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METHODS TO EXTEND THE MOLD FREE SHELF LIFE OF PIZZA CRUSTS

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ABSTRACT

Methods to extend the mold free shelf life of pizza crusts

Microbiological spoilage, particularly mold growth, is a major factor limiting the shelf life of bakery products. Therefore, methods to inhibit mold growth are of great importance to the bakery industry since mold spoilage results in over 90 million kgs of product being spoiled annually.

In this research, initial studies were done to determine the effect of various methods of preservation involving chemical preservatives, water activity (a_w), and modified atmosphere packaging (MAP) on mold growth in an agar model system. Results showed that preservatives could completely inhibit mold growth for 2-40d depending on concentration and pH used. Gas packaging (60% or 80% CO_2), oxygen absorbents, alone or in combination with potassium sorbate, could also inhibit mold growth for >40d at ambient storage temperature using a Response Surface Methodology (RSM) approach.

The effects of various methods of applying potassium sorbate into pizza crusts via direct incorporation into the batter, surface spraying, and impregnation of packaging material with potassium sorbate to control mold spoilage of pizza crusts were also investigated. Results showed that the antimicrobial effect of potassium sorbate was negligible when the packaging material was impregnated with the inhibitor but more pronounced when it was incorporated directly into the dough or sprayed onto the product's surface. The inhibitory effect of potassium sorbate increased as both the pH and the inoculum level decreased.

Shelf life studies using low concentrations of potassium sorbate (1000 and 2000 p.p.m.) and MAP, alone and in combination with each other, showed that potassium sorbate, gas packaging or oxygen absorbents (Ageless FX) could extend the shelf life of pizza crusts and decrease the growth rate of molds, bacteria and yeast. Furthermore, when pizza crusts were packaged in 60% CO_2 or with an oxygen absorbent, in combination with potassium sorbate (1000-2000 p.p.m.), a shelf life of 42d was possible without compromising the sensory shelf life of the product.

RESUME

Méthodes pour réduire le développement des moisissures et augmenter la durée de conservation à l'étalage des croûtes à pizza

La détérioration microbienne, particulièrement la croissance des moisissures, est un des facteurs qui réduit le plus la durée de conservation à l'étalage des produits de boulangerie. Les méthodes qui empêchent le développement des moisissures sont importantes pour l'industrie de la boulangerie dont les pertes annuelles à cause des moisissures sont de 90 millions de kilogrammes de produit.

Lors de cette recherche, des études préliminaires ont été faites sur des géloses (agar) pour déterminer les effets sur le développement des moisissures de différentes méthodes de conservation impliquant des préservatifs chimiques, l'activité de l'eau (a_w) et l'emballage sous atmosphère modifiée. Les résultats ont montré que les préservatifs pouvaient empêcher complètement le développement des moisissures pour une période de 2 à 40 jours selon la concentration utilisée et le pH. L'utilisation de la méthode de la surface de réponse (MSR) montre que l'emballage sous atmosphère gazeuse (60 ou 80% de CO_2) et les absorbeurs d'oxygène, seuls ou en combinaison avec du sorbate de potassium, pouvaient aussi empêcher le développement des moisissures durant plus de 40 jours à la température ambiante de conservation.

On a aussi étudié plusieurs méthodes d'application du sorbate de potassium sur des croûtes à pizza, soit en l'incorporant directement à la pâte, en le vaporisant sur la surface du produit ou en en imprégnant l'emballage, afin de contrôler la dégradation par les moisissures. Les résultats ont montré que le sorbate de potassium avait un effet négligeable lorsque les emballages avaient été imprégnés avec l'inhibiteur. Lorsque le sorbate de potassium était incorporé à la pâte ou vaporisé sur la surface du produit, l'effet anti-microbien était plus prononcé. L'effet inhibiteur du sorbate de potassium augmentait lorsque le pH et le taux d'inoculant diminuaient.

Les études sur la durée de conservation à l'étalage, où de faibles concentrations de sorbate de potassium (1000 et 2000 ppm) et l'emballage sous atmosphère modifiée sont utilisés seuls et ensemble, ont montré que le sorbate de potassium, l'emballage sous atmosphère gazeuse ou les absorbeurs d'oxygène (AGELESS FX) pouvaient augmenter la

durée de conservation des croûtes à pizza et réduire le taux de prolifération des moisissures, bactéries et levures. De plus, lorsque les croûtes à pizza étaient emballées dans 60% de CO_2 ou avec un absorbeur d'oxygène, conjointement avec du sorbate de potassium (1000-2000 ppm), la durée de conservation à l'étalage atteignait 42 jours sans que les propriétés organoleptiques du produit soient affectées.

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Table Of Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER: 1 INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Bakery products and cereals are a valuable source of nutrients in our diet providing us with most of our food calories and approximately half of our protein requirements. Cereals have been a basic food of man since prehistoric times and were consumed long before bread making was developed. Variety breads and other bakery products have increased in sales volume within the past decade. The importance of bakery products has expanded especially the use of whole and natural grains and other natural ingredients. The nutrients in bakery products are carbohydrates, proteins, lipids, vitamins and mineral. Furthermore, bakery products are considered as a source of carbohydrate because starch is the main chemical constituent (Kent, 1983).

Global consumption of bakery products and cereals have increased at an average rate of 25 % per year since 1970 and are used more than any other food source in our diet. Furthermore, consumption of bakery products in North America is estimated at $23 billion dollars annually (Smith and Simpson, 1996).

However, most bakery products are subject to spoilage problems. These include microbial, chemical and physical spoilage. Since the most common factor of bakery products is water activity, microbiological spoilage, in particular mold growth, is the major economical importance of bakery products. Modified atmosphere packaging has been used recently to overcome these problems by packaging bakery products under a mixture of gases to extend their shelf life (Smith and Simpson, 1996). Other methods which can also be used to extend the shelf life of bakery products are discussed in this chapter.

1.2. Economic importance of bakery products:

Bakery products are an important part of food expenditure. Consumption of bakery products have been falling since the end of World War Two in some industrialized countries such as the USA, Canada, the UK and Australia (Kent, 1983). According to Hunt and Robbins (1989), bakery products accounted for 9% of the average weekly food consumption. Anon (1992) estimated the consumption of bread in the UK was still 41.5 kg per person in 1990. Baur (1991) estimated the Western European bread market to be 23.000 million French francs.

In 1987, the Canadian industry sold $ 1.33 billion worth of products with bread accounting for approximately 50% of the total value of bakery product sold (Smith and Simpson, 1996). For the same year, the bakery industry in the U.S.A sold about $22 billion worth of bakery products (Smith and Simpson, 1996). In a recent study by Mclaughlin et al. (1995) the consumption of certain products, such as bagels and croissants, per capita has increased at an average annual rate of 1.5 percent from 1988 to 1992. Even the consumption of sweet goods has increased at an annual rate of approximately 1.0 percent.

1.3. Classification Of Bakery Products

Bakery products, made from wheat flour, consist of bread, unsweetened rolls, doughnuts, pies, pizza, cookies and other products. Various methods can be used to classify bakery products. Classification can be based on product type, e.g., bread or sweet goods, the method of leavening e.g., biological, chemical or unleavened, and water activity (a_w). In this section, products will be grouped based on product type.

1.3.1. Bread and Rolls:

Bread and rolls are considered as the most important type of bakery products (Table 1). They consist of many varieties according to their composition and their methods of production. These type of products are made from flour, water, salt, yeast or sour yeast, fat, sugar, milk powder, spices and flavors. The are many steps of production include mixing, fermentation, scaling, rounding, proofing, molding, panning, baking, cooling, slicing and packaging.

1.3.2. Other products:

Examples of other products are shown in Table 1

Type of bread	Shape and popularity	Processing characteristics
White pan	Most popular in the U.S. and Canada	Fermented dough
White mountain	Round or loaf form	Surface is sprinkled with water and dusted with flour
French bread and rolls	Baguettes are the most popular variety.	Lean dough with small mount of malt and sugar
Pullman	Similar to white pan.	Baked in a pan closed on four sides.
Vienna bread	Austrian bread.	Rich dough.
Bolillos	Oval-shaped, most popular in Mexico.	
Pan de agua	Popular in the Dominican Republic.	Rich dough.
Egg bread and rolls	Many shapes: oval, snail, three, four five or six twist.	High percentage of eggs : increased richness, volume and shelf life.
Hard rolls	Most popular: Kaiser roll.	Different dough but all have a firm and crisp crust.
Soft rolls	Hamburger, frankfurter, twisted, butterflake and cloverleaf.	Rich dough with more sugar and fat as compared to hard rolls.
Rye bread and rolls	Many shapes	Lean dough: rye flour, salt, water. yeast, or sour dough
Pita	Used in the Middle-East.	Lean dough, low in fat.
Bagels and Bialys	Popular in the U.S. and Canada.	Stiff dough

Adapted from Assouad (1996)

1.3.3. Classification of product based on water activity a_w

Water activity is among the chief factors influencing the shelf life of bakery products. In general, water is present in all bakery products in varying amounts. Therefore bakery products can also be classified into three major categories based on a_w:

- Low moisture bakery products with an a_w less than 0.6. The foods in this group are not affected by microbiological spoilage (Seiler, 1988).
- Intermediate moisture bakery products with an a_w 0.6-0.85. This group is subject to spoilage by molds and osmophilic yeast.
- High moisture bakery product with an a_w higher than 0.85. These products are affected by all groups of microorganisms. (Smith and Simpson, 1996).

Examples of bakery products and their a_w are shown in Table 2.

Table 2: Classification of bakery products based on moisture content and water activity

Product type	Water activity (a_w)
Low moisture content	
Cookies	0.20-0.30
Crackers	0.20-0.30
Intermediate moisture content	
Cake donut	0.85-0.87
Chocolate coated donuts	0.82-0.83
Danish pastries	0.82-0.83
Cream-filled snack cakes	0.78-0.81
pound cake	0.84-0.86
Banana cake	0.84-0.86
Soft cookies	0.50-0.78
Bean/Jam cake	0.85
High moisture content	
Bread	0. 96-0.98
Egg bread	0.90
Pumpernickel bread	0.90
Pita bread	0.90
Yeast raised donuts	0.96-0.98
Fruit pies	0.95-0.98
Soy bean pie	0.93
Carrot cake	0.94-0.96
Custard cake	0.92-0.94
Cheese cake	0.91-0.95
Butter cake	0.90
Pizza crust	0.94-0.95
Butter cake	0.99

Adapted from Doerry (1990); Smith and Simpson (1995)

1.4. Spoilage of bakery products

Bakery products, like all processed food products, are subject to spoilage. Spoilage of bakery products can be sub-divided into:

- Physical spoilage
- Chemical spoilage
- Microbial spoilage (Smith and Simpson, 1996).

1.4.1. Physical spoilage

Physical spoilage of bakery products usually involves moisture loss or gain resulting in a loss of texture or mold growth. This problem can be prevented by the use of moisture impermeable wrapping materials, such as low density polyethylene (L.D.P.E) which is a high barrier to moisture (Smith and Simpson, 1996).

Staling is a process of chemical and physical changes that occur after baking (Pomeranz and Shellenberger, 1971; Labuza, 1982). The major changes after baking are moisture redistribution, drying, starch retrogradation, increased firmness, and loss of aroma and flavor. Staling is the primary limiting factor, particular with bread and roll products. The mechanism of staling has been subject of many investigations. Bechtel et al.(1953) reported that staling may be due to moisture migration from the crumb to the crust. Therefore, it can be concluded that products with a higher moisture content stale faster than intermediate or low moisture products. Kulp (1979) postulated that staling was due to a process known as retrogradation. This is the association of the starch molecules with protein, initially the amylose molecules and later the amylopectin molecules, through hydrogen bonding. Furthermore, amylose retrogrades much faster since it is

linear and therefore association of amylose molecules with protein is easier while amylopectin is slower due to the branched nature of the molecule.

The economic importance of staling of bakery products is often underscored by statistics enumerating the millions of pounds and dollars worth of products that become unsaleable and are returned each year (Kulp, 1979). Various methods have been used to control the rate staling of bakery products. Surfactants have been used to slow staling by complexing with the starch molecules and reducing the tendency for retrogradation. Enzymes could also be used to delay staling by breaking down amylase and amylopectin molecules thereby preventing hydrogen bonding with protein (Hebeda et al., 1991).

1.4.2. Chemical spoilage

The most common form of chemical spoilage occurring in bakery products is rancidity. Two types of rancidity problems can occur oxidative and hydrolytic rancidity.

1.4.2.1. Oxidative rancidity:

Oxidative rancidity involves addition of oxygen at double bonds of unsaturated fatty acid resulting in the formation of peroxide. This peroxide is quite unstable and decomposes into aldehydes of medium molecular weight. This results in the formation of rancid odors and flavors. The free radicals and peroxide can bleach pigments, destroy vitamins A and E, break down proteins, and darken fat (Smith and Simpson,1996).

1.4.2.2. Hydrolytic rancidity:

This type of rancidity occurs in the absence of oxygen. It results in the hydrolysis of triglycerides to produce glycerol and malodorous short chain fatty acids.

1.4.3. Microbiological spoilage

Microbiological spoilage is often the major factor limiting shelf life of bakery products. Spoilage from microbial growth causes economic loss for both manufacturers and consumer (Hickey, 1980). It is estimated, that in the U.S. alone, losses due to microbial spoilage are 1 to 3% or over 90 million kg of product each year (Smith and Simpson, 1996). These losses could be due to many individual cases such as, packaging, sanitary practice in manufacturing, storage conditions and product turnover (Hickey, 1980). Microbial spoilage has been divided into three groups:

- Bacterial spoilage
- Yeast spoilage
- Mold spoilage

1.4.3.1. Bacterial spoilage

Bacteria also have a potential to contaminate baked products although their growth is more restricted by low water activity and low pH. The spores of *Bacillus subtilis* for example are heat resistant; 55% remain active in amylase after 20 min at 65 ^{o}C (Ponte et al., 1993). This microorganism, which is present in raw ingredients, e.g., flour, sugar, and yeast, causes rope in bread (Robinson, 1967; Seiler; 1989; Smith, 1993). Ropey bread is characterized by discoloration from brown to black ,the release of a rotten fruit odor and having an extremely moist, stringy bread crumb (Mountney and Gould ,1988; Rosenkvist and Hansen, 1995). This problem usually occurs in the summer season when the climate is warm and humid (Smith, 1993).

The prevention of rope problems require strict sanitary as well as good manufacturing practices designed to control the spores of *Bacillus* species. Preservatives, such as propionate, can be usually used to eliminate this problem (Barrett, 1970; Bailey and Holy, 1993).

Staphylococcus aureus is one type of bacteria known to contaminate pie fillings. This microorganism has also been implicated in food poisoning outbreaks from cream-filled bakery products (Seiler, 1978). He also noted that of the 323 outbreaks of food poisoning which occurred in Britain between 1969 and 1972, cakes and desserts contributed 3%. Other bakery ingredients, such as chocolate, desiccated coconut and cocoa powder were found to be contaminated with *Salmonella* (Seiler, 1978). For example, frozen pizza was significantly effected by *Salmonella typhimurium* (Dickson,1987).

1.4.3.2. Yeast spoilage

Yeast problems occur in bakery products. Wild yeast include *Trichosporon variable*, *Saccharomyces, Pichia,* and *Zygosaccharomyces* (Graves et al.,1967). *Saccharomyces* spp. produce white spots in bread crumb leading to the term chalk bread.

1.4.3.3. Mold spoilage

Mold growth is by far the major factor limiting shelf life of high and intermediate bakery products (Labuza, 1982). In general, most molds prefer high a_w values (>0.8) while a few xerophilic molds prefer to grow at a_w values as low as 0.65. Mold growth on bakery products is a serious problem that results in economic losses (Morlans and Sanchis, 1990). Furthermore, losses of products due to mold spoilage is between 1 and

5% depending on the type of product, season, and the method of processing (Malkki and Rauha, 1978). According to Hickey (1980), losses due to mold spoilage in the bakery industry average about 200 million pounds of product each year.

Mold spores are generally killed by the baking process in fresh bread and other baked products (Knight and Menlove, 1961). Therefore, for bread to become moldy, it must be contaminated either from the air, bakery surfaces, equipment, food handlers or raw ingredients after baking during the cooling, slicing or wrapping operations (Robinson, 1967; Jarvis,1972; Seiler,1978). This means that all spoilage problems caused by molds must occur after baking. The mold spore counts are higher in the summer months than in the winter due to airborne contamination in the warmer weather and more humid storage conditions (Smith,1994). Furthermore, moisture condensation on a product's surface, due to packaging prior to being completely cooled, may be conducive to mold growth. Jarvis (1972) found that mold spoilage caused undesirable odors and is often found on the surface of the product.

1.4.3.3.1. Types of mold in bakery products

The most common molds found in bakery products are:

***Rhizopus* sp.**

This mold, in particular *R. nigricans*, produces white cottony branches (mycelium) with black spores. It is highly proteolytic and commonly found in bread products (King, 1981).

***Aspergillus* sp.**

This mold produces greenish to black spore heads. The yellow pigment from the spore head spreads downward through the mycelium and can stain the product surface. At higher temperature, *Aspergillus* sp. accounted for 39% of molds growing on bread (Al Mohizea et al., 1987).

***Penicillium* sp.**

It is the most important mold limiting the shelf life of bakery products (Robinson, 1967). According to Legan (1993) 90-100% of loaves each month were spoiled by *Penicillium* species. Patterson et al. (1983) also observed that most fungi isolated from breads were *Penicillium* species. Furthermore, *P.expansum*, *P.notatum* and *P.virdicatum* were the predominant species producing green spore heads which spoiled bakery products with a high Equilibrium Relative Humidity (ERH).

***Monilia* sp.**

Monilia sp., in particular, *M. sitophila*, causes causing bread spoilage (Kuzyk et al., 1993). This microorganism is a very difficult mold to eliminate in bakery products as the spores are able to withstand fairly high temperatures for a reasonable length of time.

***Mucor* sp.**

This mold e.g., *M.mucedo* usually infects bread products and produces white spore heads.

***Eurotium* sp.**

Some of these molds are known to grow on the walls of bakeries, particularly in areas where condensation is a problem. The importance of this type of mold is that it can grow at lower a_w values than other molds. Therefore, *Eurotium* species e.g., *E.glaucus* and *E. amsteldami* tend to be the major molds limiting the shelf life of low a_w bakery products (Seiler, 1976).

1.4.3.3.2. Factors affecting mold free shelf life of bakery products

The main factors influencing the growth of molds on bakery products involves water activity (a_w), storage temperature, pH, and preservatives.

1.4.3.3.2.1. Water activity (a_w)

Water activity (a_w) is probably the environmental factor which most strongly affects mold growth on bakery products (Table 3). A_w is a measure of the amount of free water present in a bakery product. Scott (1957) defined a_w as a ratio of the water vapor present above the product to the ambient water vapor pressure.

In general, food with an a_w of greater than 0.90 are more susceptible to bacteriological spoilage than by yeast or molds. However, in high sugar products with a_w below 0.90, fermentation problems may occur due to yeast and mold spoilage (Smith, 1993). Molds have a wide range of a_w for growth (Table 4). For example, *Pencillium* sp. can grow at a_w range (0.80-0.99) while *Mucor* sp. require a high a_w (0.93-99). Furthermore, *Pencillium* and *Aspergillus* species comprise most of the molds capable of growth at a_w value, < 0.90 (Kushner, 1971). Some molds, termed *xerophiles*, are capable

of growth at low a_w values (Prescott et al., 1990). Among the fungi, *Xeromyces bisporous* is of special interest in that it will grow at a_w 0.65, and may have the lowest water requirement of any organism (Pitt and Christian, 1968).

Table 3: Major causes of spoilage in bakery products based on a_w

Major cause of spoilage	A_w range of product
Bacteria	0.91-0.95
Yeast	0.87-0.91
Molds	0.80-0.87
Halophilic bacteria	0.75-0.80
Xerophilic molds	0.65-0.75
Osmophilic yeast	0.60-065
Non-enzymatic browning	0.60-0.80
Enzymes (Amylase)	0.95-1.00
Lipases	0.1
Oxidation	0.01-0.50

Adapted from Doerry (1990)

Table 4 : Appropriate minimum levels of water activity permitting the growth of molds

Types of molds	A_w
Molds in general	> 0.75
Alternaria	0.84
Aspergillus niger	0.90-0.87
Other *Aspergillus* sp.	0.84-0.70
Mucor	0.93
Penicillium sp.	1.0-0.9
Xeromyces sp.	0.62-0.60

Adapted from (Earle and Putt, 1984)

1.4.3.3.2.2. Storage temperature

Temperature plays a dominant role in mold growth and in the germination of spores. The majority of molds grow within a temperature range of 18.3-29.4 ^{O}C (Frazier and Westhoff, 1978).When the temperature of bakery products is reduced from that for optimal growth, mold growth will decrease above the optimum temperature. Chamberlain (1963) reported that a reduction in the storage temperature from 27 ^{O}C to 21 ^{O}C doubled the mold free shelf life of cake and emphasized the need for care during distribution and storage. According to Jarvis (1972) small retailers who had little or no control over storage temperature were frequently responsible for the sale of moldy foods. Seiler (1965) also found that a reduction in the storage temperatures from 27 ^{O}C to 21 ^{O}C increased the mold free shelf life to a considerable extent. Generally, mold growth on bakery products was greatly affected by temperature, particularly with a low equilibrium relative humidity. For example, Seiler (1976) observed that cakes with a lower e.r.h. (82%) had a greater mold free shelf life than products with a high e.r.h (88%) at any given storage temperature.

Aspergillus niger and *Aspergillus flavus*, which can grow between 8-45 ^{O}C, are among the most destructive molds known (Pitt and Hocking, 1985). However, *Pencillium* species can grow at low temperatures i.e. 0-7 ^{O}C (Gill and Lowry, 1982). In addition, mold growth depends not only on temperature, but also on oxygen and moisture content. For example, refrigerating products at 5 ^{O}C permits storage without spoilage for longer when the water content is too high for safe storage at ambient temperature.

1.4.3.3.2.3. pH

This is an important factor governing mold free shelf life of bakery products. It plays a decisive role when molds compete with bacteria to spoil high moisture foods (Ponte et al.,1993). Molds tend to be less fastidious in their relationships to pH than bacteria. Generally, molds are tolerant of acid conditions and favor an acidic pH (3.5-5.5). Therefore, foods with pH values < 4.5 are not usually spoiled by bacteria but are more susceptible to mold spoilage. Therefore, the low pH of the sourdough breads preserves them from mold spoilage. The strongest response to pH occurs in the presence of NaCL and glucose (Ponte et al., 1993). In general, pH modification is used in combination with other methods in food preservation. Chamberlain (1963) found that the shelf life of high ratio Madeira cake increased from 5.5 to 7.5 days when the pH was reduced from 6.5 to 6.1. However, an increase in shelf life was also observed when the pH was increased from 6.5-7.2 (Seiler, 1965).

1.4.3.3.2.4. Oxygen

Molds are aerobic, i.e., they require oxygen for growth. Food spoilage molds, like all other filamentous molds, need oxygen for growth. Therefore, growth of aerobic molds can be inhibited by high concentrations of CO_2. However, molds vary widely in their tolerance to CO_2. In a CO_2 atmosphere, the growth of some molds is completely suppressed, while others are less effected (Ooraikul,1991). For example, the growth of *A.niger* and *P.expansum* could be controlled for 2-3 days in bread stored in 10 and 17% CO_2 at 28^0C (Skovholt and Bailey, 1933). *Penicillium roequefortii* could grow at 30% of the rate in air when maintained in an atmosphere of 80% CO_2 and 4.2% oxygen (Pitt and

Hocking, 1985). However, it is well documented that aerobic molds can also tolerate very low concentrations of headspace oxygen, even in the presence of elevated levels of CO_2 (Dallyn and Everton, 1969 ; Smith et al., 1986).

1.4.3.3.2.5. Preservatives

The stability of bakery products against the attack by molds is mainly due to preservatives. Sofos and Busta (1993) reported that chemical preservatives can control the growth of molds by preventing metabolism, by denaturing the protein of the cell, or by causing physical damage to the cell membrane. Among these preservatives are propionic and sorbic acid or their salts which have been shown to increase the mold free shelf life of baked products. Propionic acid and calcium propionate are usually employed at concentrations of 0.1 and 0.2% by flour weight, respectively. At these levels, molds can be inhibited for 2 days or more and the formation of rope can be prevented (Seiler, 1984). Sorbic acid is effective to control mold growth in bakery products at level of 0.125% to 0.3% on a flour weight basis.

1.5. Methods for extending the mold free shelf life of bakery products

Mold spoilage is often the major factor limiting the shelf life of high and intermediate moisture bakery products. Methods to inhibit mold growth are of significant economic importance to the bakery industry. Several basic approaches can be employed to reduce the incidence of mold growth and to extend the mold free shelf life of bakery products. These can be subdivided into three main areas:

- Prevention of post baking contamination.

- Destruction of mold contamination on bakery products.
- Control of mold growth.

1.5.1. Prevention of post baking contamination

In general, mold spoilage of bakery products is due to post baking contamination (Spicher, 1967). Breads are often contaminated by mold spores during cooling prior to packaging. The fact that the air in bakeries is often contaminated with mold spores can result in a massive infection in the bakery situation (Smith, 1993; Ooraikul et al., 1987). Therefore, extending the mold free shelf life of bakery products can be achieved by packaging as soon as possible after cooling, or slicing and wrapping under sterile conditions (Seiler, 1984).

Baking in a container and closing as soon as possible after leaving the oven has been used successfully to prevent mold growth on bakery products (Seiler 1989). This method can be used for canned cake and wrapped products in film. However, temperature of the surface of the products should not fall below 80 ^{O}C before final sealing to ensure safety (Seiler,1984). The main advantages of this method is that it is a cheap and convenient way for preservation (Seiler,1989). Care must be exercised to keep the air clean to prevent atmospheric contamination by using cleaning filters and keeping the air humid during cooling and wrapping energy (Seiler, 1989; Kyzlink,1990).

1.5.2. Destruction of mold contamination on bakery products

Heat sterilization is one of the primary methods that has been used to prevent mold growth. Therefore, the mold free shelf life of bakery products can be obtained by

destroying mold spores on the surface of products using U.V.light, infra-red irradiation or microwave energy (Smith, 1993).

1.5.2.1. U.V light

UV light is used in bakeries to prevent the growth of mold on the surface of freshly baked products, especially pies. The lethal effect of UV radiation on microorganisms is near 260 nm with a quantum energy of 4.9 electron volts (ev)(Smith, 1993). UV radiation will result in the mutation of the DNA in microorganisms. Also, the UV light causes adjacent thymine molecules to covalently link into a state known as a thymine dimer blocking further replication of the DNA (Smith, 1993). UV light has been used since before World War II to kill different types of microorganisms (Smith, 1993). However, UV light is not very effective in the extending mold free shelf life because it does not penetrate the product and mold spores still grow. Furthermore, it cannot be regarded as a viable method to extend the mold free shelf life of bakery products due to cost and its potential effect on packaging materials (Kyzlink,1990).

1.5.2.2. Infra-red irradiation

Infra-red irradiation is one food processing practice that could make the products safer microbiologically. Infra-red involves reheating the surface of products to a temperature sufficient to damage any mold spores present without markedly affecting the products or packaging materials (Seiler 1984). The mode of action of irradiation is the damage to the microbial DNA resulting in the loss of ability to reproduce (Wagner and Moberg, 1989). Removal of water and oxygen can increase the resistance of microorganisms to the lethal effect of irradiation (Wagner and Moberg, 1989). The time

to reach the desired temperature depends on the nature of the product, the distance from the infrared source, the film thickness as well as the surface of the product (Smith, 1993). Some films such QSAT and MXXT offer the ability to withstand the heat treatment. In general, the use of radiation to preserve products and reduce the incidence of mold spores could prove beneficial particularly in countries where food spoilage is always a problem. Although effective in controlling mold growth, it is quite costly, particularly if it is designed for a multi-sided product (Smith, 1993). Moreover, it may cause unacceptable organoleptic changes in food products (Wagner and Moberg, 1989).

1.5.2.3. Microwave

Treatment of wrapped bread with high frequency heat (microwave energy) for 45 to 60 second can extend the mold free shelf life (Pomeranz,1969). In a microwave facility, water molecules in the food align themselves with the electric field. This oscillation results in intermolecular friction which manifests itself as heat (Smith, 1993). The advantages and disadvantages of sterilization with microwaves are shown in Table 5.

Table 5 : Advantages and disadvantages of microwave sterilization

Advantages
Rapid
Thorough product heating
Increase to 21 day shelf life in fresh bread
Disadvantages
Emergent cake were heavy and had persistent condensation
Films, especially MSAT, adversely affected by the temperatures
Inadequate seals allow for secondary infection
Only products without coating or filling can be heated
Cakes were fragile and required a second cooling
May not give a reliable increase in mold free shelf life
Uneven heating in fruit cake due to differing dielectric loss factors

Adapted from Smith (1993)

1.5.3. Control of mold growth

Several methods can be used to control mold growth on bakery products including reformulation, freezing, and, most commonly, the use of preservatives.

1.5.3.1. Reformulation to reduce product a_w

Reformulation involves a reduction of available water e.g., a_w in bakery products to obtain a longer shelf life. Reduction in product a_w can be achieved by dehydration, either through evaporation or freeze-drying or by high osmotically active additives e.g., sugars and salts, incorporated directly into the food (Wagner and Moberg, 1989). The degree of a_w reduction is of practical significance in making a food non-perishable. The response to a given degree of a_w varies greatly among microorganisms in different environments (Kyzlink,1990).

Water contained in solutions of sugars and salt becomes unavailable to microbes due to the increased concentration of crystalloid. Furthermore, microbes are directly damaged osmotically by concentrations of these substances (Beuchat, 1983). This effect may be due to the adverse influence of lowered water availability on all metabolic activities, since all chemical reaction of cells require an aqueous environment (ICMSF, 1980)

Control of mold growth in bakery products normally relies on maintaining a sufficiently low a_w. For example, an a_w of 0.75 can give a 6 month extension in mold free shelf life. Higher a_w levels e.g., above 0.77 will only result in a short extension of shelf life (Pitt and Hocking, 1989). However, since low a_w can adversely affect the quality of the product and cause changes in shape and texture, care must be taken when reducing product a_w (Seiler,1989).

1.5.3.2. Freezing

Freezing has been used for long term preservation of bakery products particularly, cream filled products. Quick freezing is important in controlling the formation of ice crystals. Large ice crystals are formed when the rate of freezing is slower; the large crystals can disrupt membranes and internal cellular structures (Banwart, 1989).

Cakes ,cookies , short cake, and pancakes are commonly frozen and marketed in the frozen form. Bread has been held fresh for many months by storage at -22^0C (Desrosier, 1976). In contrast to fresh bread, which stales in less than a week, frozen bread stales very slowly. Therefore, the lower the temperature, the more slowly it stales. Desrosier (1976) reported that bread frozen quickly after baking and held for one year at

-18 ^{0}C, was equivalent in softness to fresh bread held for two days at 20 ^{0}C.

1.5.3.3. Chemical Preservatives

The most common method used for extending the mold free shelf life of baked products involves antimicrobial additives, specifically sorbic acid or propionic acid and their salts. However, the use of these inhibitors is limited in many countries by legislation which controls the type and concentration of antimold agent that may be used.

1.5.3.3.1. Sorbic acid and sorbates

Sorbic acid (CH_3-CH=CH-CH=CH-COOH) and its potassium salt, are recognized as effective antimold agents, and have been considered historically safe for food use (Bolin et al., 1980). Sorbic acid and potassium sorbate are Generally Regarded As Safe (GRAS) for their use in foods. This acid, or its potassium salts, has been used to retard microbial degradation in a large variety of food items (Luck, 1976). Major groups of foods in which sorbate has been used commercially because of its antimicrobial activity include baked goods, cheese, cake, chocolate coatings, fish products, fruit, butter, salad, vegetables and wine (Finol et al., 1982). The usefulness of sorbic acid as a mold inhibitor in bakery products such as cakes, cake mixes, pies, pie filling, doughnuts, etc. has also been demonstrated by Gorton (1979) and by Sofos and Busta (1993) .

In general, sorbic acid is effective against bacteria, and especially molds and yeasts. The major commercial use of sorbate is as a fungistatic (Liewen and Marth, 1985). Several studies have demonstrated the inhibitory effect of potassium sorbate on mold growth in food products. Ray and Bullerman (1981) reported that potassium sorbate exhibited a great effect on the growth of *A. niger* and *Penicillium* species. Sauer and

Burroughs (1974) observed that mold was inhibited for 2 weeks by using 0.5% potassium sorbate.

The levels of sorbate used in bakery products ranges from 0.001- 0.3% (Sofos, 1989). These concentrations have no major impact on food quality, but higher levels may cause undesirable changes in taste and flavor. Sorbates are more than twice as effective than propionates in inhibiting mold growth in bakery products, but have an adverse effect on yeast, reducing loaf volume and making dough sticky and difficult to process (Legan, 1993). This problem can be overcome by either spraying sorbate onto the product's surface after baking or mixing anhydrates of sorbic acid with fatty acids, such as palmitic. In addition, sorboyl palmitate has also been successful in controlling mold growth without interfering in the fermentation process. The heat of the baking process hydrolyses sorboyl palmitate and releases sorbic acid which inhibits molds during storage (Sofos, 1989). Sorbate acts synergistically with sodium chloride, calcium propionate, sodium propionate, citric acid and sucrose achieving a longer shelf life (Smith, 1993).

