage of Baked Products In: Blanshard, J.M.V., Frazier, P.J., Galliard, T., (Eds), *Chemistry & Physics of Baking*, The Royal Society of Chemistry, London, 216-226.

HUG-ITEN, S., HANDSCHIN, S., CONDE-PETIT, B. & ESCHER, F. 1999. Changes in Starch Microstructure on Baking and Staling of Wheat Bread, *Swiss Society of Food Science & Technology*, (32):255-260.

HUG-ITEN, S., ESCHER, F. & CONDE-PETIT, B. 2003. Staling of Bread: Role of Amylose and Amylopectin and Influence of Starch-Degrading Enzymes, *Cereal Chemistry*, (80), 6:654-661.

HUIS IN'T VELD, J.H.J. 1996. Microbial and Biochemical Spoilage of Foods: an Overview, *International Journal of Food Microbiology*, (33):1-18.

JONES, A., LAMSA, M., FRANDSEN, T.P., SPENDLER, T., HARRIS, P., SLOMA, A., XU, F., NIELSEN, J.B. & CHERRY, J.R. 2008. Directed Evolution of a Maltogenic α-Amylase from *Bacillus* spp. TS-25, *Journal of Biotechnology*, (134):325-333.

KAMEL, B.S. & PONTE, J.G. 1993. Emulsifiers in Baking. In: Kamel B.S., Stauffer, C.E., (Eds.), *Advances in Baking Technology*, Blackie Academic & Professional, London, 180-215.

KARATHANOS, V.T., KOSTAROPOULOS, A.E. & SARAVACOS, G.D. 1995. Diffusion and Equilibrium of Water in Dough/Raisin Mixtures, *Journal of Food Engineering*, (25):113-121.

KARIM, A.A., NORZIAH, M.H. & SEOW, C.C. 2000. Methods for the Study of Starch Retrogradation, *Food Chemistry*, (71):9-36.

KATINA, K., SALMENKALLIO-MARTTILA, M., PARTANEN, R., FORSSELL, P. & AUTIO, K. 2006. Effects of Sourdough and Enzymes on Staling of High-fiber Wheat Bread, *Swiss Society of Food Science & Technology*, (39):479-491.

KESTIN, S.O., SUMNU, G. & SAHIN, S. 2004. Usage of Enzymes in a Novel Baking Process, *Die Nahrung*, (48), 2:156-160.

KÖHLER, P. 2001. Study of the Effect of DATEM. 3: Synthesis and Characterization of DATEM Components, *Swiss Society of Food Science & Technology*, (34):359-366.

KNORR, D. & TOMLINS, R.I. 1985. Effect of Carbon Dioxide Modified Atmosphere on the Compressibility of Stored Baked Goods, *Journal of Food Science*, (50):1172-1173.

KOKELAAR, GARRITSEN, J.A. & PRINS, A. 1995. Surface Rheological Properties of Sodium Stearoyl-2-lactylate (SSL) and Diacetyl Tartaric Esters of Mono (and di) Glyceride (DATEM) Surfactants after a Mechanical Surface Treatment in Relation to Their Bread Improving Abilities, *Colloids & Surfaces A: Physicochemical & Engineering Aspects*, (95):69-77.

KOTSIANIS, I.S., GIANNOU, V. & TZIA, C. 2002. Production and Packaging of Bakery Products using MAP technology, *Trends in Food Science & Technology*, (13):319-324.

LABUZA, T.P., FU, B. & TAOUKIS, P.S. 1992. Prediction for the Shelf Life and Safety of Minimally Processed CAP/MAP Chilled Foods: A Review, *Journal of Food Protection*, (55),9:741-750.

LACEY, J. 1989. Pre- and Post-harvest Ecology of Fungi Causing Spoilage of Foods and Other Stored Products, *Journal of Applied Bacteriology Symposium Supplement*, (18):11S-25S.

