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SHELF-LIFE EXTENSION STUDIES ON PITA BREAD

By

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This thesis is dedicated to

My father, Adib, my mother, Aida, my sister, Sumaya, my brother, Raji

God, the Giver of all the Blessings

Abstract

Shelf-Life Extension Studies on Pita Bread

In this research, three alternative approaches to chemical preservatives to extend the mold free shelf-life and quality of pita bread were investigated namely: Modified Atmosphere Packaging (MAP) involving gas packaging, oxygen absorbents technology and ethanol vapor generators, high pressures, and direct and indirect heating.

Gas packaging using 60%CO_2 (balance N_2) inhibited the growth of *Aspergillus niger* and *Penicillium notatum* from 3d (pita bread packaged in air) to 35d at ambient temperature (~ 25°C). A longer extension in shelf-life (42d) was possible using an Ageless oxygen absorbent (Type FX100) or a Freshmax oxygen absorbent label in conjunction with gas packaging (CO_2:N_2-60:40). In all MAP products, the sensory shelf-life was terminated ~7d prior to microbiological shelf-life due to changes in odor, texture, and flavor.

Similar results were obtained with 2G-4G sachets of Ethicap, an ethanol vapor generator and 100-200S sachets of Negamold, a dual functional oxygen absorbent-ethanol vapor generator. While the mold free shelf-life could be extended to 42d, irrespective of sachet type or size, the sensory shelf-life of pita bread ranged from 30-35d. Furthermore, ethanol vapor had a plasticizing effect on film permeability as shown by an increase in the Oxygen Transmission Rate (OTR) throughout storage.

High pressures (30-400MPa) could be used to inhibit mold growth. However, higher pressures resulted in delamination of the packaging film and textural changes to the pita bread. While lower pressures (5-20 MPa) prevented these defects, the mold free shelf-life of the product could only be extended ~ 3- 4d at these pressure treatments. Even such low pressures increased the OTR of the high barrier laminated packaging film.

Other alternatives, such as direct heating and microwave processing had a minimal effect in increasing the shelf-life of pita bread due to the short processing time and low temperature within the product.

Résumé

Études sur la Prolongation de la Durée de Conservation à l'étalage du Pain Pita

Dans cette recherche, trois méthodes alternatives aux agents de conservation chimiques furent étudiées afin de retarder l'apparition de moisissure, et donc de prolonger la durée de conservation à l'étalage du pain pita et d'améliorer sa qualité. Les méthodes retenues furent: l'Emballage sous Atmosphère Modifiée (EAM), comprenant l'emballage au gaz, la technologie d'oxygène absorbeur et les générateurs de vapeur d'éthanol; les hautes pressions; ainsi que les réchauffements direct et indirect.

A 60% de CO_2 (la balance en N_2), l'emballage au gaz a retardé la croissance de l'*Aspergillus niger* et du *Penicillium notatum* de 3 jours (résultat obtenu avec l'emballage du pain pita sous air) à 35 jours à température ambiante (~25°C). Une meilleure prolongation de la durée de conservation à l'étalage du pain pita (42 jours) fut obtenue lors de l'utilisation d'un absorbeur d'oxygène Ageless (Type FX100) ou d'un absorbeur d'oxygène Freshmax, lorsque combiné à un emballage au gaz (CO_2: N_2 -60:40). Dans tous les produits de EAM, la durée de vie sensorielle du pain fut terminée près de 7 jours avant celle microbiologique, et ce à cause de changements d'odeur, de texture et de saveur.

Des résultats similaires furent obtenus avec les sachets d'Ethicap 2G-4G, un générateur de vapeur d'éthanol, et 100-200S sachets de Negamold, qui représentent simultanément des absorbeurs d'oxygène et des générateurs de vapeur d'éthanol. La durée de vie du pain avant apparition microbiologique pouvait atteindre les 42 jours, quels que soient leur type ou leur grandeurs, tandis que la durée de vie sensorielle du pain pita oscillait entre 30 et 35 jours. De plus, la vapeur d'éthanol eut un effet plastificateur sur la perméabilité de la pellicule tel que démontré par l'augmentation du Taux de Transmission d'Oxygène (TTO) tout au long du stockage.

Les hautes pressions (30-400 MPa) peuvent être utilisées afin de retarder l'apparition de moisissure. Toutefois, des pressions trop élevées entraînèrent une délamination de la pellicule de l'emballage, ainsi que des changements de texture dans le pain pita. Des pressions moindres (5-20 MPa) prévinrent ces défectuosités, par contre, la durée de vie avant

apparition microbiologique ne fut rallongée que de 3 à 4 jours. Même ces basses pressions augmentèrent le TTO de la pellicule de l'emballage.

D'autres alternatives, comme le réchauffement direct et le traitement aux micro-ondes, n'eurent que des effets minimes quant à la prolongation de la durée de conservation à l'étalage du pain pita, et ce à cause du court lapse de temps de traitement et de la basse température à l'intérieur du produit.

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Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Bread, described by Ledford and Ledford (1992) as "The Wings of Life", is still an essential component in human nutrition. The origin of bread can be linked to the cultivation of wheat and barley in an area described as the "Fertile Crescent" which extends from the Nile Valley of Egypt to Jordan, Syria, Lebanon, Tigris and the Euphrates Valley (Harlan and Zohary, 1966). Bread was considered the main food staple of that region. Wheat was first eaten unmilled mixed with water. However, it tasted better when baked and the origin of bread was discovered (Quail, 1996). Early breads were unleavened and flat. The Egyptians were accredited with discovering bread fermentation, a revolution in bread-making. This discovery spread throughout the Mediterranean region, especially to Italy, where the fermentation process was improved considerably. The Romans later removed yeast from the surface of fermenting wine and used it to leaven bread. This method of dough preparation, called a barm, gained acceptance and soon spread to other countries including Britain (Ledford and Ledford, 1992). Bread technology has improved throughout the years as our knowledge about the fermentation process and baking technology has evolved.

1.2 Economy

Bread is considered a food staple in many countries, particularly the Middle East and the Indian subcontinent (Quail, 1996).

Table 1: Annual per capita consumption of bread in several countries

Country	Bread consumption(Kg/year)
Australia	44
Egypt	180
Iran	150
Italy	73
Kuwait	98
Syria	130
U.S.A.	23

Adapted from Quail (1996)

The consumption of bakery products in North America is estimated to be $23 billion annually with ~ 50% being spent on bread (Peat Marwick Group, 1991). In 1987, the Canadian bakery industry exported $1.33 billion worth of products, with bread accounting for 50% of the total value of the bakery products shipped (Ooraikul, 1991). According to Hunt and Robbins (1989), bakery products accounted for approximately 9% of total food expenditure, with bread being the most important, accounting for 27 cents of each dollar spent. However, the consumption of white bread has decreased in the last two decades in western societies while sales of whole wheat bran bread have increased due to health concerns. Sales of flat breads, especially pita bread, have been increasing in western societies due to migration of cultures and societies. Several methods can be used to classify bread products including methods of fermentation, bread volume, and water activity.

1.3 Classification of bakery products according to volume

Faridi (1988) classified bread according to its volume into three main categories:

a. High specific volume bread e.g., pan bread
b. Medium specific volume bread e.g., rye bread
c. Low specific volume bread e.g., pita bread, lavash: flatbreads

Low specific volume bread is known for its high crust to crumb ratio and has a firm, cohesive mouth feel. These characteristics arise from a shorter final proof period and higher baking temperature than the western type breads. The classification of bakery products according to their country of origin and processing characteristics is summarized in Table 2.

Table 2: Classification of bakery products according to their country of origin and processing characteristics.

Type of bread	Country of origin	Processing characteristics
White pan	USA and Canada	Fermented dough
French baguettes	France	Lean dough with malt, sugar
Italian sticks	Italy	Similar to french baguettes
Vienna bread	Austria	Rich dough
Bolillos	Mexico	Rich dough
Pan de agua	Dominican Republic	Rich dough
Egg rolls	China	High percentage of eggs, increased richness, volume, shelf life
Pan de sal	Philippines	Yeast-raised bread
Bagels	Russia	Stiff dough
Pita bread	**Middle East**	**Pocket-like thin bread, lean dough, low in fat**

Adapted from Sultan (1990)

1.4 Classification of bakery products according to moisture content and water activity (a_w)

Water activity (a_w) is one of the main factors affecting the shelf-life of bread. It is directly related to both chemical and microbiological spoilage. The classification of bakery products according to their moisture content and water activity is summarized in Table 3.

1.5 Classification of flatbreads according to cross section

Flatbreads are an important bread product, particularly in Middle eastern countries. Qarooni (1996) also classified flatbreads according to their cross-section as

a) Single layered flatbreads e.g., Lavash, Tandoori.

b) Double layered flatbreads e.g., Arabic or Pita bread, and Balady bread

1.6 Ingredients used for pita bread formulation

1.6.1 Major ingredients

The major ingredients in the production of Pita or Arabic bread are wheat flour, yeast, salt and water. Other ingredients, such as sugars, oils or improving agents, are optional.

Table 3: Classification of bakery products based on their moisture content and water activity(a_w)

Product type	Water activity (a_w)
Low moisture content	
Cookies	0.20-0.30
Crackers	0.20-0.30
Intermediate moisture content	
Chocolate coated donut	0.85-0.87
Danish pastries	0.82-0.83
Cream-filled snack cakes	0.78-0.81
Soft cakes	0.50-0.78
High moisture content	
Bread	0.96-0.98
Egg bread	0.90
Yeast raised donuts	0.96-0.98
Fruit pies	0.95-0.98
Cheese cake	0.91-0.95
Pita bread	**0.9-0.96**

Adapted from Doerry (1985); Smith and Simpson (1995)

Table 4: Classification of flatbreads according to their type, origin, and ingredients

Type	Origin	Major ingredients
Naan	Afghanistan	Yeast, water, salt, whole milk, whole wheat flour, corn oil, sesame seed
Chapatti	India	Sifted flour, water, salt
Baladi, Shami	Egypt	Yeast, water, wheat flour, salt, olive oil
Tannor, Saage	Gulf area	Water, flour, salt, brown sugar
Lavash, Sangak	Iran	Brown sugar, water, flour, salt
Shami	Sudan	Sugar, water, flour, salt, yeast
Kmage, Khubze	Syria	Sugar, water, flour, salt, yeast
Psilli, Gomme	Turkey	Sugar, salt, water, flour, yeast
Markook, Saage	Jordan	Brown sugar, salt, water, flour
Matzo	Israel	Unleavened bread, flour, water
Gubbiz	Iraq	Water, flour, yeast, salt
Pitti	Pakistan	Hard wheat, berries, water, salt, dried apricots
Anise Bread	Morocco	Yeast, flour, salt, water, anise seed, corn
Tortilla	USA	Corn meal, flour, boiling water
Paper thin Lavash	Armenia	Water, flour, sugar, salt, yeast
Injera	Ethiopia	Water, yeast, ground flour
Fougasse	France	Yeast, water, flour, salt, olive oil
Bannock	Scotland	Flour, water, salt, baking powder, cranberries
Arabic Bread (Pita)	**Lebanon**	**Yeast, water, salt, sugar, wheat flour**

Adapted from Alford and Duguid(1995)

1.6.1.1 Wheat flour

Wheat is the main cereal used to produce flour. The wheat quality depends on its variety and growth environment. These factors affect grain pigmentation, hardness, protein quantity, and soundness when harvested. Wheat flour is a major ingredient of Arabic bread. Its major components, starch and protein, are responsible for bread structure and moisture retention. Quail (1996) reported that flour with a protein content of 9-12% is acceptable. Protein content affects the dough's strength, elasticity, and extensibility.

1.6.1.2 Water

Water is a major ingredient in dough formation and plays an important role in the changes that take place during bread production. Moisture content influences the character, softness, flexibility, and crumb texture of bread. Dough consistency can be controlled through water addition and this affects the machinability of the dough and improves bread's quality. Usually, a 2:1 ratio of flour to water is added to enhance dough formation.

1.6.1.3 Salt

Salt has a major conditioning effect on the dough. It strengthens the gluten, and reduces stickiness which improves dough handling and machinability properties. Salt also contributes to bread flavor. However, salt can also have an inhibitory effect on yeast fermentation and the rate of carbon dioxide production. The level of salt typically used in Arabic bread is <2% (w/w) (Quail, 1996). However, Williams et al. (1988) reported that lower levels i.e., 1% (w/w) were desirable for enhanced flavor.

1.6.1.4 Yeast

Baker's yeast has replaced the use of sourdough in the fermentation of Arabic bread. Yeast is used at levels of 0.3-0.4% (w/w) (Quail, 1996). Yeast fermentation is the process by which sugars are converted to carbon dioxide, water, and secondary products. The resulting carbon dioxide is required for the crumb cell structure and for the even separation of layers. Some minor components are produced during fermentation which contribute to bread flavor. Without yeast, Arabic bread would not separate into two layers. Williams et al. (1988) reported that for weak and medium strength flour, yeast levels of 1 % were optimal. However, for strong flour bread, quality improved with yeast levels up to 2% (w/w). Mizrahi and Mizrahi (1988) increased yeast levels up to 3% to reduce fermentation time and hence production time.