1.5.3.3.1.1. Methods of application of sorbate

Sorbate can be applied to bakery products in several different manners, including addition either directly by incorporating into the formulation, through spraying onto the products as an aerosol or incorporation into the packaging material (Sofos, 1989). Spraying sorbate has been used successfully to extend the mold free shelf life of bakery products i.e., pan breads, English muffins, pita bread, and tortillas (Hickey, 1980). The benefits of surface application of potassium sorbate involve lowering yeast costs up to 20% in some systems, decreasing mix times up to 25%, reducing customer complaints

due to spoilage in the home, reducing returns of unsold product, and inhibiting mold growth without adding a bad taste (Monsanto Company, 1977). Hickey (1980) found that sorbate surface treatment of bakery products had a longer shelf life by as much as 30-40% than those obtained by using calcium propionate in the dough. He also reported that low levels of sorbate, in combination with propionate on the surface of the dough, can double the shelf life produced by calcium propionate alone. Brown and serve rolls, as well as pizza crust, also showed improvements in shelf life when a sorbate surface treatment was applied (Gorton, 1979).

However, methods of preservative application can be selected based on processing, packaging procedure, and the ease of adding preservative through existing processes (Sofos and Busta,1993). The chemical properties of sorbate such as solubility and volatility, should be considered when selecting the method of application. In general, methods of sorbate application in bakery products are shown in Table 6.

Table 6: Methods application sorbate and its salt in bakery products

Products	Method of addition
Chocolate cake	Dry mix with flour or blend during creaming
Fruit cake	Dry mix with flour or blend in batter
White cake and yellow cake mixer	Blend with flour and other ingredients
Pie crust dough	Dry mix with flour or add to dough during mixing
Pie filling	Mix with fruit and syrup.If filling is cooked, add sorbic acid after cooking-on cooling cycle. This is important in custard and pudding type filling
Doughnut mixes	Pre-blend with salt and then blend in mix

Adapted Sofos (1989)

1.5.3.3.1.2. Factors influencing the antimicrobial activity of sorbate

Factors such as water activity (a_w), pH, temperature, and gas atmosphere packaging, can influence the antimicrobial activity of sorbate.

1.5.3.3.1.2.1. Water activity (a_w):

The antimicrobial action of food preservatives is generally increased as the a_w of the system is decreased. Thus, the addition of solutes to food that reduce its a_w act favorably on the inhibitory effect of preservatives (Liewen and Marth, 1985). The most common solutes present in food or added to control a_w are salt and sugar (Kushner, 1971). Both sugar and salt induce cellular swelling and make many microorganisms more sensitive to the action of inhibitors (Liewen and Marth, 1985).

Several studies have reported that the antimicrobial activity of sorbate could be increased by the addition of sugar or salt to the substrate. Acott et al. (1976) observed that *A. niger*, *A. glucose* were inhibited by sorbate and sucrose (a_w= 0.85). Furthermore, the growth of *Staphylococcus epidermidis* was also slowed under similar growth conditions. However, at an a_w of 0.88, all three organisms could grow, but at a slower rate than in the absence of sorbate. Sofos (1989) found that sorbate was effective in substrates containing high amounts of sugar or salt even at higher pH values e.g., 6.5.

1.5.3.3.1.2.2. Effect of pH:

The antimicrobial action of potassium sorbate has been associated with its undissociated fraction and not dissociated or ionized state (Sofos and Busta, 1981). The amount of undissociated form is determined by pH . The antimicrobial action of sorbate is more effective when the pH value approaches its dissociation constant (pK_a), which is

4.75 (Sofos, 1989). At this pH value, 50% of sorbic acid is in the undissociated form (Table 7). Therefore, the antimicrobial effect of sorbate in foods increases as the pH of the environment decreases (Bandelin, 1958 ; Sofos and Busta, 1981).

The maximum pH for inhibition by sorbate is in the range of 6.0 to 6.5. However, higher pH would limit the activity of sorbate. Several studies have reported the importance of using sorbate at a proper pH. Liewen and Marth (1985) reported that the growth of *Salmonella typhimurium* occurred in nutrient broth containing 0.3% sorbic acid at pH 6.7. However, the growth of these bacteria was inhibited when the pH was reduced to 5.0. Sorbate has a synergistic effect with pH levels, salt, and sugar (Sofos and Busta, 1981).

Table7: Effect of pH on sorbic acid dissociation

PH	Undissociated(%)
7.00	0.6
6.00	6.0
5.80	7.0
5.00	37.0
4.75 (pKa)	50.0
4.40	70.0
4.00	86.0
3.70	93.0
3.00	98.0

Adapted from Sofos and Busta (1981)

1.5.3.3.1.2.3. Temperature:

Sorbate is generally more effective in controlling mold growth at lower storage temperatures. In general, storage of foods at refrigerated temperatures will improve their shelf life. Therefore, to increase the potential of microbial inhibition in refrigerated products, the use of a sorbate preservative is advisable. The inhibitory effect of sorbate at lower temperatures has been demonstrated with bacteria and molds (Sofos, 1989). For example, at low temperatures i.e., 10 ^{O}C, potassium sorbate was more effective against *Salmonella* species compared to high temperatures (Monsanto, 1983). However, the combination of sorbate and a mild heat treatment (49 ^{O}C) greatly increased the shelf life of food products (Robinson and Hills, 1959). Liewen and Marth (1985) observed that the sensitivity of *A.niger* to heat increased in the presence of sorbate. They also reported that cellular injury of both molds and yeasts during heat was enhanced by sorbate.

1.5.3.3.1.2.4. Gas atmosphere packaging:

The effectiveness of potassium sorbate is also influenced by the gas atmosphere surrounding the product. Modified atmosphere packaging involving high levels of CO_2 usually enhance the inhibitory effect of sorbate. Several studies have shown that a combination of carbon dioxide and sorbate can be an effective inhibitor of microorganisms. Elliott and Gray (1981) showed that *Salmonella enteriditis* was inhibited by CO_2 in combination with sorbate. Smith et al. (1988) observed that sorbate and CO_2 acting synergistically inhibited the growth of *A.niger*.

1.5.3.3.2. Propionic acids and its salts

Propionic acid, an aminocarbolic acid (CH_3CH_2-COOH), is a naturally occurring organic acid and is an oily liquid with a slightly pungent, disagreeable rancid odor. Its salts are white, free-flowing powders with a slight cheese like flavor (Doores, 1993). Propionates were selected on the basis that higher MW fatty acids had a higher antimicrobial effect.

This acid or its salt can be used to prevent the bacterial spoilage of bread known as rope caused by certain *Bacillus* sp. (Legan, 1993). Several studies have also reported the effects of propionic acid and its salt on mold growth. Concentrations of propionate ranging from 8 to 12% have been reported effective in controlling mold growth on the surface of bakery products (Doores, 1993). However, not all molds were equally sensitive to the inhibitory effect of propionate. For example, at 0.3% calcium propionate, growth of *Monilia sitophila* and *Pencillium virdiicatum* in bread was inhibited for 2 days and 0.5 day respectively (Legan, 1993). Sauer and Burroughs (1973) observed that the mold free shelf life of corn was extended for 1 week by 0.5-1.% calcium propionate. In the United States, preservation of bread for a few days is generally accomplished by the addition of sodium or calcium propionate (Pomeranz, 1969). However, some species of *Pencillium* are resistant and can grow in media containing 5% propionic acid (Doores, 1993).

The advantage of using propionate is that it has little effect on yeast hence, propionates can be added to bread dough to prevent rope bacteria and mold growth without interfering with leavening (Grundy, 1996). Moreover, propionic acid and propionate are used at levels of 0.1 and 0.2% by flour weight, respectively (Seiler, 1984). At these levels, the mold free shelf life can be increased by 1-2 d. Compared to sodium

benzoate, propionates are generally more active against mold (King, 1981). However, propionic acid caused undesirable odors and flavors in baked goods. Therefore, the bakers prefer to use solid calcium propionate because it is easier to handle the solid salt than the corrosive liquid acid (Seiler, 1984). Sodium, potassium, and calcium propionate are the most widely used antimicrobial additives in the baking industry. They are active against molds and have little effect against bacteria (Smith, 1993). Their activity range extends from high acidity up to pH 6.5 (Barrett, 1970). Both calcium propionate and sodium propionate have been affirmed as GRAS (Grundy, 1996).

1.5.4. Modified atmosphere packaging

The natural composition of air is 78% nitrogen (N_2), 21% oxygen (O_2), and 0.03% carbon dioxide (CO_2). Modified atmosphere packaging (MAP) is rapidly becoming the food preservation technology of the future. Young et al. (1988) defined MAP as " the enclosure of food products in a high gas barrier film in which the gaseous environment has been changed or modified to slow microbial growth and retard enzymatic spoilage with the intent of extending shelf life''. Factors such as microbial growth, leakage, and the slow permeation of gases through the packaging material can change the gaseous atmosphere continuously throughout storage (Parry, 1993) .

MAP to extend the shelf life of food has been subject for many investigations in recent years. This has resulted not only from developments in the packaging technology but also from the industry's need for a less energy intensive and more economical method of short term food preservation. Currently, 300-500 European companies use MAP technology for extending food shelf life. It is estimated that in North America, 11 billion

MAP packages will be used by the year 2000 (Smith and Simpson, 1996). Several methods can be used to modify the gas atmosphere within the packaged product including vacuum packaging (VP), gas packaging, the use of oxygen absorbents and ethanol vapor generators.

1.5.4.1. Vacuum Packaging (VP)

The simplest form of MAP is Vacuum Packaging (VP). In VP, products are placed in a package made of films with low oxygen permeability, i.e., high gas barrier, air is evacuated and the package sealed. Under good packaging conditions, headspace O_2 is reduced to < 1%. This low level of oxygen helps extend the shelf life of products by inhibiting the two major spoilage agents, namely oxidative reactions and aerobic microorganisms (Smith and Simpson, 1996).

VP is unsuitable to extend the shelf life of bakery products due to its crushing effect on softer products (Smith, 1994). However, it has been used to prevent oxidative rancidity problems in short bread (American Institute of Baking, Personal Communication).

1.5.4.2. Gas Packaging

It has been known for some years now that a significant increase in the mold free shelf life of bakery products can be achieved through gas packaging. Gas packaging is simply designed to overcome some of the problems associated with vacuum packaging (Church and Parsons, 1995). This technique involves the packaging of a product in an impermeable film with a mixture of gases and heat sealing the package. For bakery products packaging under an atmosphere of various gases, such as carbon dioxide (CO_2)

and nitrogen (N_2), is always used. These gases are neither toxic nor dangerous and they are not considered as food additives (Smith and Simpson, 1995).

N_2 is found in the atmosphere. It is an inert gas, tasteless; odorless; does not have an antimicrobial effect (Farber, 1991). N_2 has two roles in gas packaging; first, it is used as filler gas to prevent the package from collapsing as CO_2 dissolves into the product because N_2 has low solubility in water. Secondly, it replaces O_2 and thus minimizes oxidative rancidity in bakery and snack food. N_2 may also inhibit the growth of aerobic microorganisms by reducing the amount of O_2 present (Smith and Simpson, 1995).

CO_2 is present in the air in trace amounts. It is the most important gas in gas packaged food products because of its bacteriostatic as well as fungistatic effect and can prevent growth of insects in the package (Farber, 1991; Smith and Simpson, 1996). It is highly soluble in water and fats, and forms carbonic acid. Its high solubility may lower the pH of the product resulting in flavor changes when used in high concentration. Collapse of the package may occur due to the absorption CO_2 by the product. CO_2 is effective when dissolved into the liquid phase of the food product (Hotchkiss, 1988). In general, the inhibitory effect of CO_2 depends on a number of interrelated factors including concentration, temperature, volume of headspace gas, water activity, type and initial number of microorganisms present in food at the time of application (Lambert et al,.1991; Farber, 1991).

1.5.4.2.1. Antimicrobial effect of CO_2

The exact mechanism of carbon dioxide's antimicrobial action is not yet known. Coyne (1933) has postulated that the antimicrobial effect of CO_2 was due to O_2 being

replaced by CO_2. This mechanism was later discounted by the fact that the growth of aerobic microorganisms was not inhibited by 100% N_2. Another theory suggested that the inhibitory effect of CO_2 may be due to lowering the intracellular pH which could interfere with enzymatic activities associated with cell metabolism (Wolfe, 1980; Daniels et al.,1985). Other theories suggested that the permeability of the bacterial cell membrane may be affected by CO_2 and the fluidity and toxicity of carbonic acid in its undissociated form (Finne, 1982). A further theory is that CO_2 appears to exert an effect on certain enzyme systems (Smith and Simpson, 1996).

1.5.4.2.2. Use of gas packaging for shelf life extension of bakery products

The use of CO_2 enriched atmosphere packaging for the extension of food shelf life is not a new method in food preservation. As early as the 19th. century, high levels of CO_2 and low levels of O_2 were recognized as a potent inhibitor of aerobic spoilage microorganism and retarded catabolic reaction in respiring foods (Smith and Simpson, 1996). In 1933, Skovolt and Bailey reported that CO_2 enriched atmospheres extended the mold free shelf life of bread. Extensive studies carried out in England with bread and cakes packaged in various concentrations of CO_2 at 21 and 27 ^{O}C, demonstrated that the mold free shelf life of products increased as the concentrations of CO_2 increased and temperature decreased (Smith, 1994). These studies also showed that the inhibitory effect of CO_2 was independent of ERH. In a study carried out in Europe in 1979, it was demonstrated that the shelf life of bread and cake could be extended by packaging in a mixture of CO_2 and N_2 as well as in 100% N_2 (Bogadtke, 1979). However, mold growth became visible in bread packaged in N_2 after 5d compared to 100d for bread packaged in

99% CO_2 and 1% O_2 (Bogadtke, 1979). Smith et al. (1990) reported that the mold free shelf life of baked products can be extended for 1-3 months by packaging in 60% CO_2 (balance N_2). Presently in Europe, more than 200 bakery firms use gas packaging technology to extend the mold free shelf life and keeping quality of rolls, cake, pizzas, and bread. Example of other possible gas mixtures to extend the shelf life of bakery products are shown in Table 8

Table 8: Typical gas mixtures for bakery products

Product	Gas % (%v/v)		
	CO_2	N_2	O_2
Sliced bread	100	-	-
Rye bread	100	-	-
Buns	100	-	-
Brioches	100	-	-
Cakes	100	-	-
Madeira cake	65	35	-
Madeira cake	80	20	-
Tea cakes	50	50	-
Danish pastries	50	50	-
Crepes	60	40	-
Croissants	100	-	-
Crumpets	100	-	-
Crumpets	60	40	-
Pita bread	99	1	-
Pita bread	73	27	-

Adapted from (Smith, 1994)

1.5.4.3. Oxygen absorbents:

Oxygen absorbents are defined as a range of chemical compounds introduced into the MAP package (not the product) to alter the atmosphere within the package (Smith et al.,1996). Oxygen absorbents technology was developed in Japan in 1976 and distribution in Japanese supermarkets under the name Ageless. The most frequently used oxygen scavengers are in the form of small sachets containing a metallic reducing agent, such as

powdered iron, which in suitable humid conditions (a_w >0.85) uses up residual oxygen to form non-toxic iron oxide. These sachets come in a variety of sizes capable of absorbing 2000 ml of O_2 from 10000 ml of air (Church, 1994). When placed in sealed packed containers, they reduce the O_2 levels to approximately 0.01% within 1-4 days at room temperature, ensuring a longer shelf life for foods by preventing the growth of molds and aerobic bacteria, insect damage and oxidation of unsaturated fatty acids (Smith et al., 1996). The absorbing reaction is the following (Smith, et al., 1990).

$$Fe \longrightarrow Fe^2 + 2e$$

$$1/2\ O_2 + H_2O + 2e \longrightarrow 2OH$$

$$Fe^2 + 2(OH) \longrightarrow Fe(OH)_2$$

$$Fe(OH)_2 + 1/2\ O_2 + 1/2\ H_2O \longrightarrow Fe(OH)_3$$

Several types and sizes of Ageless sachets are applicable to many types of foods including those high, intermediate or low in moisture and those containing or treated with oil. The main types of oxygen absorbents are shown in Table 9.

Table 9: Types of Ageless oxygen absorbents

Type	Function	Product		Absorption Speed (Day)
		Moisture Status	Water Activity	
Z	Decreases O_2	Medium moisture (Self working)	<0.65	1-3
S	Decreases O_2	High moisture (Self working)	>0.65	0.5-2
SS	Decreases O_2	High moisture (Self working)	>0.85	2-3
FX	Decreases O_2	High moisture (Moisture dependent)	>0.85	0.5-1
FM	Decreases O_2	Microwaveable (Moisture dependent)	>0.85	0.5-1
G	Decreases O_2 increase CO_2	Nuts (Self working)	0.3-0.5	1-4
SE	Decreases O_2 increase CO_2	High moisture (Moisture dependent)	>0.85	1-2

Adapted from (Smith, 1994)

Ageless type FX is used extensively to extend the shelf life of bakery products. These absorbents are moisture dependent and absorb oxygen after 12-24 hours. Ageless type SE is another absorbent of interest, especially to the bakery industry since it is self-reacting and absorbs oxygen and generates ethanol vapor. Therefore, it is commonly used to extend the mold free shelf life of bakery products in Japan.

Oxygen absorbers have been used in the shelf life of pizza crusts, sponge cake and other bakery products (Mitsubishi Gas Company, Personal Communication). The mold free shelf life of white bread packaged in high barrier film can be extended from 5 to 45 days at ambient temperature by using oxygen absorbents (Smith, 1994). Studies by Smith et al. (1986), have shown that when Ageless type FX was packaged with crusty rolls, either alone or in conjunction with gas packaging, headspace O_2 never increased beyond 0.05% and the product remained mold free for >60 days at ambient storage temperature. Results indicated that oxygen absorbents offer the food industry a viable

alternative to gas packaging to extend the mold free shelf life of bakery products (Smith et al., 1986).

1.5.4.3.1. Advantages and disadvantages of oxygen absorbent

Oxygen absorbents have several advantages for the food industryas following:

- Increased shelf life
- Inexpensive and easy to use.
- Non-toxic and safe to use.
- Improved food quality without additives.
- Reduced distribution losses.

The disadvantages of oxygen absorbents are:

- Need to have a free flow of air surrounding the sachet to scavenge oxygen in package headspace.
- Consumer resistance or misuse of sachet (Smith and Simpson, 1996).

1.5.4.4. Ethanol vapor

A novel and innovative way of generating ethanol vapor involves the use of sachets. These sachets sold under the trade name Ethicap or Antimold 102 are manufactured by the Freund Co. Ltd., Japan. Ethanol vapor generators consist of 55% ethyl alcohol (by weight) 35% silicon dioxide, and 10% moisture are contained in a sachet made of a copolymer of paper/ethyl vinyl acetate copolymer. Sachets come in various sizes ranging from 0.6 to 6 g or 0.33 to 3.3 g of ethanol evaporated (Smith et al., 1987; Smith and Simpson, 1996). The size of sachet used depends on (i) the weight of

food (ii) a_w of the food, and (iii) the desired shelf life of products (Smith and Simpson, 1996). When a food is packed with a sachet of Ethicap, moisture is absorbed from the food and the ethanol vapor is released into the package headspace. 0.5-2.5% of ethanol vapor is released from encapsulation and then condenses on the product surface and acts as an antimicrobial agent (Labuza and Breene, 1989). According to Smith et al. (1987) the main benefit of ethanol vapor generators include controlling mold spoilage, delaying staling in bakery products and the elimination of the need for preservatives, such as benzoic acid or sorbic acid, to control yeast. The main disadvantage of ethanol vapor is its absorption from package headspace. However, the maximum level of ethanol vapor found experimentally and permitted by the U.S. was 2% by product weight (Labuza and Breene, 1989).

One of the most important factors influencing the antimicrobial effect of ethanol vapor is to package the food product in a low or medium barrier film to ethanol vapor (ethanol permeability of <2g/m/day@30 ^{o}C). The level of ethanol in various bakery products was less than 1% after 20 days (Freund, 1985). However, heating the products at 375 ^{o}F prior consumption to reduce the ethanol level to less than 0.1% is recommended (Smith et al., 1987).

Freund (1985) found that ethanol vapors had an antimicrobial effect against at least 10 species of mold, including *Aspergillus* and *Penicillium*; 15 species of bacteria including *Staphylococcus*, *Salmonella*, and *E. coli*; and three species of yeast. Several studies have shown the effect of ethanol vapor to extend the mold free shelf life of bakery products. Seiler (1978) found that the mold free shelf life of pizza could be extended by spraying ethyl alcohol onto the product surface prior to packaging. The shelf life of

Madeira cake could also be extended for three weeks at room temperature using a 3 g sachet of Ethicap (Smith and Simpson, 1996).

1.6. Objectives of research

Since bakery products are usually spoiled by mold growth within a few days at room temperature, the bakery industry is seeking additional alternatives to extend the shelf life of bakery products. Food preservatives (potassium sorbate and calcium propionate) act to delay and decrease the rate of growth of molds. However, since the effectiveness of preservatives is limited mainly to products with pH below 6.0, additional methods i.e., modified atmosphere packaging involving gas packaging and oxygen absorbent, may be necessary to control mold growth. Therefore, on the basis of the above comments, the specific objectives of this research are:

1. To study the effect of a_w, pH, mold inhibitors, and MAP, alone, and in combination with each other, on the growth of *Aspergillus niger, Pencillium expansum,* and *Pencillium notatum*, in model agar systems.

2. To determine the optimal levels of CO_2, pH and the concentration of potassium sorbate to prevent the growth *of Aspergillus niger*, by using an RSM approach.

3. To determine the effect of potassium sorbate in combination with MAP on mold growth of pizza crusts.

4. To study the effect of methods of application of potassium sorbate to inhibit mold growth in pizza i.e., direct incorporation into batter, surface spraying, and impregnation of packaging material with potassium sorbate.

5. To determine the effect of sorbate in combination with MAP on the shelf life of pizza crusts.

CHAPTER 2
FACTORS INFLUENCING MOLD GROWTH

2.1. Introduction

Various factors, such as antimicrobial agents, pH, water activity (a_w), and modified atmosphere packaging (MAP), influence the growth of the common spoilage molds of bakery products. Chemical preservatives are widely used to extend the shelf life and keeping quality of bakery products, snack food products, pizza, meat, and cheese. Sorbic acid, and its potassium salt, have been used successfully to inhibit the growth of yeast, molds, and many bacteria in food. Propionic acid and its salts are also highly effective mold inhibitors, without inhibiting yeast activity, and are used to prevent mold growth and ropiness in bakery products.

From a food technologist's view point, water activity (a_w) i.e., the amount of available water in food for microbial growth, is the most important parameter influencing the growth and metabolic activity of microorganisms (Scott, 1957). Microbial spoilage and food poisoning occur if the a_w of the substrate is favorable for the growth of microbial contaminants. Most microorganisms contaminating food grow at a high a_w, while a few, such as osmophilic yeasts, require a low a_w for growth. Thus, if a_w decreases, only a few microorganisms are capable of growth in, and spoilage of, a specific food.

Modified atmosphere packaging (MAP) is becoming an increasingly popular method of food preservation. MAP, involving various gas mixtures of CO_2, O_2, and N_2, has been used to extend both the chemical and microbial shelf life of meat (Clark and Lentz,1969), peanuts and pecans (Holaday et al.,1979), fish (Banks et al.,1980), and rice

(Ory et al.,1980), However, it has been used sparingly as a method for extending the mold free shelf life of baked products.

Objectives:

In view of the above comments, the objectives of this study were:

To determine the effect of a_w, pH, mold inhibitors, and MAP, alone, and in combination with each other, on the growth of the common contaminants of bakery products *Aspergillus niger, Pencillium expansum,* and *Pencillium notatum*, in model agar systems.

2.2. MATERIALS AND METHODS

2.2.1. Microorganisms and inoculum preparation

Mold strains of *Aspergillus niger* (*16404*), *Pencillium notatum* (*10108*), and *Pencillium expansum* (*7861*) were obtained from the American Type Culture Collection (ATCC). Cultures were grown on Potato Dextrose Agar (PDA)(Difco, Michigan,USA) at 27 ^{o}C and transferred every three weeks to ensure viability. The inoculum was prepared by growing molds on PDA at 27 ^{o}C for 5 to 7 days until sporulation had occurred. Mold spores were harvested by dislodging spores into 9 ml of 0.1% (v/v) peptone water containing 1-2 drops of Tween 80 to prevent spore clumping. Spores were enumerated using an Improved Neubauer haemacytometer (Fisher Scientific). Appropriate dilutions of each stock solutions were made using 0.1% peptone water to give working suspensions containing an inoculum level of ~10^4 spores/ml throughout this study.

2.2.2. Antimold activity of sorbate and propionate

2.2.2.1. Preparation of potassium sorbate and calcium propionate

A 10% (w/v) stock solution of each preservative was prepared by dissolving 10 g of potassium sorbate (KS) and calcium propionate (CP) in 100 ml water and stirring for approximately 2 min. The preservatives were then filtered sterilized using a seitz filtration unit (Nalge Company, Sybron International, N.Y., USA). Each preservative solution was stored in a dark bottle under refrigeration until use.

2.2.2.2. Antimicrobial effect of preservatives at various pH levels

Experimental flasks were filled with appropriate amounts of Potato Dextrose Agar (Difco, Michigan,USA), sterilized by autoclaving at 121 ^{O}C for 15 min., and then cooled in a water bath to 50 ^{O}C. The pH of the cooled media was then adjusted to pH 5,5.5,6.0 and 7.0 using 1 N NaOH or 1 N lactic acid. The pH of the media was checked using a previously calibrated pH meter (Model 220, Corning Glass N. Y.) with an agal filled polymer body combined electrode with agal Ag/ AgCl reference (Fisher Scientific, Model (13-620-104). Appropriate amounts of each stock solution of potassium sorbate, calcium propionate, and equal mixtures of each stock solution were then added aseptically to the pH adjusted media to give final concentrations ranging from 0-2000 p.p.m. at increments of 250 p.p.m. i.e., levels commonly used commercially as shown in Table 10-12 respectively. The media was then dispensed into petri dishes (150 x 150 mm) and allowed to solidify in a sterile laminar flow cabinet (Labconco Corporation). Plates were inoculated in triplicate at 5 points with 25µl of each mold suspension using an automatic

pipette (Fisher Scientific). Inoculated plates were incubated aerobically at 25 ^{o}C and checked daily for visible signs of mold growth .

Table 10: The amount of 10% (w/v) stock solution of potassium sorbate (KS) and PDA media to obtain a final concentrations of preservative ranging from 0-2000 p.p.m. in media at various pH levels.

Vol. of stock solution of KS	Vol. of media (ml)	Final concentration (p.p.m.)
0	100	0
0.25	99.75	250
0.50	99.50	500
0.75	99.25	750
1.0	99.0	1000
1.50	98.50	1500
1.75	98.25	1750
2.0	98.0	2000

Table 11: The amount of 10% (w/v) stock solution of calcium propionate (CP) and PDA media to obtain a final concentration of preservative ranging from 0-2000 p.p.m. in media at various pH levels.

Vol. of stock solution of CP	Vol. of media (ml)	Final concentration of p.p.m.
0	100	0
0.25	99.75	250
0.50	99.50	500
0.75	99.25	750
1.0	99.0	1000
1.50	98.50	1500
1.75	98.25	1750
2.0	98.0	2000

Table 12: The amount of 10% (w/v) stock solution of potassium sorbate (KS) and calcium propionate (CP) and PDA media to obtain a final concentrations of equal levels of KS and CP ranging from 0-2000 p.p.m. in media at various pH levels.

Vol. of stock solution (ml)			
KS	CP	Vol. of media (ml)	Final concentration p.p.m.
0	0	100	0
0.125	0.125	99.75	250
0.250	0.250	99.50	500
0.350	0.350	99.25	750
0.500	0.500	99.00	1000
0.750	0.750	98.50	1500
0.875	0.875	98.25	1750
1.00	1.00	98.00	2000

2.2.3. Effect of water activity (a_w) on mold growth

A preliminary study was done in a model agar system to determine the effect of a_w on growth of selected molds. PDA was again used as the test medium to which appropriate amounts of glycerol were added to reduce the a_w from 0.99 to 0.85 (Table 13). The a_w of the prepared media was checked using a Decagon a_w meter (Decagon Instrument Company, New York, USA) previously calibrated with a saturated solution of sodium chloride (a_w 0.75). All measurements were carried out at a constant temperature (25 ^{O}C) and had an accuracy of ± 0.1%. The a_w adjusted media was then autoclaved at 121^{0}C for 15 min and dispensed into petri dishes (150 x150 mm). Triplicate plates were inoculated with mold spores as described in section 2.2.2.2. and packaged in 210 x 210 mm high gas barrier Cryovac bags. Bags were heat sealed using an Impulse heat sealer (UL Sealing Equipment, Taiwan), incubated at 25 ^{O}C, and checked daily for visible signs of mold growth.

Table 13 : The amount of glycerol added, PDA media and a_w of the adjusted media

Vol.of of glycerol (ml)	Vol. of media (ml)	Water activity (a_w)
0	100	0.99
15	90	0.98
20	80	0.95
25	75	0.92
30	70	0.90
35	65	0.87
40	60	0.85

2.2.4. Effect of MAP on mold growth in an agar system

To determine the effect of MAP on the growth of *A.niger*, *P.notatum*, and *P.expansum*, PDA plates were prepared and sterilized according to the manufacture's specifications. Upon cooling, the pH of all plates was adjusted to 7.0 by adding appropriate amounts of 1N Lactic acid or 1N NaOH to the medium.Triplicate plates were then inoculated with each of the mold suspensions as described previously. All plates were packaged in 210 x 210 mm high gas barrier Cryovac bags (oxygen transmission rate :3-6cm^3 /day at 4.4 ^oC, 0%RH). One set of packages was flushed with levels of CO_2 ranging from 60 to 80% (balance N_2) using a Multivac chamber type, heat seal packaging machine (Model KM100-3M). The proportion of gases was regulated by a Smith's level gas mixer (Tescom, Minneapolis,MN) to give the desired proportion of gases in the package headspace. In the second set of packages, the atmosphere was modified using an Ageless 100 type FX oxygen absorbent (Mitsubishi Gas Co., Tokyo, Japan). Absorbents (1 or 2) were taped to the inside of each package prior to heat sealing with an Impulse heat sealer. All packages were incubated at 25 ^oC and checked daily for visible signs of mold growth. Headspace gas was monitored in all packages using a

previously calibrated portable oxygen analyzer (Teledyne Analytical Instrument, City of Industry ,CA, U.S.A).

2.2.5. Combined effect of chemical preservatives with various gas atmospheres on mold growth

In this study, the combined effect of preservatives [potassium sorbate (KS) and equal parts of potassium sorbate and calcium propionate (CP)] and MAP on mold growth were investigated in a model agar system. PDA was again used as the basal medium and prepared as described previously. KS, and equal parts of KS and CP, were aseptically added to the media to give a final concentrations of 1000 p.p.m., of each preservative. The pH of the media was then adjusted to 6.0 using appropriate amounts of 1 N Lactic acid or 1 N NaOH. Plates were inoculated as described in section 2.2.1. The inoculated plates were then packaged in Cryovac bags under various gas atmospheres. Packages were filled with 60% and 80% CO_2 (balance N_2). All plates were incubated at 25 ^{O}C and checked daily for visible sign of mold growth.

2.2.6. Headspace Gas Analysis

Samples were analyzed for changes in headspace gas when visible signs of mold growth were observed. Gas samples were withdrawn using a 0.5 ml gas tight Pressure-lok syringe (Precision Sampling Corp., Baton Rouge, La.) through silicone seals attached to the package exterior. The gas samples were injected into a Varian gas chromatograph (Model 3400, Varian Canada Inc.) equipped with a thermal conductivity detector (TCD) and using Porapak Q (80-100 mesh) and Molecular Sieve 5A (80-100 mesh) columns in series (Supelco Canada Ltd). The carrier gas was helium set at a flow rate of 30 ml/min.

The column oven was set at 80 ^{O}C, the injector at 100 ^{O}C and the detector filament at 150 ^{O}C. Peaks were recorded using a Hewlett Packard integrator (Model 3390A, Hewlett Packard Co., Avondale, PA.).

2.2.7. Statistical analysis

Statistical analysis (GLM, ANOVA) was computed using the statistical analysis system on a McGill University mainframe.

2.3. RESULTS AND DISCUSSION

2.3.1. Antimicrobial effect of potassium sorbate (KS)

The effect of various concentrations of KS (0-2000 p.p.m.) on the growth of *A.niger*, *P.notatum*, and *P.expansum* on PDA at pH 5.0, 5.5, 6.0 and 7.0 at 25 ^{O}C are shown in Tables 14-17, respectively. At pH 5.0, mold growth was observed in control samples after 1-2d respectively (Table 14). Potassium sorbate (250 p.p.m) inhibited mold growth from 2 to 10d, depending on mold species, with *A.niger* being more resistant to KS than either *P.notatum* and *P.expansum*. While the growth of these two latter species was inhibited for up to 40d by concentrations of KS as low as 500 p.p.m., at least twice this concentration (1250-1500 p.p.m) was required for the complete inhibition of *A.niger* at pH 5.0. These results are in agreement with the studies of Seiler (1962) who also observed that *A.niger* was more resistant to mold inhibitors than other mold species.

Similar trends were observed for *A.niger*, *P.notatum*, and *P.expansum* on PDA at pH 5.5 and 25 ^{O}C (Table 15). While potassium sorbate levels, ranging from 250 to 1000 p.p.m., delayed the growth of *A.niger* for 2-4d, growth of *A.niger* still occurred even with high concentrations of potassium sorbate (1000-2000 p.p.m) after 5-6d (Table 15). While growth of both *P.notatum* and *P.expansum* occurred after 2-5d with low concentrations of KS (250-1000 p.p.m.) at pH 5.5, growth of both these molds was completely inhibited for >40d using higher levels of KS in the growth media (Table 15).