LAINEZ, E., VERGARA, F. & BÁRCENAS, M.E. 2008. Quality and Microbial Safety of Partially Baked Bread during Refrigerated Storage, *Journal of Food Engineering*, (89):414-418.

LARSEN, D.M., SETSER, C.S. & FAUBION, J.M. 1993. Effects of Flour Type and Dough Retardation Time on the Sensory Characteristics of Pizza Crust, *Cereal Chemistry*, (70), 6:647-650.

LASSOUED, N., DELARUE, J., LAUNAY, B. & MICHON, C. 2008. Baked Product Texture: Correlations Between Instrumental and Sensory Characterization Using Flash Profile, *Journal of Cereal Science*, (48):133-143.

LEGAN, J.D. & VOYSEY, P.A. 1991. A Review, Yeast Spoilage of Bakery Products and Ingredients, *Journal of Applied Bacteriology*, (70):361-371.

.LEÓN, A.E., DURÁN, E. & BENEDITO DE BARBER, C. 1997. Firming of Starch Gels and Amylopectin Retrogradation as Related to Dextrin Production by α-Amylase, *Z Lebensmittel Unters Forsch* A, (205):131-134.

LEÓN, A.E, DURÁN, E. & BENEDITO DE BARBER, C. 2002. Utilization of Enzyme Mixtures to Retard Bread Crumb Firming, *Journal of Agriculture & Food Chemistry*, (50):1416-1419

LEUSCHNER, R.G.K., O'CALLAGHAN, M.J.A. & ARENDT, E.K. 1999. Moisture Distribution and Microbial Quality of Part Baked Bread as Related to Storage and Rebaking Conditions, *Journal of Food Science*, (64), 3:543-546.

LI, Y., KLOEPPEL, M. & HSIEH, F. 1998. Texture of Glassy Corn Cakes as a Function of Moisture Content, *Journal of Food Science,* (63), 5:1-4.

LIBEROPOULOS, G. & TSAROUHAS, P. 2005. Reliability Analysis of an Automated Pizza Production Line, *Journal of Food Engineering*, (69):79-96.

LODI, A., ABDULJALIL, A.M. & VODOVOTZ, Y. 2007. Characterization of Water Distribution in Bread During Storage Using Magnetic Resonance Imaging, *Magnetic Resonance Imaging*, (25):1449-1458.

MANI, K., TRÄGÅRDH, C. & ELIASSON, A.-C. 1992. Water Content, Water Soluble Fraction, and Mixing Affect Fundamental Rheological Properties of Wheat Flour Doughs, *Journal of Food Science*, (57), 5:1198-1200.

MANDALA, I., KARABELA, D. & KOSTAROPOULOS, A. 2007. Physical Properties of Breads Containing Hydrocolloids Stored at low Temperatures. I. Effect of Chilling, *Food Hydrocolloids*, (21), 8:1397-1406.

MILLER, M.W. 1979: Yeast in Food Spoilage: an Update, *Food Technology*, (33):76-80.

MIYAZAKI, M., MAEDA, T. & MORITA, N. 2004. Effect of Various Dextrin Substitutions for Wheat Flour on Dough Properties and Bread Qualities, *Food Research International*, (37):59-65.

NIELSEN, P.V. & RIOS, R. 2000: Inhibition of Fungal Growth on Bread by Volatile Components From Spices and Herbs and the Possible application in Active Packaging, with Special Emphasis on Mustard Essential Oil, *International Journal of Food Microbiology*, (60):219-229.

PATERAS, I.M.C. 1998. Bread Spoilage and Staling, In: Cauvain, S.P., Young, L.S., (Eds), *Technology of Breadmaking*, Springer, New York, 240-254.

PIAZZA, L. & MASI, P. 1995. Moisture Redistribution Throughout the Bread Loaf During Staling and its Effect on Mechanical Properties, *Cereal Chemistry,* (72), 3:320-325.

PINHO, B.H. & FURLONG, E.B. 2000. The Occurrence of Mould, Yeasts and Mycotoxins in Pre-cooked Pizza Dough Sold in Southern Rio Grande Do Sul, *Brazilian Journal of Microbiology*, (31):99-102.