1.6.2 Other Optional ingredients

Other ingredients can be added to assist in dough processing and baking operations or to enrich bread quality. These ingredients include fats, oils, emulsifiers, and dried gluten. Additives which help strengthen or soften dough through chemical action on gluten are called gluten modifying agents or improvers. Some of the optional ingredients used in pita or Arabic bread production are now discussed.

1.6.2.1 Ascorbic acid

Ascorbic acid acts as an oxidizing agent to strengthen or toughen the dough. Qarooni et al. (1989a) examined ascorbic acid levels at 50, 70, 100 p.p.m. (flour weight basis) and observed that bread scores were reduced with increasing levels of ascorbic acid. Faridi and Rubenthaler (1983) also found that the addition of 30 p.p.m. ascorbic acid improved the quality of Arabic bread made from low protein quality flour.

1.6.2.2 Potassium bromate

Potassium bromate also acts as an oxidizing agent on dough. Qarooni et al. (1989b) examined bromate levels of 10, 20, 30 p.p.m. At levels of 30 p.p.m , bread had poor rolling and folding characteristics with uneven layers and uneven crumb on the second day.

1.6.2.3 L-cysteine hydrochloride

L-cysteine hydrochloride is a chemical reducing agent which has a softening action on gluten, allowing shorter dough mixing times and a softer dough. Approximately 25p.p.m. of this reducing agent is adequate .

1.6.2.4 Bran

Bran is sometimes added to enhance the color of the bread crust. It also increases water absorption in the dough and adds flavors.

1.6.2.5 Fats and Oils

For Arabic or pita bread, it is recommended that only vegetable fat and oil are used. This will ensure that bread meets both Halal and Kosher requirements (Quail, 1996). Futhermore, doughs were softer and easier to machine. However, fat at a level of 0.6% (w/w), is adequate for good dough preparation.

1.6.2.6 Emulsifiers

Emulsifiers which are used as dough conditioners and anti-staling agents include mono and di-glycerides, diacetyl tartaric esters, sodium and calcium stearoyl lactylate, sorbitans and lecithin. Qarooni et al. (1989b) examined sodium steroyl lactylate (SSL) at levels of 0.2,

0.3, 0.4% (w/w). They showed that 0.3% gave the optimum results in terms of bread softness after two days.

1.6.2.7 Gluten

If the flour is weak or below an optimal protein content of <9%, gluten can be added to strengthen the flour. Addition of gluten increases dough strength and water absorption. Williams et al. (1988) found that the addition of 3% (w/w) gluten improved loaf score.

1.6.2.8 Sugar

Sugar is added to enhance fermentation in flour that has a low maltose figure. It is also added to darken the crust color due to the Maillard reaction which occurs during baking. At levels of 1% (w/w), bread has a sweet taste. Increasing levels of sugar to more than 5% decreases the fermentation process through yeast inhibition (Quail, 1996).

1.6.2.9 Milk Powder

Milk powder contains the sugar lactose which is not fermented by Baker's yeast. However, it will influence crust color. Approximately 1% (w/w) is recommended.

1.6.2.10 α-amylase

These enzymes are derived from bacterial, fungal, or cereal sources. They are used to assist in fermentation, to increase loaf volume, and to delay staling. Although the addition of fungal amylases does not alter mixing time, doughs tended to be sticky after a bulk fermentation of one hour (Qarooni, 1989a). The optional ingredients levels used and their function in the bread making process are summarized in Table 5.

Table 5: Levels and functions of optional ingredients used in the bread making process

Optional ingredient	Recommended level	Function
Ascorbic acid	20 p.p.m.	Strengthens dough
Potassium bromate	10-20 p.p.m.	Powerful oxidizing agent
L- cysteine HCL	25 p.p.m.	Decreases dough mixing time, Improves machinability
Bran	< 0.05%	Adds flavor, color
Emulsifiers	0.3%	Crumb softening agent Anti-staling
Fats and Oils	0.6%	Anti-staling agent
Dried gluten	1%	Improves loaf score
Sugar	<1%	Darkens crust color Enhances flavor
Milk powder	1%	Improves crust color, flavor
α-amylase	1 unit/g flour	Delays staling

Adapted from Quail (1996)

1.7. Commercial processing of pita bread

According to Quail (1996), the commercial processing of pita bread involves two processes: (a) Divided dough sheeted individually and (b) Die cut doughs. Nowadays, the first process is most commonly used commercially. The second process is used to produce pita bread of uniform shape for use in fast food outlets. Both processes are described in Figure 1. The unit operations include mixing of ingredients, primary proofing (floor time), rolling or extruding in the die cut method, a second intermediate proof, sheeting or die cutting and a final proof. The bread is then baked at a high temperature (> 500°F) for a short time (<10 seconds) to aid the puffing process which results from the accumulation of steam and carbon dioxide. The bread is then cooled and packaged in polyethylene bags. The local baking of pita bread is shown in Figure 3 (Chapter 2, section 2.2.1).

1.8 Spoilage of pita bread

Traditionally, pita bread was purchased and consumed within hours of baking. However, due to urbanization and changing lifestyles, bread may have to be distributed over long distances and so a longer shelf-life is desirable. Like other processed food products, pita bread is subject to spoilage.

Spoilage of pita bread can be subdivided into three main groups:

1. Chemical;
2. Physical;
3. Microbiological; (Smith and Simpson,1995)

Each of these areas will be briefly discussed.

1.Divided Doughs Sheeted Individually *2.Die Cut Douhgs*

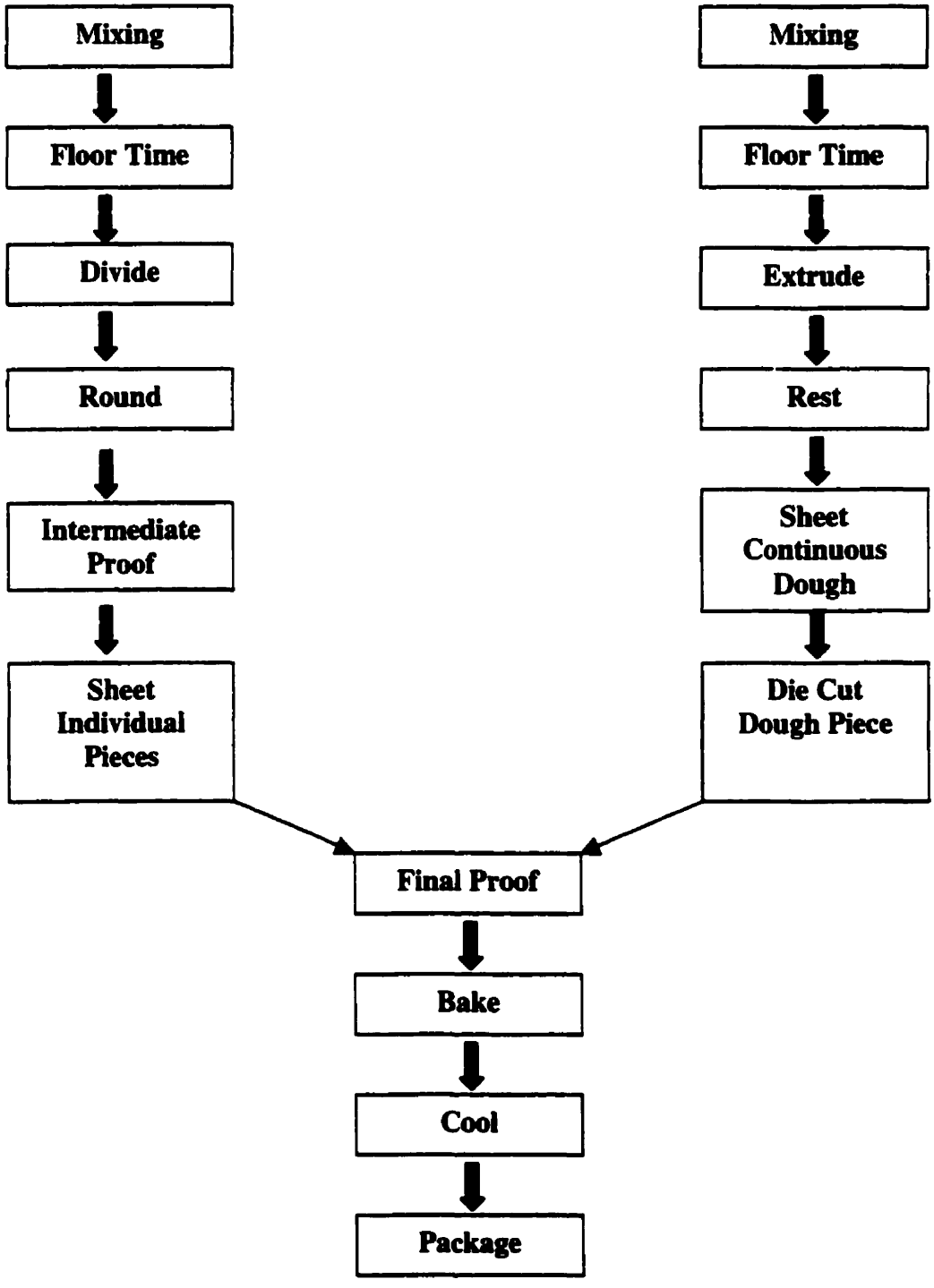


Figure 1. Production of pita or Arabic bread on a commercial basis

1.8.1 Chemical spoilage

Chemical spoilage of pita bread arises from lipid degradation and production of off-flavors and off-odors known as rancidity. Rancidity can be either oxidative or hydrolytic. Oxidative rancidity occurs in bakery products with a water activity < 0.3. Products turn rancid when the fat is oxidized and decomposed to short chain fatty acids, aldehydes, and ketones through autolytic free radical mechanism. These radicals can bleach pigments, breakdown proteins, destroy fat-soluble vitamins, and darken fat (Smith, 1992). Rancid products will produce a musty, disagreeable odor and taste. The addition of commercial anti-oxidants, such as butylate hydroxy toluene (BHT) and butylate hydroxy anisole (BHA), in addition to natural antioxidants, such as vitamin C, can extend the shelf-life and protect essential nutrients (Mahan and Arlin, 1992). The a_w of pita bread is ~ 0.96 making it more susceptible to hydrolytic rancidity. Hydrolytic rancidity occurs mostly in the absence of oxygen. It results in the hydrolysis of triglycerides and the release of glycerol and short-chain fatty acids. Unless fat is added, hydrolytic rancidity will not occur in pita bread.

1.8.2. Physical spoilage

Physical spoilage includes:

a) Moisture loss or gain.

b) Staling

1.8.2.1 Moisture loss or gain

Moisture loss or gain affects the texture and the firmness of bakery products. This problem can be prevented by using a high moisture barrier film e.g., low or high density polyethylene.

1.8.2.2 Staling

Staling is defined as "almost any change, short of microbiological spoilage, that occurs in bread during the post baking period, making it less acceptable to the consumer" (Kulp and Zobel, 1996). Such changes can be sensory (loss of aroma, mouth feel) or physical (loss of crumb softness, development of crumbliness). Any loss of freshness in bread will lead to staleness. In a market of approximately 20 billion pounds of bread produced annually, the return of 3% (600 million pounds) due to staling is an economic loss to both producer and consumer (Hebeda and Zobel, 1996). The major changes that occur after baking are moisture redistribution, starch retrogradation, increased bread firmness associated with loss of flavor and aroma (Quail, 1996). Pita bread, if packaged soon after cooling, is considered fresh for up to 8 hours. After 8 hours, staling begins, however, after 24 hours, pita bread is still acceptable. Beyond 24 hours, pita bread tends to stale (Quail, 1996). Staling is divided into crust and crumb staling. Crust staling is due to moisture redistribution from the crumb to the crust rendering the crust moist, leathery, and soft (Kulp, 1979). Crust staling is important in hard rolls and hearth breads which are palatable only if the crust is fresh. If these breads are left completely unwrapped, they will totally dry out. If they are packaged, the crust will soon stale (Kulp,1979). The use of high moisture barrier films prevents moisture loss from the crust to the atmosphere thus making the crust soft and leathery. Crumb staling results in a firmer, less elastic, dry, harsh textured crumb with a change of aroma and flavor. All of these changes result in staling.

Several factors influence staling. These are (a) time and temperature of storage, (b) flour proteins, (c) flour pentosans, (d) mixing conditions (e) other ingredients, such as yeast levels and olive oil.

1.8.2.3 Compounds used to prevent staling

Several compounds can be used to prevent or delay staling. These include lipids and shortening, surfactants, emulsifiers, amylotic enzymes, mono and diglycerides. Some of the ingredients used to delay staling, their maximum levels permitted, and their mode of action are summarized in Table 6.