The response of mold growth on agar media containing 0-2000 p.p.m. KS, at pH 6.0 is shown in Table 16. At concentrations of 250-1000 p.p.m. KS, growth of *A.niger* was visible after 1d, i.e., similar to control samples. However, when the concentration of

potassium sorbate was increased to 1000-2000 p.p.m., growth of *A.niger* was only inhibited for 2d at pH 6.0 (Table 16). Growth of *P.notatum* occurred on PDA after 1d at pH 6.0 in the both control samples and in plates containing 250-1000 p.p.m. KS. When the level of KS increased to 1500-2000 p.p.m., growth was visible only after 3-4d at pH 6.0. *P.expansum* was more sensitive to all concentrations of KS in the media. While growth was inhibited for 5d at low concentrations of KS, growth was completely inhibited in plates containing >500 p.p.m. for > 40d at pH 6.0. These results confirmed the early observations of Hoeckst (1976) who reported that sorbate, in contrast to the other preservatives acids, can be used for the preservation of food with a relatively high pH value. However, the growth of *A.niger*, *P.notatum*, and *P.expansum* on PDA at pH 7.0. was visible after 1-2d on PDA at all concentrations of KS under investigation (Table 17).

The effect of pH on the antimicrobial action of potassium sorbate on mold growth is shown in Figures 1-4 respectively. It is evident from all figures that the activity of potassium sorbate increased as pH decreased. With the exception of *A.niger*, mold growth was completely inhibited in an agar system containing >500 p.p.m. KS at pH 5.0. However, three times this concentration of KS (1500 p.p.m.) was required to completely inhibit the growth of *A.niger* at this pH (Figure 1).

Similar trends were observed at both pH 5.5 and 6.0 indicating that inhibition was due to the combined effect of pH and sorbate concentrations. These results agree with those observed by Sofos and Busta in (1981) who found that potassium sorbate was more effective against the molds at lower pH values (<6.5). Growth of *P.expansum* was

completely inhibited in agar at pH 5.0, 5.5 and 6.0 and at concentrations of KS of 500 p.p.m., indicating that it was the most sensitive mold to KS.

Statistical analysis of the data showed that pH had a highly significant effect ($P < 0.0001$) on the antimicrobial activity of potassium sorbate. The results showed that the antimicrobial activity of potassium sorbate at pH 5.0 against both *A.niger* and *P.notatum* was significantly different compared to pH 5.5, 6.0, and 7.0. It is evident that pH played a significant role on the activity of potassium sorbate; however this effect is dependent on other interrelated factors, such as levels of sorbate and species of microorganism. This study agrees with the results of Earle and Putt (1983) and Liewen and Marth (1985) who reported that the initial number and type of mold present in food will influence the ability of potassium sorbate to prevent microbial growth and spoilage. As a consequence, potassium sorbate should not be used for preservation when high mold counts are expected, since a high level of mold contamination can metabolize potassium sorbate thereby reducing its fungistatic effect. This may explain why some molds in this study grew in the presence of high concentrations of potassium sorbate. Overall, it is critical to use potassium sorbate in products manufactured under good manufacturing practices and with the appropriate pH value.

The concentration of potassium sorbate also had a highly significant effect ($P < 0.0001$) on mold growth. A comparison between concentrations showed that a level 1500 p.p.m. potassium sorbate would be the most adequate level to inhibit mold growth. It is evident from the results obtained in this study that the minimal inhibitory (MIC) concentration of potassium sorbate for inhibiting mold growth varied with mold species and pH. The MIC in this study of potassium sorbate against test molds ranged between

750 to 1500 p.p.m. at pH 6.0 and 500-1500 p.p.m., at pH 5.0 and 5.5. The results indicated that the MIC of potassium sorbate at pH 5.0 was slightly contrary to the observations of Bandelin (1958). He reported that the minimum antimicrobial activity of sorbate was 800 p.p.m. at pH 5.0. However, Sofos in (1989) observed that the MIC of potassium sorbate was in the range between 100-1000 p.p.m. and was dependent on the pH of the substrate, types and numbers of molds and their environmental factors.

Table 14: Antimicrobial effect of potassium sorbate (KS) on mold growth in PDA at pH 5.0 & 25 ^{O}C

Level of KS (p.p.m.)	Days to visible mold growth			Extent of growth		
	A. niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^{e}	1^{c}	2^{c}	++	++	++
250	2^{d}	4^{b}	10^{b}	++	+	++
500	2^{d}	NG^{a}	NG^{a}	++	-	-
750	3.5^{c}	NG^{a}	NG^{a}	++	-	-
1000	5^{b}	NG^{a}	NG^{a}	-	-	-
1500	NG^{a}	NG^{a}	NG^{a}	-	-	-
1750	NG^{a}	NG^{a}	NG^{a}	-	-	-
2000	NG^{a}	NG^{a}	NG^{a}	-	-	-

+=Slight growth, ++=Light growth, +++=Medium growth, NG= No growth (>40d)

Table 15: Antimicrobial effect of potassium sorbate (KS) on mold growth in PDA at pH 5.5 & 25 ^{O}C

Level of KS (p.p.m.)	Days to visible mold growth			Extent of growth		
	A. niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^{f}	1^{e}	2^{c}	++	++	++
250	2^{e}	2^{d}	7^{b}	++	++	++
500	2^{e}	3^{c}	NG^{a}	++	++	-
750	3^{d}	4^{b}	NG^{a}	+	++	-
1000	4^{c}	4^{b}	NG^{a}	+	-	-
1500	5^{b}	NG^{a}	NG^{a}	+	-	-
1750	6^{a}	NG^{a}	NG^{a}	+	-	-
2000	6^{a}	NG^{a}	NG^{a}	+	-	-

+=Slight growth, ++=Light growth, +++=Medium growth, NG= No growth (>40d)

Table 16: Antimicrobial effect of potassium sorbate (KS) on mold growth in PDA at pH 6.0 & 25 ^{O}C

Level of KS p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^{b}	1^{d}	2^{c}	++	++	++
250	1^{b}	1^{d}	5^{b}	++	++	+
500	1^{b}	1^{d}	NG ^{a}	++	++	-
750	1^{b}	1^{d}	NG^{a}	+	+	-
1000	2^{a}	2^{c}	NG^{a}	+	+	-
1500	2^{a}	3^{b}	NG^{a}	+	+	-
1750	2^{a}	4^{a}	NG^{a}	+	+	-
2000	2^{a}	4^{a}	NG^{a}	+	+	-

+=Slight growth, ++=Light growth, +++=Medium growth, NG= No growth (>40d)

Table 17: Antimicrobial effect of potassium sorbate (KS) on mold growth in PDA at pH 7.0 & 25 ^{O}C

Level of KS p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^{a}	1^{a}	2^{a}	++	++	++
250	1^{a}	1^{a}	2^{a}	++	++	+
500	1^{a}	1^{a}	2^{a}	+	+	+
750	1^{a}	1^{a}	2^{a}	+	+	+
1000	1^{a}	1^{a}	2^{a}	+	+	+
1500	1^{a}	1^{a}	2^{a}	+	+	+
1750	1^{a}	1^{a}	2^{a}	+	+	+
2000	1^{a}	1^{a}	2^{a}	+	+	+

+=Slight growth, ++=Light growth, +++=Medium growth

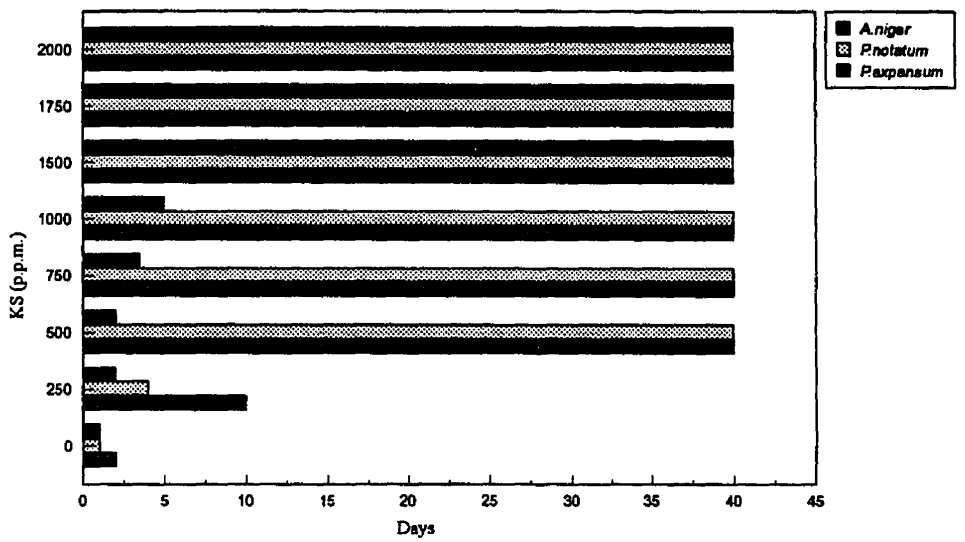

Figure 1: The antimicrobial action of potassium sorbate (KS) on mold growth at pH 5.0

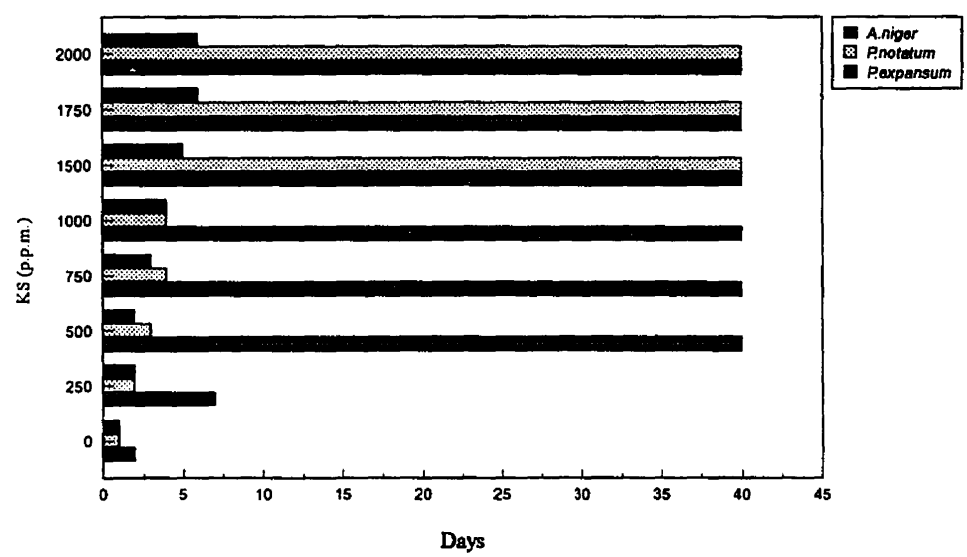

Figure 2 : The antimicrobial action of potassium sorbate (KS) on mold growth at pH 5.5

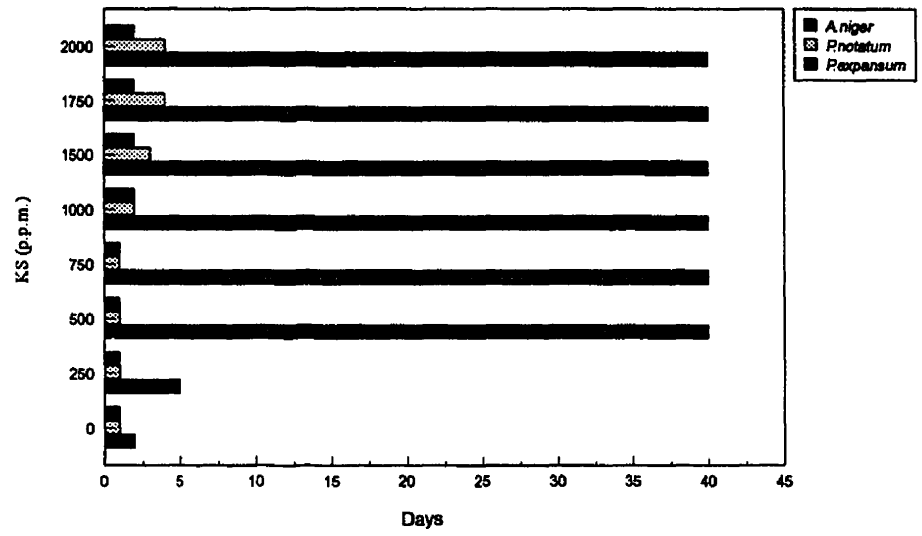

Figure 3 : The antimicrobial action of potassium sorbate (KS) on mold growth at pH 6.0

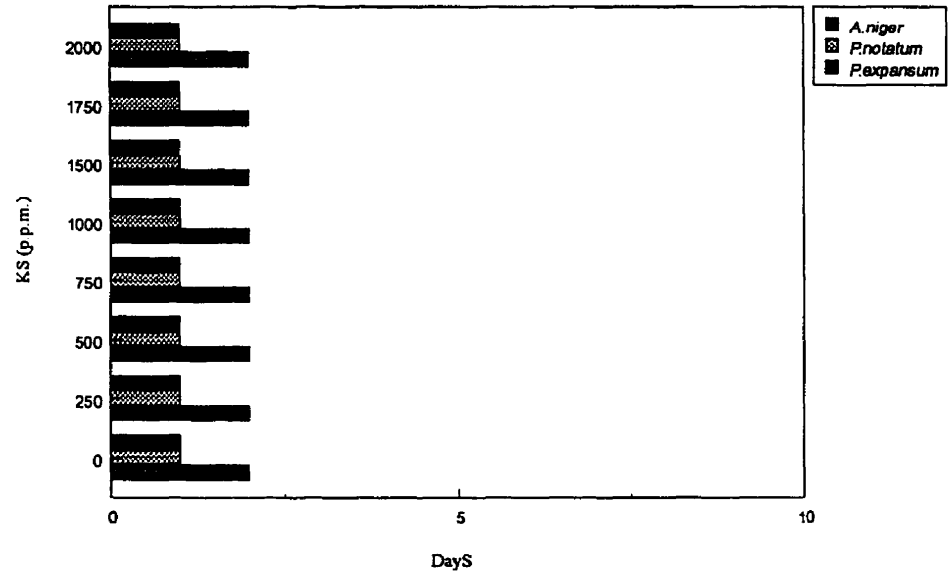

Figure 4 : The antimicrobial action of potassium sorbate (KS) on mold growth at pH 7.0

2.3.2. Antimicrobial effect of calcium propionate (CP)

The effect of various concentrations of CP on the growth of *A.niger, P.notatum*,and *P.expansum* on PDA at various pH levels are shown in Tables 18-21 respectively. As shown in Table 18, low concentrations of CP were unable to inhibit mold growth. At pH 5.0, growth of *A.niger* was visible after 1d on PDA containing 250-750 p.p.m. of CP i.e., similar to control plates. However, the effectiveness of calcium propionate was proportional to its concentration in the agar medium. At higher concentrations of CP (1000-2000 p.p.m.) growth of *A.niger* was delayed for 2-4d respectively (Table 18).

The response of *P.notatum* on PDA to various concentrations of CP at pH 5.0 was similar to that observed for *P.expansum*. For *Penicillium* species, CP almost doubled the time for visible growth compared to *A.niger*. At concentrations ranging from 1000-2000 p.p.m., growth of *P.notatum* was inhibited for 4-10d respectively (Table 18). Similar results were observed for *P.expansum*. However, at higher concentrations of CP (1750-2000 p.p.m.) growth of this mold was inhibited for 11-13d. These results confirmed that higher concentrations of CP had a more inhibitory effect on mold growth and that *Penicillium* species were more sensitive to these higher concentrations than *Aspergillus* species i.e., similar to previous observations with potassium sorbtate.

The antimicrobial activity of various concentrations of CP at pH 5.5, 6.0, and 7 is shown in Tables (19-21). In all cases, growth of *A.niger, P.notatum*, and *P.expansum* occurred after 1-2d respectively on PDA, irrespective of the levels of CP in the media. These results confirm that both the inhibitory effect of CP and KS is also pH dependent with the inhibitory effect being enhanced at lower pH values.

This pH effect is shown in Figures 5-9 respectively. It is apparent that the activity of CP increased as pH decreased. Growth of *A.niger* was visible after 4d even with at high concentration of CP (2000 p.p.m.) on PDA at pH 5.0. However, growth occurred after only 1d at all CP concentrations at pH 5.5, 6.0 and 7.0, respectively.

Complete inhibition of *Pencillium* species was observed at pH 5.0 and at high concentrations of CP (2000 p.p.m.). At pH 5.5 , 6.0 and 7.0, similar levels of CP were unable to control mold growth. The results show that molds may have the ability to develop resistance to CP, particularly at higher pH levels. Similar results were observed by Ray and Bullerman (1982) who reported that while calcium propionate exhibited antimycotic activity, its use was limited to lower pH food since no antimycotic activity was observed in food with neutral or near neutral pH values.

Statistical analysis of the data (GLM) showed that pH had a highly significant effect ($P<0.0001$) on the activity of CP. A comparison between the pH levels investigated showed that pH 5.0 was the most effective pH for the antimicrobial action of CP. This is again due to the greater amount of undissociated of CP at more acid pH levels.

The concentration of CP also had a highly significant effect ($P<0.0001$) on mold growth. At pH 5.0, higher levels of CP (1000-2000 p.p.m.) had a more significant effect on mold growth compared to lower levels at all pH values under investigation. These results confirm the earlier finding of Seiler (1962) who observed that the minimum inhibitory concentration of calcium propionate in PDA required to prevent the growth of *A.niger* and *P.notatum* at pH 6.0 was 4000 p.p.m. and 3000 p.p.m. respectively. Acott and Labuza, (1975) reported that, if foods were higher in pH, more calcium propionate than the FDA allowance of 2000 p.p.m. would be necessary for preservation.

Furthermore, these results clearly indicate that, on a weight to weight basis, KS is a far more effective antimycotic agent against the molds tested in this study.

Table 18: Antimicrobial effect of calcium propionate (CP) on mold growth on PDA at pH 5.0 & 25 °C

Level of CP p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^{d}	1^{g}	2^{f}	++	++	++
250	1^{d}	1^{g}	2^{f}	++	++	+
500	1^{d}	2^{f}	3^{e}	++	++	+
750	1^{d}	3^{e}	3^{e}	++	+	+
1000	2^{c}	4^{d}	5^{d}	+	+	+
1500	3^{b}	8^{c}	8^{c}	+	+	+
1750	4^{a}	9^{b}	11^{b}	+	+	+
2000	4^{a}	10^{a}	13^{a}	+	+	+

+=Slight growth, ++=Light growth

Table 19: Antimicrobial effect of calcium propionate(CP) on mold growth on PDA at pH 5.5 & 25 °C

Level of CP p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^{a}	1^{a}	2^{a}	++	++	++
250	1^{a}	1^{a}	2^{a}	++	++	++
500	1^{a}	1^{a}	2^{a}	++	++	++
750	1^{a}	1^{a}	2^{a}	++	++	++
1000	1^{a}	1^{a}	2^{a}	++	++	++
1500	1^{a}	1^{a}	2^{a}	++	++	++
1750	1^{a}	1^{a}	2^{a}	++	++	++
2000	1^{a}	1^{a}	2^{a}	++	++	++

+=Slight growth, ++=Light growth

Table 20: Antimicrobial effect of calcium propionate (CP) on mold growth on PDA at pH 6.0 & 25 °C

Level of CP p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1[a]	1[a]	2[a]	++	++	++
250	1[a]	1[a]	2[a]	++	++	++
500	1[a]	1[a]	2[a]	++	++	++
750	1[a]	1[a]	2[a]	++	++	++
1000	1[a]	1[a]	2[a]	++	++	++
1500	1[a]	1[a]	2[a]	++	++	++
1750	1[a]	1[a]	2[a]	++	++	++
2000	1[a]	1[a]	2[a]	++	++	++

+=Slight growth, ++=Light growth

Table 21: Antimicrobial effect of calcium propionate (CP) on mold growth in PDA at pH 7.0 & 25 °C

Level of CP p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1[a]	1[a]	2[a]	++	++	++
250	1[a]	1[a]	2[a]	++	++	++
500	1[a]	1[a]	2[a]	++	++	++
750	1[a]	1[a]	2[a]	++	++	++
1000	1[a]	1[a]	2[a]	++	++	++
1500	1[a]	1[a]	2[a]	++	++	++
1750	1[a]	1[a]	2[a]	++	++	++
2000	1[a]	1[a]	2[a]	++	++	++

+=Slight growth, ++=Light growth

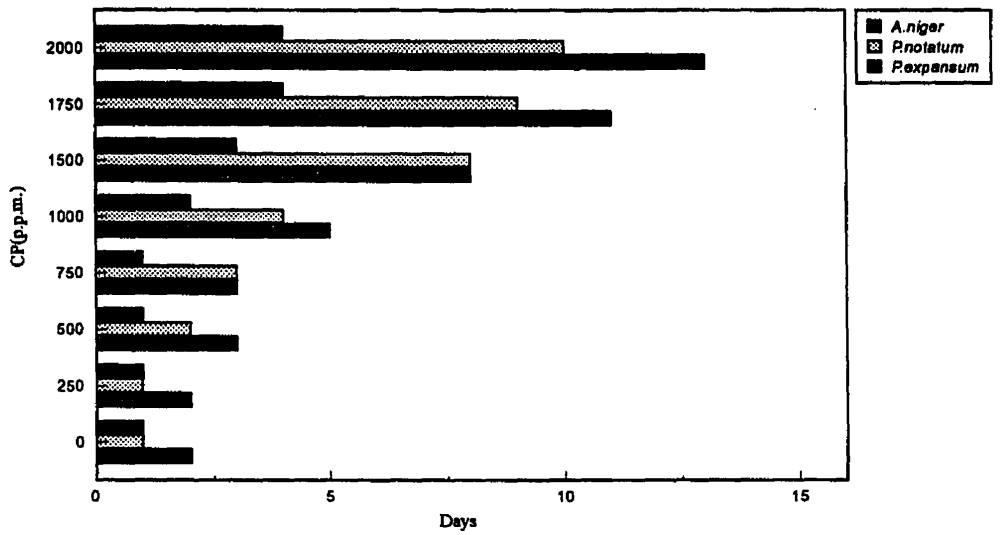

Figure 5: The antimicrobial action of calcium propionate (CP) on mold growth at pH 5.0

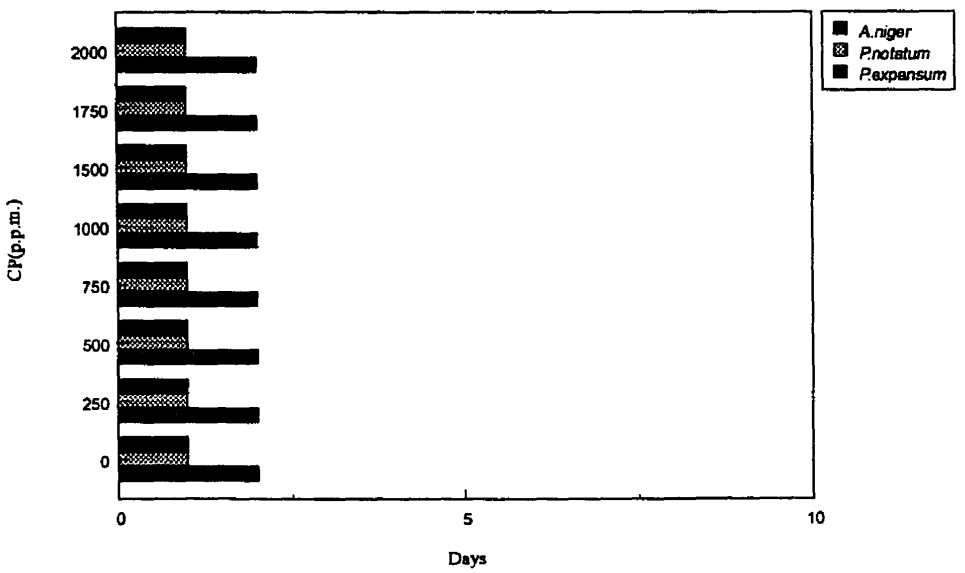

Figure 6: The antimicrobial action of calcium propionate (CP) on mold growth at pH 5.5

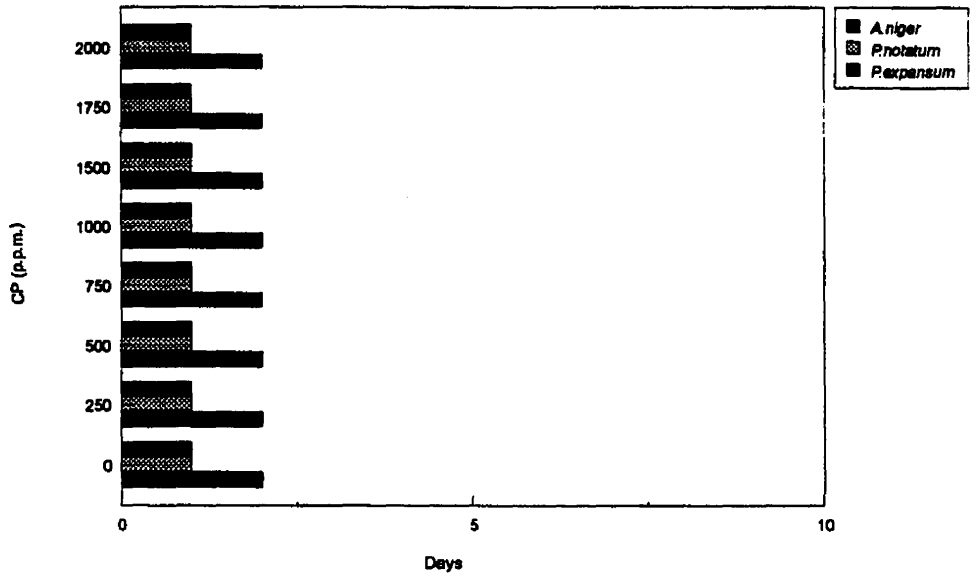

Figure 7: The antimicrobial action of calcium propionate (CP) on mold growth at pH 6.0

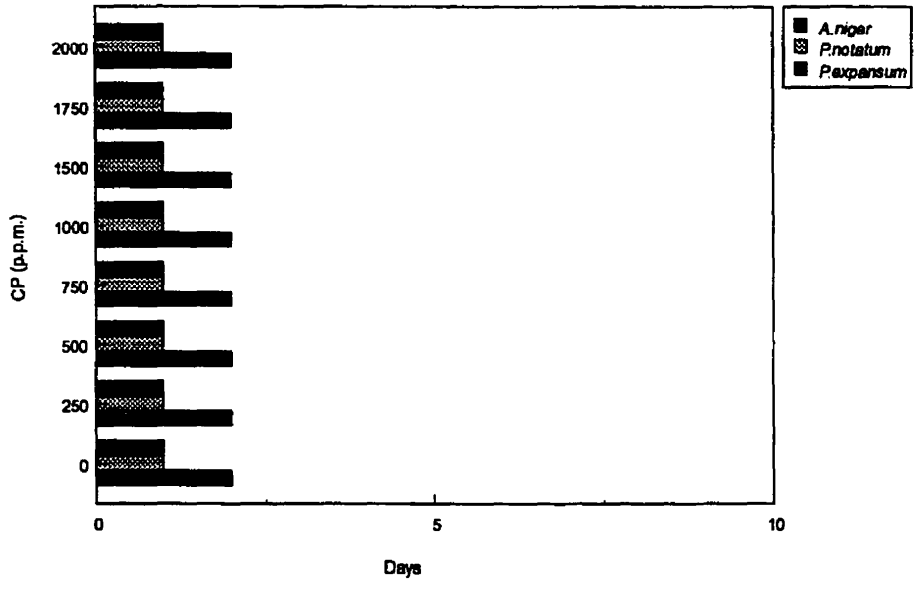

Figure 8: The antimicrobial action of calcium propionate (CP) on mold growth at pH 7.0

2.3.3. The combined antimicrobial effect of equal concentrations of KS and CP on mold growth

The effect of equal concentrations of potassium sorbate (KS) and calcium propionate (CP) (0-2000 p.p.m.) on the growth of *A.niger*, *P.notatum*, and *P.expansum* on PDA at pH 5.0-7.0 is shown in Tables 22-25, respectively. It is evident that growth of *A.niger* was visible after 2-3d using equal levels of KS and CP ranging from 250-1000 p.p.m. in PDA at pH 5.0 (Table 22). However, complete inhibition of *A.niger* was observed for >40d in agar containing 1500-2000 p.p.m. of equal levels of both preservatives (Table 22) i.e., similar to results observed when KS was used alone.

As observed previously, *Pencillium* species were again more sensitive to these antimycotic agents than *A.niger*. While visible mold growth occurred after 3-6d for both *P.notatum* and *P.expansum* respectively, the inhibitory effect of KS and CP in combination with each other greatly inhibited the growth of both *Pencillium* species as the concentrations of inhibitors increased. Complete inhibition of both *P.notatum* and *P.expansum* was observed for >40d in media containing equal levels of (750-2000 p.p.m.) of both sorbate and propionate (Table 22).

Similar trends were observed at both pH 5.5 and 6.0 (Tables 23-25). At pH 5.5 growth of *A.niger* occurred within 1-5d in PDA containing 250-2000 p.p.m. of equal levels of both KS and CP (Table 23). While growth of *P.notatum* was inhibited for 3-5d respectively at low concentrations of KS and CP (250-1000 p.p.m.), complete inhibition for *P.notatum* was again observed in PDA with 1500-2000 p.p.m. of KS and CP for >40d. Similar results were observed for *P.expansum* although lower concentration of both

KS and CP i.e., 500 p.p.m. were required for complete inhibition of this mold at pH 5.5 (Table 23).

Once again, equal levels of sorbate and propionate (0-2000 p.p.m.) were less effective against both *A.niger* and *P.notatum* at all pH levels under investigation (Table 22-25). These results also showed that *P.expansum* was again more sensitive to equal levels of KS and CP than either of the two other molds under investigation. Complete inhibition of *P.expansum* (>40d) was observed in agar containing lower levels of KS in combination with CP at pH levels i.e., similar to previous results with KS alone.

The inhibitory effect of KS in combination with CP against mold growth at pH 7.0 is shown in Table 24. It is evident that the inhibitory effect of these preservatives was reduced as mold growth was visible after 1-2 d in PDA containing 0-2000 p.p.m. of equal levels of both preservatives at pH 7.0.

The effects of pH on the antimicrobial action of equal levels of sorbate and propionate in PDA plates are shown in Figures 9-12 respectively. The results from these investigations confirmed our previous observations. Growth of both *Aspergillus* and *Pencillium* species was completely inhibited in PDA at pH 5.0 using 750 p.p.m. of equal amounts of each inhibitor. Mold growth was inhibited to a lesser extent at pH 6.0-7.0 than pH 5.5, again indicating of the effect of pH on the antimicrobial action of preservatives.

Statistical analysis of the data also showed that pH had a highly significant effect ($P<0.0001$) on the antimicrobial action of sorbate in combination with propionate. A comparison between pH showed that pH 5.0 had again the most significant effect on the

antimicrobial action of preservatives i.e., confirming previous observations that the organic acids are mainly in the undissociated state at this pH.

The concentrations of sorbate in combination with propionate also had highly significant effect ($P<0.0001$) on mold growth. The minimum inhibitory effect of sorbate in combination with propionate was between 500 and 1500 p.p.m. or 250 and 750 p.p.m. of each inhibitor depending on mold type and pH. It is evident in this study that equal concentrations of both sorbate and propionate were more effective in controlling mold growth than when CP was used individually but almost identical to results obtained when KS was used alone. Liewen and Marth (1985) reported that when two compounds react synergistically, only 40% of the minimum inhibitory concentration of each compound is required to give 100% inhibition of the test microorganism. This is very desirable in food products since lower concentrations of each inhibitor may reduce any potential undesirable effects on products, such as adverse flavor effects, as well as reducing cost.