PIZZA Crust Production. 1996. *Lallemand Baking Update*, Canada, (2), 1.

POWERS, E.M. & BERKOWITZ, D. 1990. Efficacy of an Oxygen Scavenger to Modify the Atmosphere and Prevent Mould Growth on Meal, Ready-to-Eat Pouched Bread, *Journal of Food Protection*, (53), 9:767-771.

PRIMO-MARTÍN, C., HAMER, R.J & DE JONGH, H.H.J. 2006. Surface Layer Properties of Dough Liquor Components: Are Key Parameters in Gas Retention in Bread Dough? *Food Biophysics*, (1), 2:1-22.

PRIMO-MARTÍN, C., DE BEUKELAER, H., HAMER, R.J. & VAN VLIET, T. 2008. Fracture Behaviour of Bread Crust: Effect of Ingredient Modification, *Journal of Cereal Science*, (48):604-612.

RIBOTTA, P.D. & LE BAIL, A. 2007. Thermo-physical Assessment of Bread during Staling, *Swiss Society of Food Science & Technology*, (40):879-884.

RIBOTTA, P.D., PÉREZ, G.T., LEÓN, A.E. & AÑÓN, M.C. 2004. Effect of Emulsifier and Guar Gum on Micro Structural, Rheological and Baking performance of Frozen Bread dough, *Food Hydrocolloids,* (18):305-313.

RODRIGUEZ, V., MEDINA, L. & JORDANO, R. 2000. Effect of Modified Atmosphere Packaging on the Shelf Life of Sliced Wheat Bread, *Die Nahrung*, (44), 4:247-252.

RODRIGUEZ, V., MEDINA, L. & RAFAEL, J. 2003. Influence Modified Atmosphere Packaging on the Shelf Life of Pre-baked Pizza Dough with and without Preservatives Added, *Die Nahrung*, (47), 2: 122-125.

ROJAS, J.A., ROSELL, C.M. & BENEDITO DE BARBER, C. 2001. Role of Maltodextrins in the Staling of Starch Gels, *European Food Research & Technology*, (212):364-368.

SAHLSRÖM, S. & BRÅTHEN, E. 1997. Effects of Enzyme Preparations for Baking, Mixing Time and Resting time on Bread Quality and Bread Staling, *Food Chemistry*, (58), 1-2:75-80.

SELOMULYO, V.O. & ZHOU, W. 2007. Frozen Bread Dough: Effects of Freezing Storage and Dough Improvers, *Journal of Cereal Science,* (45):1-17.

SI, J. 1997. Synergistic Effect of Enzymes for Breadbaking, *Cereal Food World*, (42), 10:802-807.

SI, J.Q. & LUSTENBERGER, Q. 2001. Novamyl® – A true Anti-staling Enzyme, *Cereal Food*, Novozymes, 1699-01.pdf, 1-20.

SIDHU, J.S., AL-SAQER, J. & AL-ZENKI, S. 1997. Comparison Methods for the Assessment of the Extend of Staling in Bread, *Food Chemistry*, (58), 1-2:161-167.

SINGH, H., ROCKALL, A., MARTIN, C.R., CHUNG, O.K. & LOOKHART, G.L. 2006. The Analysis of Stress Relaxation Data of Some Viscoelastic Foods using a Texture Analyzer, *Journal of Texture Studies*, (37):383-392.

SISWOYO, T.A., TANAKA, N. & MORITA, N. 1999. Effect of Lipase Combined with α-Amylase on Retrogradation of Bread, *Food Science Technology Research*, (5), 4:356-361.

SLUIMER, P. 2005. Functionality of Raw Materials and Processing Steps, Piet Sliumer (Ed) In: *Principles of Breadmaking*, American Association of Cereal Chemist.