1.8.2.4 Effect of CO_2 on bread staling

The effect of high levels of CO_2 as an anti-staling agent needs more research as results have been contradictory. Doerry (1985) showed that the bread crumb became firmer irrespective of storage atmosphere i.e., 100% carbon dioxide or 100% nitrogen. Brody (1989) reported that the staling rate of white and whole wheat bread was not significantly reduced when packaged in carbon dioxide or nitrogen compared to air. Black et al. (1993) also reported no uniform pattern of firming over time in pita bread packaged under various atmospheres. On the other hand, Knorr and Tomlins (1985) showed that the compressibility of bread packaged in 100% carbon dioxide was lower than bread packaged in air suggesting that CO_2 decreased the rate of bread firming.

Table 6: Ingredients used to delay staling, their maximum levels permitted, and their mode of action.

Product	Maximum permitted levels	Strengtheners	Softeners
Surfactants			
Calcium steroyl-2-lactylate	0.5%	Excellent	Good
Sodium steroyl-2-lactylate	0.5%	Excellent	V.good
DATEM	0.5%	Excellent	Fair
Mono, di-glycerides	0.5%	No	Excellent
Succinylated monoglycerides	0.5%	Good	Good
Polysorbate 60	0.5%	Fair	V. good
Ethoxylated monoglycerides	0.5%	V. good	Poor
Sucrose fatty acid esters	0.5%	V. good	V. good
Lipid and shortening	2-3%	No	V. good
Gums	0.5%	No	V. good
Enzymes			
Amylases (α, β)	0.2-0.3%	No	Excellent

Adapted from Hebeda and Zobel (1996)

Avital et al. (1990) also showed that CO_2 delayed bread firming. They proposed that changes in the sorption properties of modified atmosphere packaged (MAP) baked goods were responsible for this effect. Amylose is found in its crystalline state after one day while amylopectin has water binding sites. It is suggested that carbon dioxide blocked some of these sites, thus causing a reduction in the hydrogen bonding between the amylopectin branches resulting in a reduced water sorption capacity. Since hydrogen bonding is implicated in bread staling, blockage of water binding regions may explain bread firming. The effect of carbon dioxide was found when water was in the solute state. The solubility of carbon dioxide in water is 35x higher than oxygen in water. Thus, it might be possible that when water was in the solute stage, carbon dioxide dissolved easily and bound strongly to amylopectin, thus preventing H-bonding.

1.8.3 Microbiological spoilage

Microbiological spoilage is often the major factor limiting the shelf-life of baked products. It has been estimated that in the USA alone, losses due to microbiological spoilage are 1-3% or over 90 million kg of product each year (Betchell et al., 1953). The most important factor influencing the microbiological spoilage of bakery products is the water activity (a_w). For low-moisture bakery products (a_w<0.6), microbiological spoilage is not usually a problem. In intermediate moisture products, (a_w=0.65-0.85), osmophilic yeasts and molds are the predominant spoilage microorganisms. According to Legan and Voysey (1991), yeast problems in bakery products can be divided into two types: (a) visible yeast which grow on the surface of the bread in white or pinkish patches; and, (b) fermentative spoilage associated with alcoholic and esteric odors and hence osmophilic yeasts. Yeasts, which cause surface spoilage of bread, are mainly *Pichia burtonii* ("Chalk mold"). Contamination of products by osmophilic yeasts normally results from unclean utensils and equipment. Therefore, maintaining good manufacturing practices will minimize contamination by osmophilic yeasts.

For high-moisture products (a_w=0.94-0.99) e.g., pita bread (a_w=0.96) almost all bacteria, yeasts, and molds are capable of growth. According to Malkki and Rauha (1978), losses due to mold spoilage vary between 1-5% of products depending on season, type of product, and method of processing. Although fresh bread and other baked products are free of viable vegetative mold spores, products soon become contaminated as a result of post baking contamination from the air, bakery surfaces and equipment, food handlers, and raw ingredients such as glazes, nuts, spices, and sugar (Seiler, 1988). Mold problems are most troublesome during the summer months due to airborne contamination and the warmer, more humid, storage conditions. According to a one year study with bread obtained from a whole number of bakeries and stored in plastic bags for 5-6d at 22°C, *Penicillium* species were present in nearly all the loaves while *Aspergillus* species and *Cladosporium* species occurred in approximately half the loaves (Smith and Simpson, 1995). Chalk mold was also isolated from the loaves, especially in September. While the total water content of pita bread may be lower than that of sliced and wrapped pan bread, its a_w is sufficient to support the growth of molds and bacteria (Quail, 1996). Avital and Manneheim (1988) found that the a_w of Arabic bread ranged from 0.9 - 0.96 and was conducive to mold and bacterial growth. The main types of molds causing spoilage of Arabic or pita bread are *Monilia stolonifer*, and members of *Aspergillus, Rhizopus, and Penicillium* species particularly *Aspergillus niger, Penicillium expansum* and *Rhizopus stolonifer.* Al-Mohizea et al. (1987) studied the shelf-life of Arabic bread purchased from commercial bakeries in Saudi Arabia. Two to four hours after baking the microbial counts were:

- Aerobic plate count: 11-850 cfu/g
- Coliforms: Absent
- Spore formers : 12 spores/ g.
- Yeasts, molds: 6 cfu/g

These authors reported that the sanitation of bakeries varied considerably. For bread stored at ambient temperature and relative humidity (RH) of 43%, the mold free shelf-life ranged from 9-12 days. *Penicillium* and *Aspergillus* species were the most predominant mold contaminants (39-42%) of pita bread. Approximately one third of *Aspergillus* isolates were identified as *Aspergillus niger*, *Rhizopus* and *Neurospora* (13-15%) were also isolated from pita bread. Mold spoilage results from post-processing contamination during cooling and packaging (Black et al., 1993). Contamination can also occur from food handlers and raw ingredients such as sugar, salt, water (Smith, 1994). Shelf-life extension methods for bakery products can be subdivided into traditional and novel methods.

Traditional methods include;

(a) Good manufacturing practices (GMPs): cleanliness, sanitation, hygiene (Jenkins,1975)

(b) Ultra-violet light and microwave heating (Black et al., 1993)

(c) Use of preservatives e.g. sorbic and propionic acid (Seiler, 1989)

(d) Freezing (Matz, 1992)

(e) Modified atmosphere packaging (MAP) involving gas packaging with mixtures of carbon dioxide and nitrogen, oxygen absorbents, and ethanol vapour generators (Smith and Simpson, 1995).

Novel Methods of extending the shelf-life of bakery products include;

(a) Ultra High Pressure Processing (HPP)

(b) High Intensity Pulsed Light (HIPL)

Each of these methods will be briefly reviewed.

1.8.3.1 Traditional methods

1.8.3.1.1 Good manufacturing practices

Good manufacturing practices reflect the cleanliness in the plant or in any local or commercial bakery. There is no substitute for good sanitation practices to eliminate or to control spoilage. The slicing equipment or slicer blades should always be kept clean to prevent the accumulation of molds on its edges and prevent post processing contamination (Jenkins, 1975). Humidity should also be controlled as much as possible by using an air conditioning system in an enclosed area. However, this is considered extremely expensive. Some of the important sanitation programs used in the bakery industry to increase the mold free shelf-life of products are the following:

(a) Adequate clean-up procedures to reduce dust particles and unclean equipment;

(b) Supervision of personal hygiene;

(c) Supervision of warehousing of raw ingredients and finished products;

(d) Supervision of lighting, heating, and ventilation of the plant: If possible, bakery facilities should be operated under positive pressure with filtered air to reduce the chance of microorganisms entering the plant from the outside.

1.8.3.1.2 U.V. light

Ultra-violet light is used in bakeries to prevent mold growth on the surface of freshly baked products. It is non-ionizing and is absorbed by proteins and nucleic acids which may lead to cell death (Jay, 1996). The lethal effect of UV radiation on microorganisms is near 260nm with a quantum energy of 4.9 electron volts (ev) (Smith, 1993). The mechanism of UV results in DNA mutations in microorganisms. In addition, 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). However, UV light is not very effective in extending the mold free shelf-life because it does not penetrate the product and molds spores can still grow in the products. Usually, UV light is used to treat the surface of bread prior to packaging. Furthermore, it cannot be regarded as a viable method to extend the mold free shelf-life of bakery products due to its cost, its potential effect on packaging material, and effect on workers' eyes (Kyzlink, 1990).

1.8.3.1.3 Microwave heating

Microwave energy causes food molecules with a dipole or charge to oscillate when placed in an electromagnetic field creating an intermolecular friction which is manifested as heat. Most microwave food research has been carried out at two frequencies: 915 and 2450 megacycles (Jay, 1996). Treatment of wrapped bread with high frequency microwave energy for 45 to 60 seconds further extended the mold free shelf-life of bread (Pomeranz, 1969). Microwave heating is rapid and it may increase the shelf-life of fresh bread for 21 days (Smith, 1993). Some of the disadvantages of microwave heating are that it may not give the required extended shelf-life in some products and some packaging films may be adversely affected by high temperatures (Smith, 1993).

1.8.3.1.4 Irradiation

The effect of gamma irradiation on Arabic bread was studied by Gretz et al. (1985). The flour was found to contain spore forming bacteria, yeasts, and molds. The microbial count in flour, dough, and bread, prior to and after irradiation is summarized in Table 7.The most abundant organisms in Arabic bread after baking were spore-formers ($3.5x10^3$ cfu/ g) of which 70% were thermophilic. Baking essentially killed all yeasts and partially reduced the number of spore-formers. When gamma irradiation was applied within the acceptable range of 0.2Mrads, it inactivated all molds and yeasts and decreased the spore-forming bacteria to $1x10^1$ cfu/g (Gretz et al., 1985). Over a 7 day period, irradiated bread was not spoiled by mold, while bread, which had not been irradiated, developed visible mold growth within one to two days. Sensory evaluation found no difference between non-irradiated and irradiated bread (Gretz et al., 1985).

1.8.3.1.5 Preservatives

Preservatives are most commonly used to control mold growth in baked goods. The Code of Federal Regulations (CFR) defines a preservative "as an antimicrobial agent used to preserve food by preventing growth of microorganisms and subsequent spoilage" (CFR, 1990). There are two classification of preservatives: chemical and natural. Permitted chemical mold inhibitors in bread include acetic, sorbic, propionic acid and their salts. Most countries have regulations limiting the addition of preservatives to food (Quail,1996).These preservatives affect the flavor of bread and this may be a factor limiting their addition. Natural food preservatives, such as cultured products, raisins, vinegar, are identified by their common name on the ingredient statement. A good chemical or natural food preservative should possess several factors to maximize efficacy, cost efficiency, and ease of handling while minimizing adverse effects on the products in which they are used.

Table 7: The microbial count in flour, dough, and bread prior to and after irradiation

Ingredient	Irradiation dose (Mrad)	CFU/g*			
		Spore-formers	Yeast	Molds	Total
Flour	-	2.1×10^1	3.7×10^1	4.0×10^2	4.5×10^1
Dough	-	4.5×10^4	2.7×10^7	-	1.2×10^7
Bread	-	3.5×10^3	2.0×10^1	1.0×10^1	2.0×10^4
Bread	0.2	1.0×10^1	-	-	-

Adapted from Gretz et al. (1985)

*CFU: Colony Forming Units

- : no value

The characteristics of a food preservative are (a) a broad antimicrobial spectrum, (b) non-toxic to humans, (c) effective at low temperatures, (d) not affected by pH, (e) free of odor, color, and flavors at levels used, (f) available in dry form, (g) water soluble, (h) non-corrosive, (i) stable during storage, and (j) cost effective (King, 1981). The most common preservatives used to extend the mold free shelf-life of baked products are sorbic acid or propionic acid. Sorbic acid, and its potassium salts, (sorbates) are antimicrobial agents which are Generally Regarded as Safe (GRAS) if applied in a range of 0.001-0.3% (w/w) basis. Sorbic acid and its potassium salt have a broad spectrum of activity against yeasts and molds but limited activity against bacteria with the exception of rope forming *Bacillus mesentericus*. Sorbates act as preservatives in food products having a pH between 4 and 7. However, the antimicrobial effect of sorbate increases as the pH of the food decreases (Hassan, 1997). The antimicrobial action of sorbates has been associated with the undissociated form rather than the dissociated (Sofos and Busta, 1981). Sorbates should not be used with yeast leavened products due to their inhibitory effect on yeast (Hebeda and Zobel, 1996). Propionic acid and its potassium salt, known as the propionates, were patented in 1939 as mold inhibitors for bread. This preservative acts as an antimicrobial agent and also prevents rope in bread. This chemical is GRAS if applied within a range of 0.1-0.2% (w/w) basis. Propionates have no effect on yeast and are widely used with yeast leavened products. The antimicrobial activity of propionates is again optimized when used within a pH range of 4.5-6.0 (Hebeda and Zobel, 1996).