Table 22: Antimicrobial effect of equal levels of potassium sorbate (KS) and calcium propionate (CP) on mold growth in PDA at pH 5.0 & 25 ^{O}C

Level of KS & CP p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^{d}	1^{d}	2^{c}	++	++	++
250	2^{c}	3^{c}	6^{b}	++	++	+
500	2^{c}	29^{b}	NG^{a}	++	+	-
750	2^{c}	NG^{a}	NG^{a}	++	-	-
1000	3^{b}	NG^{a}	NG^{a}	++	-	-
1500	NG^{a}	NG^{a}	NG^{a}	-	-	-
1750	NG^{a}	NG^{a}	NG^{a}	-	-	-
2000	NG^{a}	NG^{a}	NG^{a}	-	-	-

+=Slight growth, ++=Light growth, NG= No growth (>40d)

Table 23: Antimicrobial effect of equal levels of potassium sorbate (KS) and calcium propionate (CP) on mold growth in PDA at pH 5.5 & 25 ^{O}C

Level of KS & CP p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^{e}	1^{e}	2^{c}	++	++	++
250	2^{d}	3^{d}	5^{b}	++	++	+
500	2^{d}	4^{c}	NG^{a}	++	+	-
750	2^{d}	5^{b}	NG^{a}	++	+	-
1000	3^{c}	5^{b}	NG^{a}	+	+	-
1500	4^{b}	NG^{a}	NG^{a}	+	-	-
1750	5^{a}	NG^{a}	NG^{a}	+	-	-
2000	5^{a}	NG^{a}	NG^{a}	+	-	-

+=Slight growth, ++=Light growth, NG= No growth (>40d)

Table 24: Antimicrobial effect of equal levels of potassium sorbate (KS) and calcium propionate (CP) on mold growth in PDA at pH 6.0 & 25 °C

Level of KS & CP p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^b	1^e	2^c	++	++	++
250	1^b	1^e	3^c	++	++	+
500	1^b	1^e	30^b	++	+	-
750	1^b	2^d	NG^a	++	+	-
1000	1^b	3^c	NG^a	+	+	-
1500	2^a	4^b	NG^a	+	-	-
1750	2^a	5^a	NG^a	+	-	-
2000	2^a	5^a	NG^a	+	-	-

+=Slight growth, ++=Light growth, NG= No growth (>40d)

Table 25: Antimicrobial effect of equal levels of potassium sorbate (KS) and calcium propionate (CP) on mold growth in PDA at pH 7 & 25 °C

Level of KS & CP p.p.m.	Days to visible mold growth			Extent of growth		
	A.niger	*P.notatum*	*P.expansum*	*A.niger*	*P.notatum*	*P.expansum*
0	1^b	1^a	2^a	++	++	++
250	1^b	1^a	2^a	++	++	+
500	1^b	1^a	2^a	++	+	+
750	1^b	1^a	2^a	++	+	+
1000	1^b	1^a	2^a	+	+	+
1500	1^b	1^a	2^a	+	+	+
1750	1^b	1^a	2^a	+	+	+
2000	2^a	1^a	2^a	+	+	+

+=Slight growth, ++=Light growth

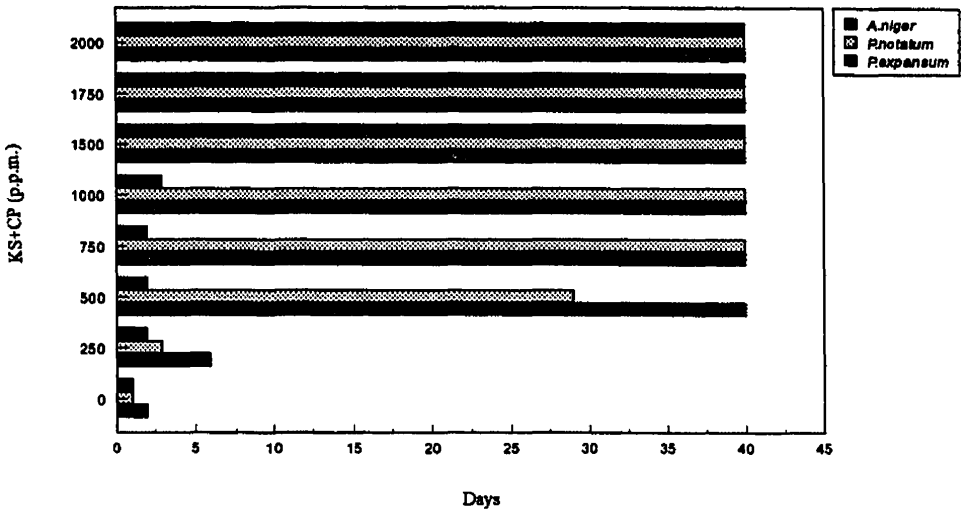

Figure 9: The antimicrobial action of equal levels of potassium sorbate and calcium propionate (KS + CP) on mold growth at pH 5

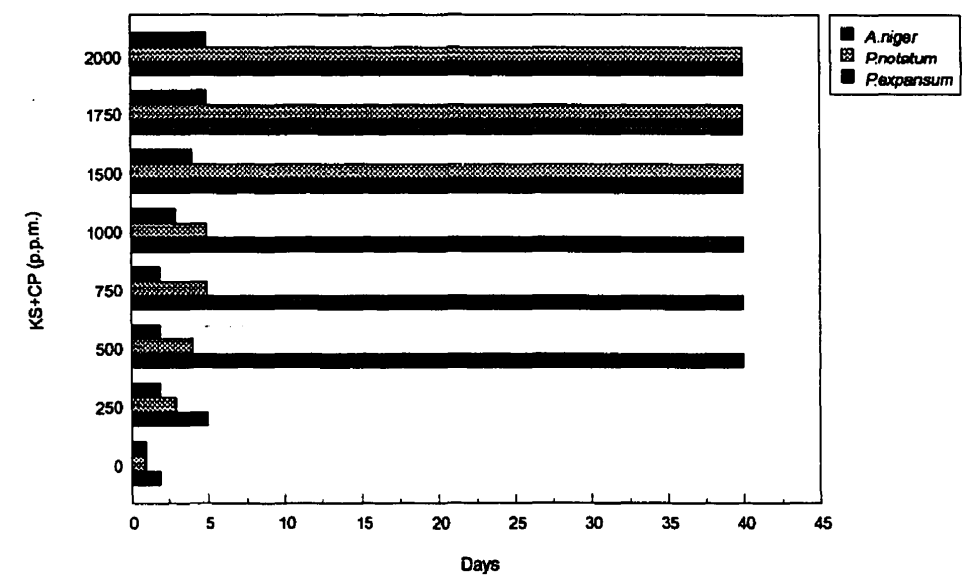

Figure 10 : The antimicrobial action of equal levels of potassium sorbate and calcium propionate (KS + CP) on mold growth at pH 5.5

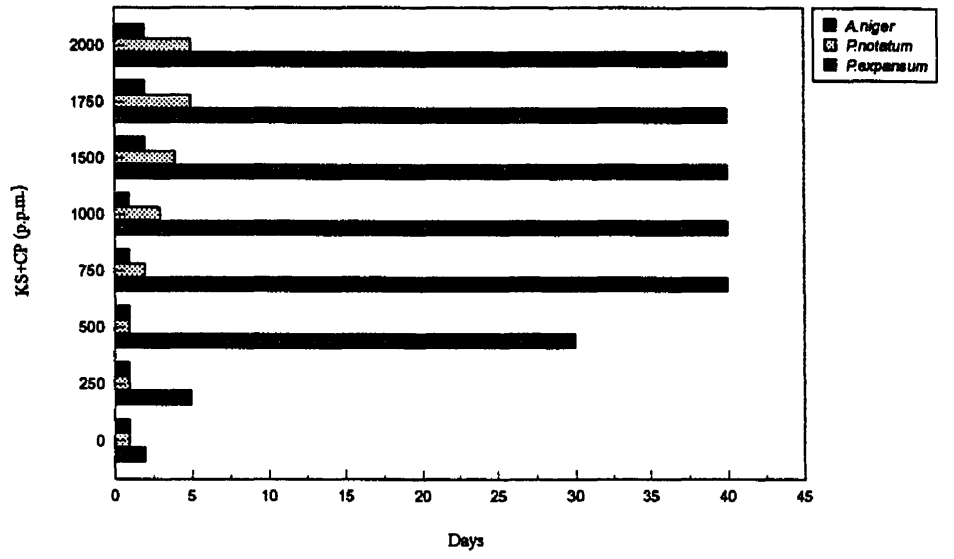

Figure 11: The antimicrobial action of equal levels of potassium sorbate and calcium propionate (KS + CP) on mold growth at pH 6.0

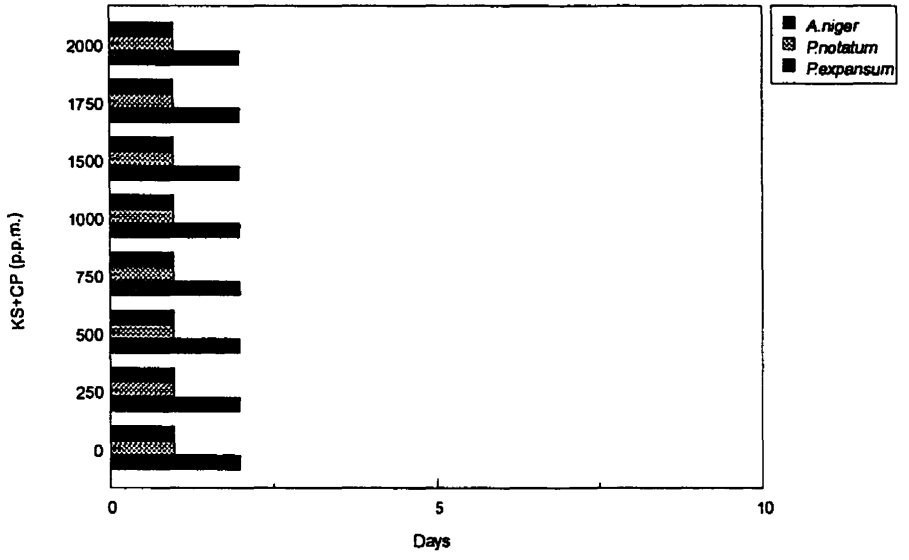

Figure 12: The antimicrobial action of equal levels of potassium sorbate and calcium propionate (KS+ CP) on mold growth at pH 7.0

2.3.4. The inhibitory effect of water activity (a_w) on mold growth on agar model system at pH 7.0 & 25 ^{O}C

The effect of a_w on the growth of *A.niger,P.notatum*, and *P.expansum* on PDA at pH 7.0 & 25 ^{O}C is shown in Figure 13. This pH was chosen since most antimicrobial agents are not effective at neutral or near neutral pH levels. Glycerol was chosen to reduce a_w since it has been shown to have no antimicrobial effect per se (Smith et al., 1987). It is evident that growth was influenced by a_w levels i.e., as a_w was reduced by the addition of glycerol, mold growth was delayed. In PDA at a_w 0.994 and at pH 7.0, growth of *A.niger* was visible after 1d at 25 ^{O}C. However, as a_w decreased from 0.994 to 0.923, mold growth was further delayed for 2-3 d at a_w 0.902. At a_w 0.872, growth of *A.niger* occurred after 3 d at 25 ^{O}C and after 4 d at a_w 0.854.

These results also showed that *P.notatum* and *P.expansum* were the most tolerant molds to decreased a_w. This may be due to differences in proteins and to changes in intracellular conditions such that the adverse effect of the environment is reduced (Avari and Allsopp, 1983). Both *P.notatum* and *P.expansum* were able to grow over the a_w ranges used in this study. Growth occurred on media after 1d at a_w 0.994 and after 2-3 d at a_w 0.872 and 0.854, respectively. For *P.expansum*, growth was visible after 1d at a_w 0.994 and after 3 d at a_w 0.872 and 0.854.

This study has shown that a_w affected the initiation of growth or the length of time before growth was visible. It is also evident that the minimum a_w of *Penicillium* was slightly higher compared to *Aspergillus* species. These results showed that molds can grow under a wide range of a_w and that a_w reduction alone would not be a viable approach to extend the mold free shelf life of baked products. Many mold species are tolerant of

reduced a_w when other factors such temperature, pH, and nutrients are optimal (ICMSF, 1980).

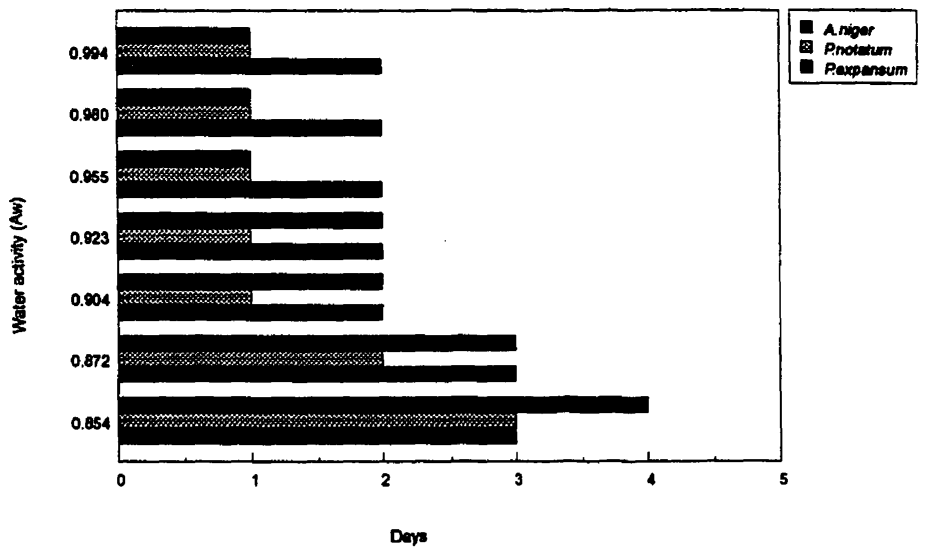

Figure 13: The antimicrobial effect of water activity (Aw) on mold growth at pH 7.0

2.3.5. Effect of MAP on mold growth in model agar system at pH 7.0 and 25 ^{O}C

The effect of modified atmosphere packaging on the growth of *A.niger*, *P.notatum*, and *P.expansum* in PDA plates at pH 7.0 are shown in Tables 26-28. The percentage of O_2, CO_2 and N_2 in the package headspace was the headspace gas composition of the packages when growth of *A.niger*, *P.notatum*, and *P.expansum* was visible.

Molds tend to vary in their tolerance of CO_2, with *Pencillium* species being more sensitive than *Aspergillus* species. For plates packaged in air, growth of *A.niger*, *P.notatum*, and *P.expansum* was evident in control plates (air packaged) after 1-2 d, respectively at pH 7.0 (Tables 26-28) . Addition of CO_2 (approximately 60 %) inhibited the growth of *A.niger*, with growth appearing within 5d in all plates. This agrees with previous studies that have shown growth of *A.niger* in agar plates packaged in 60% CO_2 to occurred within 5d (Smith et al., 1986). When plates were packaged in higher CO_2 levels (approximately 80%) growth appeared after 8d at 25 ^{O}C (Table 26).

Similar results were observed for both *P.notatum* and *P.expansum*. Growth of *P.notatum* was observed after 7d in PDA plates packaged in 60% CO_2, while growth was delayed for 10d in plates packaged in approximately 80% CO_2 (Tables 27). However, growth of *P.expansum* was delayed for ~ 30d in plates packaged in 60% CO_2 while no growth appeared in plates packaged in 80% CO_2 (Table 28). Several studies have reported that a concentration of 60% CO_2 has been used to extend the mold free shelf life of bakery product (Ooraikul,1991). Therefore, the earlier appearance of mold growth in this study maybe due to residual O_2 in the package headspace. The level of residual O_2 in

gas packaged products could be due to a number of factors such as (i) oxygen permeability of the packaging material, (ii) ability of the product to trap air, and (iii) leakage of air through poor sealing (Smith, 1994).

It is evident from the results obtained in this study that mold growth was suppressed when the level of O_2 decreased and CO_2 increased in the package headspace. These results are in agreement with the previous observations of Seiler (1989) who reported that the time (days) to detect visible mold growth increased as levels of CO_2 increased. The results also indicated that gas packaging can be used to extend the mold free shelf life of products. However aerobic spoilage i.e., mold growth can still occur in these products, depending on the level of residual oxygen, in the package headspace. It is clear that *A.niger* can tolerate and grow in low levels of O_2 resulting in mold spoilage. These results confirm the earlier findings of Smith et al. (1986) who observed that *A.niger* could grow in a gas packaged bakery product containing 60% CO_2 and low oxygen levels (<1% O_2). Dallyn and Everton (1969) also reported that *Xeromyces bisporus*, a xerophilic mold, which causes spoilage in food products with a low water activity, grew in an atmosphere of 90% CO_2 and 10% O_2. These authors also reported growth of *Aspergillus* species in 80% CO_2 and 2.5% O_2. Possibly the only means to reduce headspace oxygen in products to levels inhibitory to mold growth is through the incorporation of oxygen absorbents into the packaged products.

Table 26: Effect of gas packaging on the growth *A.niger* at pH 7.0 and 25 °C

Levels of CO_2 (%)	Days to visible growth of *A.niger*	* Headspace gas composition (%)		
		CO_2	O_2	N_2
Air	1	1.0	20.4	78.6
60	5	50	2.0	48
80	8	69.8	3.4	26.8

All values are the average of triplicates
* At onset of mold growth

Table 27: Effect of gas packaging on the growth of *P.notatum* growth at pH 7.0 & 25 °C

Levels of CO_2 (%)	Days to visible growth of *P.notatum*	* Headspace gas composition (%)		
		CO_2	O_2	N_2
Air	1	1.5	18.8	79.7
60	7	49.2	1.7	49.1
80	10	69.8	2.2	28

All values are the average of triplicates
* At onset of mold growth

Table 28: Effect of gas packaging on the growth of *P.expansum* growth at pH 7.0 & 25 °C

Levels of CO_2 (%)	Days to visible growth of *P.expansum*	* Headspace gas composition (%)		
		CO_2	O_2	N_2
Air	1	1.5	19.7	78.8
60	30	39	1.5	59.5
80	**NG	45	2.5	52.5

All values are the average of triplicates
* At onset of mold growth
**NG=No growth after (42d)

2.3.6. Effect of oxygen absorbents on mold growth

Oxygen is often a major factor limiting the shelf life of packaged food products. Such products are more susceptible to chemical, mold and bacterial spoilage. Oxygen absorbents are used extensively in the Japanese market to extend the chemical and microbial shelf life of products (Smith et al., 1986). However, few studies have examined the use of oxygen absorbents to control spoilage of bakery products. Removal of oxygen from the atmosphere of a packaged product using oxygen scavengers may be a viable method to inhibit mold spoilage (Smith et al., 1986). Furthermore, the combined use of oxygen absorbent and CO_2 enriched atmosphere could be used effectively to control mold growth of *A.niger* and *Pencillium* species which appear to be more resistant to elevated levels of CO_2 alone. However, this combination means additional costs to the processor. Therefore, preliminary studies were done to determine the effect of oxygen absorbents alone to control the growth of common mold contaminant of baked products.

The effect of oxygen absorbents on the growth of *A.niger*, *P.notatum*, and *P.expansum* in PDA plates packaged in high gas barrier film is shown in Tables 29-31 respectively. In control plates, growth of *A.niger*, *P.notatum*, and *P.expansum* was evident after 1-2d respectively. While the growth of *A.niger* and *P.notatum* was observed after 2d in plates packaged with one oxygen absorbent, the growth of *P.expansum* was completely inhibited for >40d in plates packaged with only one oxygen absorbent.

Addition of two oxygen absorbents inhibited the growth of all molds for >40d. This can be attributed to the effect of lower levels of headspace oxygen. Several studies have reported significant extension in the chemical and microbiological shelf life of intermediate and high moisture bakery products using oxygen absorbent technology (Abe

and Kondoh, 1989). More recently Powers and Berkowitz (1990) reported that two oxygen absorbents delayed the growth of *Aspergillus* species and *Penicillium* species for 30d while growth was extensive within 4d in plates packaged with one only oxygen absorbent which is in agreement with our observations.

A comparison between oxygen absorbent technology and gas packaging, showed that using oxygen absorbents was a more effective method for controlling the mold growth. These results confirmed the earlier observations of Smith (1994) who reported that oxygen absorbents were three times more effective than gas packaging for increasing the mold free shelf life of crusty rolls. These results have also demonstrated that the use of oxygen absorbents can give longer inhibition of mold growth compared to CO_2. It can be concluded from this study that the use of oxygen absorbents alone is a simple, effective method of controlling mold growth and extending the mold free shelf life of products.

Table 29: Effect of oxygen absorbent on the growth of *A.niger* at pH 7.0 & 25 °C

Number of Sachets	Days to visible growth of *A.niger*	* Headspace gas composition (%)		
		CO_2	O_2	N_2
Air	1	1.0	20.4	78.6
1	2	0.98	1.26	97.7
2	**NG	0.1	0	99.9

All values are the average of triplicates
* At onset of mold growth
**NG= No growth (>40d)

Table 30 : Effect of oxygen absorbent on the growth of *P.notatum* at pH 7.0 & 25 °C

Number of Sachets	Days to visible growth of *P.notatum*	* Headspace gas composition (%)		
		CO_2	O_2	N_2
Air	1	1.4	20.6	79
1	2	0.88	1.8	97.3
2	**NG	0.1	0	99.9

All values are the average of triplicates
* At onset of mold growth
**NG= No growth (>40d)

Table 31: Effect of oxygen absorbent on the growth of *P.expansum* at pH 7.0 &25 °C

Number of Sachets	Days to visible growth of *P.expansum*	* Headspace gas composition (%)		
		CO_2	O_2	N_2
Air	1	1.5	19.5	79
1	**NG	0.3	1.0	98.7
2	**NG	0.1	0	99.9

All values are the average of triplicates
* At onset of mold growth
**NG= No growth (>40d)

3.2.4. Combination effect of MAP and preservatives on mold growth

One of the main reasons for the preliminary gas packaging study was to extend mold free shelf life. However, gas packaging did not totally eliminate mold spoilage on an agar system, even with high levels of CO_2 and low levels of O_2. Therefore, preliminary studies were done to determine the inhibitory effect of MAP in combination with KS and equal levels of sorbate and propionate to control mold growth .

The levels of potassium sorbate and pH in the agar media and the packaging atmospheres used were selected on the basis of previous studies. The antimicrobial activity of potassium sorbate (1000 p.p.m.) in combination with different gaseous environments at pH 6.0 is shown in Figure 14.

Packaging in 60% CO_2 in conjunction with 1000 p.p.m. of KS delayed the growth of *A.niger* and both *Penicillium* species for >40d. This is probably due to the synergistic effect between potassium sorbate and CO_2. Several studies have shown that KS in combination with CO_2 was effective in inhibiting *Staphylococcus aureus* in meat (Tomlins and Gray, 1982). Smith (1988) observed that KS, in combination with CO_2, prevented the growth of *Aspergillus* species and *Penicillium* species in bakery products.

The combined effect of packaging in a CO_2 enriched atmosphere with equal levels of sorbate and propionate on the growth of *A.niger and P.notatum*, and *P.expansum* at pH 6.0 is shown in Figure 15. Again, growth of *Aspergillus* and *Penicillium species* was completely inhibited for >40d in plates packaged in either 60% or 80% CO_2 at pH 6.0 with equal levels of sorbate and propionate i.e., superior results when either KS or equal mixtures of KS and CP or CO_2 were used alone.

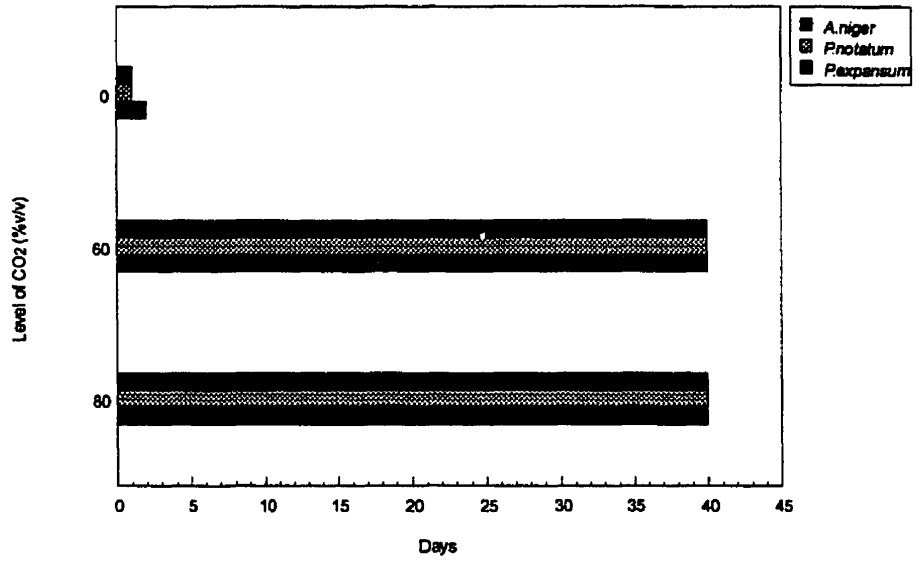

Figure 14: The combined effect of KS (1000 p.p.m.) and CO2 on mold growth at pH 6.0

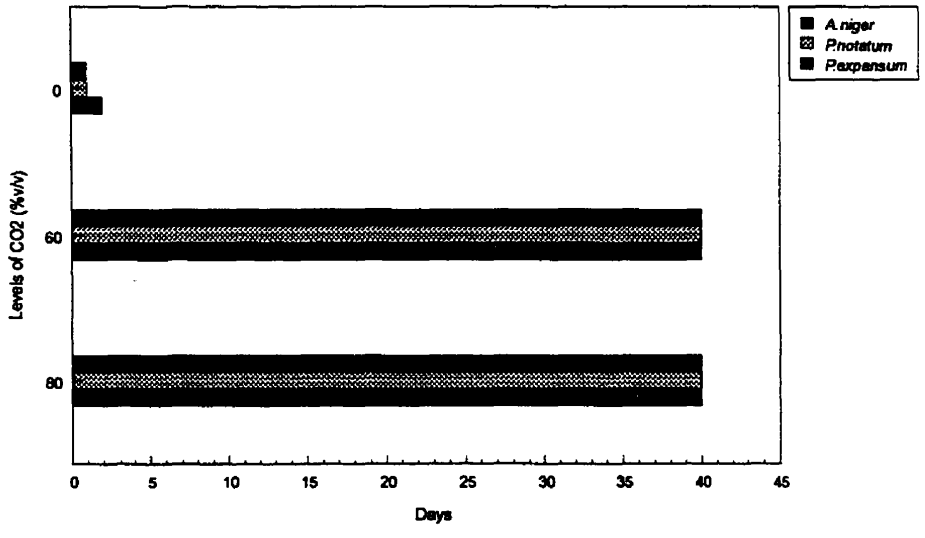

Figure 15: The combined effect of equal levels of KS and CP (1000 p.p.m.) and CO2) on mold growth at pH 6.0

Conclusion

In conclusion, methods to control mold growth in foods include chemical preservatives, water activity reduction, and modified atmosphere packaging. This study has clearly shown that preservatives can be used effectively to retard mold growth and that the effectiveness of these preservatives is enhanced by increased presence of the undissociated form of the chemical, achieved at low substrate pH. Since metabolic activity and mold growth depends on available free water, reduced water activity (a_w) could also be used to control the growth of *A.niger* and *Penicillium* species. Minimum a_w levels allowing growth can vary depending on the type and strain of mold. However, a_w reduction may also result in textural changes to the product and is not a viable method to extend the mold free shelf life of bakery products.

The use of gas packaging is, without doubt, one of the most exciting interactive packaging technologies available to the food industry. This study shows that MAP comprised of 60% or 80% CO_2 (balance N_2) can be used to inhibit mold growth. However, oxygen absorbents, alone can also be used to inhibit mold growth for >40d. Furthermore, the efficiency of oxygen absorbent to inhibit *A.niger* and *P.notatum* can be achieved by using two absorbents in the package. The use of oxygen absorbent technology offers to the food industry a viable alternative to gas flushing for shelf life of foods (Smith et al., 1986).

Generally, a combination of chemical preservatives and other preservation method is needed to control biological deterioration and extend the shelf life of food products. Therefore, the combination of preservatives with MAP could result in a longer mold free shelf of products, particularly in gas packaged products where low levels of residual

headspace O_2 could support mold growth and limit shelf life. The major factors limiting the use of this combined technology is (i) additional cost and (ii) consumer concern about preservatives.

CHAPTER 3
USE OF RESPONSE SURFACE METHODOLOGY IN SHELF LIFE EXTENSION STUDIES

3.1. INTRODUCTION

Mold spoilage of baked products is of considerable economic importance to the bakery industry. Numerous studies have been done to prevent mold growth by controlling factors influencing growth. In previous studies, factors affecting mold growth were thoroughly investigated. Factors investigated included pH, concentration of inhibitors (potassium sorbate and calcium propionate) and modified atmosphere packaging (MAP) involving CO_2 enriched atmospheres and oxygen absorbent technology. The influence of pH, inhibitor, and CO_2 enriched atmospheres on mold growth has usually been investigated by evaluating the variables individually (Seiler, 1978; Bogadtke, 1979; Pomeranz, 1969). The disadvantages of using the one variable at a time approach are: (i) laborious and time consuming, (ii) it gives large quantities of data which are difficult to interpret and (iii) it fails to measure interaction effects (Smith et al., 1988).

To overcome the limitations of the one variable at a time approach, and to adequately describe the effect of several environmental factors, including their important interactions, a technique involving factorial experimental designs and multiple regression analyses termed Response Surface Methodology (RSM) was used in this study. The main advantage of RSM is that it permits factors of interest to be examined, not simply one at a time, but simultaneously in an experimental design. Mathematical models, generally first order and second order polynomials are generated to define the optimal levels of the most significant factors to give the desired response (Box et al., 1978). Developed initially for

process optimization studies in chemical engineering, RSM has recently been applied to extend the shelf life of bagels (Assouad, 1996) and aflatoxin production in MAP peanuts (Ellis et al ., 1994).

Objective

The objective of this study was using an RSM approach to determine the optimal levels of CO_2, pH and concentration of inhibitor (potassium sorbate) to prevent the growth *of Aspergillus niger*, the common and most resistant mold contaminant of bakery products.

3.2. MATERIALS AND METHOD

3.2.1. Experimental design

To determine the effect of pH, potassium sorbate, and CO_2 enriched atmospheres simultaneously on the growth of *A.niger* in an agar model system, a 3 factor, 5 level central composite rotatable design (CCRD) was used (Box et al., 1978). The levels of each factor used in the study included pH (5.5-7.5), concentration of inhibitor, potassium sorbate (250-1250 p.p.m.), and CO_2 (0-80% v/v). The values of each level of environmental factor selected were based on the results of the previous study (Chapter 2). The CCRD design and coded levels of each factor used in this design are shown in Table 32. To facilitate statistical analysis, variable levels were coded -2, -1, 0, +1, +2. The coded and actual values of levels used in the CCRD are shown in Table 33.

3.2.2. Adjustment and inoculation of media

Potato Dextrose Agar was again used as the basal medium throughout this study. Potassium sorbate (10% w/v) was added in appropriate amounts as preservative to give concentrations ranging from 250-1250 p.p.m. Media were autoclaved at 121 ^{o}C for 15 min and the pH adjusted after cooling with 1 N Lactic acid or 1 N NaOH. Measurement of pH was done as described previously in (Chapter 2, section 2.2.2). The adjusted media were then dispensed into 150X150 mm Petri dishes (Fisher Scientific).

A.niger, a common contaminant of bakery products and the most resistant mold to the antimicrobial action of preservatives, was chosen as the test mold. All plates were inoculated with 10^4 spores/ml of *A. niger* using the enumeration method as described in Chapter 2, section 2.2.1. All inoculated plates were then packaged in 210 x 210 cm high gas barrier Cryovac bags (Cryovac Div., W.R. Grace & Co. Canada Ltd., Mississauga, Ontario, Canada). Bags were flushed with various levels of CO_2 (balance N_2) ranging from 0 to 80% using a Multivac chamber type, heat seal packaging machine (Model KM100-3M). All packages were incubated at 25^{0}C and examined daily for visible signs of mold growth.

3.2.4. Statistical analysis

Statistical analysis (regression coefficient, analysis of variance) were computed using the Statistical Analysis System (SAS, 1988). All 3 dimensional graphs were done using the SAS/Graph program and Word Perfect Graph on a McGill University IBM computer.

Table 32: Coded level combinations for a 3 variable Central Composite Rotatable Design (CCRD) to control the growth of *A.niger*

Run No.*	**Variables		
	X_1	X_2	X_3
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-2	0	0
10	2	0	0
11	0	-2	0
12	0	2	0
13	0	0	-2
14	0	0	2
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

*** Each run done in duplicate**

****X1= pH; X2= potassium sorbate (p.p.m.); X3=CO_2 (%v/v)**

Table 33: Values of coded levels used in the CCRD

Variables	Levels				
	-2	-1	0	1	2
pH	5.5	6.0	5.0	7.0	7.5
*KS (p.p.m.)	250	500	750	1000	1250
** CO_2 (%v/v)	0	20	40	60	80

*KS=Potassium sorbate (p.p.m.)

** (Balance N_2)

3.3. RESULTS AND DISCUSSION

In this study, an RSM approach was used to determine the optimum levels of pH, inhibitor, and CO_2 to inhibit the growth of *A.niger*. A 2^k+2k+n CCRD was used as the experimental design (Table 32) and the actual levels of variables used in each experimental run are shown in Table 33. The combined effects of inhibitor, pH, and CO_2 on the growth of *A.niger* on PDA plates packaged in a high gas barrier film are shown in Table 34. Mold growth was either completely inhibited or was visible after 3 d (Table 34). Generally, growth was visible after 3-5d in plates containing preservatives at higher pH values (pH 6.0-7.5) and packaged in a low level of CO_2 (20%). Growth of *A.niger* was delayed for 10-40d in certain treatment combinations i.e., plates containing lower levels of inhibitor and pH (500 p.p.m., pH 6.0) but packaged in higher levels of CO_2 (60%). However, it is widely accepted that microorganisms show greatest tolerance to a single environmental factor, such as CO_2, pH or temperature when all other conditions are optimal for growth (ICMSF, 1980). Conversely, two or more environmental conditions will be more inhibitory than each parameter considered separately. In this study, mold growth was completely inhibited when pH was reduced and level of KS increased or when the CO_2 concentration was increased from 20% to 80%. Thus inhibition can be attributed to the synergistic effect of several hurdles on mold growth .