SMITH, J.P., OORALKUL, B., KOERSEN, W.J., JACKSON, E.D. & LAWRENCE, R.A. 1986. Novel Approach to Oxygen Control in Modified Atmosphere Packaging of Bakery Products, *Food Microbiology*, (3):315-320.

SMITH, J.P., DAIFAS, D.P., EL-KHOURY, W., KOUKOUTSIS, J. & EL-KHOURY, A. 2004. Shelf Life and Safety Concerns of Bakery Products – A Review, *Critical Review in Food Science & Nutrition*, (44):4419-4455.

STAMPFLI, L. & NERSTEN, B. 1995. Emulsifiers in Bread Making, *Food Chemistry*, (52):353-360.

SULTAN, W. J. 1990. Basic Ingredients, and Natural Bakery Products and Pizza, In Sultan W.J., (Ed), *Practical Baking,* 5th Ed, Van Nostrand Reinhold, New York, 15-48 & 774-777.

THERMOFORMING Packaging for Pizza, Ulma Packaging, Barrio Garibai. Available on the Internet at: www.ulmapackaging.com (accessed on 26/06/2009).

THORMAR, H. & HILMARSSON, H. 2007. The role of Microbicidal Lipids in Host Defence Against Pathogens and Their Potential as Therapeutic Agents, *Chemistry & Physics of Lipids*, (150):1-11.

TOUFEILI, I., SHADAREVIAN, S., MISKI., A.M.A. & HANI, I. 1995. Effect of Shortening and Surfactants on Selected Chemical/Physicochemical Parameters and Sensory Quality of Arabic Bread, *Food Chemistry,* (53):253-258.

VANOS, V. & COX, L. 1986. Rapid Routine Method for the Detection of Lactic Acid Bacteria among Competitive Flora, *Food Microbiology*, (3):223-234.

VLAEMUNCK, G.M. 1994. Comparison of Petrifilm™ and Plate Count Methods for Enumerating Molds and Yeasts in Cheese and Yoghurt, *Journal of Food Protection*, (57), 10:913-914.

VULICEVIC, I.R., ABADEL-AAL, E-S.M., MITTAL, G.S. & LU, X. 2004. Quality and Storage Life of Par-baked Frozen Breads, *Swiss Society of Foods Science & Technology*, (37):205-213.

WAI LEE, T.S., SCHALLER, J., ANDERSON, S.N., MAZER, T.B., HILTY, M.D., GUZMAN-HARTY, M., MEULBROEK, J.A., EWING, P.J. & LUI, J. 1999. Water-miscible Esters of Mono-and-Diglycerides Having Antibacterial Activity and Their use in Inhibiting Infection, *United States Patent*, 5,908,862.

WANG, X., CHOI, S. & KERR, W.L. 2004. Water Dynamics in White Bread and Starch Gels as Affected by Water and Gluten Content, *Swiss Society of Foods Science and Technology*, (37), 3:377-384.

WILLIAMS, T. & PULLEN, G. 2007. Funtional Ingredients In: Cauvain, S.P., Young, L.S., (Eds), *Technology of Breadmaking*, 2nd ed, Springer, New York, 51-92.

YING, B., JUN, L., YUNZI, F., ZHUO, C. & ZAIGUI, L. 2008. Application of DATEM for Chinese Steamed Bread Made from Flours of Different Gluten Qualities, *Journal of Science Food & Agriculture*, (89):227-231.

The aerobic plate count for par-baked pizza packaged in air, MAP and MAP +OA, stored at 10 ºC for 16 d