1.8.3.1.6 Freezing

Freezing can be used to prevent mold growth if applied directly after packaging (Matz, 1992). However, mold growth may occur after the bread has thawed. Freezing is not commonly used for bread, but it is more commonly used for filled products such as meat, fruits, and cream pies where microbial growth, particularly pathogens or spoilage is a concern.

1.8.3.1.7 Modified atmosphere packaging

Modified atmosphere packaging (MAP) is a relatively new packaging technology used to increase the mold free shelf-life of bakery products. In high moisture products, such as pita bread, (a_w=0.96) almost all organisms are capable of growth, but mold growth is often the main spoilage problem (Smith, 1994). MAP is defined as "the enclosure of food products in a high barrier film in which the gaseous environment has been changed or modified to slow the respiration rates, reduce microbiological growth, and retard enzymatic spoilage with the intent of extending the shelf-life" (Young et al., 1988). It is estimated that the demand for MAP foods in North America could reach 11 billion packages by the year 2000 (Smith and Simpson,1995). Several methods can be used to modify the atmosphere within the packaged product, including

1. Vacuum packaging;
2. Gas packaging;
3. Oxygen absorbents;
4. Ethanol vapor generators;

1.8.3.1.8 Vacuum packaging

Vacuum packaging involves evacuating most of the oxygen present in the package to levels less than 1%. This low oxygen concentration will help prevent the growth of aerobic organisms and reduce the rate of oxidative rancidity (Smith and Simpson,1995). The use of vacuum packaging is limited in the baking industry due to its crushing effect on products (Smith, 1994).

1.8.3.1.9 Gas packaging

Gas packaging is an extension of vacuum packaging technology. It involves packaging a product in an impermeable film, flushing with appropriate gas mixtures, and heat sealing the package. Gases used in MAP include nitrogen and carbon dioxide. These gases are used since they are neither toxic nor dangerous and they are not considered as food additives (Smith and Simpson, 1995). Nitrogen is an inert, tasteless gas, and is used as a filler gas. Because of its insolubility in water, the presence of nitrogen in MAP food can prevent pack collapse that occurs when high concentrations of carbon dioxide are used. In addition, nitrogen on its own can delay oxidative rancidity in low water activity products. Carbon dioxide is the most important gas in gas packaged bakery products. It is both fungistatic and bacteriostatic and prevents insect growth in packaged and stored food products. Carbon dioxide is highly soluble in fat and water where it forms carbonic acid. The solubility may lower the product pH resulting in slight flavor changes. The most common applied levels of CO_2 to N_2 in bakery products are 60: 40; however, higher levels of carbon dioxide are sometimes used (Smith, 1994). Typical gas mixtures to extend the shelf-life of bakery products are shown in Table 8.

Several studies have been done to determine the mode of action of carbon dioxide and its antimicrobial effect which may be due to;

(a) The exclusion of oxygen by replacement with carbon dioxide may contribute to the overall antimicrobial effect by slowing the growth of aerobic spoilage microorganisms.

(b) The CO_2 /HCO_3^- may affect the permeability of the cell membrane.

(c) CO_2 may reduce the internal pH of the cell and influence certain enzyme systems and metabolic activities (Smith and Simpson,1995).

The mold free shelf-life of products increases as the concentration of carbon dioxide in the atmosphere increases. The effect is further enhanced at lower storage temperature due to the greater solubility of carbon dioxide. The advantages and the disadvantages of gas packaging are summarized in Table 9.

1.8.3.1.10 Oxygen absorbents

Oxygen absorbents are gas scavenger products designed to reduce the oxygen content of the package to less than 1%. They are defined "as a range of chemical compounds introduced in a food package to alter the atmosphere within the package"(Smith, 1992). The basic system consists of finely divided iron powder particles contained in a sachet similar to a desiccant. The sachet material is highly permeable to oxygen and water vapor and under appropriate humidity conditions; it scavenges residual oxygen to form non-toxic iron oxide. This reaction decreases the oxygen headspace to as low as 0.01% provided a film of the correct permeability is used. The oxidation reaction mechanism can be summarized as follows (Smith et al., 1990).

$$Fe \rightarrow Fe^{2+} + 2e^-$$

$$1/2O_2 + H_2O + 2e^- \rightarrow 2\ OH^-$$

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2$$

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

$$2Fe(OH)_2 + O_2 + H_2O \rightarrow 2Fe(OH)_3$$

Table 8: Typical gas mixtures to extend the shelf-life of bakery products

Product	$\%CO_2$	$\%N_2$	$\%O_2$
Sliced Bread	100	-	-
Rye Bread	100	-	-
Croissants	100	-	-
Buns	100	-	-
Cakes	80	20	-
Pastries and Muffins	50	50	-
Crumpets	80	20	-
Crepes	80	20	-
Sausage Rolls	80	20	-
Pizza	80	20	-
Pita bread	99	1	-

Adapted from Smith and Simpson (1996)

Table 9: Advantages and disadvantages of gas packaging

Advantages	Disadvantages
Increased shelf-life	High intial cost of packaging films
Increased market rate	Leakage
Reduction in production, storage costs	Secondary fermentation products
Improved presentation of products	Potential growth of microorganims of public health concern.
Fresh appearance of products	
Clear view of products	
Easy separation of slices	

Adapted from Smith and Simpson (1995)

Substances normally used in the sachet are iron or ascorbic acid (Smith and Simpson, 1995). The first oxygen absorbent was an iron based absorber developed in 1977 by Mitsubishi Gas Company under the trade name of Ageless. Until now, 7000 million sachets have been sold in Japan. The sale of absorbents is expected to grow at a rate of 20% per year (Smith and Simpson,1995). Other types of absorbents are now available on the market. These include Freshilizer and Freshpax absorbents which act in a similar manner to Ageless (Smith and Simpson, 1995). Oxygen absorbents must meet specific criteria to be accepted scientifically and succeed commercially. These are:

(a) The ingredients should be non-toxic.
(b) They should absorb oxygen at an appropriate rate.
(c) There should not be any unfavorable side reactions.
(d) They should be of uniform quality.
(e) They must be compact and uniform in size. (Brody,1989)

Several factors influence the choice of oxygen absorbents including:

(a) The nature of the food i.e., size, shape, weight.
(b) The a_w of the food.
(c) The amount of dissolved oxygen in the food.
(d) The desired shelf-life of the product.
(e) The initial level of oxygen in the package headspace.
(f) The oxygen permeability of the packaging material.
(Smith and Simpson,1995).

Studies at McGill University have shown that oxygen absorbents are three times more effective than gas packaging at increasing the mold free shelf-life of bread rolls. Ageless S and FX were packaged along side the rolls and the oxygen never increased above 0.05%. The product remained mold free for over 60 days at ambient storage temperature. Studies at Cryovac, a leading company in packaging materials and packaging equipment, have shown that oxygen absorbents can be used to prevent mold growth, rancidity problems, and flavor changes in pasta and pizza crusts thereby enhancing consumer appeal for such a product (Smith and Simpson, 1995). The mold free shelf-life of white bread packaged in polypropylene film can be extended from 5 to 45 days at room temperature by incorporating an oxygen absorbent sachet in the package. Pizza crusts, usually became moldy within 2-3 days at 30°C; however, they can remain mold free for more than 14 days at this temperature using an appropriate oxygen absorbent. The main problems with oxygen absorbents are consumer resistance to their use in food, the fear of ingesting the absorbent and the spillage of sachet contents in the foods thus adulterating the product (Smith and Simpson, 1995). The advantages and the disadvantages of oxygen absorbers are summarized in Table 10.

Table 10: Advantages and disadvantages of oxygen absorbers

Advantages	Disadvantages
Inexpensive, easy to use	Need to have a free flow of air surrounding the sachet to scavenge the headspace oxygen
Non-toxic, fast to use	Consumer resistance, misuse of sachets
Prevent aerobic microbial growth	May promote growth of potentially harmful anaerobic bacteria
Arrest development of rancid off-flavors of fats and oils	
Increases product shelf-life and distribution	
Reduces distribution losses	

Adapted from Smith and Simpson (1995)

1.8.3.1.11 Ethanol vapor generators

Despite its widespread use as a germicidal agent, few studies have evaluated ethanol as a preservative for food products. Ethyl alcohol has been shown to increase the shelf-life of bread when sprayed onto the surface of the product prior to packaging indicating its potential as a vapor phase inhibitor (Seiler, 1978). Another model of atmosphere modification is manufactured by the Freund Company Ltd.(1985) of Japan and sold under the name of Ethicap or Antimold 102. Ethicap is a sachet placed alongside food and it releases ethanol vapor into the package headspace. The released ethanol vapor (0.5-2.5%(v/v)) then condenses on the food surface and acts as a microbial inhibitor (Labuza and Breene, 1989). Ethicap consists of food grade alcohol adsorbed on to silicon dioxide powder and contained in a sachet made up of a copolymer of paper and ethyl vinyl acetate (Smith and Simpson, 1995).Vanilla and other compounds are used to mask the alcohol flavor. Ethicap sachets come in various sizes ranging from 0.6 to 6g or 0.33 to 3.3g of ethanol evaporated. The size of the sachet used depends on the weight of the food, the a_w of the food, and the desired shelf-life of the product. Another kind of ethanol vapor generator produced by Freund, Japan is termed Negamold. This compound, like Ethicap, is moisture dependent. Both work with products having an a_w >0.85. On the other hand, Negamold also acts as an oxygen absorbent as well as an ethanol vapor generator. The types of ethanol vapor generators are summarized in Table 11. Studies done by Smith et al. (1990) have shown that ethanol vapor generators are effective in controlling 10 species of molds including *Aspergillus* and *Penicillium* species, 15 species of bacteria including *Salmonella*, *Staphylococcus*, and *Escherichia coli*, and three species of spoilage yeast. Other studies conducted by Smith et al. (1987) investigated the effect of ethanol vapor on the growth of *Saccharomyces cerevisiae*, the main spoilage microorganism in gas packaged apple turnovers.

The results showed that when Ethicap was incorporated into the packaged product, yeast growth was completely suppressed and all the packages appeared normal at the end of the 21day storage period. This study demonstrated the usefulness of ethanol vapor for the shelf-life extension of a fruit filled bakery product subject to secondary spoilage by yeast.

The advantages of Ethicap as a preservative are:

(a) Ethanol vapor can be generated without spraying ethanol solutions directly onto products prior to packaging.

(b) Sachets can be conveniently removed from packages and discarded at the end of the storage period.

(c) It eliminates the need to use preservatives such as benzoic acid or sorbic acid to control yeast fermentation.

(d) They are inexpensive.

The main disadvantage of using ethanol vapor for shelf-life extension of food is its absorption from the package headspace by the product (Smith et al., 1987). Ethanol has been generally regarded as safe (GRAS) in the USA as a direct human food ingredient. As a permitted additive, there is no objection to its use at levels up to 2% by product weight (CFR, 1990). It is suggested that ethanol also prevents or delays staling by acting as a plasticizer of the protein network the bread crumb. The anti-staling effect of ethanol has been observed in Japanese confectionary products and in doughnuts (Russell, 1991).

Table 11: Types of ethanol vapor generators

Type	Function	Types	a_w of product
Ethicap	Generates ethanol vapor	Moisture-dependent & Self-reacting	> 0.85
Negamold	Generates ethanol vapor Absorbs Oxygen	Moisture-dependent & Self reacting	> 0.85

Courtesy of Y.Ohno, Freund Technical Co., Japan.

1.8.3.2 Novel Methods

1.8.3.2.1 High Pressure Processing

The possibility of using high pressure to process and preserve food has been known and studied for almost a century (Hite, 1899). However, in the last few years, there have been tremendous developments in engineering aspects of high pressure equipment so that it is now both economically and technically feasible to subject foods to the desired high pressures. The first food products processed with high pressure were introduced on the Japanese market in April 1990 (Rovere, 1995). High pressure processing (HPP), also called ultra high pressure processing, is considered complementary to traditional heat treatment. One basic difference between high pressure and thermal processing is that pressure does not affect any of the desired components that may be reduced or destroyed by traditional processing; e.g., vitamins, chlorophyll and most aroma substances remain intact. Physical damage to the food is also less using HPP than by heat treatment (Rovere, 1995). Jams and fruit drinks were the first food products treated with high pressure since the beginning of the decade (Ledward et al., 1995). When HPP is used on fruits and vegetable products, it is often possible to achieve several weeks or months of refrigerated shelf-life with a product quality equal to, or very close to the fresh raw material. Furthermore, color, flavor, and texture are all well preserved. The food product must be in a package before it is subjected to high pressure. The choice of the package type and packaging material will influence the quality of the final product. The choice of the packaging material must meet the following criteria:

(a) The package must be able to withstand the stress of high pressure without physical damage, leakage or loss of barrier properties;
(b) The package must be able to physically protect the processed food while under pressure;

(c) The package must be able to preserve the superior quality of the food product in terms of flavor, color, texture and nutritional value during storage and distribution to the final consumer (ABB Labs, 1996).