Table 34: Effect of pH, inhibitor, and gas atmosphere on mold growth

Run No.*	Variables			Days to visible mold growth
	X_1(pH)	X_2(KS)	X_3(CO_2)	
1	6	500	20	4
2	7	500	20	3
3	6	1000	20	5
4	7	1000	20	4
5	6	500	60	35
6	7	500	60	13
7	6	1000	60	NG**
8	7	1000	60	29
9	5.5	750	40	20
10	7.5	750	40	6
11	6.5	250	40	6
12	6.5	1250	40	13
13	6.5	750	0	6
14	6.5	750	80	NG**
15	6.5	750	40	8
16	6.5	750	40	9
17	6.5	750	40	8
18	6.5	750	40	7
19	6.5	750	40	8
20	6.5	750	40	8

* Each run done in duplicate

** NG= No visible growth after 42 days

To quantify the combined synergistic effect of pH, inhibitor, and CO_2 on the growth of *A.niger*, second order models were generated from the multiple regression analysis of the uncoded as shown in Equation 1:

$$\mathbf{Y=224.70-68.09*X_1-0.01*X_2+1.07X_3+6.09*X_1*X_1-0.006*X_2*X_1+0.0002*X_2*X_2-0.43*X_3*X_1+0.0005*X_3*X_2+0.02X_3*X_3}$$

The analysis of variance (ANOVA) for this design is shown in Table 35. The ANOVA for Y, days to visible growth, indicated that the model was highly significant ($P<0.0005$) and had an R^2 value of 0.78. Examination of the fitted coefficients using the F-test showed that the two linear terms pH (X_1) and CO_2 (X_3), one quadratic term (CO_2^2, X_3^2) and one interaction term pH*CO_2 (X_1*X_3) were highly significant, and therefore influenced the growth of *A.niger*. Similar trends were observed by Smith (1988) who reported that pH, inhibitor and CO_2 had a significant effect on mold growth. However, the significant effect of pH in this study was contrary to the observations of Seiler (1965) who reported that molds could grow over a pH range of 5-7.5 in cake. He concluded that pH had little or no effect to control mold spoilage of bakery products. The lack of significance of inhibitor (potassium sorbate) in this study can also be attributed to pH of the test medium since sorbate has been shown to be more effective at more acid pH values than at neutral pH values where it is more dissociated (Monsanto Technical Bulletin, 1984). The results are again consistent with the previous observations of the increased inhibitory effect of potassium sorbate at lower pH values.

Table 35: Analysis of variance for (2^K+2k+n) factorial design of factor influencing growth of *A.niger*

Source	d.f	Sum of	Mean	*F*
Model	9	5503.71	611.52	12.46***
X_1	1		413.28	8.42**
X_2	1		26.60	0.46^{ns}
X_3	1		1237.53	25.22***
X_1*X_1	1		0.11	0.00^{ns}
X_2*X_2	1		28.25	0.58^{ns}
X_3*X_3	1		3373.64	68.75***
X_1*X_2	1		0.15	0.00^{ns}
X_1*X_3	1		297.56	6.6**
X_2*X_3	1		126.56	2.5^{ns}
Error	30	1472.06	49.06	
Total	39	6975.77		
R^2	0.78			

Level of significance *** P<0.0005
Level of significance ** P<0.005
Level of significance * P<0.05
ns= not significance

Only the significant variables influencing mold growth were used to generate 3 dimensional response surface graphs of days to visible mold growth (shown in Equation 2).

Equation 2: $\mathbf{Y= -38.1+10.0*X_1+2.0*X_3+0.01*X_3*X_3-0.0.43X_1*X_3}$

Examples of response surface graphs for CO_2 vs KS (with pH held constant at 5.5-7.0) and for pH vs CO_2 (with inhibitor held constant at 500-1000 p.p.m.) and pH vs KS (with CO_2 held constant at 40-80% v/v), and their effect on mold free shelf life are shown in Figures 17 -25 respectively. As these figures illustrate, days to visible mold growth increased by increasing both CO_2 and KS concentrations and decreasing pH. For example, mold growth was delayed for 52d on PDA plates containing 1240 p.p.m., KS at pH 5.5 and packaged with 80% CO_2 (Figure 16). However, the ability of KS and CO_2 to control mold growth decreased slightly when pH of the medium increased i.e., pH 6.0 and 6.5, with mold growth being visible after 40 and 27d on the PDA plates containing 1240

p.p.m. KS and packaged with 80% CO_2 (Figures 17-18). Mold was completely inhibited for >52d by adjusting pH to 5.5 and adding KS (500-1000 p.p.m.) and packaging plates in 80% CO_2. It is evident from Figures 22-24 that *A.niger* was inhibited for 18d in plates containing 1240 p.p.m., KS and packaged with 40% CO_2 at pH 5.5. However, shelf life can be extended for 31 and 52d with similar levels of sorbate and pH value, and packaged with increasing levels of CO_2 i.e., 60% and 80% CO_2. It is evident from Figures 22-24, that the inhibitory effect of KS was enhanced when the pH decreased. These results are also in agreement with previous studies reported in the literature (Smith et al., 1988).

The response surface graphs shown are examples of a saddle point where the optimum response is either a long the sides or in one or more of the four corners (Box et al., 1978). Canonical analysis for this set of experimental data indicated that the stationary point on the fitted surface was neither minimum or maximum. The actual values of variables at the stationary point are shown in Table 36. All of these values are well within the experimental range used in this study. The predicted value at the stationary point was a mold free shelf life of ~7 d. When these variables were tested in vitro, the actual shelf life obtained was ~ 6 d, indicating the reliability of the model to predict mold free shelf life.

Table 36 : Actual values of variables at stationary point *X_0 (Point of mold inhibition)

Variable	Actual Value (%)
pH (X_1)	6.3
KS (X_2)	1225 p.p.m.
CO_2 (X_3)	23.7

Balance N_2

* Predicted shelf life at X_0=7.1d.

CONCLUSION

In conclusion, this study has shown that RSM approach is an elegant statistical technique when several variables are to be examined simultaneously. This study also has shown that by using RSM, the manufacturer can use several factors to control mold spoilage and achieve a longer shelf life would normally be possible than by using the one variable at a time approach.

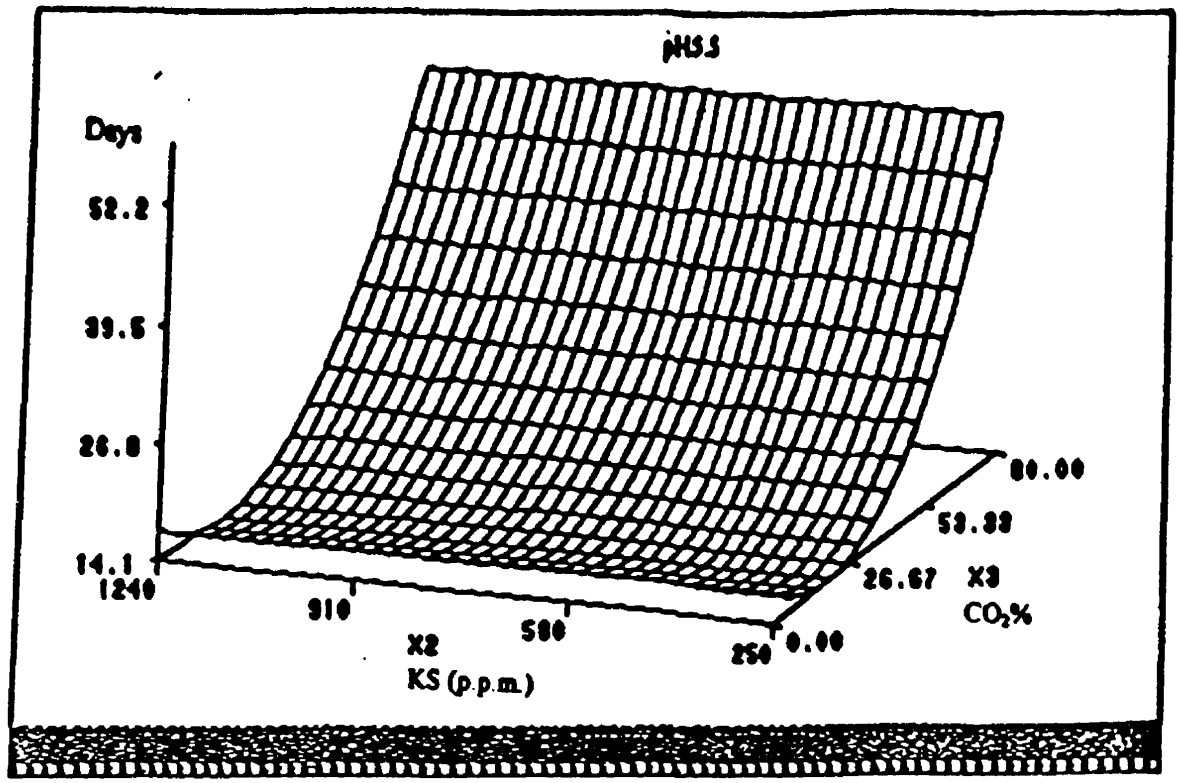

Figure 16: Three dimensional response surface plot showing the effect of potassium sorbate (KS) and CO_2 with pH held constant at 5.5 on the growth of *A.niger*.

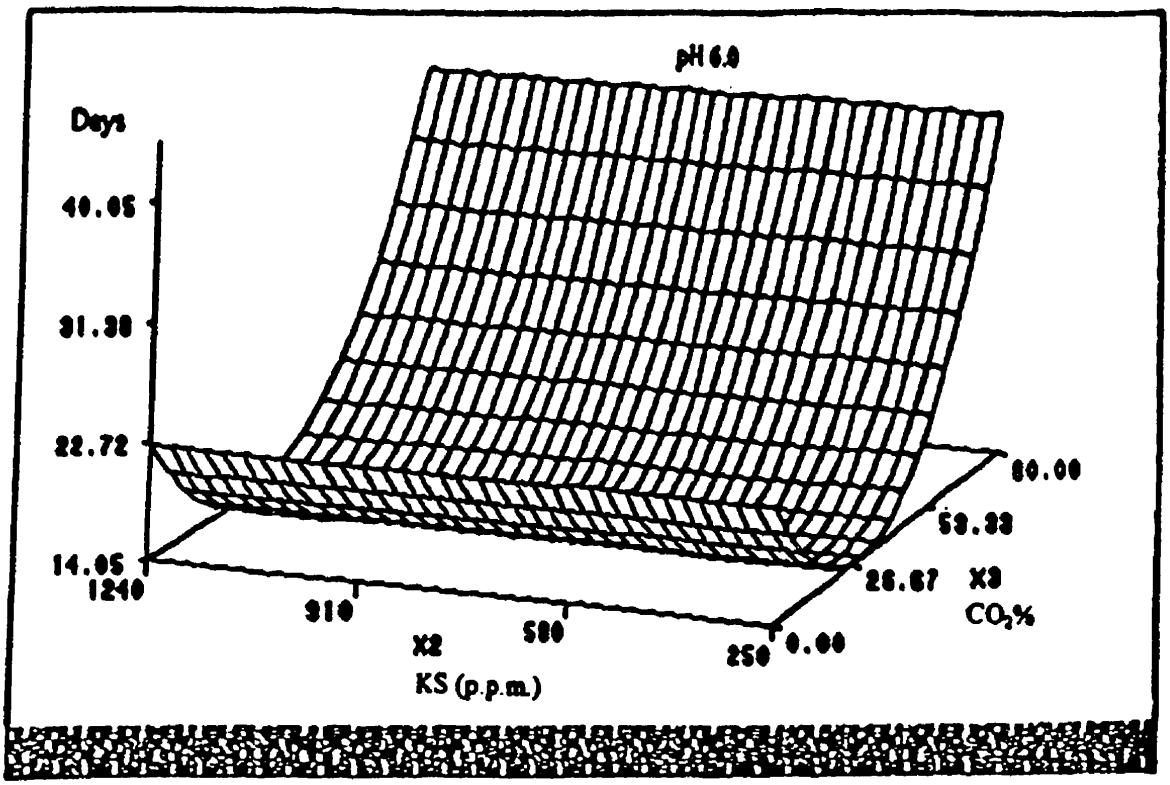

Figure 17: Three dimensional response surface plot showing the effect of potassium sorbate and (KS) CO_2 with pH held constant at 6.8 on the growth of *A.niger*.

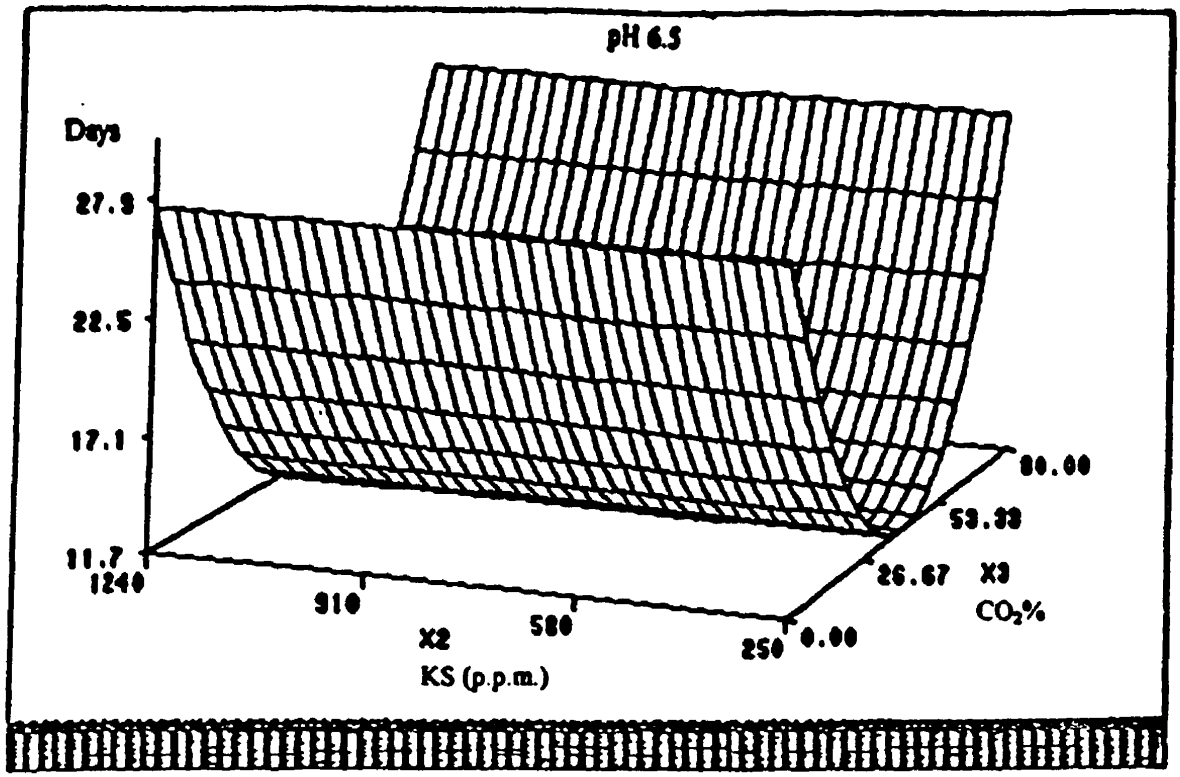

Figure 18: Three dimensional response surface plot showing the effect of potassium sorbate and (KS) CO_2 with pH held constant at 6.5 on the growth of *A.niger*.

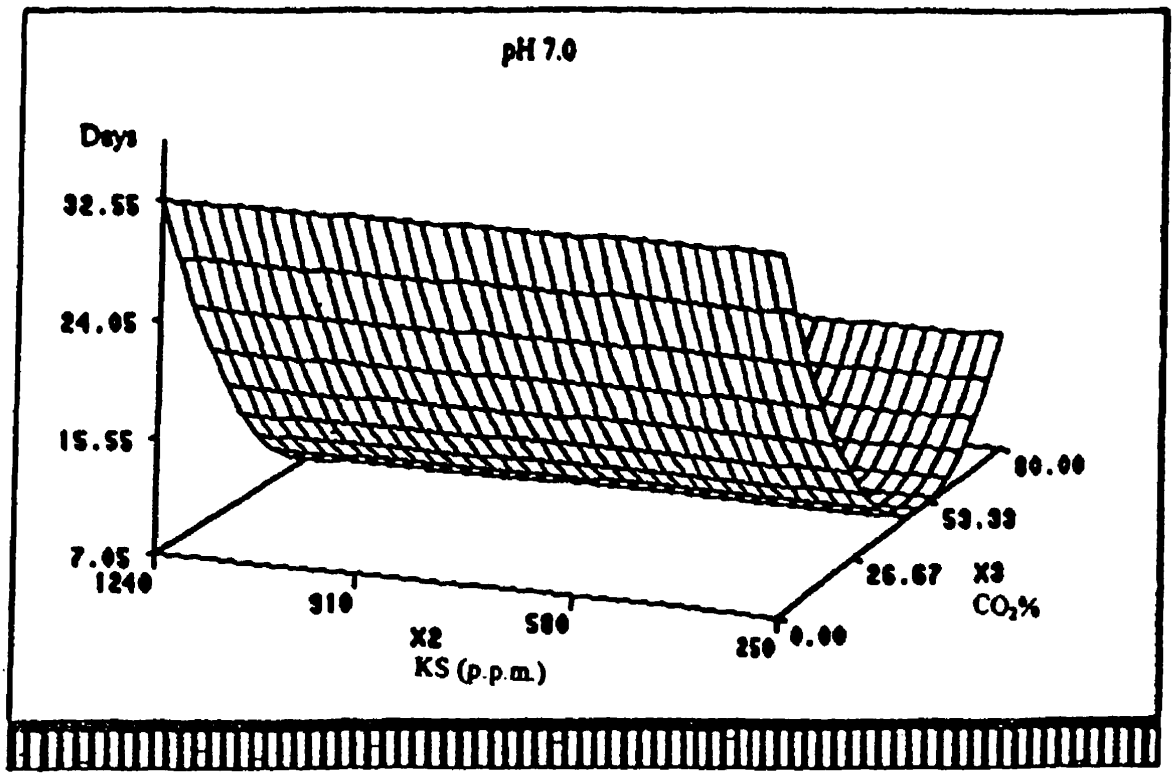

Figure 19: Three dimensional response surface plot showing the effect of potassium sorbate (KS) and CO_2 with pH held constant at 7.0 on the growth of *A.niger*.

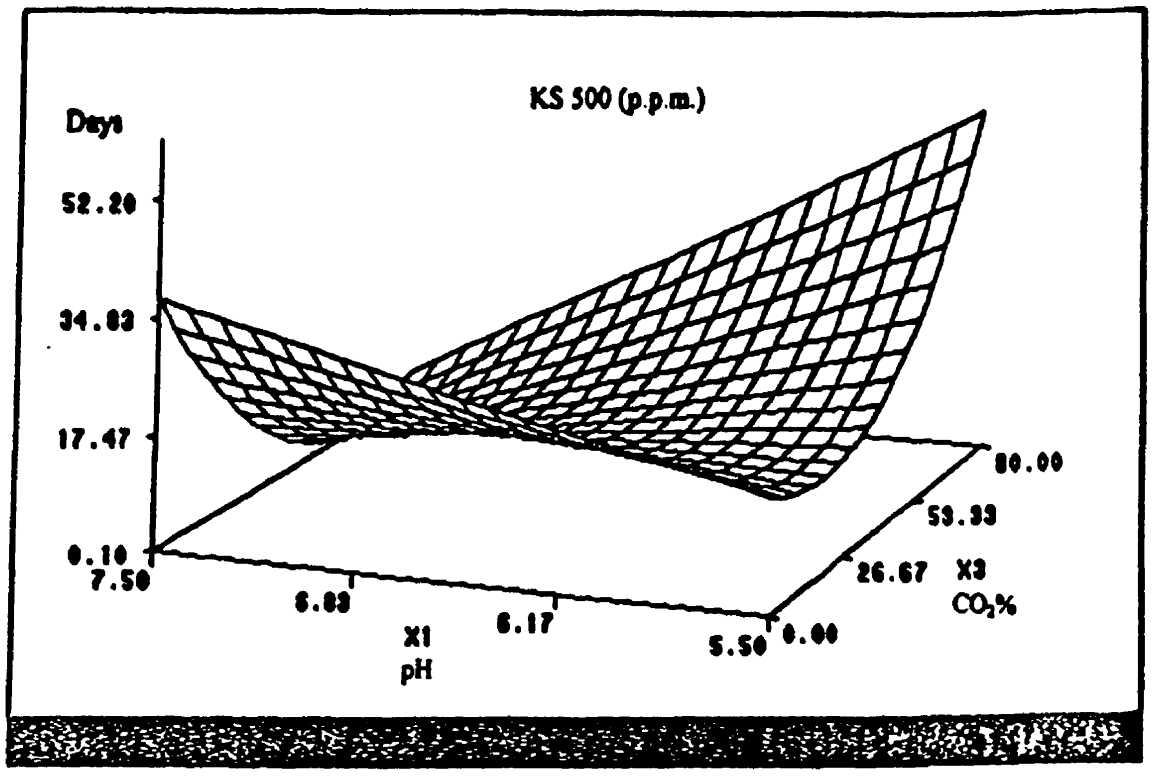

Figure 20: Three dimensional response surface plot showing the effect of pH and CO_2 with potassium sorbate (KS) held constant at 500 p.p.m. on the growth of *A.niger*.

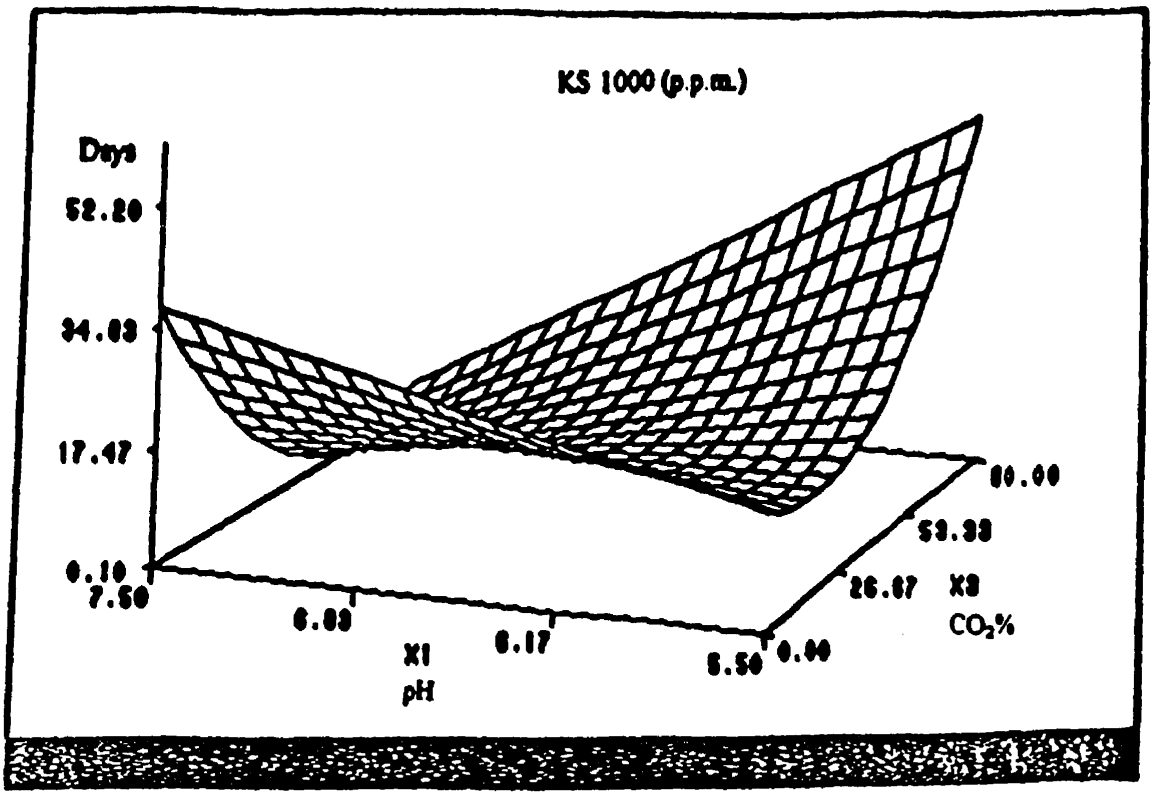

Figure 21: Three dimensional response surface plot showing the effect of pH and CO_2 with potassium sorbate (KS) held constant at 1000 p.p.m. on the growth of *A.niger*.

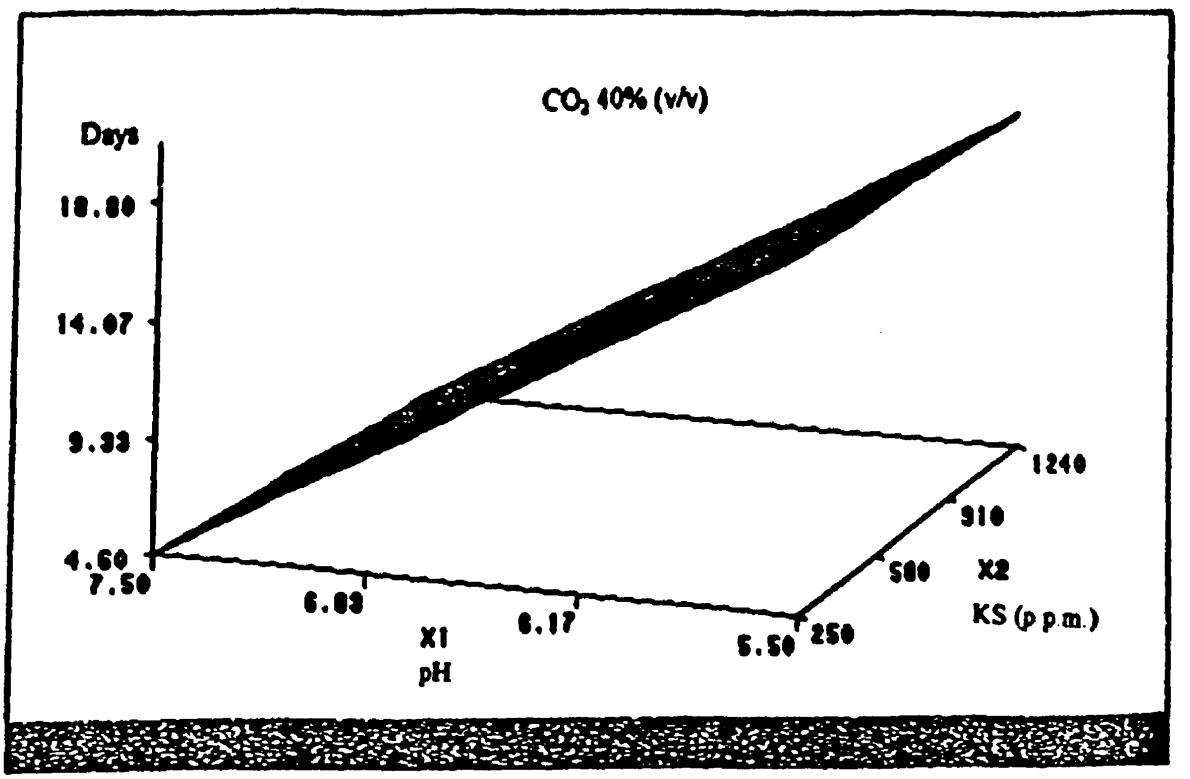

Figure 22: Three dimensional response surface plot showing the effect of pH and potassium sorbate (KS) with CO_2 held constant at 40% (v/v) on the growth of *A.niger*.

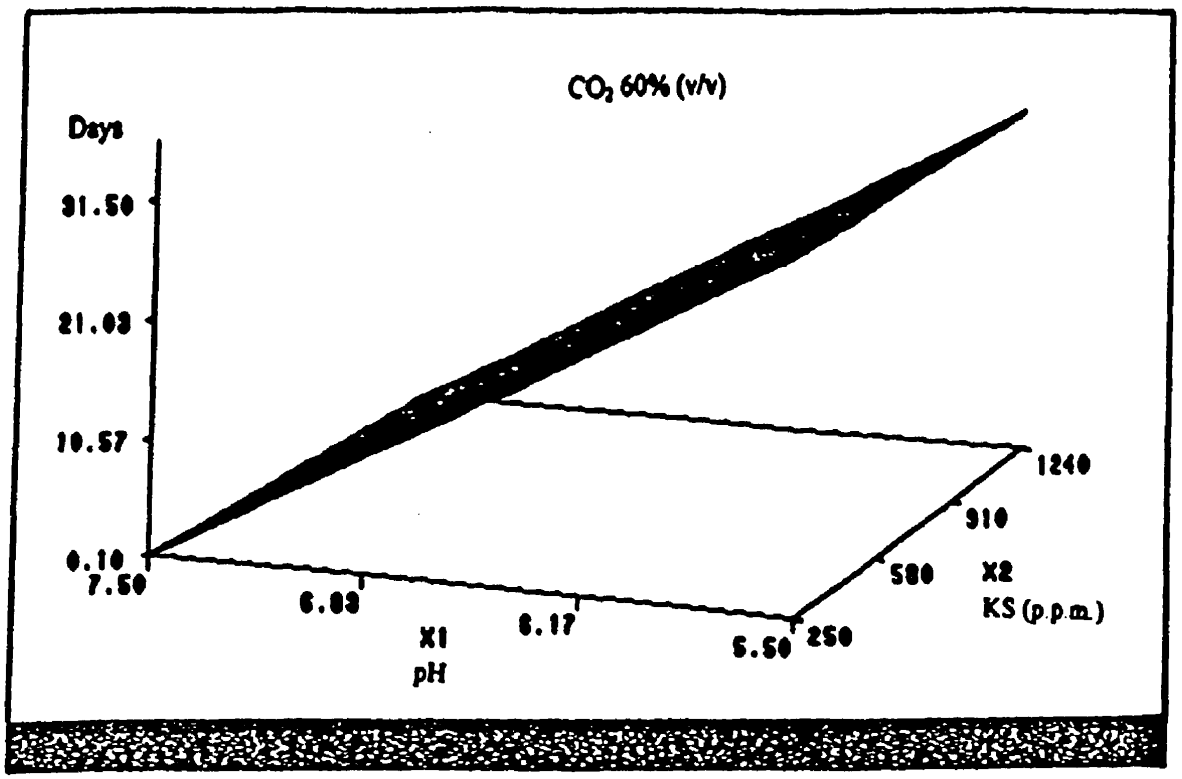

Figure 23: Three dimensional response surface plot showing the effect of pH and potassium sorbate (KS) with CO_2 held constant at 60% (v/v) on the growth of *A.niger*.

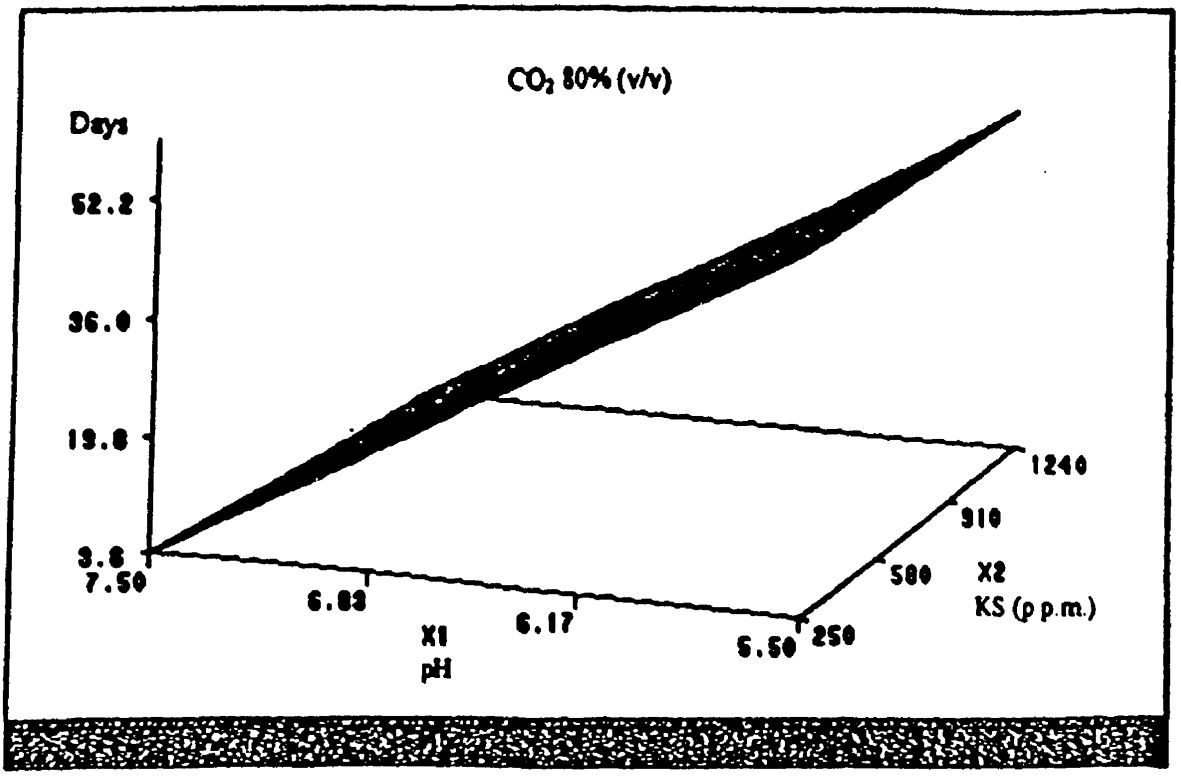

Figure 24: Three dimensional response surface plot showing the effect of pH and potassium sorbate (KS) with CO_2 held constant at 80% (v/v) on the growth of *A.niger*.

CHAPTER 4

SHELF LIFE EXTENSION OF PIZZA CRUSTS

4.1. INTRODUCTION

Pizza crusts are yeast leavened bakery products made from flour and water and have an $a_w \sim 0.954$ and a pH ~ of 6.2. Since they are high moisture products which are not completely sterilized on baking, post-contamination is unavoidable and mold spoilage can occur within a few days. In order to extend shelf life, pizza crusts have been traditionally marketed frozen and stored in a frozen display case for retail sale (Dickson, 1987). In an effort to reduce production and storage costs sales, associated with freezing, this study was done to examine the use of both preservatives and MAP to extend the mold free shelf life of partially baked pizza crusts at ambient storage temperature.

Objectives

The specific objectives of this study were:

To determine the effect of various methods of application of potassium sorbate to inhibit mold growth on pizza i.e., direct incorporation into batter, surface spraying, and impregnation of packaging material with potassium sorbate; to determine the effect of MAP on mold growth of pizza crusts; and to determine the effectiveness of potassium sorbate in combination with MAP on mold growth on pizza crusts.

4.2. MATERIALS AND METHODS

4.2.1. Ingredients

The ingredients used in pizza crust formulation consisted of wheat flour, yeast, sugar, salt, water, and oil as shown in Table 37 .