| Additive | Days | Packaging treatment | | | Main effect of additive[d] |
|---|---|---|---|---|---|
| | | AIR | MAP | MAP + OA | |
| No additives (C) | 0 | 0.85 (± 0.21) | 0.85 (± 0.21) | 0.85 (± 0.21) | 1.53[a] |
| | 4 | 1.65 (± 1.35) | 0.70(± 0.00) | 1.24(± 0.09) | |
| | 8 | 1.96(± 0.11) | 1.42(± 1.01) | 1.09(± 0.55) | |
| | 12 | 2.78(± 0.53) | 2.02 (± 0.42) | 0.35(± 0.49) | |
| | 16 | 3.52(± 0.05) | 2.25(± 0.09) | 1.35(±1.91) | |
| DATEM | 0 | 1.17(± 0.66) | 1.17(± 0.66) | 1.17(± 0.66) | 1.40[a] |
| | 4 | 2.0(± 0.31) | 0.00 | 0.59(± 0.71 | |
| | 8 | 0.70(± 1.0) | 0.35(± 0.49) | 0.70(± 0.00) | |
| | 12 | 1.92(± 0.25) | 1.82(± 0.59) | 1.44(± 0.63) | |
| | 16 | 2.91(± 1.60) | 3.30(± 0.69) | 1.88(± 1.67) | |
| Enzyme combination (EC) | 0 | 1.65(± 0.49) | 1.65(± 0.49) | 1.65(± 0.49) | 1.14[a] |
| | 4 | 0.59± 0.83) | 1.00(± 0.43) | 0.94(± 0.34) | |
| | 8 | 1.41(± 2.0) | 0.50(± 0.71) | 0.70(± 0.00) | |
| | 12 | 0.00 | 2.36(± 0.78) | 0.00 | |
| | 16 | 2.1(± 1.13) | 0.65(± 0.92) | 1.29(± 1.82) | |
| Main effect of packaging[e] | | 1.74[b] | 1.34[ab] | 1.01[a] | |
| Main effect of time | | | | | |
| Days | 0 | 4 | 8 | 12 | 16 |
| Main effect [e] | 1.22[a] | 0.96[a] | 0.98[a] | 1.49[a] | 2.14[b] |

[d] Mean values with different letters in same column differ significantly from each other ($p<0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p<0.05$)

The lactic acid bacterial count for par-baked pizza packaged in air, MAP and MAP +OA, stored at 10 ºC for 16 d

| Additive | Days | Packaging treatment | | | Main effect of additived |
|---|---|---|---|---|---|
| | | AIR | MAP | MAP + OA | |
| No additives (C) | 0 | 1.74 (± 0.12) | 1.74 (± 0.12) | 1.74 (± 0.12) | 2.41[a] |
| | 4 | 0.90 (± 1.27) | 2.02 (± 0.40) | 1.5 (± 0.28) | |
| | 8 | 2.75 (± 0.92) | 2.02 (± 0.40) | 2.96 (± 0.11) | |
| | 12 | 2.78 | 2.22 (± 0.95) | 2.76 (± 0.29) | |
| | 16 | 4.17 (± 0.02) | 3.59 (± 0.08) | 2.96 (± 0.53) | |
| DATEM | 0 | 1.83 (± 0.13) | 1.83 (± 0.13) | 1.83 (± 0.13) | 2.42[a] |
| | 4 | 1.59 (± 0.27) | 1.98 (± 0.44) | 1.94 (± 0.77) | |
| | 8 | 2.04 (± 0.16) | 2.36 (± 0.10) | 2.79 (± 0.44) | |
| | 12 | 3.31 | 2.27 (± 0.40) | 2.00 (± 0.74) | |
| | 16 | 3.96 (± 0.24) | 4.15 (± 0.08) | 2.91 (± 0.93) | |
| Enzyme combination (EC) | 0 | 1.72 (± 0.10) | 1.72 (± 0.10) | 1.72 (± 0.10) | 2.09[a] |
| | 4 | 1.58 (± 0.14) | 1.83 (± 1.60) | 1.30 (± 1.82) | |
| | 8 | 3.25 (± 0.10) | 2.32 (± 0.30) | 3.00 (± 0.15) | |
| | 12 | 0.94 (± 0.34) | 1.09 (± 1.55) | 1.98 (± 0.09) | |
| | 16 | 2.94 (± 1.22) | 2.94 (± 1.13) | 3.00 (± 0.51) | |
| Main effect of packaging[e] | | 1.53[a] | 1.40[a] | 1.14[a] | |
| Main effect of time | | | | | |
| Days | 0 | 4 | 8 | 12 | 16 |
| Main effect[e] | 1.77[ab] | 1.63[a] | 2.61[c] | 2.11[b] | 3.40[d] |