Many types of packages can be used with HPP and include plastic bottles and films, cups, trays, and pouches. However, they must be flexible to allow for a 10-20% volume decrease of the product under pressure. Headspace in packages should generally be avoided, although if the package design is correct a certain headspace is possible (ABB Labs, 1996). Provided production is efficient, and production volumes reasonable, the total costs for HPP treatment is around $ 0.1- 0.5U.S/liter or kilogram of food. Isostatic pressing means the compression of a material obtained through the use of a pressure transmitting fluid. In practice, this means, the placement of food in flexible packages into pressure vessels which contain a pressure transmitting fluid, in most cases water (Rovere, 1995). According to Pascal's Law, pressure has a uniform effect on everything inside the pressure vessel. Therefore, the food due to the container's flexibility enables it to compensate during the product change for the external pressure (Rovere, 1995). The processed product will be compressed by a uniform pressure in all directions and then return to its original shape when the pressure has been released. During this process, temperature inside the pressure vessel increases. This increase is constant for a product high in water content. Since many food products are basically made up of water, high pressure processing does not result in large changes in volume or mechanical damage.

Once the product is loaded and the vessel closed, the pressure is increased by pushing in a piston until the desired pressure is reached. The unit pumping fluid into the press consists of standard components working at relatively low pressure, assuring high reliability and minimum downtime. After the pressurising and holding sequence, the vessel is decompressed and the product is unloaded. Material handling and process control is fully automated.

High pressure processing has several effects on the reactions on food involving microbial inactivation (bacteria, yeast, mold), and enzyme kinetics, and chemical reactions (Ledward et al.,1995). High pressure processing can inactivate microorganisms by damaging the cell membranes and inactivate key enzymes involved in DNA replication and transcription (Hoover et al., 1989). The extent of inactivation achieved at a particular pressure depends on a number of interrelated factors including level and duration of pressure, microbial species, processing temperature and substrate (Ledward et al., 1995).

The advantages of high pressure processing are the following:

(a) Avoids or limits heat treatment;

(b) Preserves nutritional value and vitamin content;

(c) Preserves natural taste, color, and texture of many products especially fruits and vegetables;

(d) Extends the shelf-life of refrigerated high acid products;

(e) Increases food safety at a given shelf-life for certain food products;

(f) It enables high productivity as the processing time does not depend on the dimensions of the product processed (isostatic);

(g) The impact on the environment is minimal: it requires only electrical energy and there are no waste products (ABB Labs, 1996)

The kinetics of enzyme catalysed reactions can be altered by the influence of high pressure on the binding site or the catalytic step which will result in either decreased or increased activity. If enzymes are exposed to very high pressures, they will be inactivated due to protein denaturation (Ledward et al., 1995). However, recent work by Rovere et al. (1994) showed that enzymes may recover some of their activity on subsequent storage at normal pressures.

In addition, some of them will renature to reproduce the native molecule. Chemical reactions affected by high pressure include the Maillard reaction. Depending on the kinetics of the reaction, reaction velocities which may be either accelerated or retarded by high pressure. It has also been shown that an increase in temperature results in an increase in reaction rate. Two examples illustrate this point. The Maillard reaction is strongly inhibited by high pressure; however, the oxidation of unsaturated lipids is accelerated by high pressure. As far as biological systems are concerned, high pressure denatures proteins, solidifies lipids, and breaks biomembranes resulting in the inactivation of bacteria (Ledward et al., 1995). High pressure influences biomolecular structures causing changes in hydrophobic interactions and hydrogen bonding. The effect of pressure on these weak interactions will cause proteins to unfold completely leading to denaturation, aggregation and precipitation. The sensitivity of vegetative pathogens to high pressure is summarized in Table 12.

Table 12: Sensitivity of vegetative pathogens to high pressure

Microorganisms	Pressure (MPa)	Reduction (Log cfu/g)
Salmonella	500	6
Escherichia coli O157:H7	700	6
Listeria monocytogenes	340	6
Staphylococcus aureus	400	8

Adapted from Ledward et al. (1995)

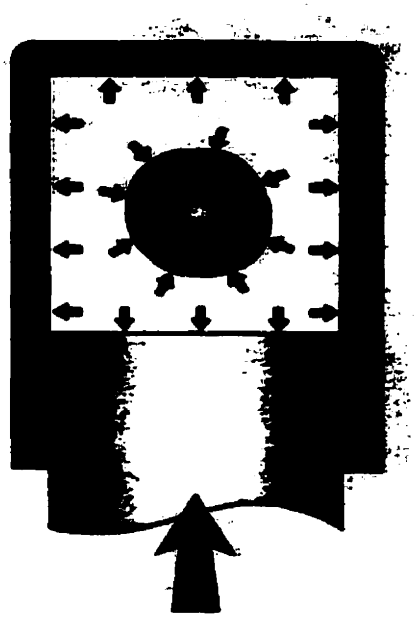


Fig 2. High pressure processing systems: Principle of isostatic pressing

1.8.3.2.2 Pulsed light technology

Pulsed light processing is a new technology discovered by PurePulse Technologies, San Diego, California in the 1990s. PurePulse Technologies have developed two new processes, i.e., intense pulses of light or electric fields. The process that uses intensive pulses of light on solid food products is known as PureBright^{R}. On the other hand, the process using electric fields on pumpable products is termed as CoolPure^{R}. These technologies use a technique known as pulsed energy processing. PureBright^{R} process uses short duration flashes of broad spectrum white light of wavelengths ranging from ultraviolet spectrum (200nm) to the infra-red spectrum (1000nm) to kill a wide range of microorganisms, including microbial and fungal spores. Each pulse or flash of light lasts a few hundred microseconds with a light intensity 20,000 times more intense than sunlight at the earth's surface. For most applications, a few flashes, applied in a fraction of a second, inactivates microorganisms (Dunn et al.,1997). This destruction can be achieved on the surfaces of packaging materials, foods, and medical devices. The bactericidal effect of pulsed light provide a new and highly effective process for sterilization and reducing bacterial contamination associated with many different products. The PureBright^{R} system consists of two main parts:(a) a power unit and (b) a lamp unit. The power unit generates high voltage, high current pulses which are used to give energy to the lamps. The unit operates by converting line voltage AC power into a high DC voltage power. This high power is used to charge the capacitor. Once the capacitor has been charged to a set point, a high voltage switch discharges the capacitor into a cable connected to the lamp. Control of the lamp firing can be done by an internal controller (Dunn et al., 1997). The power unit is mounted in a cabinet which can either stand alone or be an integrated product packaging/processing machine. The lamp unit consists of one or more inert, non-ionizing xenon gas lamps arranged to illuminate the desired treatment area. To flash the lamps, a high voltage, high current pulse is applied. The high current passing through the gas in the lamp causes it to emit an intense pulse of light.

The intense light pulse lasts for a few hundred microseconds. The number of lamps, the flashing configuration, and the flash rate depend on the application. Two diagnostics are monitored on every flash to ensure the lamps are operating correctly. These include the lamp fluence, which is defined as the measure of the incident light energy per unit surface area in joules/cm^2, and the lamp current. Normal operating ranges for PureBright^R treatment are from 0.1-0.3J/cm^2 with total accumulated fluences of 0.1-12J/cm^2. Flashes are applied at a rate of 0.5-10Hz.

Pulsed light can extend the shelf-life of a variety of foods. Studies conducted Dunn et al. (1997) on baked goods, seafood, meats, fruits, and vegetables showed significant reduction in the microbial load resulting in an extended product shelf-life. Bread, treated with pulsed light, had an extended mold free shelf-life of more than two weeks, while control loaves had a maximum shelf-life of a week at ambient temperature. Samples of chicken wings inoculated with three strains of *Salmonella* at levels of 5 log/cm^2 decreased to less than 2 log/cm^2 after pulsed light treatment. PurePulse is currently working worldwide with many companies to establish applications in food, medical, packaging, and environmental markets.

1.9 Objectives of Research

Pita bread is an important functional food in the Lebanese diet. Due to the migration of societies and cultures, pita bread has become a worldwide food commodity in many diets. However, like other bakery products, pita bread is subject to mold spoilage. The objectives of this research are to examine various alternatives to preservatives to extend the mold free shelf-life of pita bread.

The specific objectives of this research were to determine:

1. The effect of various gas atmospheres, ethanol vapor generators, high pressure processing, and direct and indirect heating on the mold free shelf-life of pita bread.

2. The effect of these packaging and processing technologies on the sensory quality of pita bread.

3. The effect of ethanol vapor generators and high pressures on the permeability of the packaging films to oxygen and water vapor used to package pita bread.

CHAPTER 2

EFFECT OF GAS PACKAGING ON THE SHELF-LIFE OF PITA BREAD

2.1. INTRODUCTION

Pita bread is a double layered flatbread enjoyed worldwide due to the migration of societies and cultures. However, like other bakery products, pita bread is subject to spoilage by staling and mold growth. Preservatives are commonly used to extend the mold free shelf-life of many bakery products. However, with increasing consumer concerns about preservatives, and the demand for preservative free products, the baking industry is seeking alternative methods to extend the mold free shelf-life of products. One such method is Modified Atmosphere Packaging (MAP) which is widely used in Europe to extend the shelf-life of bakery products. The objectives of this study were to determine the effect of various methods of atmosphere modification to extend the mold free shelf-life of pita bread and to determine the effect of MAP on the sensory characteristics of the bread.

2.2. MATERIALS AND METHODS

2.2.1. Pita bread formulation

Pita bread was formulated according to a commercial recipe obtained from a Lebanese local bakery in Beirut. All ingredients were weighed on a Mettler Toledo Balance (PB-3001) in a round metallic bowl. The ingredients (Table 13) and the luke warm water (50°C) were placed in a Hobart Mixer (D-300, Hobart Canada Inc., Don Mills, Ontario) and mixed at medium speed for 12 minutes until a dough was formed. The dough was then removed from the mixer, kneaded, and allowed to proof for 25-30 minutes at room temperature. The dough was then cut into small pieces (~40 grams each) and then passed twice through a pasta maker to make the dough as thin as possible. The long, thin pieces of dough were then flattened out with a wooden rolling pin prior to being cut into circular pieces.

The circular thin pieces of dough were then placed on flour dusted trays for a final proof of 5 minutes at room temperature. The dough was then placed, six at a time, on a griddle and baked at 500°F allowing the dough to puff into a double layered flatbread known as pita bread. The pita bread was then stacked on top of each other and covered with a damp J-cloth to prevent dehydration. The pita bread was cooled for 45 minutes at room temperature prior to packaging. In the initial study, pita bread was formulated with and without enzyme. The enzyme used for processing was SuperFresh™Plus Enzyme produced by Bio-system Ltd. (Beloit, Wisconsin) at 0.2% (w/w), that is, 2.6 g (w/w) in order to delay staling throughout storage. However, in all subsequent studies, only enzyme treated pita bread was used. Figure 3 summarizes the flow process of pita bread on a laboratory scale.

2.2.2 Inoculation

Two mold species, *Aspergillus niger* and *Penicillium notatum*, were used as the molds of study. These molds were selected since they are the most common spoilage molds of bakery products. Both molds were grown on Potato Dextrose Agar (PDA, Difco, Michigan, USA) then harvested from plates by washing with 0.1% (v/v) peptone water (Difco, Michigan, USA). Spores were enumerated by using a Haemacytometer and appropriate dilutions were made again with 0.1% (v/v) peptone water to give a stock suspension of 10^5 spores/ml. Pita bread was inoculated with a total of 100μl of each mold suspension at random spots on the bread surface to give a final inoculum of 10^3 spores/g. Control pita bread was inoculated in a similar manner with 100μl of sterile 0.1% peptone water. All pita bread was inoculated aseptically under a laminar flow safety cabinet (Labconco Corporation, Kansas City, Missouri 64132, purifier™ class II Safety Cabinet).

Table 13: A summary of the ingredients and their weights in the pita bread formulation.