Table 37: Ingredients for a standard pizza dough recipe.

Basic ingredients	Brand Name
All Purpose Flour	Robin Hood
Sugar	Lantic
Dried Yeast	Fleischman's
Salt	Windsor
Potassium sorbate	Monsanto
Vinegar	Allen's
Vegetable oil	Crisco

4.2.2. Pizza dough formulation

A standard pizza recipe, obtained from (Lindsay, 1984) was used as the standard recipe for this study. Ingredients, based on a percentage of flour weight basis, are shown in Table 38. This formula produced 12 slices of pizza per batch of standard dimension (9 X 8.5 X 1.5 cm) which weighed ~ 50g.

Table 38: Standard pizza recipe

Ingredients	*Percentage	Weight (g)
Flour	100	400
Water	62.50	250
Yeast	3.62	14.5
Sugar	1.05	4.2
Salt	1.12	4.5
Oil	5.75	23

*Based on a flour weight basis

4.2.3. Standard pizza preparation

To prepare the pizza dough, flour was dissolved in warm water, yeast added and the mixture left to stand for 10 minutes until frothy. All other ingredients were then added to a Kitchen Aid mixer and mixed at medium speed for 3 mins until the dough was properly developed i.e., tacky. The dough was then removed from the mixing bowl and kneaded for ~ 3 mins to incorporate enough of the remaining flour to make a smooth elastic dough. The dough was then rolled out using a rolling pin to make a 5 mm thick shell and transferred to a lightly oiled baking sheet. The dough was then left for 15 mins to rise and then baked for 5 mins in a convection oven at 177 ^{o}C (Garland Convection Oven (T) TE-3,4CH, Commercial Ranges Ltd., Mississauga, Ontario) . After baking, the pizza dough was cooled to room temperature and divided into 12 slices, each weighing 50 g with the following dimensions L= 9 cm, b=8.5, and h=1.5 cm. A flow diagram of pizza crust production is shown in Figure 25.

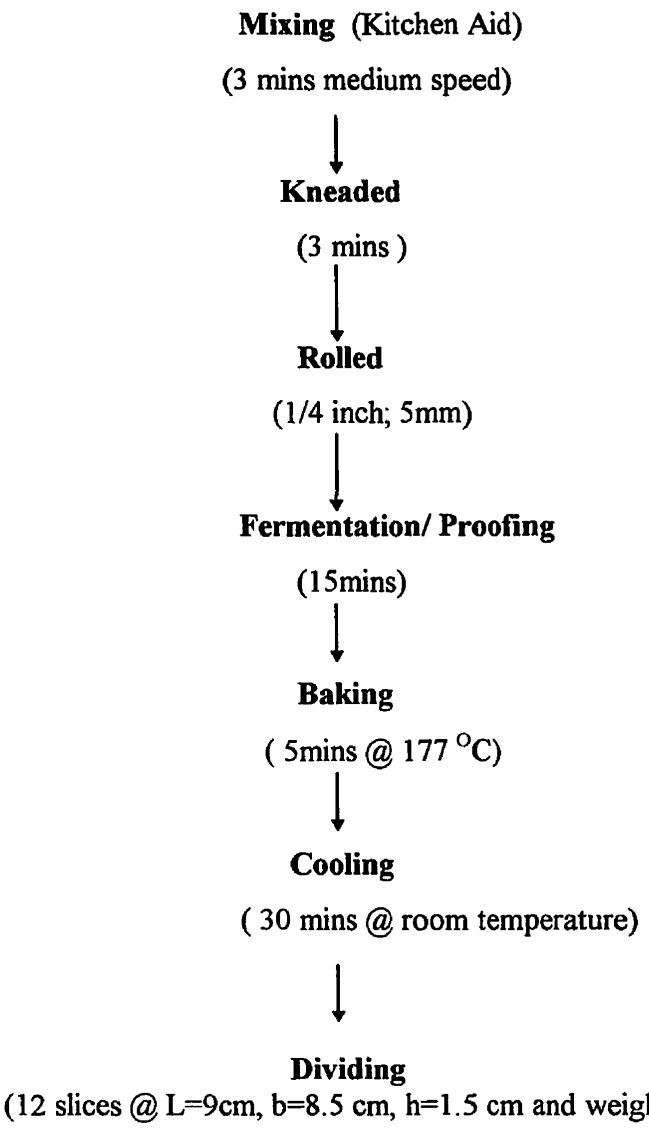

Figure 25: Pizza dough preparation

4.2.4. Preparation of inoculum

A mold strain of *Aspergillus niger* (*16404*) was obtained from American Type Culture Collection (ATCC). The culture was grown on Potato Dextrose Agar (PDA)(Difco, Michigan,USA) at 27 °C and transferred every three weeks to ensure

viability. The inoculum was prepared by growing *A.niger* on PDA at 27 ^{o}C for 5 to 7d until sporulation had occurred. Spores of *A.niger* were harvested by dislodging spores into 9 ml of 0.1% (v/v) peptone water containing 1-2 drops of Tween 80 to prevent spore clumping. Spores were then enumerated using an Improved Neubauer Haemacytometer (Fisher Scientific) and then diluted to give a spore inoculum of 10^4 spores/ml.

4.2.5. Methods of addition of Potassium sorbate

4.2.5.1. Preparation of potassium sorbate

A 40% stock solution of potassium sorbate (KS) was prepared by dissolving 40 g into 100 ml water and stirring for approximately 10 min to obtain a 40% w/v of KS. The potassium sorbate was then filtered and sterilized as described previously in section 2.2.2.1 of Chapter 2.

4.2.5.2. Direct incorporation of potassium sorbate (KS) into the pizza dough

In this study, potassium sorbate (KS) was incorporated directly into the various batches of pizza dough to give final concentrations of 1000 and 2000 p.p.m. in each dough (based on flour weight basis). The pH of pizza dough was adjusted to 5.5 and 6.0 using 5% acetic acid . Similar levels of distilled water were added to the control samples. Pizza dough was then shaped, baked and cooled as described previously (Chapter 2, section 2.2.2).

4.2.5.3. Surface application of potassium sorbate (KS)

To determine the effect of surface application of KS on mold growth, pizza dough was prepared as described previously and the pH adjusted to 5.5 and 6.0 using 5% acetic acid prior to baking. In this trial, appropriate amounts of a 40% stock solution of potassium sorbate were sprayed on to the surface of the pizza crust slices to give final concentrations of 1000 and 2000 p.p.m, again based on flour weight. Similar levels of distilled water were sprayed directly onto the surface of control samples. All crusts were air dried at ambient temperature prior to inoculation.

4.2.5.4. Impregnation of packaging material with potassium sorbate

In this study, pizza dough was prepared as described previously and the pH adjusted to 5.5 and 6.0 using 5% acetic acid. Appropriate amounts of stock solution equivalent to (1000 & 2000 p.p.m.) were then sprayed directly onto the surface of 1.5 x1.5 m of aluminum foil i.e., sufficient to cover the whole pizza crust and air dried for 15 min. Similar amounts of distilled water were sprayed onto the aluminum foil for control samples. All treated foil was dried at room temperature prior to wrapping the pizza crusts.

4.2.6. Inoculation and packaging

Pizza crusts were either inoculated with *A.niger*, the most common contaminant of bakery products or indirectly through airborne contamination. Indirect contamination was achieved by leaving slices exposed to air for ~30 mins at room temperature (25 ^{O}C). For inoculated crusts, each slice of pizza crust was inoculated with a mold suspension containing ~ 10^4 spores/ml as described in (Chapter 2.0 section 2.2.2) or with an

equivalent amount of distilled water (control). For pizza with KS added directly to the dough or sprayed on the surface, slices were packaged in high gas barrier Cryovac bag in triplicate. All slices were stored at 25 ^{O}C and examined daily for visible signs of mold growth. In the last experiment, the whole pizza crust was overwrapped with the dried, impregnated aluminum foil. All slices were stored at 25 ^{O}C. All slices were unwrapped and examined daily for visible signs of mold growth.

4.2.7. Effect of modified atmosphere packaging on mold growth on pizza crusts

In this study, pizza crust was again prepared and adjusted to pH 6.0 as described previously. All slices of pizza were either inoculated with 10^4/ml spores of *A.niger* or inoculated indirectly as described previously (Chapter 2.0 section 2.2.2) or with a similar level of distilled water (Control). Packaging and sealing of packages was done using a Multivac type vacuum/gas packaging unit (model AG500) (W.R. Grace and Co., Ajax, Ont. Canada) . Pizza slices were packaged in 210 x 210 Cryovac bags (O_2 transmission rate 3-6 cc/m^2 /24 hr, atm @ 4.4 ^{O}C, 0% RH) under various gas atmospheres i.e., with 60% and 80% CO_2 (balance N_2). A Smith's proportional mixer (Tescom, Minneapolis, Minnesota) was used to give the desired proportions of CO_2 and N_2 in each package. Anaerobic conditions (0% O_2) were obtained and maintained within the package by placing an Ageless type FX-100 oxygen absorbent (Mitsubishi Gas Chemical Co., Japan) inside the appropriate packages prior to gas packaging and sealing.

4.2.8. Combined effect of potassium sorbate and MAP

Pizza crusts were prepared as described previously and the pH adjusted to 5.5 and 6.0 with 5% acetic acid. Potassium sorbate was incorporated directly into the dough to give concentrations of 1000 p.p.m. based on flour weight basis. Pizza crusts were inoculated with either 10^4 spores/ml of *A.niger* as described previously (Chapter 2.0 section 2.2.2) or with appropriate amounts of distilled water (Control) or left uninoculated. All pizza slices were packaged under the following conditions: Air (control), CO_2:N_2 (60:40); and (80:20). All samples (control and inoculated) were stored at 25 ^{O}C and examined daily for visible signs of mold growth.

4.2.9. Headspace gas analysis

Headspace gas analysis was done immediately after packaging to ensure that the desired concentration of gases had been obtained and to monitor changes at the end of the storage trial i.e., when visible signs of mold growth occurred. Gas sampling and headspace gas analysis was done by gas chromatography as described in Chapter 2 section 2.2.7.

4.2.10. pH measurement

The pH of the pizza crusts was measured using a previously calibrated (pH 4.0 and pH 7.0) Corning pH meter (Model 2220, Corning Glass Works, Corninig N.Y.). A 1:2 slurry of the pizza crusts was made by combining 15 g of sample with 30 g deionized water in a stomacher bag and blended for 2 min (Lab Blender 400 BA 6021, Seward Medical, London). A pH electrode (Fisher Scientific Model 13-620-104) was then

immersed directly into the mixture. The pH recorded was the mean result of triplicate samples.

4.2.11. Statistical analysis

The data were analyzed by the Statistical Analysis System (SAS, 1988) using General Linear Model Procedure and Comparison of means was done using a Duncans and Least Square Methods.

4.3. RESULTS AND DISCUSSION

4.3.1. Effect of potassium sorbate incorporated into dough on mold growth

The effects of adding KS directly to the dough at levels of 1000 and 2000 p.p.m. on mold growth in both uninoculated i.e., indirect contamination and inoculated i.e., direct contamination of pizza crust slices at pH 5.5 and 6.0 is shown in Tables 39-42. In general, KS inhibited mold growth in the uninoculated pizza for 5-20d depending on both concentrations of KS and pH of the dough. Significant differences ($P<0.05$) were found between the control dough (no KS) and the sorbate treated samples with mold growth being observed after 5d at both pH 5.5 and 6.0 in control samples. However, in samples containing 1000-2000 p.p.m. KS mold growth was inhibited in the uninoculated pizza crusts for 14-20d and for 10-13d at pH 5.5 and 6.0 respectively (Table 39 and 40).

The effect of various levels of KS on the growth of *A.niger* in pizza crusts at pH 5.5 and 6.0 is shown in Tables 41-42. The most significant effect of KS on the growth of *A.niger* in the inoculated pizza was again observed in crusts at pH 5.5. In control samples, growth of *A.niger* was evident after 3d in pizza crust at both pH levels. Addition of 1000 and 2000 p.p.m. KS significantly inhibited the growth of *A.niger* compared to the control dough with growth of *A.niger* being inhibited for 8-11d in pizza crust at pH 5.5 (Table 41). This study also confirmed that the antimicrobial effect of KS decreased as pH increased as growth of *A.niger* occurred after 4 d in pizza dough containing both 1000 & 2000 p.p.m. KS at pH 6.0 (Table 42).

These studies confirmed that 2000 p.p.m. KS resulted in a longer mold free shelf life of pizza crusts at pH 5.5. However, the effect was less as the initial number of mold spores in pizza crusts increased (uninoculated and inoculated). Therefore, good

manufacturing practices can influence both the inhibitory effect of KS and the mold free shelf life of pizza crusts. These results are in agreement with previous studies which showed that the antimicrobial activity of KS was reduced with increasing levels of contamination (Sofos, 1989).

Table 39: Effect of KS incorporated into the dough on mold growth of uninoculated pizza crusts at pH 5.5

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	5^c	+++
1000	14^b	++
2000	20^a	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 40 : Effect of KS incorporated into the dough on mold growth of uninoculated pizza crusts at pH 6

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	5^c	+++
1000	10^b	++
2000	13^a	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 41: Effect of KS incorporated in to the dough on mold growth of inoculated pizza crusts at pH 5.5

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	3^{c}	+++
1000	8^{b}	++
2000	11^{a}	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 42: Effect of KS incorporated in to the dough on mold growth of inoculated pizza crusts at pH 6.0

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	3^{b}	+++
1000	4^{a}	++
2000	4^{a}	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

4.3.2. Effect of surface application of potassium sorbate on mold growth

The post-contamination of pizza crusts occurs after baking and packaging of products. Under favorable conditions, mold spores will grow and render the product unacceptable.Therefore, application of KS to the products surface through surface spraying after baking may be a more effective method to extend the mold free shelf life of pizza crusts. The effect of surface spraying of KS to pizza crusts at both pH 5.5 and 6.0 are shown in Tables 43-46. In general, preservation of pizza crusts varied with the concentration of KS, pH, and level of contamination. In control samples (no KS), mold growth was evident after 4-5d at both pH 5.5 and 6.0. Addition of 1000 p.p.m., to the uninoculated pizza crusts resulted in mold growth being inhibited for 11d at pH 5.5 and for 9d at pH 6.0 (Table 43). However, when the concentration of KS was increased to 2000 p.p.m. the shelf life of the uninoculated pizza was extended to 17 d at pH 5.5. while at pH 6.0 it was not extended beyond 9d (Table 43). It is evident from Table 44, that there was no significant difference ($P>0.05$) between 1000 & 2000 p.p.m., on mold growth of uninoculated pizza crusts. These results are in agreement with Baldock et al. (1979) who reported that KS was only partly effective when applied to a product's surface due to the removal of mold spores by the rinsing action of the spray.

The antimicrobial action of KS (1000 & 2000 p.p.m.) on the growth of *A.niger* in pizza crusts at pH 5.5 and 6.0 is shown in Tables 45-46. It is again evident that the mold free shelf life of pizza crusts decreased as the inoculum level increased, even with high concentrations of KS at both pH 5.5 and 6.0 (Tables 45-46). Growth of *A.niger* occurred after 3 d in control samples at both pH 5.5 and 6.0. However, at pH 5.5 the growth of *A.niger* was inhibited for 6d in pizza crusts sprayed with 1000 p.p.m. KS and for 9d with

2000 p.p.m. KS (Table 45). At pH 6.0, growth was evident after 3 d in control crusts (no KS), while growth was delayed for 4 d in samples containing both 1000 & 2000 p.p.m. KS i.e., almost similar to control samples (Table 46).

Several studies have shown that surface application of potassium sorbate was effective in inhibiting mold growth in bakery products (Monsanto, 1977). Hickey (1980) observed that the shelf life of inoculated English muffins were extended for 10-17 d by spraying KS (1000 & 2000 p.p.m.) onto the surface. These results confirm previous studies that inhibition was greater as the sorbate concentration increased and pH decreased. This study has also shown that, while application of KS to the product's surface can control mold spoilage, the shelf life extension of the product was not as long compared to direct incorporation of KS.

Table 43: Effect of using surface application of sorbate on mold growth of uninoculated pizza crusts at pH 5.5

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	5^{c}	+++
1000	11^{b}	++
2000	17^{a}	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 44: Effect of using surface application of sorbate on mold growth of uninoculated pizza dough at pH 6

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	5^{b}	+++
1000	9^{a}	++
2000	9^{a}	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 45: Effect of surface application of sorbate on mold growth of inoculated pizza dough at pH 5.5

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	3^{c}	+++
1000	6^{b}	++
2000	9^{a}	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 46: Effect of surface application of sorbate on mold growth of inoculated pizza crusts at pH 6

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	3^{b}	+++
0.1	4^{a}	++
0.2	4^{a}	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

4.3.3 Effect of KS impregnated packaging materials on mold growth

Previous studies showed that potassium sorbate could extend the mold free shelf life of pizza crusts when incorporated directly into the product or applied to the product's surface as a spray. In this study, the effect of potassium sorbate impregnated to the packaging material was investigated to determine its effect on mold growth of pizza crusts at various pH levels.

The inhibitory effect of KS impregnated packaging material on mold growth in both the uninoculated and inoculated pizza crusts at pH 5.5 and 6.0 is shown in Tables 47-50 respectively. It is evident that similar patterns of inhibition of mold growth was observed with 1000 and 2000 p.p.m., KS at pH 5.5 and 6.0 (Table 47-48). In control samples, mold growth was visible after 4d at pH 5.5 and 6.0. However, when pizza crusts were packaged in KS impregnated film, mold growth was only delayed for ~ 1-2d irrespective of the inoculation method, pH or level of inhibitor. This study showed that this method of applications of KS gave significantly lower shelf life extensions compared to the other two methods of application of KS to pizza crusts. These results are probably due to the fact that KS did not diffuse into the pizza crusts but remained at the interface of the wrapper and pizza crust.

Table 47: Effect of sorbate impregnated film on mold growth of uninoculated pizza crusts at pH 5.5

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	4[b]	+++
1000	5[a]	++
2000	5[a]	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 48: Effect of sorbate impregnated film on mold growth of uninoculated pizza crusts at pH 6

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	4[b]	+++
1000	5[a]	++
2000	5[a]	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 49: Effect of sorbate impregnated film on mold growth of inoculated pizza crusts at pH 5.5

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	3[b]	+++
1000	4[a]	++
2000	4[a]	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

Table 50 : Effect of sorbate impregnated film on mold growth of inoculated pizza crusts at pH 6

Levels of KS (p.p.m.)	Days to visible mold growth	Extent of mold growth
0 (Control)	3[b]	+++
1000	4[a]	++
2000	4[a]	++

All values are the average of triplicate samples

+=Slight growth, ++=Light growth, +++=Medium growth

4.3.4. Effect of Modified Atmosphere Packaging on mold growth of pizza crusts

In this study, the effect of Modified Atmosphere Packaging (MAP) was investigated as an alternative method of extending the mold free shelf life of pizza crusts. The inhibitory effect of MAP in uninoculated and inoculated pizza crusts packaged in 60% and 80 %CO_2 (balance N_2) or with an Ageless FX-100 oxygen absorbent and stored at 25 ^{0}C is shown in Tables 51-52. In air packaged samples, mold growth was visible after 5d. However, the mold free shelf life of uninoculated pizza crusts was significantly extended by packaging in either 60% or 80% CO_2. For pizza crusts packaged in 60% CO_2 , mold growth occurred after 26d, while packaging in 80% CO_2 extended the mold free shelf life for 30d (Table 51). This inhibition may be attributed to the antimicrobial action of CO_2 and to lower levels of oxygen in the package headspace. These results are in agreement with the earlier observations of Ooraikul (1982) who observed that a concentration of 60% CO_2 increased the mold free shelf life of crumpets for more than 14d at ambient temperature. Recently, Smith et al. (1990) reported that mold could be inhibited for ~19d in crusty rolls when packaged in 60 % CO_2 (balance N_2) .

Since molds require very low oxygen levels for growth, a significant increase and mold free shelf life was observed for products packaged with an oxygen absorbent. When pizza crusts were packaged with Ageless FX-100, growth was completely inhibited for >42d (Table 51). The lack of visible signs of mold growth for samples packaged with an oxygen absorbent is due to the complete reduction of O_2 in the package headspace. Oxygen absorbents are used extensively in Japan to reduce mold spoilage in intermediate and high moisture bakery products (Smith, et al., 1996). These studies confirm the

observation of Alarcon and Hotchkiss (1993) who reported that oxygen absorbents can prevent mold growth in white bread for 8 weeks at ambient temperature.

The effect of MAP on the growth of *A.niger* in pizza crusts packaged under various MAP conditions is shown in Table 52. In general, the antimicrobial effect of CO_2 was influenced by the level of mold contamination present in pizza crusts. In control samples, the growth of *A.niger* was visible after 3d, while the growth of *A.niger* was observed after 14d in samples packaged in 60% CO_2 and after 18d in samples packaged in 80% CO_2. The growth of *A.niger* in gas packaged pizza can again be attributed to (i) higher level of mold contamination and (ii) the residual headspace O_2 in these packages which was between 1-2%. Golding (1949) reported that molds can grow at very low levels of oxygen and the growth of *Aspergillus* and *Penicillium* species was only inhibited when the concentration of oxygen was < 0.5. Furthermore, several studies have shown that mold can grow in relatively low levels of oxygen (0.5-2%) even in the presence of inhibitory levels of CO_2 (Smith et al.,1986) These results confirm the earlier observations of Smith (1993) who reported that gas packaged baked products contaminated with a high mold spore load had a reduced shelf life compared to similarly packaged products contaminated with a low mold spore load emphasizing the need for good hygiene and good manufacturing practices to ensure the antimicrobial effect of CO_2 enriched atmospheres.

It is known that aerobic microorganisms can tolerate very low concentrations of residual oxygen in the package headspace and additional control measures are necessary to completely inhibit the growth of aerobic spoilage microorganisms in gas packaged products. Therefore, when oxygen absorbents were placed inside the packaged pizza

growth of *A.niger* was inhibited for >42 d at ambient temperature (Table 52). These studies agree with those obtained by Smith et al. (1990) who reported that the mold free shelf life of bread could be extended to 45 d at room temperature by incorporating an oxygen absorbent into the package.

It can be concluded from this study that CO_2 enriched atmospheres i.e., 60% and 80% CO_2 could be used to prevent the mold growth and to extend the shelf life of pizza crusts.On the other hand, oxygen absorbents are a more effective and simpler mean of extending the mold free shelf life of pizza crusts and are less expensive to use.

Table 51: The effect of MAP on mold growth of uninoculated pizza crusts at 25 °C

Treatment	Days to visible mold growth	Headspace gas composition (%v/v)		
		CO_2	O_2	N_2
Air	5[d]	6.5	15	78.5
CO_2:N_2 (60:40)	26[c]	67	1.4	31.6
CO_2:N_2(80:20)	30[b]	85	1.0	14
Agless(FX-100)	*NG[a]	9	0.5	90.5

*NG= no growth after 42 days

All values are the average of triplicate samples.

Table 52: The effect of MAP on mold growth of the inoculated pizza crusts at 25 °C

Treatment	Days to visible mold growth	Headspace gas composition (%v/v)		
		CO_2	O_2	N_2
Air	3[d]	7	15	78
CO_2:N_2 (60:40)	14[c]	57	2.0	41
CO_2:N_2(80:20)	18[b]	74	1.3	24.7
Agless(FX-100)	*NG[a]	9	0.5	90.5

*NG= no growth after 42 days

All values are the average of triplicate samples

4.3.5. Combined effect of MAP and potassium sorbate on mold growth

Since it is well established that the combined effect of two antimicrobial agents is greater than the effect of either alone, this study examined the combined effect of MAP and KS on mold growth in pizza crusts. The antimicrobial effect of MAP in combination with 1000 p.p.m., KS on mold growth of both uninoculated pizza and inoculated pizza is shown in Tables 53-54. Similar trends were observed for samples containing 1000 p.p.m. KS and packaged in either 60% CO_2 or 80% CO_2 (balance N_2). When pizza crusts (pH 6.0) containing 1000 p.p.m., KS and packaged in 60% or 80% CO_2, mold growth was inhibited for >42d. This inhibition can be attributed to the synergistic effect of both inhibitor and high CO_2 concentrations.

The combination effect on the growth of *A.niger* is shown in Table 54. It is again evident that there was no significant difference between 1000 p.p.m, in combination with CO_2 levels i.e., 60% and 80% on mold growth of pizza crusts. In control samples, growth of *A.niger* appeared after 3d. However, growth of *A.niger* was completely inhibited for >42 d in samples packaged in 60% or 80% CO_2 with a sorbate concentration of 1000 p.p.m. at pH 6.0. These studies showed that concentrations of CO_2 i.e., 60% and 80% enhanced the effectiveness of 1000 p.p.m. KS and were vice versa particularly at pH 6.0. Elliott and Gray (1985) reported that exposing organism to various concentrations of CO_2 can reduce the required amount of KS for mold inhibition by as much as 50%.

Table 53: The effect of MAP in combination with potassium sorbate (1000 p.p.m.) on mold growth of the uninoculated pizza dough at pH 6.0

Treatment	Days to visible mold growth	Headspace gas composition (%v/v)		
		CO_2	O_2	N_2
Air	5[b]	4	16	80
CO2:N_2 (60:40)	*NG[a]	63	0.5	36.5
CO2:N_2 (80:20)	*NG[a]	78	0.3	21.7

*NG= no growth after 42 days

All values are the average of triplicate samples

Table 54: The effect of MAP in combination with potassium sorbate (1000 p.p.m.) on mold growth of the inoculated pizza crusts at pH 6.0

Treatment	Days to visible mold growth	Headspace gas composition(%v/v)		
		CO_2	O_2	N_2
Air	3[b]	4	16	80
CO2:N2 (60:40)	*NG[a]	65	0	35
CO2:N2 (80:20)	*NG[a]	80	0.5	19.5

*NG= no growth after 42 days

All values are the average of triplicate samples

Conclusion

In conclusion, pizza dough containing potassium sorbate (1000-2000 p.p.m.) inhibited mold growth and increased the shelf life of pizza crusts. This effect was negligible when pizza crusts were packaged in KS impregnated aluminum foil but more pronounced when KS was incorporated directly into the dough or sprayed onto the product's surface. However, the antimicrobial efficacy of sorbate to control mold growth was dependent on several variables including (i) concentration of KS (ii) pH of product and (iii) level of mold contamination. This study also showed that MAP with 60% or 80% CO_2 (balance N_2) or with an oxygen absorbent (Ageless FX) can also be used to inhibit mold growth and extend the shelf life of pizza crusts at ambient temperature. It also demonstrated that packaging with 60% or 80% CO_2 in combination with potassium sorbate (1000 p.p.m.) can also be used to extend the shelf life particularly at higher pH levels where KS is less effective. However, preservatives could be eliminated entirely by using an Ageless FX oxygen absorbent to extend the mold free shelf life of pizza crusts.

CHAPTER 5
SHELF LIFE STORAGE STUDIES

5.1. INTRODUCTION

In previous studies (Chapter 4), a 42d mold free shelf life was possible for pizza crusts using either preservatives or MAP alone or in conjunction with each other. However, little is known about the combined effect of preservatives and MAP on the shelf life stability of packaged pizza crusts. Therefore, this study was undertaken to determine the effect of various concentrations of sorbate and MAP alone, in combination with each other, on the physical, chemical, microbiological and sensorial changes in packaged pizza crusts throughout a 42d storage period at ambient storage temperature (25 ^{o}C).

5.2. MATERIALS AND METHODS

5.2.1. Preparation of potassium sorbate

A 10% (w/v) of stock solution of potassium sorbate was prepared as described previously in Chapter 2 section 2.2.2.1.

5.2.2. Preparation and packaging of pizza crusts

Pizza crusts were prepared, with and without potassium sorbate KS (1000 and 2000 p.p.m.), as described in Chapter 4 section 4.3.3. All slices (50 g each) were packaged in high gas barrier bags under the following conditions: air (AP), 60% CO_2 (balance N_2) and Air+Ageless Type FX 100 Oxygen absorbent as described previously

(Chapter 4, section 4.2.3.). All packaged samples were stored at 25 ^{o}C for 42 d and examined daily for visible signs of mold growth.

5.2.3. pH measurement

The pH of pizza crusts was measured; in duplicate, with a previously calibrated (pH 4.0 and pH 7.0) Corning pH meter (Model 2220, Corning Glass Works, Corning N.Y.) by making a 1:2 slurry (10 g of pizza dough + 20 g deionized water) and immersing a pH electrode directly into the slurry. PH measurements were carried out on the first day and at the termination of shelf life.

5.2.4. Headspace analysis

All packages were analyzed for headspace gas composition at regular intervals throughout the 42 d storage period. Gas samples were again taken through an adhesive septum placed on the surface of the package using a gas tight syringe (Precision Sampling Corp., Baton rouge, LA). Headspace gas analysis was done using a Varian Gas chromatograph (Model 3400, Varian Canada Inc.) equipped with a thermal conductivity detector (TCD) as described in Chapter 2 section 2.2.7.

5.2.5. Sensory analysis

At day 0, and at each subsequent sampling period (day 3,7,14,21,28,35, and 42), the packaged pizza crusts were evaluated subjectively by 6 untrained panel members. Odor, color and overall acceptability were evaluated using a hedonic scale of 1 to 5 where 1= dislike extremely and 5= like extremely (Larmond, 1977). Products were considered

unacceptable for each parameter when an average score of 3 was reached. Fresh samples were used as controls at each sampling day.

5.2.6. Microbiological analysis

At each sampling period (0,3,7,14,21,28,35,and 42), bags were aseptically opened and portions cut using a sterile knife at random from the pizza crusts to give a sample size of 25g. This was then placed in a stomacher bag and stomached with 225 ml of 0.1% buffered peptone water (Difco, Michigan, USA) for 1 min. From this 10^{-1} dilution, appropriate dilutions were again made using 0.1% peptone .

Total aerobic plate counts (APC) were determined by spreading 0.1 ml of the appropriate dilution onto duplicate plates of Tryptic Soy Agar (TSA, Difico). All plates were incubated aerobically at 35 ^{0}C for 48 hours (Health Protection Branch, HPB, 1989).

Lactic acid bacteria (LAB) were enumerated using Lactobacillus MRS agar (Difco) and incubating plates under anaerobic conditions in anaerobic jars (BBL, Gas Pak jar system, Cockeysville, MD) at 35 ^{0}C for 48 hours (Smith et al., 1986).

Counts of heat resistant *Bacillus* spores present in pizza crusts were determined by heating 1 ml of the appropriate dilutions in microcentrifuge tubes in a water bath set at 80^{0}C for 15 minutes. After heating, 0.1 ml of the appropriate dilution was spread plated onto Tryptic Soy Agar (Difico). Duplicate plates were incubated aerobically and colonies were counted after 48 hours at 35 ^{0}C.

Yeast counts were determined using oxytetracycline glucose yeast extract agar (OGYE) (Difco) by plating appropriate dilutions onto the agar using a spread plate

technique (Mossel et al., 1970). All plates were stored aerobically at room temperature for 5 days .

5.2.7. Statistical analysis

The data were analyzed by the Statistical Analysis System (Sas 1988) using General Linear Model Procedure and Comparison of means was done using a Duncans and Least Square Methods.

5.3. RESULTS AND DISCUSSION

5.3.1. pH

The initial pH of pizza crusts ranged from 6.0-6.2. In most packaged products without KS, pH decreased from 6.0 at day 1 to pH ~5.5 at day 42 (results not shown) This decrease in pH may be due to either growth of lactic acid bacteria or yeasts in the packaged products or slight dissolution of headspace CO_2 into the aqueous phase of the product packaged in 60% CO_2 (Gill, 1988). However, the pH changes of pizza crusts containing 1000 and 2000 p.p.m. of KS were minimal due to inhibition of LAB and yeasts and remained within pH 5.8-5.9 throughout the 42d storage period, irrespective of packaging treatments.

5.3.2. Changes in headspace gas composition

Changes in headspace gas composition for pizza crusts, with or without KS (1000 and 2000 p.p.m.), and packaged in air and under various gas atmospheres are shown in Figures 26-34 respectively.

For air packaged pizza crusts without KS (control), O_2 decreased from 21% to 15% with a corresponding increase in headspace CO_2 to 4.4% after 4d (Figure 26). Headspace N_2 remained constant i.e., approximately 79.9%. These changes can be attributed to mold growth which was visible on all products after 4d storage at 25 ^{O}C. For samples without KS packaged in air and with an Ageless FX oxygen absorbent, headspace O_2 decreased to <1% within 24 hours and remained at this level throughout the storage period. In these samples, CO_2 increased gradually to 20% after 28d and then

decreased throughout storage to 17% (Figure 27). Since mold growth was completely inhibited products headspace changes can be attributed to facultative aerobic microorganisms which utilize O_2 and produce CO_2 e.g., lactic acid bacteria (Smith, 1983).

For gas packaged samples without KS (Figure 28), headspace oxygen remained at <1% throughout storage while headspace CO_2 increased gradually throughout storage to approximately ~ 70% resulting in all packages having a slightly swollen appearance (Figure 28). This increase can again be attributed to the growth of facultative spoilage microorganisms i.e., LAB in pizza crusts. Several studies have shown that lactic acid bacteria can grow in low levels of O_2, even in the presence of elevated levels of CO_2 (Smith et al., 1983; Ooraikul, 1991). Thus, the trends and changes in headspace gas composition are consistent with previous studies on modified atmosphere packaging of crumpets (Ooraikul, 1982).