[d] Mean values with different letters in same column differ significantly from each other ($p < 0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p < 0.05$)

The yeast count for par-baked pizza packaged in air, MAP and MAP +OA, stored at 10 ºC for 16 d

| Additive | Days | Packaging treatment | | | Main effect of additive[d] |
|---|---|---|---|---|---|
| | | AIR | MAP | MAP + OA | |
| No additives (C) | 0 | 1.0 (± 0.43) | 1.0 (± 0.43) | 1.0 (± 0.43) | 2.07[b] |
| | 4 | 1.39 (± 0.13) | 1.42 (± 1.02) | 1.54 (± 0.51) | |
| | 8 | 2.76 (± 1.19) | 2.39 (± 0.16) | 2.12 (± 1.33) | |
| | 12 | 3.8 | 1.56 (± 0.11) | 3.00 (± 0.48) | |
| | 16 | 2.58 (± 1.13) | 2.92 (± 0.44) | 3.50 (± 0.41) | |
| DATEM | 0 | 1.78 (± 0.25) | 1.78 (± 0.25) | 1.78 (± 0.25) | 1.91[b] |
| | 4 | 1.35 (± 0.07) | 1.39 (± 0.55) | 1.42 (± 1.01) | |
| | 8 | 1.78 (± 0.00) | 1.58 (± 0.40) | 1.26 (± 0.79) | |
| | 12 | 3.20 | 1.53 (± 0.18) | 1.94 (± 0.13) | |
| | 16 | 2.79 (± 1.33) | 3.20 (± 0.39) | 2.47 (± 0.73) | |
| Enzyme combination (EC) | 0 | 0.85 (± 0.21) | 0.85 (± 0.21) | 0.85 (± 0.21) | 1.11[a] |
| | 4 | 0.50 (± 0.71) | 1.59 (± 0.27) | 0.50 (± 0.71) | |
| | 8 | 1.10 (± 0.55) | 0.70 (± 0.99) | 1.10 (± 0.13) | |
| | 12 | 0.00 | 0.85 (± 0.21) | 0.80 (± 1.13) | |
| | 16 | 3.30 (± 1.01) | 1.11 (± 1.56) | 2.10 (± 0.63) | |
| Main effect of packaging[e] | | 1.82[a] | 1.59[a] | 1.69[a] | |
| Main effect of time | | | | | |
| Days | 0 | 4 | 8 | 12 | 16 |
| Main effect[e] | 1.21[a] | 1.23[a] | 1.64[ab] | 1.76[b] | 2.66[c] |

[d] Mean values with different letters in same column differ significantly from each other ($p < 0.05$)
[e] Mean values with different letters in same row differ significantly from each other ($p < 0.05$)

# APPENDIX D

The average gas composition (%) of the head space of the par-baked pizza packaged in air, 100 % $CO_2$ modified atmosphere (MAP) with and without an oxygen absorber (MAP + OA), during the storage period of 16 d at 10ºC

| **Packaging** | **Air** | **MAP** | | **MAP + OA** | |
|---|---|---|---|---|---|
| **Days** | **$O_2$** | **$O_2$** | **$CO_2$** | **$O_2$** | **$CO_2$** |
| 0 | 16.9 | 0.9 | 77.0 | 0.8 | 77.5 |
| 4 | 16.5 | 1.6 | 75.3 | 1.4 | 76.8 |
| 8 | 16.7 | 1.7 | 78.4 | 1.9 | 80.5 |
| 12 | 16.9 | 4.6 | 58.6 | 2.8 | 69.1 |
| 16 | 14.6 | 3.0 | 65.9 | 2.4 | 68.9 |