Ingredient	Brand name	Amount (g)
All Purpose Flour	Robin Hood Inc.	1300
Water	Tap water	675
Granular Sugar	Redpath	20
Salt	Windsor	10
Yeast	Fleischmanns (Quick-Rise)	24
SuperFresh™Plus Enzyme	Bio-system Ltd.	2.6

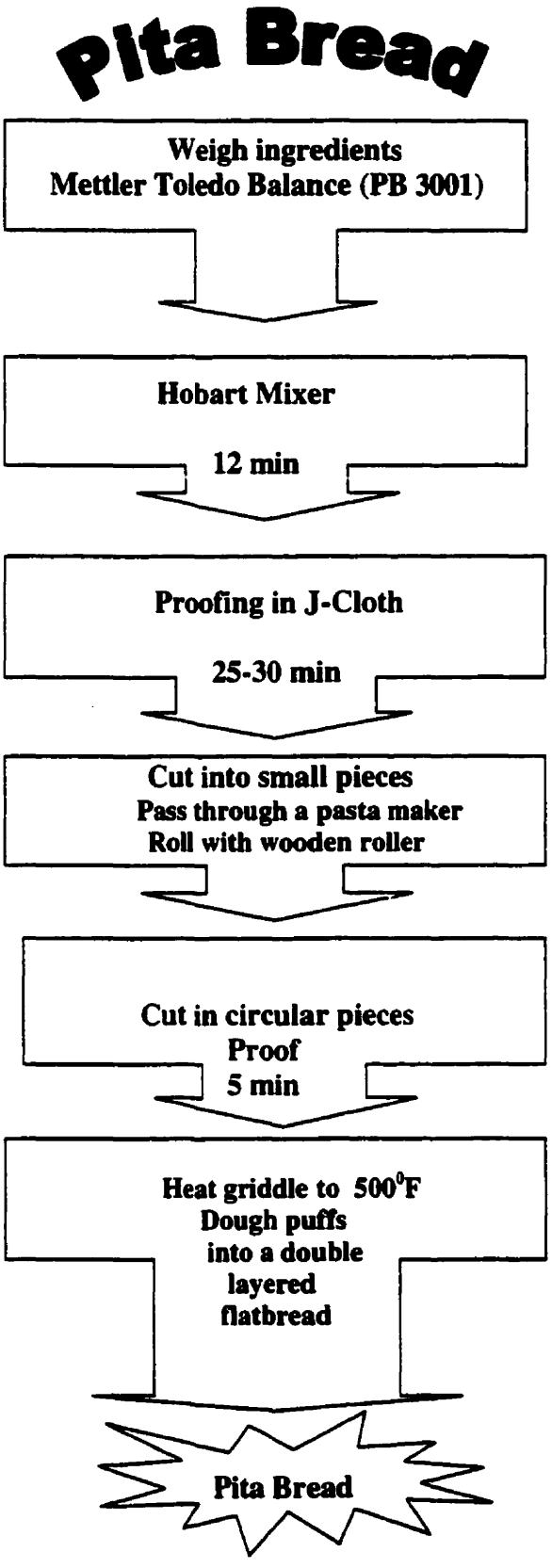


Figure 3: Flow process of pita bread on a laboratory scale

2.2.3. Packaging and storage experiments

Pita bread (~40g) was placed (1 per bag) in 210 x 210 mm Cryovac bags (oxygen transmission rate of 3-5 cc/m^2/day at 4.4°C, 0%RH) and packaged under the following different gaseous conditions: (1) Air, (2) gas packaging (60%CO_2:40%N_2), (3) gas packaging (60%CO_2 : 40%N_2 with a Freshmax Label (Multisorb Technology Buffalo, N.Y.) and (4) with an Ageless Type FX 100 oxygen absorber (Mitsubishi Gas Chemical Co., Tokyo, Japan). Air packaged samples were heat sealed using an Impulse heat sealer. Gas packaged pita bread was flushed with a gas mixture of CO_2:N_2 (60:40) using a Smith's proportional gas mixer (Model 299-028, Tescom Corp., Minneapolis, MN). For gas packaged samples with a Freshmax label, labels were taped inside appropriate bags prior to gas flushing. For the oxygen absorbent treatments, an Ageless Type FX 100 oxygen absorbent was taped inside the bag prior to heat sealing. Triplicate samples per treatment per sampling time were placed in large containers and stored at room temperature and analyzed at weekly intervals for six weeks.

2.2.4. Analysis

On the appropriate sampling day, three bags of pita bread, with and without enzyme corresponding to each packaging treatment, were removed from their respective containers for analysis. Bags were analyzed for visible mold growth (recorded in days) and headspace gas composition. Bags were then opened and the pita bread was analyzed for water activity (a_w), pH, and for sensory changes (flavor, texture, color, odor) by six untrained panelists.

2.2.4.1 Days to visible mold growth

Pita bread was observed for mold growth throughout storage. When pita bread showed any visible signs of mold growth, recorded in days, shelf-life was terminated, as this bread would eventually be rejected by consumers.

2.2.4.2 Head space gas analysis

Headspace gas composition was analyzed on packaged samples of pita bread prior to opening. Gas samples were taken using a gas tight syringe (Precision Sampling Corp., Baton Rouge, LA) through an adhesive septum placed on the surface of the package. Samples were injected into a Varian Gas Chromatograph (Model 3400, Varian Canada Inc.) equipped with a thermal conductivity detector equipped with a Poropak Q (80-100 mesh) and a Molecular Sieve 5A (80-100 mesh) columns in series (Supelco, Canada Ltd.). Helium was used as the carrier gas with a flow rate of 30ml/minute. The oven temperature was preset at 60°C , the injector port was at 100°C and the detector filament temperature was 170°C. The gas concentrations of CO_2, N_2, and O_2 were determined by a Hewlett Packard Integrator (Model 3390 A, Hewlett Packard Co., Avondale PA).

2.2.4.3 Water activity determination

At each sampling interval, water activity(a_w) measurements were made, in triplicate, on each pita bread. Packages were aseptically opened and representative samples (5g) were taken from the center of the bread, crumbed and then placed in a small round container. A_w measurements were done using a Decagon Water Activity meter (Decagon Devices Inc., Pollman, Washington 99163, USA) previously calibrated with a solution of saturated NaCl (a_w 0.75) prior to testing. All measurements were made at room temperature ~ 25°C.

2.2.4.4 pH measurements

The pH of pita bread was determined using a previously calibrated (pH 4.0 & 7.0) Corning pH meter (Model 2220, Corning Glassworks, Corning N.Y.). A 1:2 slurry of pita bread was made by using 15 g of pita bread with 30 g of distilled water in a stomacher bag and blending for 2 minutes in a stomacher (Lab Blender 400 BA, 6021, Seward Medical, London). A pH electrode (Fisher Scientific Model 13-620- 104) was then immersed directly into the slurry. The recorded pH was the mean result of triplicate samples.

2.2.4.5 Sensory analysis

At each sampling time, day 0 to day 42 the packaged pita bread was evaluated sensorially by 6 untrained panelists. Texture, odor, color, flavor and overall acceptability were evaluated using a hedonic scale from 1-5 where 1=Like very much, 2= Like Somewhat, 3= Neither Like nor Dislike, 4= Dislike somewhat, 5= Dislike very much. For each sensory parameter, an average score of 3 was considered the minimum of acceptability, implying that the shelf-life was terminated when this sensory score was reached.

2.3 RESULTS & DISCUSSIONS

2.3.1 Days to visible mold growth

The days to visible mold growth of inoculated pita bread packaged with and without enzyme under the various packaging conditions and stored at 25 °C are shown in Tables 14-15. Air packaged samples developed mold growth in 3-4 days for all inoculated samples. It is evident that mold growth of both *Aspergillus niger* and *Penicillium notatum* appeared after 14-30 days (Table 14-15) in all gas packaged pita bread with and without a label confirming earlier studies by Smith and Simpson (1995) and Ooraikul (1982). While CO_2 can be used to delay mold growth, inhibition depends on several factors including inoculum level, level of CO_2 and residual oxygen within the packages. Smith et al. (1990) showed that mold can grow in <1% headspce O_2 even in the presence of high CO_2 levels. These observations confirmed earlier studies of Ooraikul (1982) who reported that a concentration of 60%CO_2 increased the mold free shelf-life of crumpets for more than 14 days at ambient temperature. Recently, Smith et al. (1990) reported that mold growth could be inhibited for ~ 19 days in crusty rolls when packaged with 60% CO_2 (balance N_2). It is interesting to note that inoculated pita bread developed no mold growth when packaged with an oxygen absorber FX100. These results support the results obtained by Hassan (1997) who used oxygen absorbents to extend the mold free shelf-life of pizza crusts.

2.3.2 Changes in the headspace gas composition

Changes in headspace gas composition for pita bread, with or without enzyme, and packaged under various atmospheres and stored at 25 °C are shown in Figures 4-7 A, B respectively. In the air packaged pita bread, with and without enzyme, headspace oxygen and nitrogen remained fairly constant during storage until mold growth appeared after 3-4 days when headspace oxygen decreased and carbon dioxide concentration increased due to mold metabolism (Figures 4 A,B).

For gas packaged pita bread (60% CO_2:40%N_2) with and without enzyme, headspace O_2 remained at less than 1% while headspace CO_2 increased to 70% resulting in some packages having a swollen appearance. For gas packaged samples containing 60%CO_2 and a Freshmax label, residual headspace oxygen remained at less than 1% throughout storage while head space CO_2 again increased to 70% (Figures 5-6 A,B). Increases in headspace CO_2 in MAP pita bread can again be attributed to the growth of facultative microorganisms, such as *Bacillus* species, or post- baking contaminants, such as lactic acid bacteria, which have been implicated in the secondary spoilage of crumpets (Smith et al., 1983). For pita bread packaged in air and an Ageless FX 100 oxygen absorber, headspace oxygen decreased to less than 1-2% within 3 days (Figures 7 A,B) and remained at this level throughout storage. The lack of visible signs of mold growth for all pita bread packaged with an oxygen absorbent is due to the 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 and Simpson, 1996). These studies confirm the observations of Alarcon and Hotchkiss (1993) who reported that oxygen absorbents can prevent mold growth in white bread for 8 weeks at ambient temperature. Headspace CO_2 increased to 20% (v/v) by day 28 for both enzyme and non-enzyme treated pita bread packaged with an absorbent. While mold growth was completely inhibited throughout storage, headspace CO_2 production was probably due to the growth of facultative microorganisms, specifically *Bacillus* species which survive the baking process (Smith and Simpson, 1995).

Table 14: Growth of *A. niger* in pita bread with and without enzyme packaged under various gas atmospheres

Treatment	Visible mold growth (Days)
Air	3
60% CO_2	14
60% CO_2 + L[a]	25
Oxygen Absorbent FX 100	NG[b]

a: L: Freshmax[R] oxygen absorbent label , b: NG: No Growth

Table 15: Growth of *P. notatum* in pita bread packaged with and without enzyme under various gas atmospheres.

Treatment	Visible mold growth (Days)
Air	4
60% CO_2	17
60% CO_2 + L[a]	30
Oxygen Absorbent FX 100	NG[b]

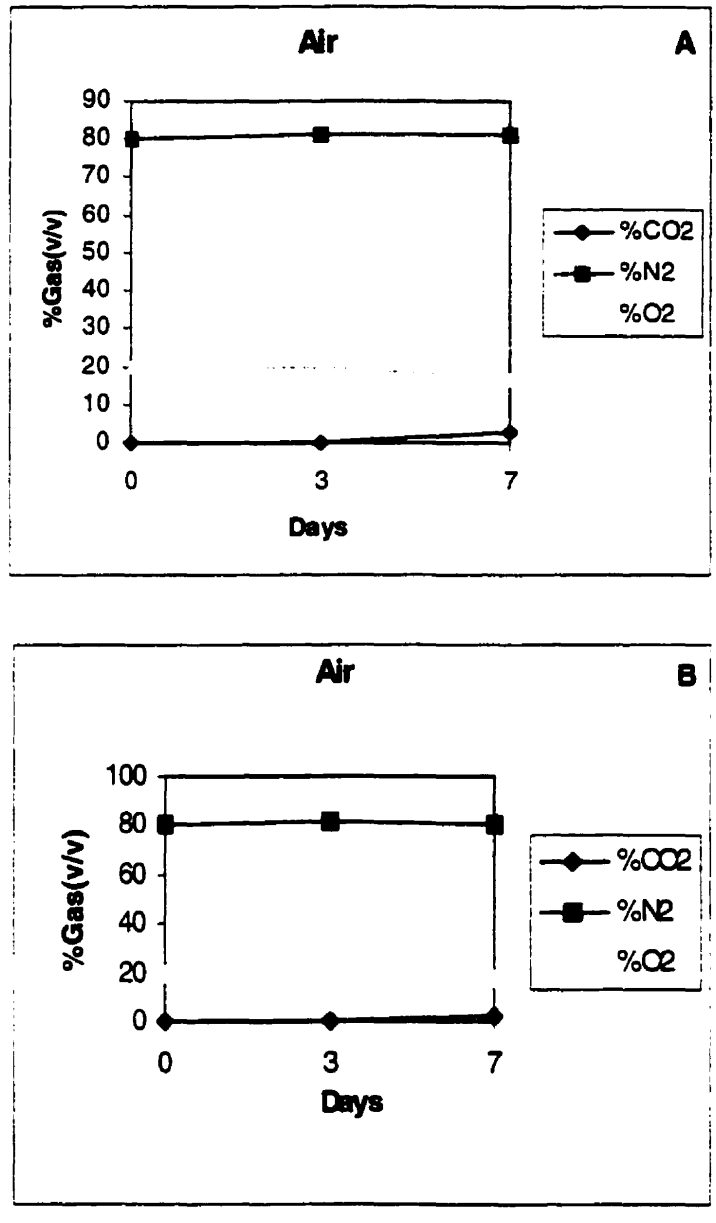


Figure 4. Changes in headspace gas composition of air packaged pita bread without enzyme (A) and with enzyme (B) and stored at 25°C.