Changes in headspace gas composition for the various packaging treatments of pizza crusts containing 1000 and 2000 p.p.m. of KS are shown in Figures 29-34. Generally, differences in the gaseous concentrations (O_2 CO_2 and N_2) were observed between samples containing 1000 and 2000 p.p.m. KS. In the air packaged samples with 1000 p.p.m. KS, O_2 decreased to 16.9% after 7d while CO_2 increased to 6.5% (Figure 29). Similar, but more dramatic changes, were observed for the air packaged samples with 2000 p.p.m. KS. Here, O_2 was depleted to 10.6% with an increase in headspace CO_2 to 14.4% after 14d (Figure 30). These changes in the headspace gas composition can again be attributed to the growth of facultative aerobic spoilage microorganism, such as lactic acid bacteria, which are resistant to both high levels of CO_2 and KS.

For crusts containing 1000 p.p.m. KS and packaged with an Ageless oxygen absorbent, headspace O_2 decreased to 1% or less after 3d of storage and remained unchanged throughout the storage period while headspace CO_2 increased to ~ 9% after 42d (Figure 31). However, the changes in headspace gas composition for products containing 2000 p.p.m. KS and packaged with an oxygen absorbent were significantly different compared to samples containing 1000 p.p.m. KS. While headspace O_2 decreased to less than 1% within 3d and remained at this level throughout the storage period due to the oxygen scavenging capacity of the type FX-100 O_2 absorber, CO_2 was not detected in the package (Figure 32). Headspace N_2 remained at ~ 99% (Figure 33). Therefore, higher levels of KS in conjunction with Ageless FX and low residual O_2 may have inhibited the growth of LAB in the packaged product.

For products containing 1000 p.p.m. KS and packaged in 60% CO_2, the initial headspace O_2 was negligible and remained unchanged throughout the storage. However, CO_2 increased from 60% to 64% after 21d storage and then declined ~ to 59% after 42d (Figure 33). Similar trends were also observed for all samples containing 2000 p.p.m. KS and packaged in 60% CO_2 (Figure 34). This decrease in CO_2 may be due to either its absorption by the product or loss through the packaging film (Banks et al., 1980; and Seideman et al., 1979). Such changes in headspace gas composition have been previously observed by Ooraikul (1991). He reported an initial increase in headspace CO_2 in the first two weeks of storage of crumpets followed by a decrease in CO_2 and then a further increase. These changes in headspace gas composition were attributed to bacterial metabolism, mainly heterofermentative lactic acid bacteria (Ooraikul, 1991).

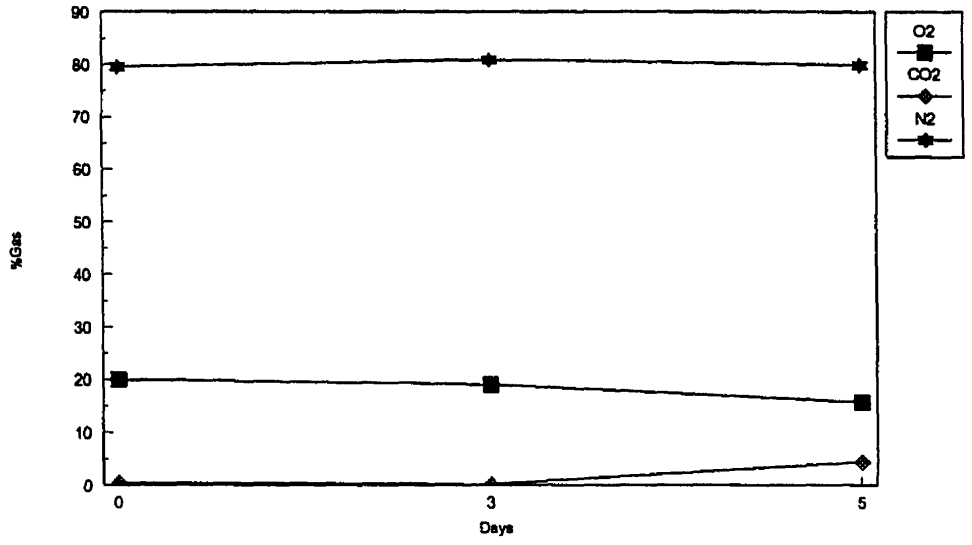

Figure 26: Changes in headspace gas composition of pizza crusts without potassium sorbate (KS) and packaged in air

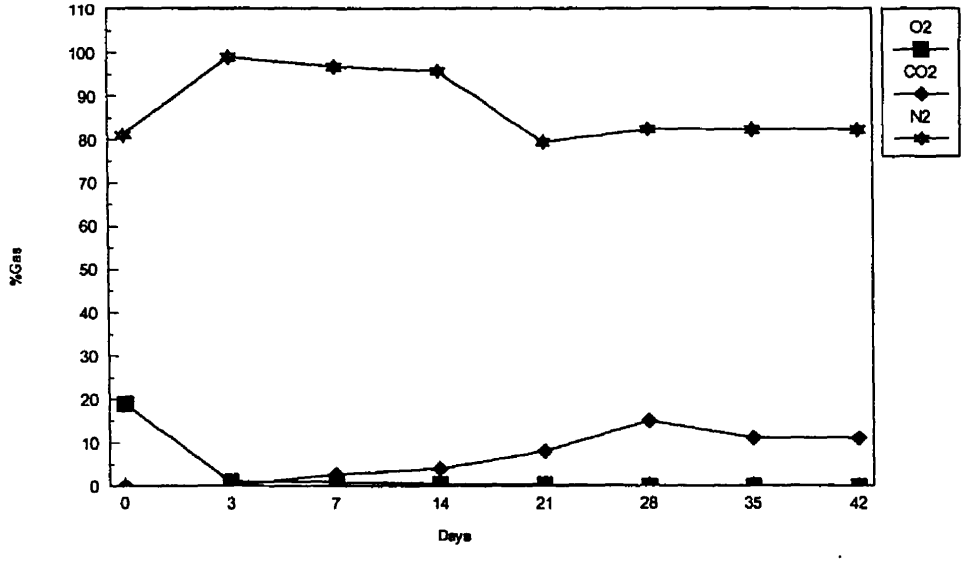

Figure 27: Changes in headspace gas composition of pizza crusts without potassium sorbate (KS) and packaged with an oxygen absorbent (Agless FX-100)

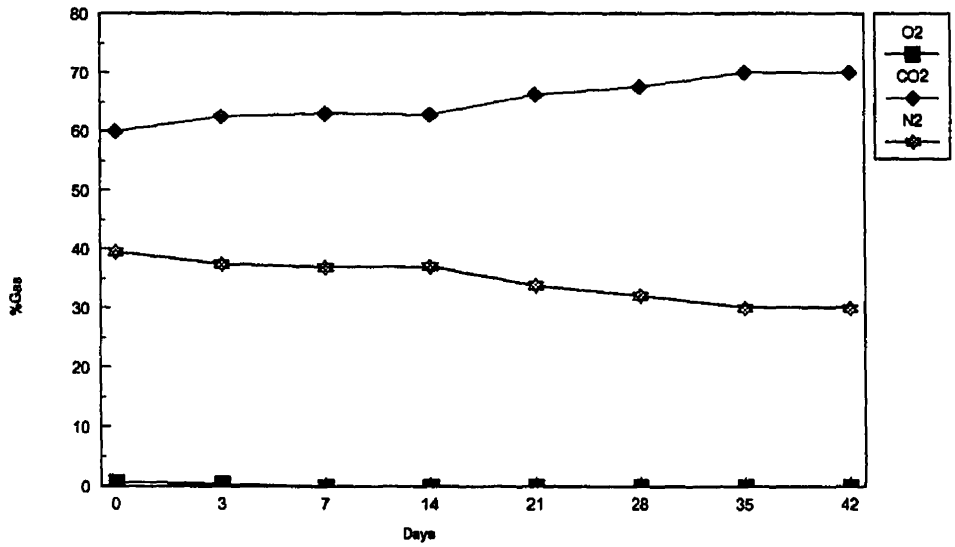

Figure 28: Changes in headspace gas composition of pizza crusts without potassium sorbate (KS) and packaged in 60% CO2 (balance N2)

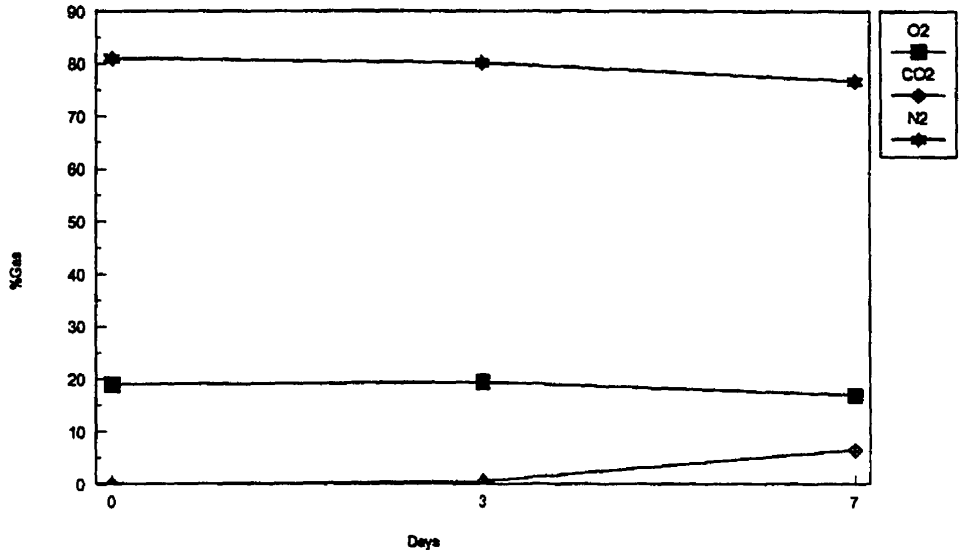

Figure 29: Changes in headspace gas composition of pizza crusts containing 1000 p.p.m. potassium sorbate (KS) and packaged in air

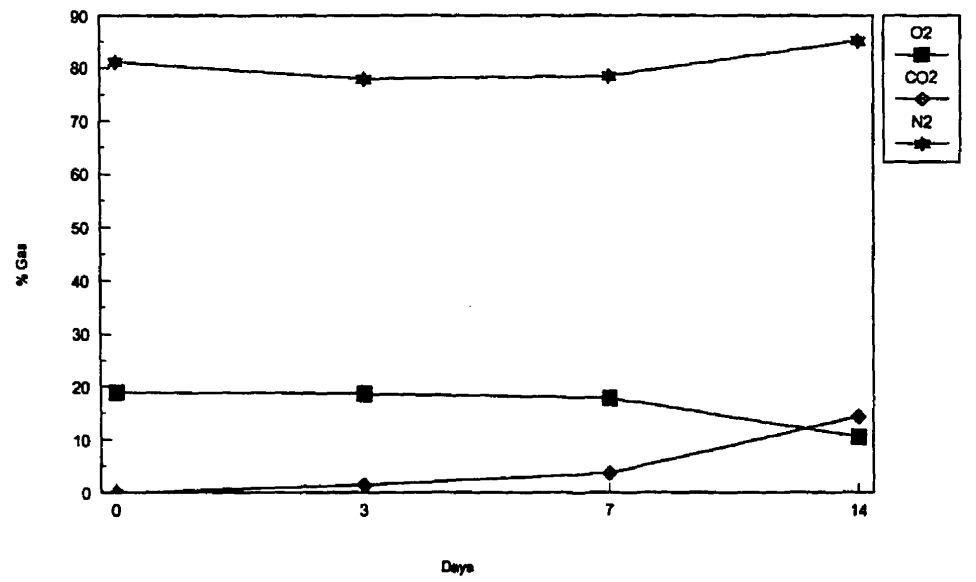

Figure 30: Changes in headspace gas composition of pizza crusts containing 2000 p.p.m. potassium sorbate (KS) and packaged in air

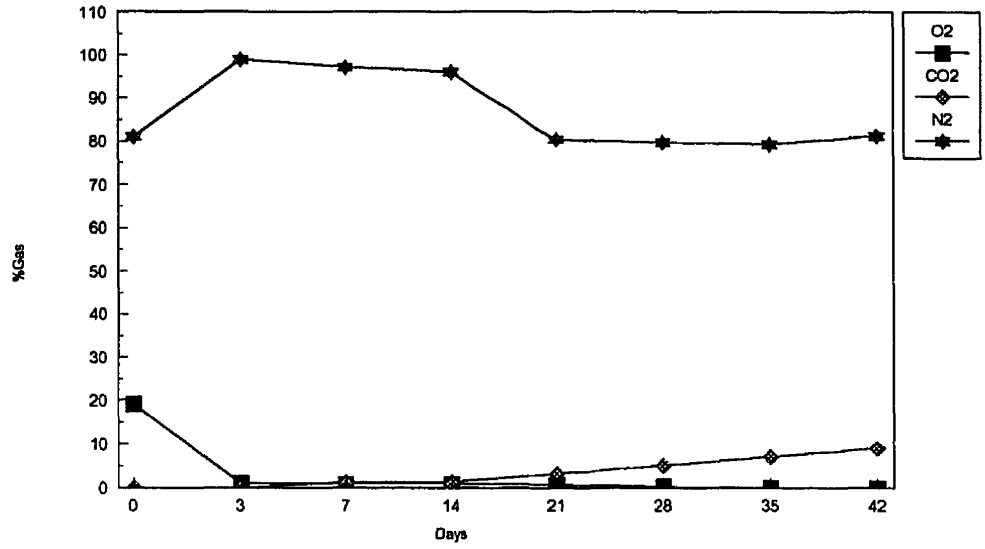

Figure 31: Changes in headspace gas composition of pizza crusts containing 1000 p.p.m. potassium sorbate (KS) and packaged with an oxygen absorbent (Ageless FX-100)

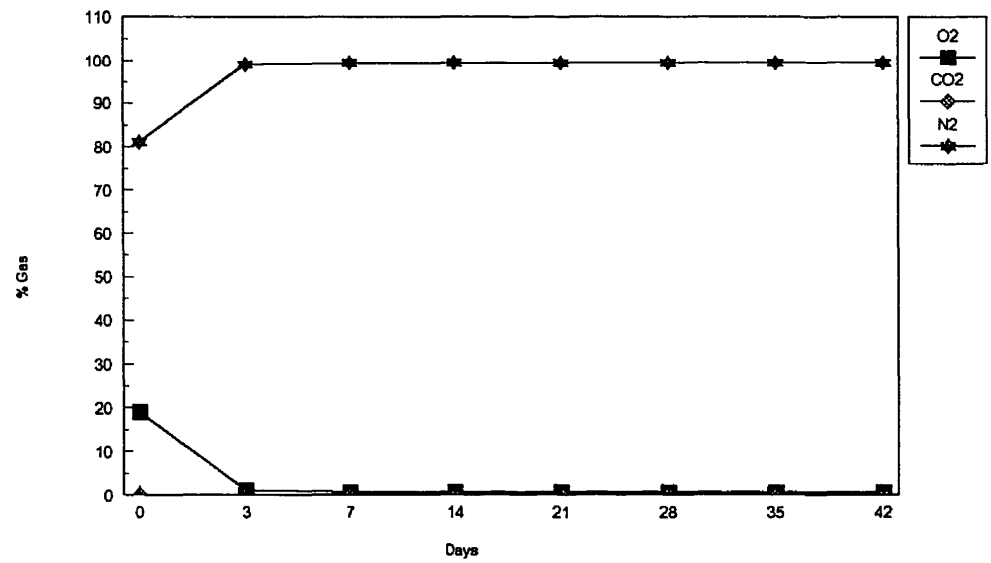

Figure 32: Changes in headspace gas composition of pizza crusts containing 2000 p.p.m. potassium sorbate (KS) and packaged with an oxygen absorbent (Ageless FX-100)

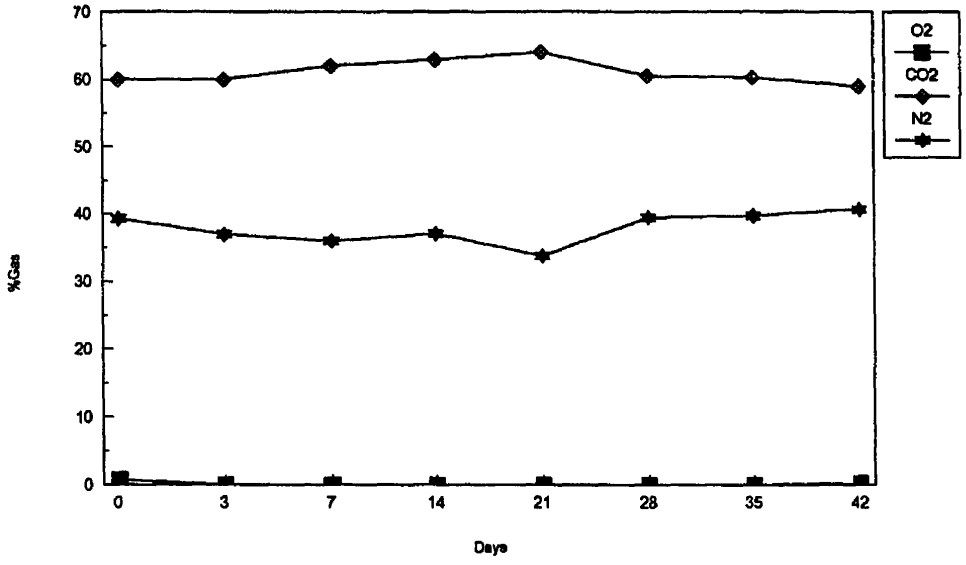

Figure 33: Changes in headspace gas composition of pizza crusts containing 1000 p.p.m. potassium sorbate (KS) and packaged in 60% CO2 (balance N2)

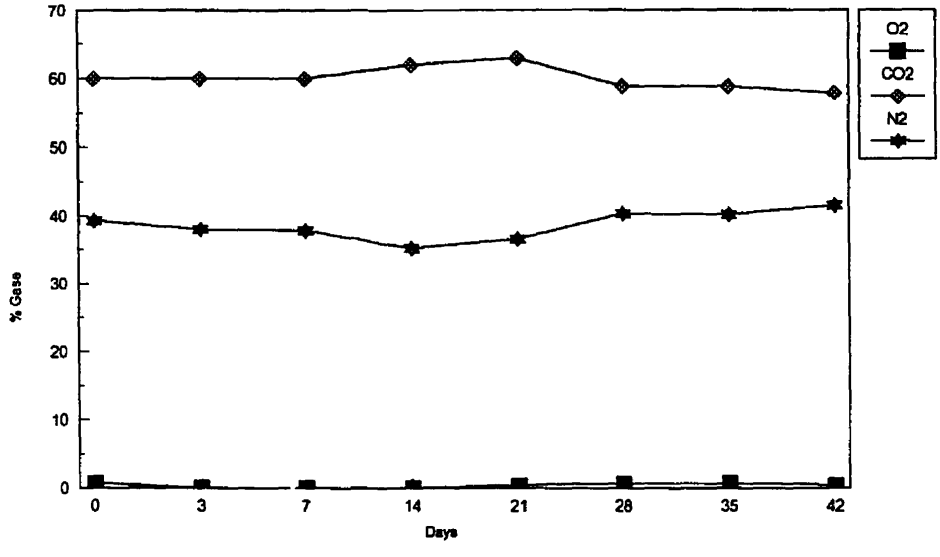

Figure 34: Changes in headspace gas composition of pizza crusts containing 2000 p.p.m. potassium sorbate (KS) and packaged in 60% CO2 (balance N2)

5.3.3. Sensory analysis.

Changes in sensory evaluation (color & odor) attributed to the various packaging conditions of pizza crusts, with and without KS (1000 and 2000 p.p.m.), and stored at 25^0C are shown in Figures 35-40. Products were regarded as unacceptable when a score of 3 on a scale of 5 was reached and hence, termination of shelf life. Sensory analysis was discontinued on air packaged crusts , with or without KS (1000 and 2000 p.p.m.), after 4, 7, and 14 d as shelf life was terminated due to mold growth.

In general, optimum sensory results were obtained by either packaging under MAP conditions, alone or in combination with, various concentrations of KS (1000 and 2000 p.p.m.). It is evident that the score for color was significantly lower ($P<0.05$) in air packaged samples after 4d compared to all other MAP treatments at 25 ^{o}C. Furthermore, most MAP samples had an acceptable color score even after 42d. The rejection of the air packaged pizza crusts without KS after 4d was due to visible growth of mold on the surface of the crusts.

The combined effect of KS and MAP atmosphere had a significant effect ($P<0.05$) on both the color/odor of packaged pizza crusts. After day 7, the KS (1000 p.p.m.): air packaged samples were rejected due to mold developing on the crust's surface. With 2000 p.p.m. KS, the air packaged samples were rejected after 14d, again due to mold growth. However, from a sensory viewpoint, the effect of KS in combination with MAP had a pronounced effect on shelf life. For example, crusts containing 1000 p.p.m. KS and packaged under MAP conditions, had acceptable color after 42 d (Figure 36). Statistical analysis showed that there was no significant difference ($P>0.05$) in the crust color in products containing 2000 p.p.m. KS and packaged with 60% CO_2 or with an oxygen

absorbents. Overall, crusts packaged in 60% CO_2 or with an Ageless FX oxygen absorbent and containing 2000 p.p.m. KS were acceptable in color after 42d (Figure 37).

Changes in the sensory odor for the various packaging treatments are shown in Figures 38-40 respectively. Significant differences were observed between the air packaged samples and the MAP products ($P<0.05$). For example, samples packaged with air were rejected after 4d, again due to visible mold growth on the crust's surface and a higher microbial count. Some panelists observed off-odors (yeast odor) in samples packaged under MAP conditions. Similar trends were obtained for samples packaged with oxygen absorbents. Undesirable odors were detected when the gas packaged pizza crusts were opened after 21d (Figure 38). These results indicate that microbial growth was primarily responsible for the low odor score in the MAP samples without KS. Smith et al. (1983) reported that members of lactic acid bacteria and *Bacillus* species have shown to be the predominant spoilage organisms in crumpets packaged in 60% CO_2.

Samples packaged in air with 1000 and 2000 p.p.m. KS were rejected after 7 and 14d due to mold growth. In general, the odor shelf life of pizza crusts was extended when MAP was used in combination with KS, particularly at high concentrations (Figures 39-40). For example, samples containing 1000 p.p.m. KS and packaged in 60% CO_2 were rejected after 28d. However, samples packaged in 60% CO_2 with 2000 p.p.m. KS had acceptable odor scores even after 42d. The results also showed that products containing 2000 p.p.m. KS and packaged with an oxygen absorbent had a longer odor shelf life than samples containing 1000 p.p.m. and packaged under similar conditions. These results confirm previous observations that MAP in conjunction with higher levels of KS had a more beneficial effect on a product's shelf life.

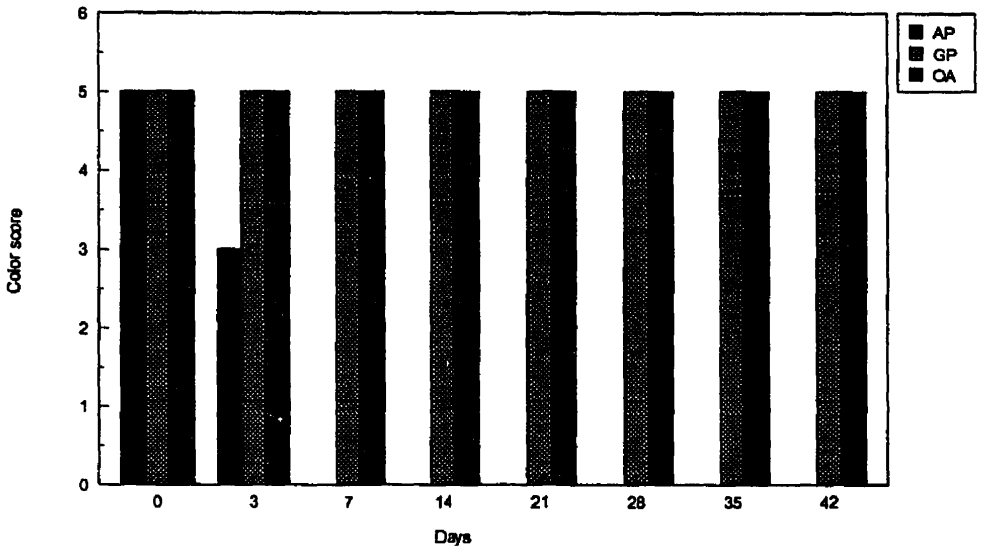

Figure 35 : Changes in sensory color of pizza crusts without potassium sorbate (KS) and packaged under various gas atmospheres

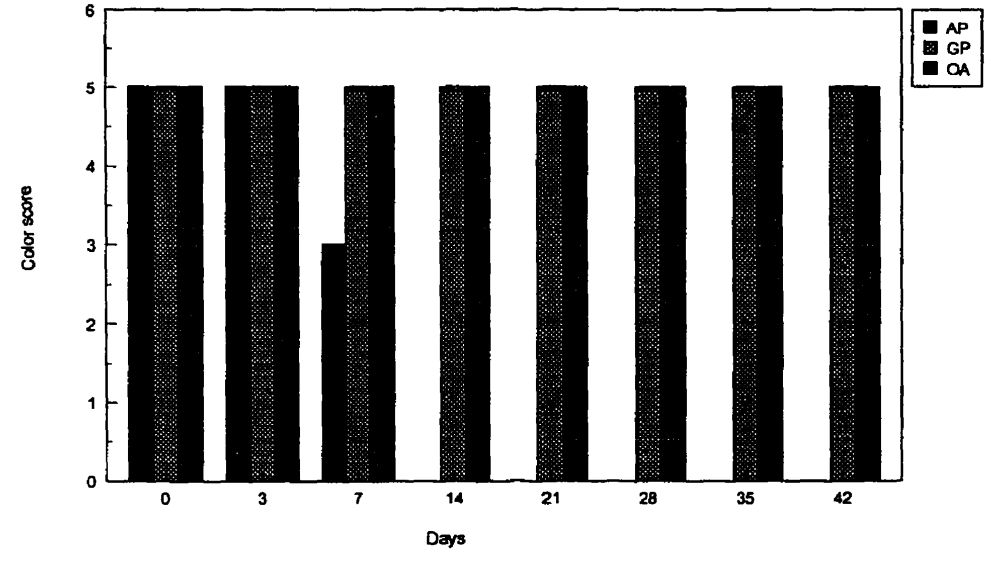

Figure 36 : Changes in sensory color of pizza crusts containing 1000 p.p.m.potassium sorbate (KS) and packaged under various gas atmospheres

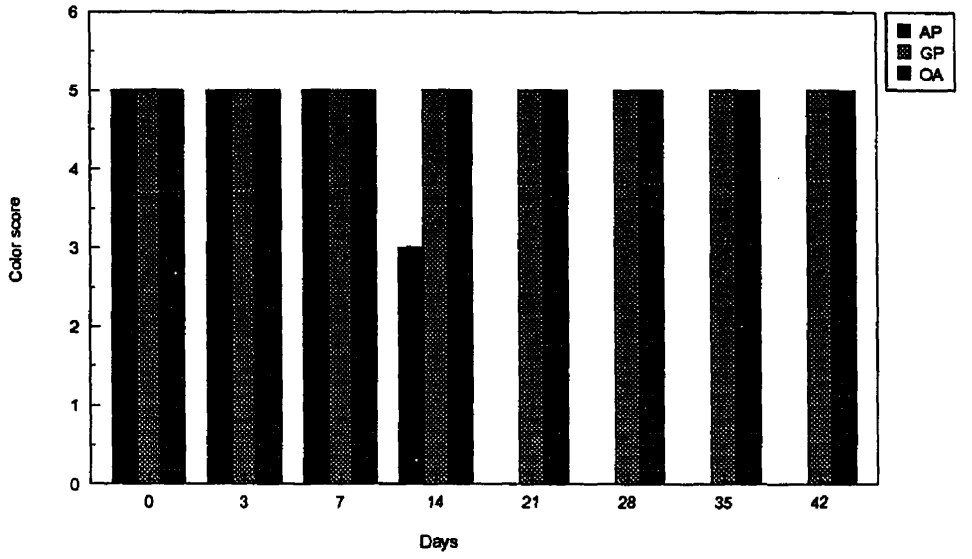

Figure 37 : Changes in sensory color of pizza crusts containing 2000 p.p.m. potassium sorbate (KS) and packaged under various gas atmospheres

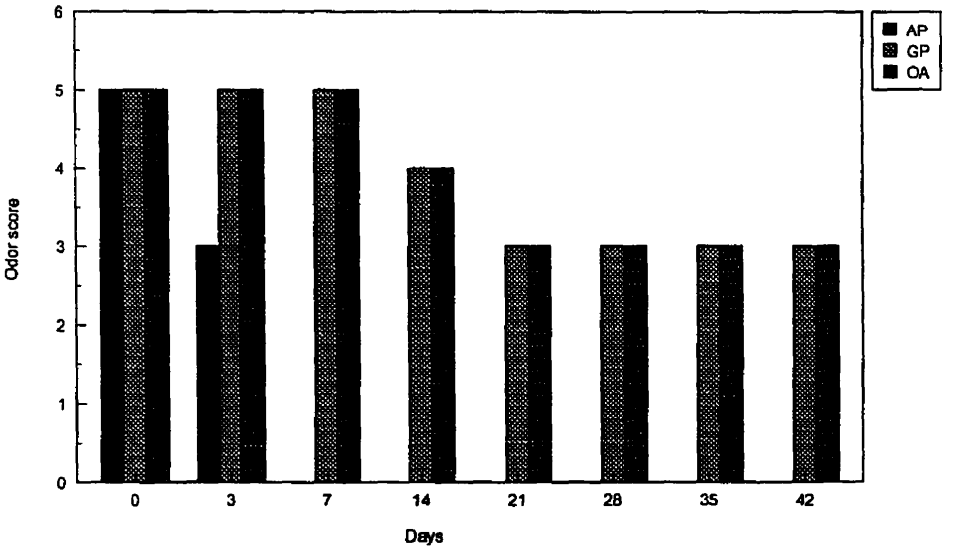

Figure 38: Changes in sensory odor of pizza crusts without potassium sorbate (KS) and packaged under various gas atmospheres

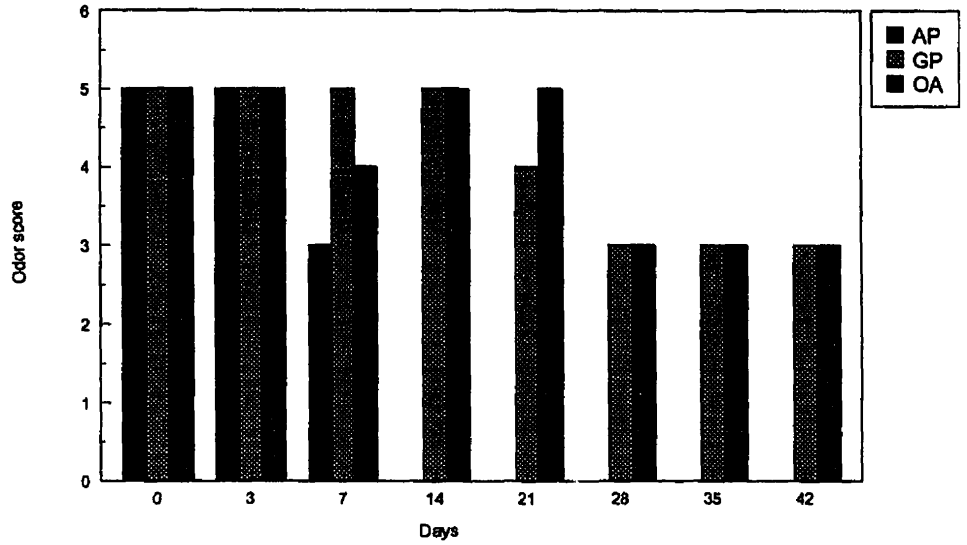

Figure 39 : Changes in sensory odor of pizza crusts containing 1000 p.p.m. potassium sorbate (KS) and packaged under under various gas atmospheres

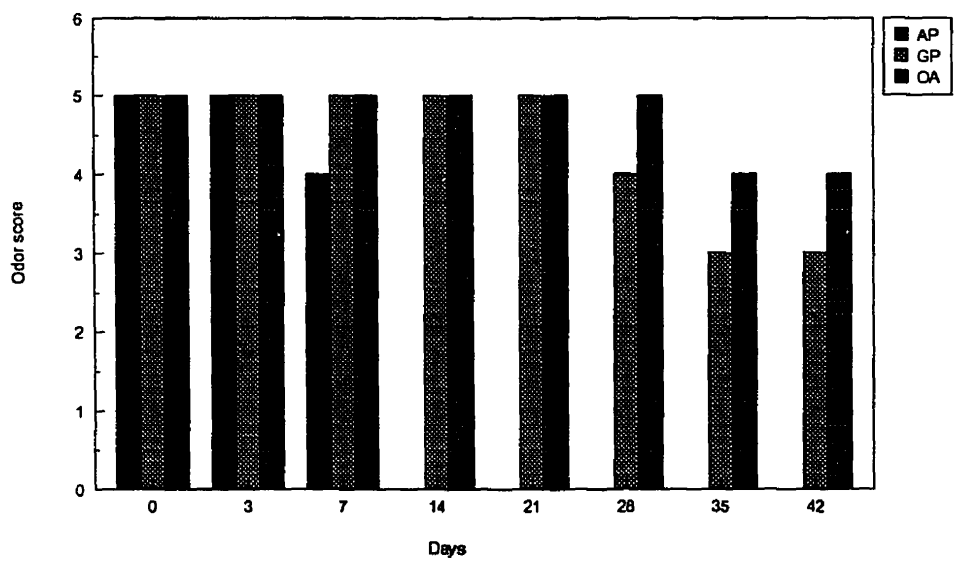

Figure 40 : Changes in sensory odor of pizza crusts containing 2000 p.p.m. potassium sorbate (KS) and packaged under under various gas atmospheres

5.3.4. Microbiological analysis:

Changes in the microbiological composition of pizza crusts with or without KS, packaged under various gas atmospheres and stored at 25 ^{o}C are summarized in Table 55 and Figures 40-48 respectively. Changes in sorbate free pizza crusts (control) and packaged under various gaseous conditions will be discussed first (Table 55 and Figure 41-44).