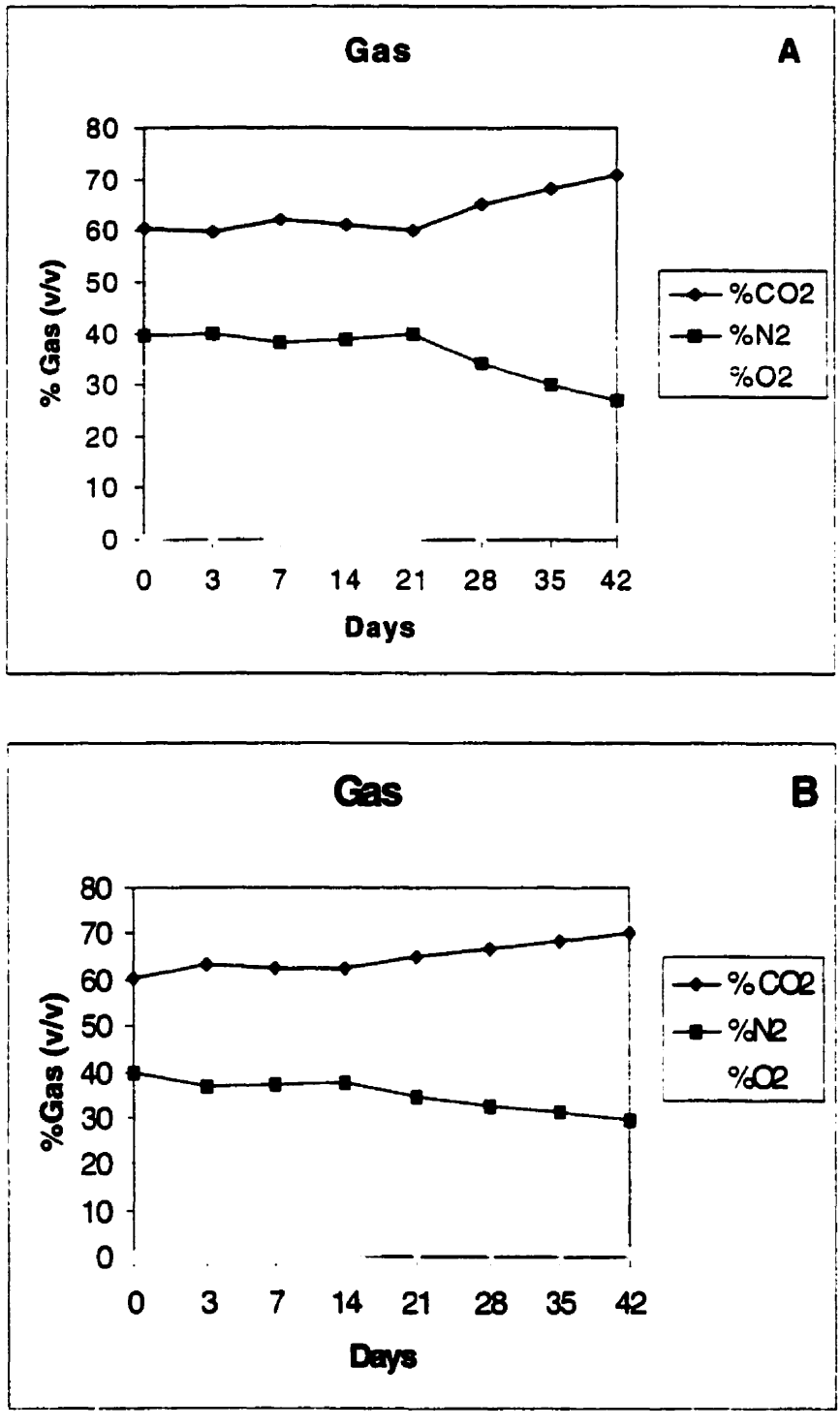


Figure 5. Changes in headspace gas composition of gas packaged pita bread without enzyme (A) and with enzyme (B) and stored at 25°C.

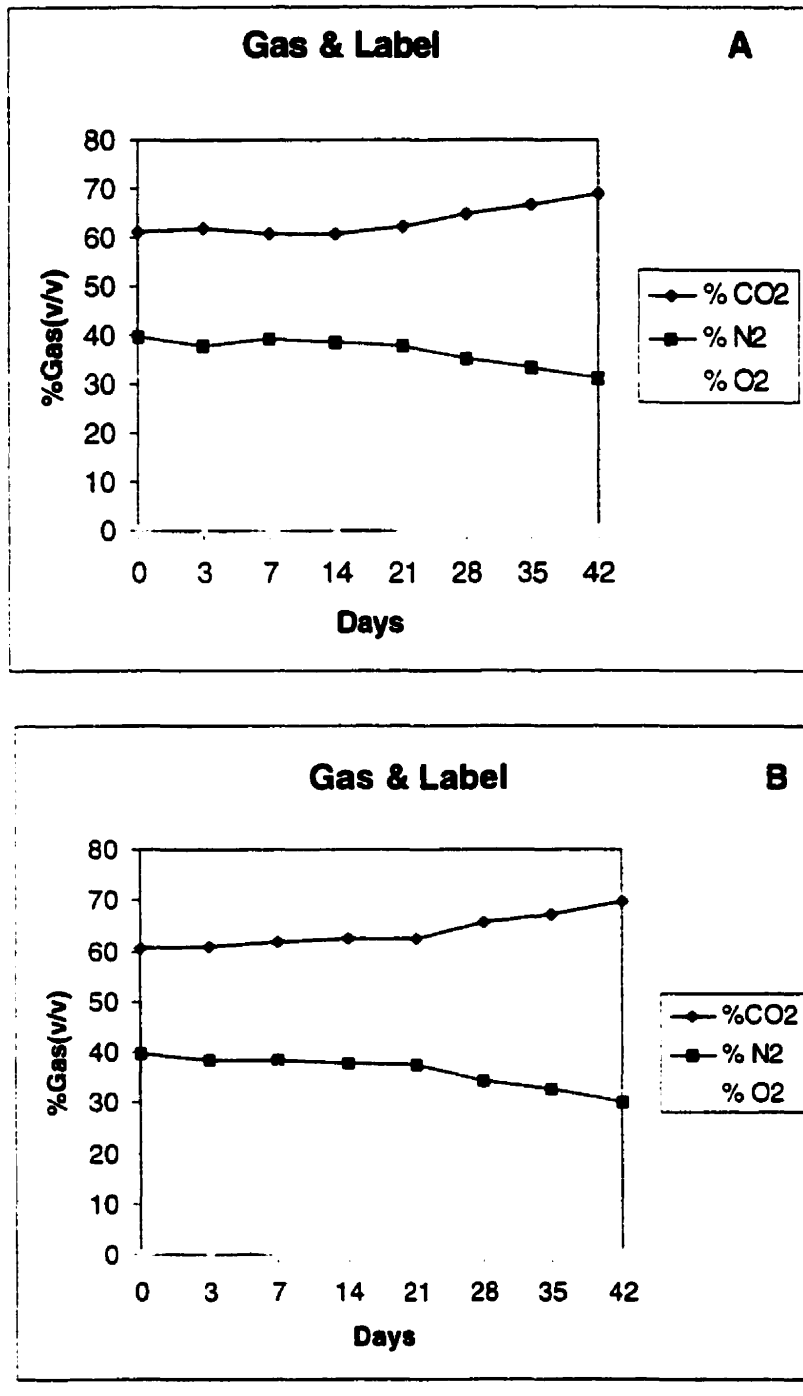


Figure 6. Changes in headspace gas composition of gas packaged pita bread & an oxygen absorbent label without enzyme (A) and with enzyme (B) and stored at 25°C.

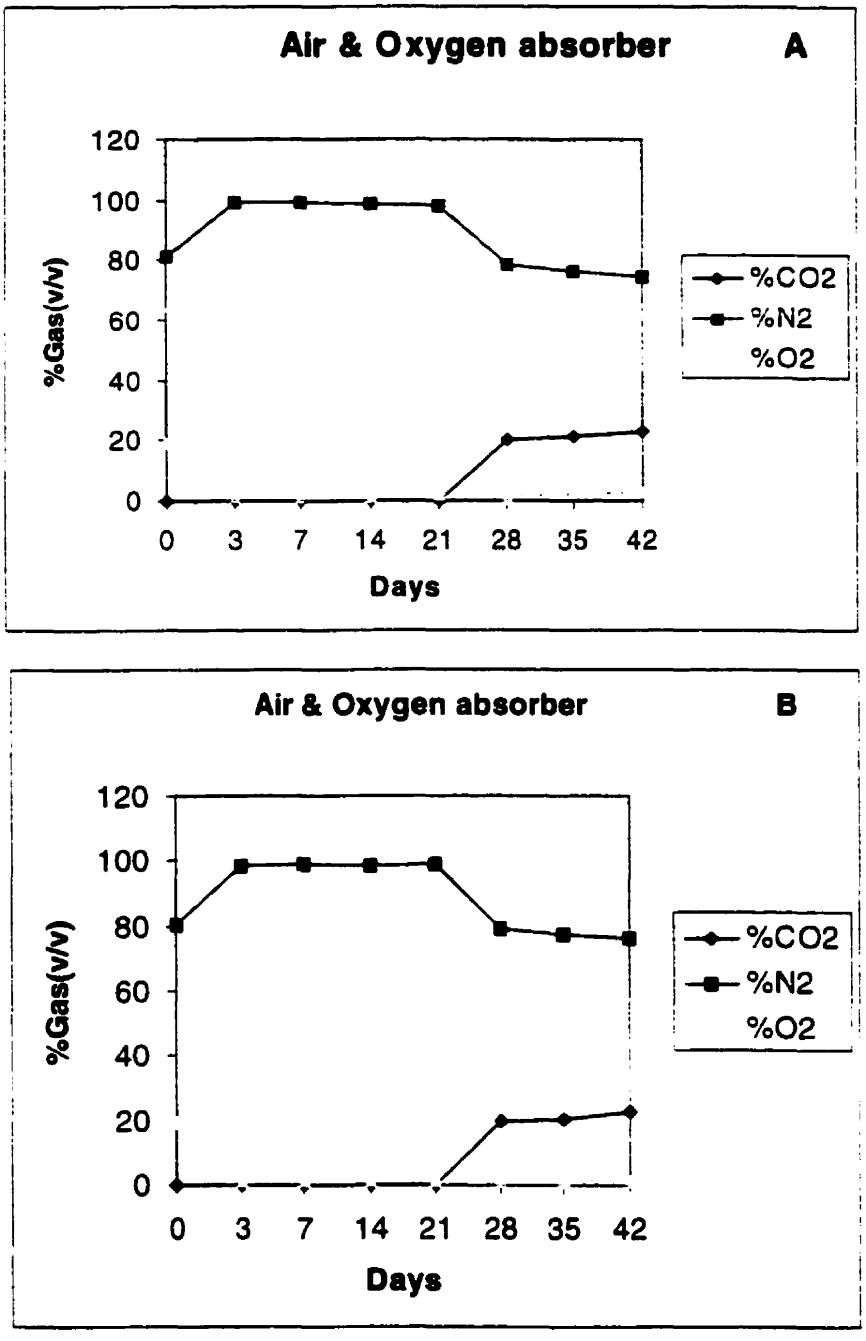


Figure 7. Changes in headspace gas composition of packaged pita bread with an Ageless FX oxygen absorber without enzyme (A) and with enzyme (B) stored at 25°C.

2.3.3 a_W and pH analysis of pita bread

Changes in the a_w of pita bread, with and without enzyme, packaged under different gaseous atmospheres and stored at 25 °C are shown in Figures 8-11. Differences in a_w were observed for pita bread with and without enzyme. The initial average a_w of pita bread without enzyme was ~ 0.915; however, the average a_w of pita bread with enzyme was ~ 0.940. These differences may be attributed to the enzyme which acts as an anti-staling agent, thus softening bread. These enzymes cleave amylose and the amylopectin branches resulting in less starch-protein interactions. They also create low molecular weight sugars and dextrins thus improving the water retention capacity of baked goods (Assouad, 1996). With the exception of air packaged pita bread, a_w increased from day 0 to day 28 in both enzyme and non-enzyme treated pita bread. However, after day 28, a_w decreased to ~ 0.9 by day 42 in non-enzyme treated bread, while a_w also decreased to ~0.92 in enzyme treated pita bread (Figures 9-11). However, these a_w levels are still conducive to mold and yeast growth.

The intial pH of pita bread ranged from 5.85-6.02 in most packaged bread. During storage, pH decreased slightly from 5.85 to a minimum of 5.60 (results not shown). This decrease can be attributed either to the growth of lactic acid bacteria or yeasts in the packaged product or to a slight dissolution of headspace carbon dioxide in the aqueous phase of the product packaged under 60% CO_2 (Gill, 1988).