In all air packaged products without KS, mold growth was significant ($P<0.05$) compared to products packaged under modified atmospheres (Table 55). For example, mold growth was visible in all air packaged crusts after only 4d (Table 54). However, in products without KS the mold free shelf life could be extended to 42d through gas packaging (60% CO_2 balance N_2) or by packaging with an Ageless FX oxygen absorbent (Table 55). These studies showed that the shelf life of gas packaged crusts was longer than in previous gas packaging studies. These results may be attributed to the reduced level of O_2 in the package headspace to less than 1% which would enhance the antimicrobial effect of CO_2. It has been demonstrated that the inhibitory effect of CO_2 is dependent on oxygen concentration in gas packaged products (Ooraikul, 1991). Furthermore, these results confirm previous studies in our laboratory which showed that mold growth can be inhibited for >42d in pizza crusts packaged with an oxygen absorbent.

Changes in total aerobic plate count (APC) of pizza crusts for various packaging treatments are shown in Figures 41-43. Generally, the APC was discontinued after 3d in air packaged products and after 7- 14d in air packaged samples with 1000 p.p.m. and 2000 p.p.m. KS due to visible mold growth and hence termination of shelf life. Initially,

the APC increased more rapidly for all air packaged samples compared to products packaged under MAP conditions. For example, counts increased from an initial level of <10 CFU/g to 10^5 CFU/g after 3d (Figure 41). However, a dramatic effect was observed for samples packaged in 60% CO_2, with counts reaching 10^7 CFU/g after 21d and then decreasing to 10^6 CFU/g throughout storage due to bacteria entering their stationary growth phase (Figure 41). Similar, but less rapid trends were observed for pizza crusts packaged with an oxygen absorbent. Small, white colonies, resembling LAB, were evident in all plates and comprised > 90% of the total APC (Figure 41).

Changes in lactic acid bacteria (LAB) counts for pizza crusts without KS and packaged under various gas atmosphere are shown in Figure 42. In the air packaged crusts, LAB counts increased to log 10^4 CFU/g after 3d. While the counts of LAB reached 10^7 CFU/g in samples packaged in 60% CO_2 after 28d, counts never reached 10^7 CFU/g in samples packaged with an oxygen absorbent. Previous studies have reported that LAB are tolerant, and even stimulated by, high CO_2 concentrations, and can become the major spoilage microorganism in many gas packaged food products (Smith and Simpson, 1996).

Bacillus counts in all treatments were consistently low (<10 CFU/g) even in the air sample packages at 25 ^{0}C (results are not shown). The absence of *Bacillus* may be due to the presence of acetic acid which is commonly used to control rope caused by *Bacillus subtilus* in pizza crusts (Rosenkvist and Hansen, 1995).

Yeast counts in preservative free pizza crusts packaged in air and various modified atmospheres and stored at 25 ^{O}C are shown in Figure 43. It is evident from Figure 43 that yeast counts increased in all treatments. After day 3, yeast counts reached

~10^4 CFU/g in air packaged products.This may be due to depletion of headspace O_2 by molds enhancing yeast growth. Similar trends were observed for pizza crusts packaged in 60% CO_2 and with an oxygen absorbent. Furthermore, yeast counts increased to 10^6 CFU/g after 35d and remained at this level throughout the storage period.

The effect of incorporating KS (1000 and 2000 p.p.m.) into the pizza crusts and packaging under various gaseous conditions on the growth of indigenous microorganisms (mold, total aerobes, lactic acid bacteria, *Bacillus*, and yeasts) are shown in Table 54 and Figures 44-50 respectively. After day 7-9, the mold growth was visible in samples containing 1000 p.p.m. KS and packaged in air (Table 54). Similar trends were observed for all air samples containing 2000 p.p.m. KS with mold growth being evident after 14d. This data is in agreement with our previous studies for air packaged crusts with KS. However, significant differences ($P<0.05$) were observed for samples packaged under various gas atmospheres. For example, mold growth was completely inhibited for 42d in sorbate treated samples packaged under MAP conditions. These results are consistent with our previous studies which showed that KS, in combination with MAP, extended the mold free shelf life of pizza crusts to more than 42d.

Changes in APC and LAB counts are shown in Figures 44-47. Generally, the APC increased rapidly as storage progressed. As shown in Figure 44, the APCs exceeded 10^4 CFU/g after 7d for products packaged in air with 1000 p.p.m. KS. Furthermore, higher APCs were observed in gas packaged pizza crusts containing 1000 p.p.m. KS compared to crusts packaged with an oxygen absorbent. This again could be due to the level of residual headspace oxygen but is more likely due to the stimulating effect of CO_2 as all colonies again resemble lactic acid bacteria. However, the inhibitory effect of KS

increased as the concentrations increased (Sofos, 1989). For example, in air packaged pizza crusts containing 2000 p.p.m. KS, APCs reached 10^7 CFU/g after 14d (Figure 45). However, while the APC increased in gas packaged (60% CO_2:N_2), crusts never reached 10^7 CFU/g even after 42d. It is evident that aerobic bacteria decreased significantly in products packaged with oxygen absorbents, with counts reaching only 10^5 CFU/g after 42d (Figure 45). This is probably due to the synergistic effect of KS and lower levels of residual oxygen.

Changes in lactic acid bacteria (LAB) of pizza crust samples are shown in Figures 46-47. It is again evident that the inhibitory effect of KS was greater with 2000 p.p.m. than 1000 p.p.m. For example, in pizza crusts containing 1000-2000 p.p.m. KS and packaged in air, LAB counts increased to 10^5 CFU/g after 7d and 14d respectively (Figures 46-47). LAB counts increased gradually throughout storage in products containing 1000 p.p.m. KS and packaged under MAP conditions. Again, this may be due to the fact that LAB are facultative anaerobes and therefore become the predominant spoilage microorganism in pizza crusts stored under anaerobic conditions(Figure 46). The results also showed that the inhibition of LAB increased significantly ($P<0.05$) in samples containing 2000 p.p.m. KS and packaged with an oxygen absorbent compared to gas packaged samples (Figure 47). In all samples packaged with an oxygen absorbent, LAB counts reached 10^4 CFU/g after 42d (Figure 47). These results emphasize the importance of preservatives i.e., KS and low residual O_2 to control the growth of spoilage microorganisms of pizza crusts packaged under various gas atmospheres.

Bacillus counts in pizza crusts packaged in air and under various modified atmospheres, with or with out KS, were similar (results not shown). In all cases, *Bacillus* counts were not detected throughout storage.

Changes in yeast counts of pizza crusts packaged under various gaseous conditions with various concentrations of KS are shown in Figures (48-49). In air packaged samples with 1000 p.p.m. KS, yeast counts were higher compared to aerobic packaged crusts with 2000 p.p.m. KS (Figures 48-49). Yeast counts in crusts packaged in 60% CO_2 with 1000 p.p.m. KS were lower than counts found in products containing similar levels of KS but packaged with an oxygen absorbent (Figure 48). Again, this is probably due to the combined inhibitory effect of both CO_2 and KS. It is evident that there was no significant difference ($P>0.05$) in yeast counts between samples containing 2000 p.p.m. KS and packaged in 60% CO_2 or packaged with an oxygen absorbent, with counts being >10 CFU/g after 42d (Figure 49).

Table 55: Mold growth in pizza crusts with or without potassium sorbate (KS) and packaged in air and under MAP conditions at 25 ^{o}C

Packaging treatment	pH of pizza crusts	Days to visible mold growth
Without KS		
Air	6	4
60% CO_2	6	*NG
Ageless FX	6	*NG
With 1000 p.p.m. KS		
Air	6	7-9
60% CO_2	6	*NG
Ageless FX	6	*NG
With 2000 p.p.m. KS		
Air	6	14
60% CO_2	6	*NG
Ageless FX	6	*NG

*NG=No growth after 42d

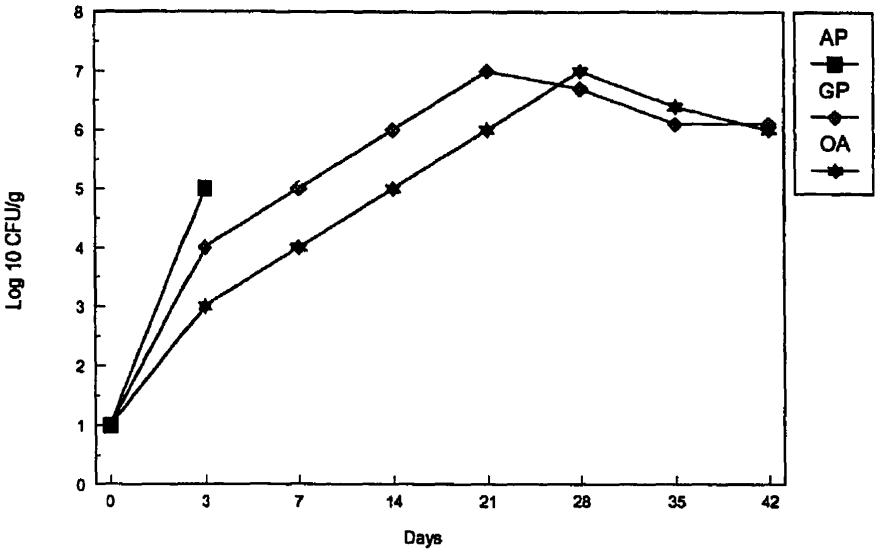

Figure 41: Changes in aerobic plate count (APC) of pizza crusts without potassium sorbate (KS) and packaged under various gas atmospheres

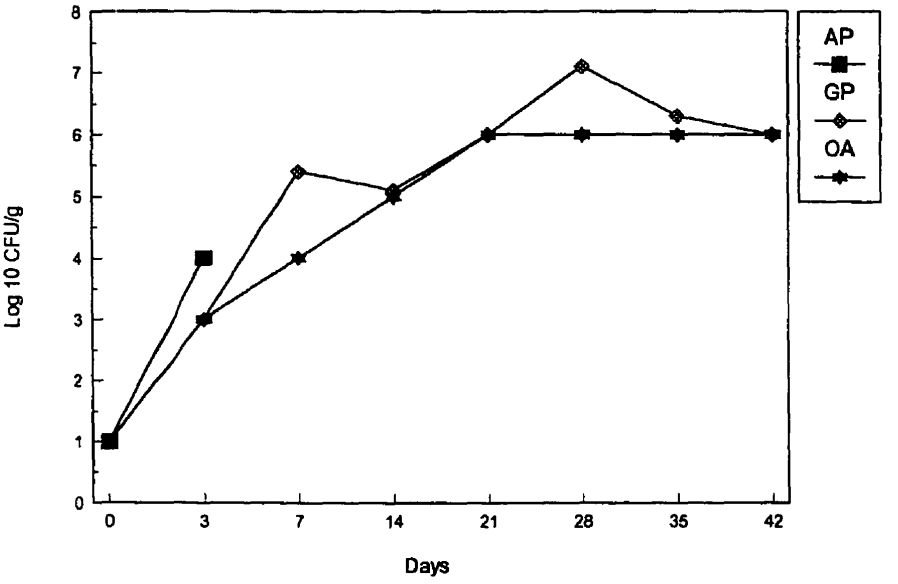

Figure 42: Changes in lactic acid bacteria counts (LAB) of pizza crusts without potassium sorbate (KS) and packaged under various gas atmospheres

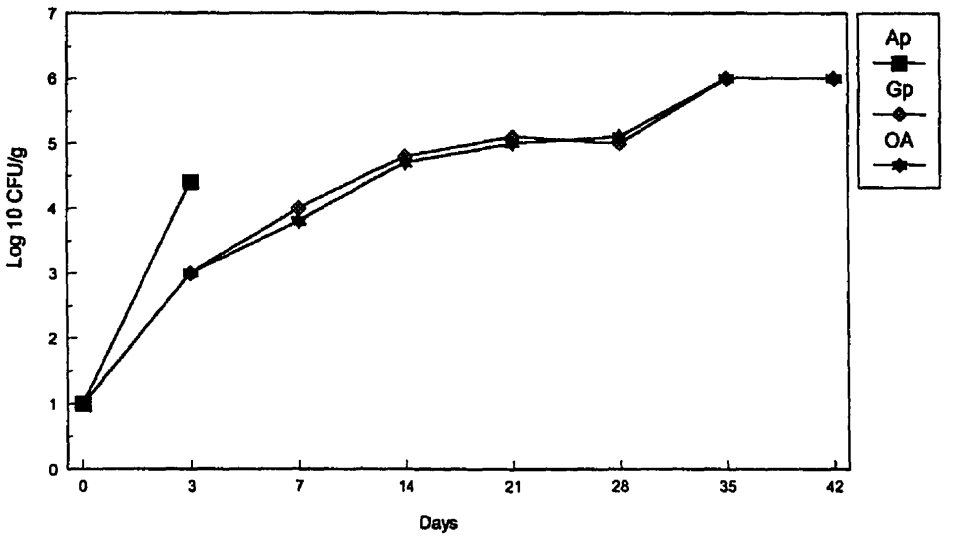

Figure 43: Changes in yeast counts of pizza crusts without potassium sorbate (KS) and packaged under various gas atmospheres

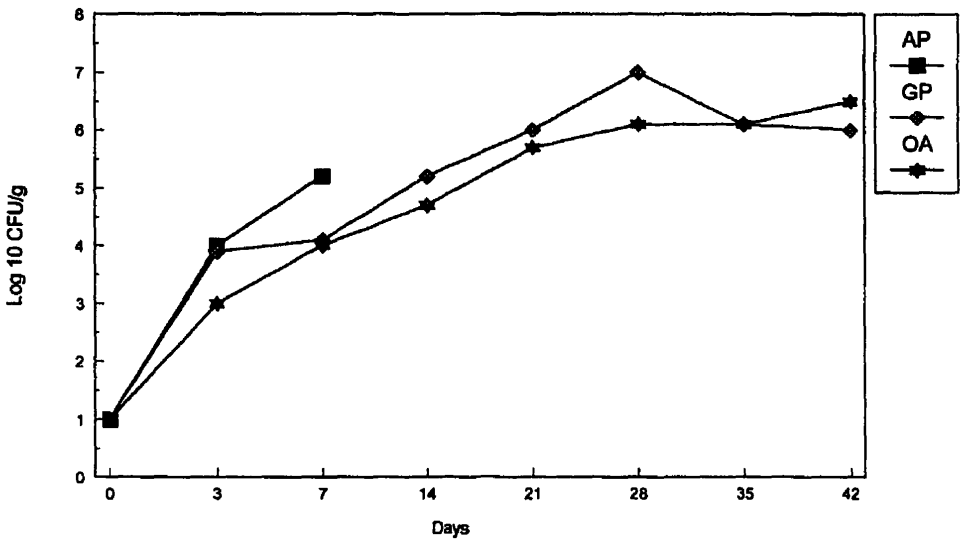

Figure 44: Changes in aerobic plate counts (APC) of pizza crusts containing 1000 p.p.m. potassium sorbate (KS) and packaged under various gas atmospheres

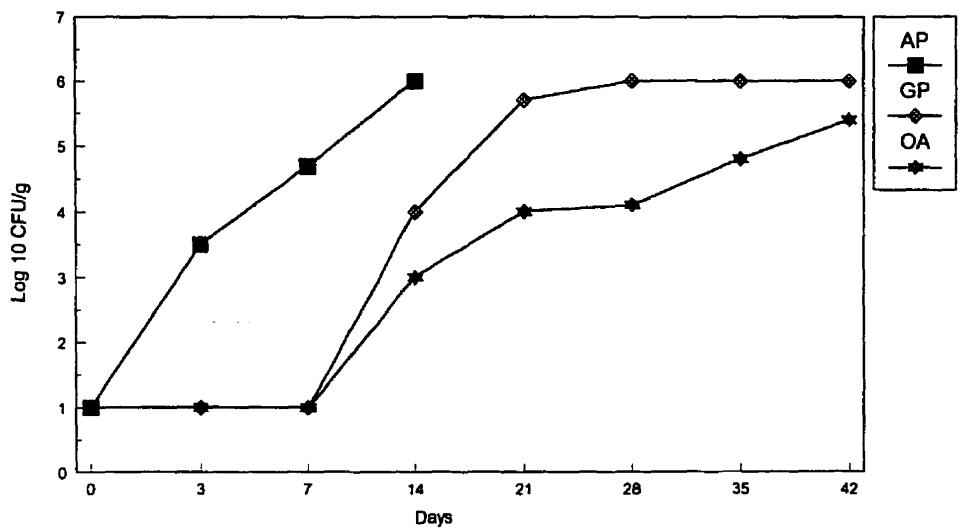

Figure 45: Changes in aerobic plate counts (APC) of pizza crusts containing 2000 p.p.m. potassium sorbate (KS) packaged under various gas atmospheres

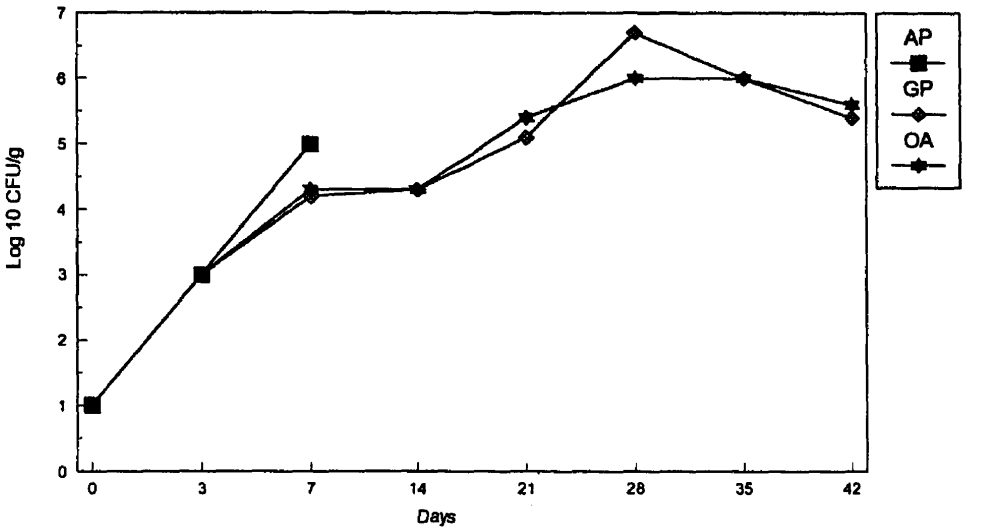

Figure 46: Changes in lactic acid bacteria counts (LAB) of pizza crusts containing 1000 p.p.m. potassium sorbate (KS) and packaged under various gas atmospheres

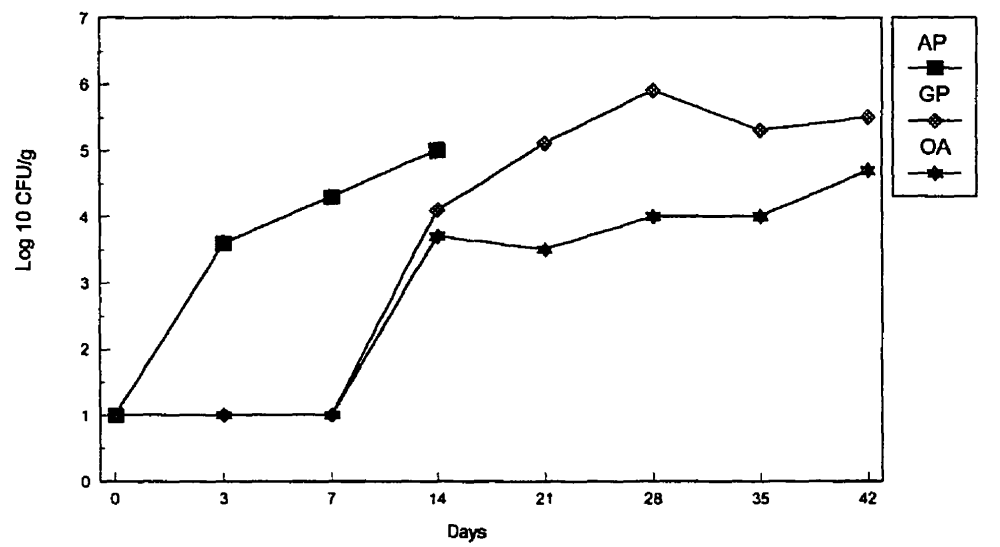

Figure 47: Changes in lactic acid bacteria counts (LAB) of pizza crusts containing 2000 p.p.m. potassium sorbate (KS) and packaged under various gas atmospheres

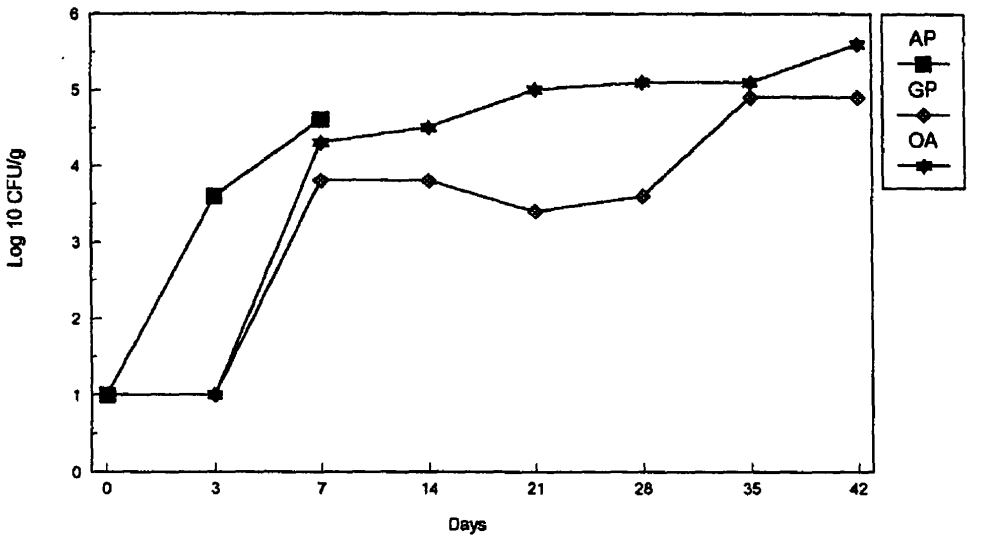

Figure 48: Changes in yeast counts of pizza crusts containing 1000 p.p.m. potassium sorbate (KS) and packaged under various gas atmospheres

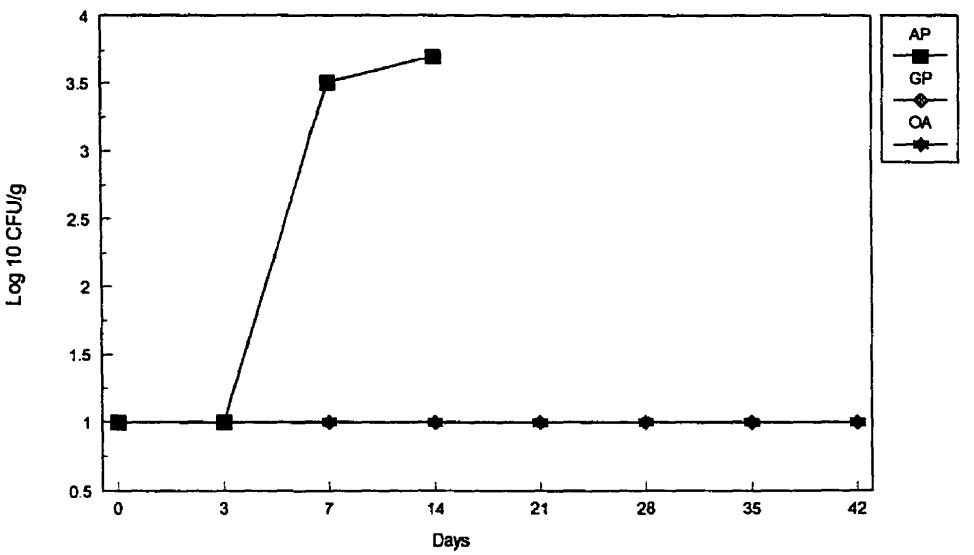

Figure 49: Changes in yeast counts of pizza crusts containing 2000 p.p.m. potassium sorbate (KS) and packaged under various gas atmospheres

5.4. Shelf life

The sensory shelf life of pizza crusts with or without KS and packaged under various gaseous conditions at 25 ^{o}C is summarzied in Table 56. The storage shelf life of pizza crusts was based on the days to visible mold growth or the time to reach a sensory score (odor/color) of 3 (rejection point). The overall shelf life extension of pizza crusts was directly proportional to storage time, packaging treatment and the inhibitory effect of KS. Air packaged crusts, with or without KS (1000 and 2000 p.p.m.), had unacceptable color and odor scores after 3, 7, and 14d respectively due to mold growth. Generally, the mold free shelf life of pizza crusts, with or without KS, and packaged under MAP was similar. Furthermore, packaging of pizza crusts under various MAP condition substantially inhibited mold growth and increased shelf life of pizza crusts to ~ 42d. It is evident from Table 56 that, in gas packaged crusts without KS, odor scores were unacceptable after 21d due to mold growth and a high aerobic plate count (>10^7 CFU/g) causing products to be rejected. Similar trends were observed for all pizza crusts packaged with an oxygen absorbent. In all products containing 1000 p.p.m. KS, and packaged under both 60% CO_2 and with an oxygen absorbent, pizza crusts were rejected on a sensory basis by the panelists after 28d. This again may possibly be due to the high LAB which can cause an acid sharp odor in packaged products. However, at 25 ^{o}C, the shelf life of pizza crusts extended to 42d using MAP technology i.e., gas flushing or oxygen absorbent in combination with 2000 p.p.m. KS.

Table 56: Estimated shelf life of pizza crusts with or without KS and stored under various packaging conditions at 25 ^{0}C

Packaging treatment	Rejection point/color[a]	Rejection point/odor[a]	Microbial shelf life (days)[b]	Overall shelf life (Days)[c]
Without KS				
Air	4	4	4	4
60% CO_2	42	21	42	30
Ageless FX	42	21	42	30
With 1000 p.p.m. KS				
Air	7	7	7-9	7
60% CO_2	42	28	42	37
Ageless FX	42	28	42	37
With 2000 p.p.m. KS				
Air	14	14	14	14
60% CO_2	42	42	42	42
Ageless FX	42	42	42	42

[a] Time (Days) to reach a score of three

[b] Time (Days) to visible mold growth

[c] Earlest rejection point on terms of odor, color and microbial growth

Conclusion

In conclusion, MAP, alone or with KS (1000 and 2000 p.p.m.), can be used to substantially improve the shelf life of pizza crusts with minimal changes to color and odor of the packaged products. The increase in shelf life was greater in samples containing 2000 p.p.m. KS and packaged under MAP conditions. Microbiological analyses of pizza crusts, with or without KS using different packaging treatments, showed that MAP, alone or with KS, completely inhibited mold growth and decreased bacterial counts throughout the storage period. Counts were consistently lower in products containing higher levels of KS (2000 p.p.m.) and packaged under MAP conditions using CO_2 enriched atmospheres or oxygen absorbent technology compared to air packaged products.

GENERAL CONCLUSION

Mold spoilage is still a major problem limiting the shelf life of many high and intermediate moisture bakery products. Losses due to mold spoilage have been estimated at 1-5% of the annual production, resulting in millions of dollars in lost revenue to the baking industry. Therefore, methods to control mold growth and to extend the shelf life of bakery products is of great economic importance to the baking industry where an increased demand in global consumption exists.

Preliminary studies clearly indicated that chemical preservatives i.e., potassium sorbate, calcium propionate and a combination of potassium sorbate and calcium propionate could be used effectively to control mold growth in an agar model system. The effectiveness of these preservatives was enhanced by increased presence of the undissociated form of the chemical, achieved by lowering the pH of the substrate. Further studies in agar media indicated that MAP alone or in conjunction with preservatives could also be used to control mold growth for a substantial time i.e., >42d.

However, when potassium sorbate was incorporated into pizza dough, some conflicting results were obtained. While potassium sorbate (1000 & 2000 p.p.m.) could be used to delay mold growth in an agar model system at pH 5.5, the shelf life observed was significantly less than observed in pizza crusts at pH 5.5. This may be due to the nature of the substrate, i.e., pizza crusts vs. agar, the latter enhancing microbial spoilage.

The results of this study showed that methods of applying potassium sorbate played an important role in controlling mold growth and extending the shelf life of pizza. While mold growth was inhibited for 14-20d in pizza crusts containing potassium sorbate directly incorporated into the dough, growth was inhibited for 11-17d by spraying

potassium sorbate onto the surface of dough. However, the effect of potassium sorbate impregnated films was negligible, with growth being visible after only 4-5d depending on the concentrations of potassium sorbate. In all cases the inhibitory effect was enhanced as the level of contamination and pH decreased.

Mold growth could also be inhibited for 26-30d in pizza crusts packaged in. 60% and 80% CO_2, i.e., slightly higher than observed in an agar model system. These results are probably due to various residual levels of oxygen in the package headspace or being trapped in the product. However, when headspace oxygen was reduced in the gas package to <1%, mold growth was completely inhibited for 42d. Therefore, it is critical to control the level of residual oxygen in MAP foods if mold growth is to be inhibited. Oxygen absorbent technology can be therefore used to reduce and maintain headspace oxygen to low levels result in a extension shelf life to >42d.

In conclusion, this study has shown that control of mold growth in pizza crusts can be obtained by using preservatives , by MAP, and by a combination of preservatives with MAP. Optimum shelf life extension could be achieved through combinations of potassium sorbate with MAP or with MAP alone without adversely effecting sensorial qualities of the packaged product. Oxygen absorbent technology is a simple and cost effective method of modifying the atmosphere within packaged products. This technology could be of potential use in countries such as Libya where mold spoilage is a major problem in many baked products, including pizza.

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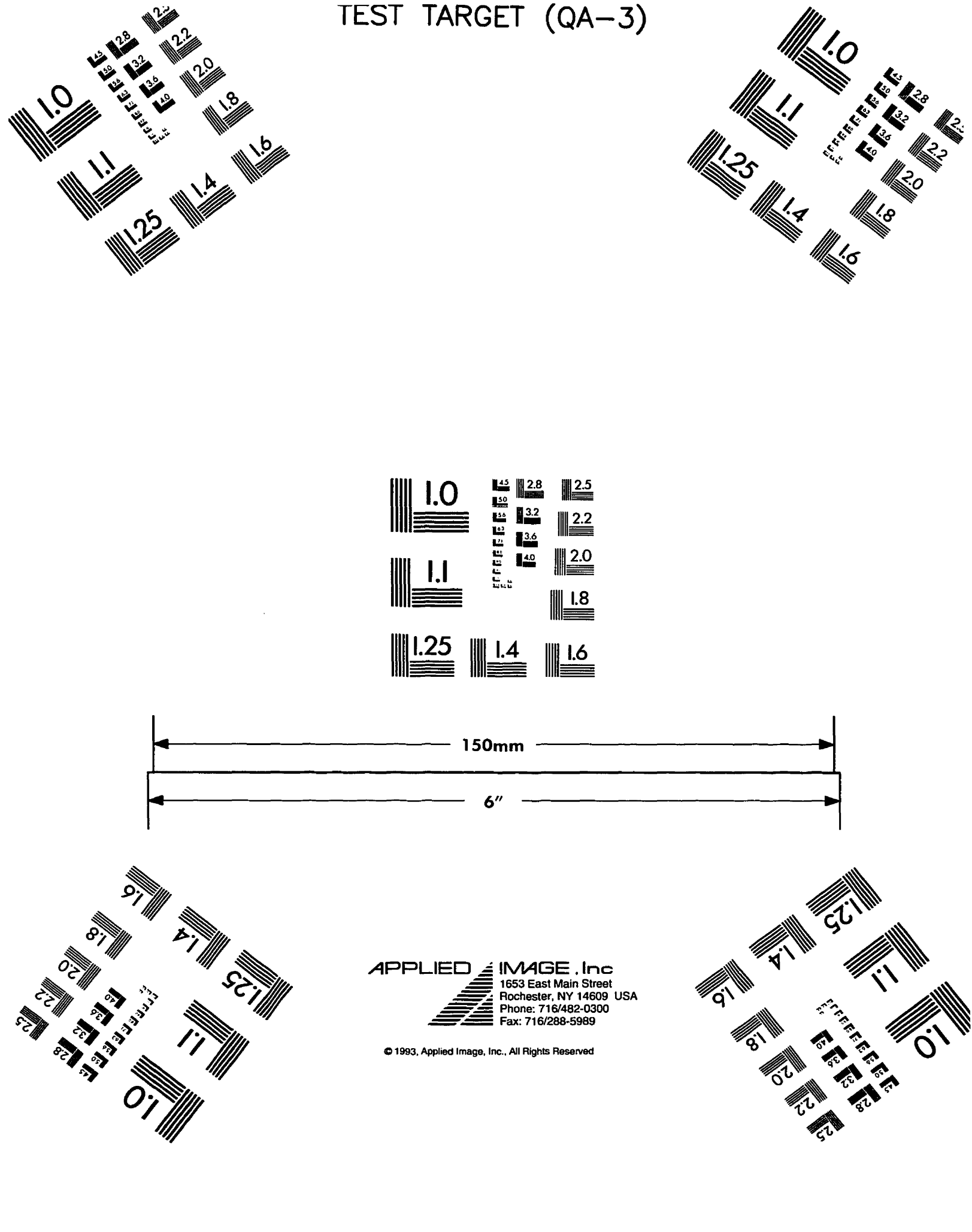
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