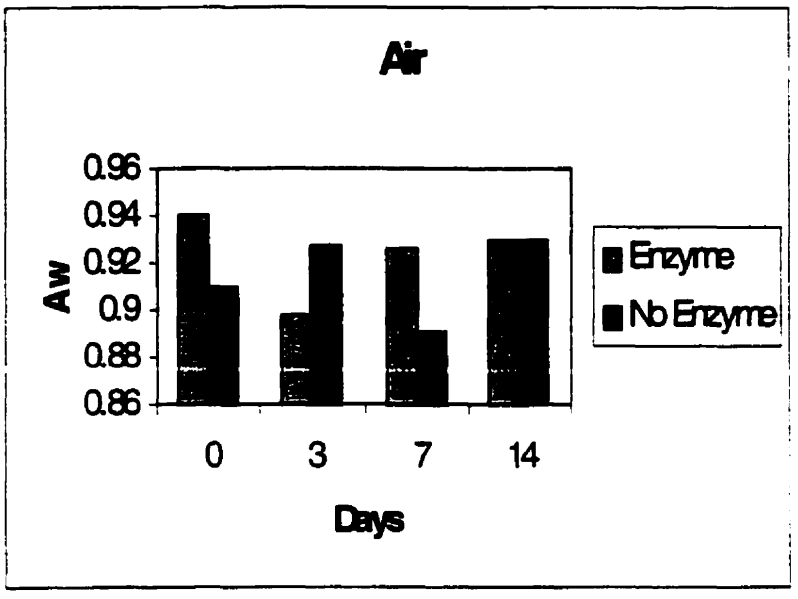


Figure 8. Changes in a_w of pita bread packaged in air and stored at 25°C.

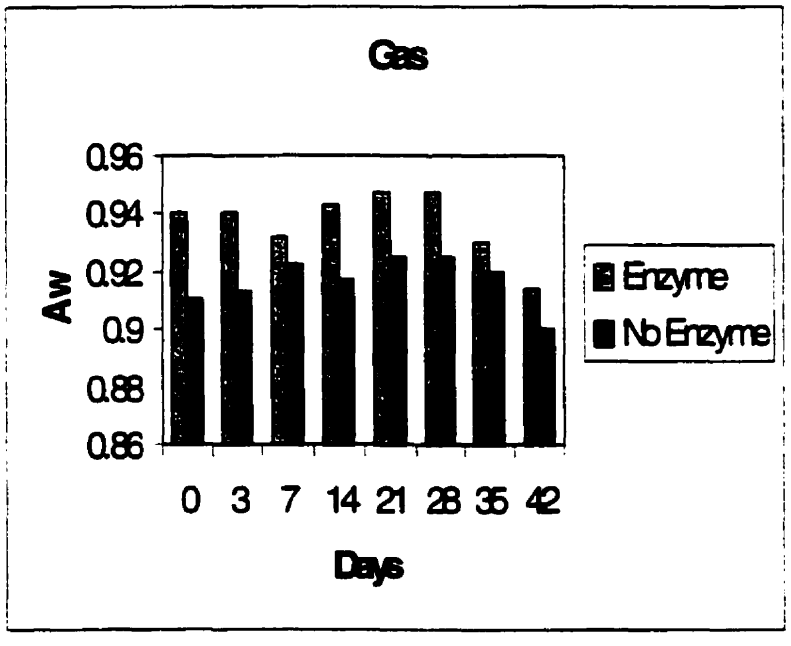
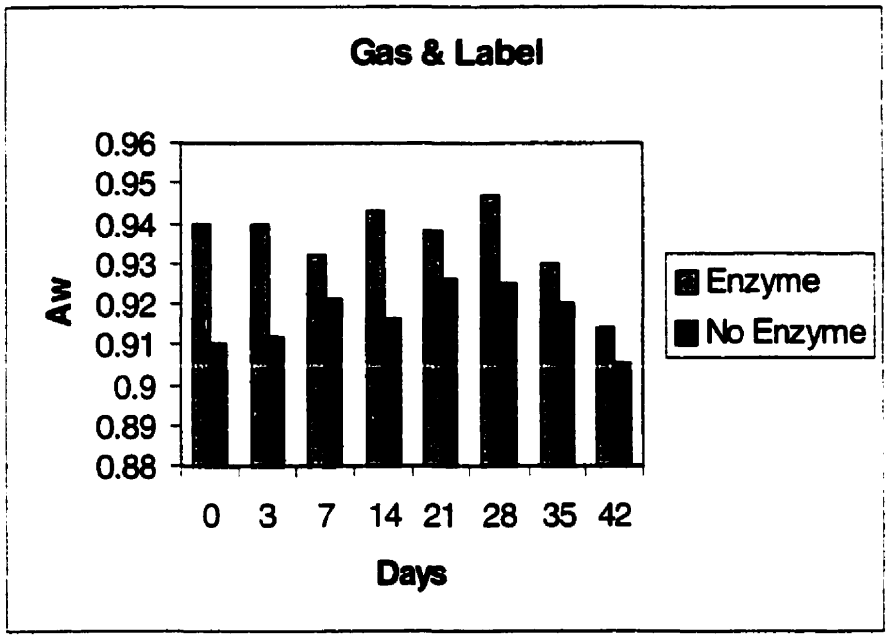


Figure 9. Changes in a_w of gas packaged pita bread and stored at 25°C.



Figures 10. Changes in a_w of gas packaged pita bread with a Freshmax label and stored at 25°C.

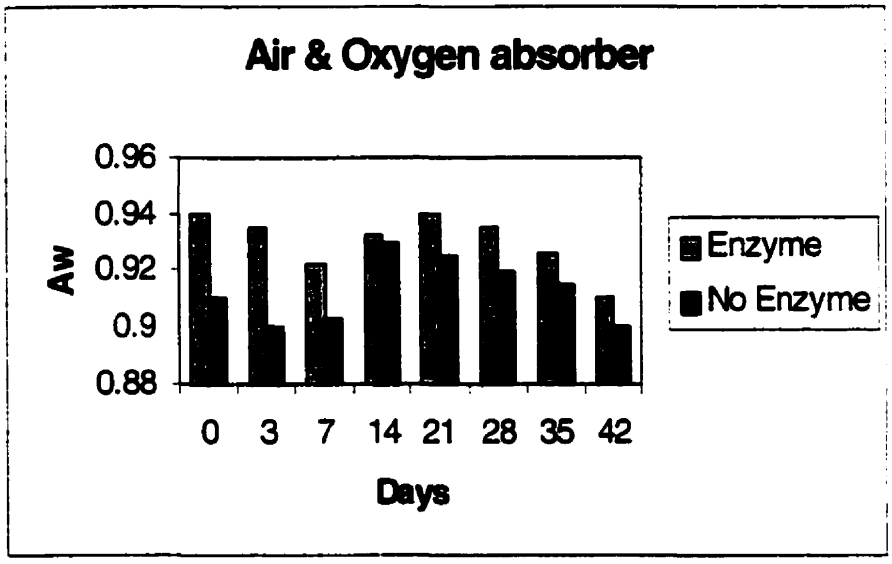
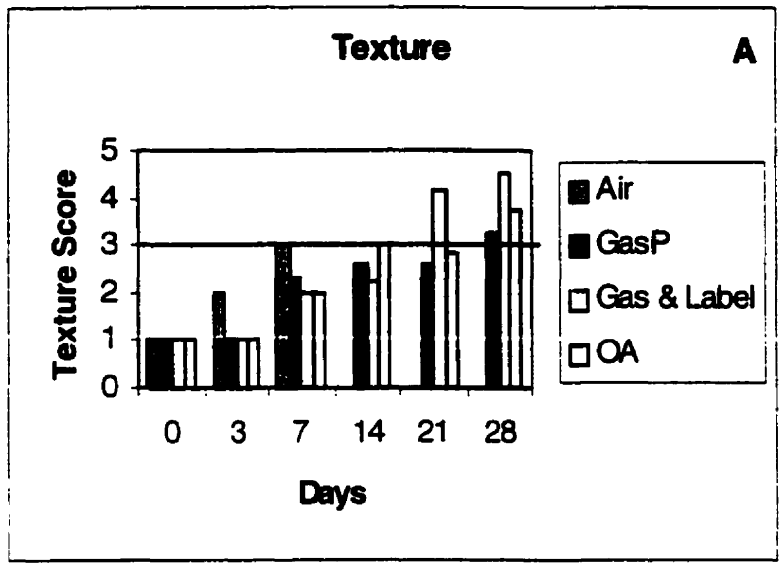


Figure 11. Changes in a_w of pita bread packaged in air with an Ageless FX oxygen absorber and stored at 25°C

2.3.4 Changes in sensory analysis

Changes in texture, color, odor, and flavor of both enzyme and non-enzyme treated pita bread packaged under various gas conditions and stored at 25°C are shown in Figures 12-14 A, B. These changes in sensory analysis were attributed to two major factors, i.e., staling and mold growth (Hebeda and Zobel, 1996). Pita bread was regarded as unacceptable when an average parameter (score of 3 out of 5) was reached and hence, the shelf-life is considered terminated. All air packaged samples were rejected on day 3 due to the development of visible mold growth on the surface of pita bread. Optimum results were obtained when pita bread was packaged under MAP conditions. Changes in texture of pita bread were more obvious in non-enzyme treated pita bread than pita bread containing enzyme (Figure 12). These changes in texture can be observed in pita bread packaged under 60%CO_2 and 60%CO_2 and an oxygen absorbent label where the product was rejected at day 21 in non-enzyme treated bread and at day 28 in the enzyme treated bread (Figure 12). These results support the observations by Black et al. (1993) as well as the importance of enzymes in delaying staling. This is again attributed to the ability of the enzyme to cleave the amylose and amylopectin rigid branches of starch resulting in smaller branches which will prevent starch-protein interaction (Assouad, 1996). However, pita bread packaged with an oxygen absorbent FX 100 sachet were rejected by day 28 for both enzyme and non-enzyme treated samples (Figure 12). No observable changes in the color of pita bread were detected in the MAP samples (results not shown). Similar results were observed by Hassan (1997) who observed no color changes in pizza crusts packaged under modified atmosphere throughout storage at ambient temperature. Changes in the sensory odor and flavor for the various packaging treatments are shown in Figures 13 and 14 respectively. Differences in these attributes were observed in the air and MAP packaged breads. Again, all the samples packaged in air were rejected by day 3 due to the visible mold growth on the surface of the bread. Some panelists detected off-odors (probably due to yeast /lactic acid bacteria) in samples packaged under MAP conditions. These off-odors were detected by panelists at the end of the storage trials, i.e., by day 15 (Figure 13).

Smith et al. (1983) reported that lactic acid bacteria and *Bacillus* species have been shown to be the predominant spoilage organisms in crumpets packaged in 60% CO_2. These results are in contrast to the gas packaged pizza crusts were panelists detected off-odors by day 21 (Hassan, 1997). According to Figure 14, the panelists detected a change in the flavor of pita bread packaged with 60% CO_2 at day 28 which might be due to the dissolution of carbon dioxide in the bread and imparting a slight bitter, acidic taste to the bread. Similar results were detected in samples packaged with 60%CO_2 and an oxygen absorbent label. Pita bread packaged with an oxygen absorbent FX100 had an acceptable flavor until day 28 (Figure 14).



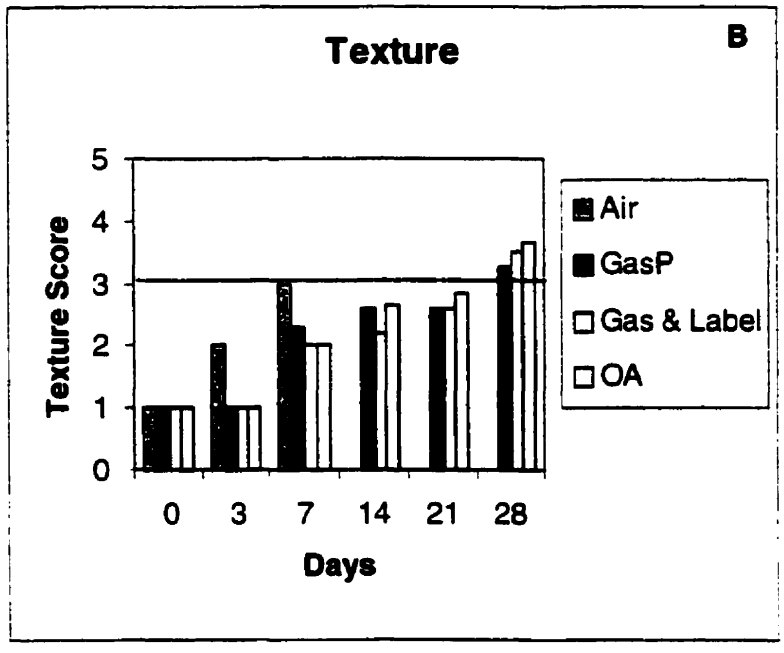


Figure 12. Changes in texture of pita bread without enzyme (A) and with enzyme (B) packaged under various gaseous atmospheres and stored at 25°C.

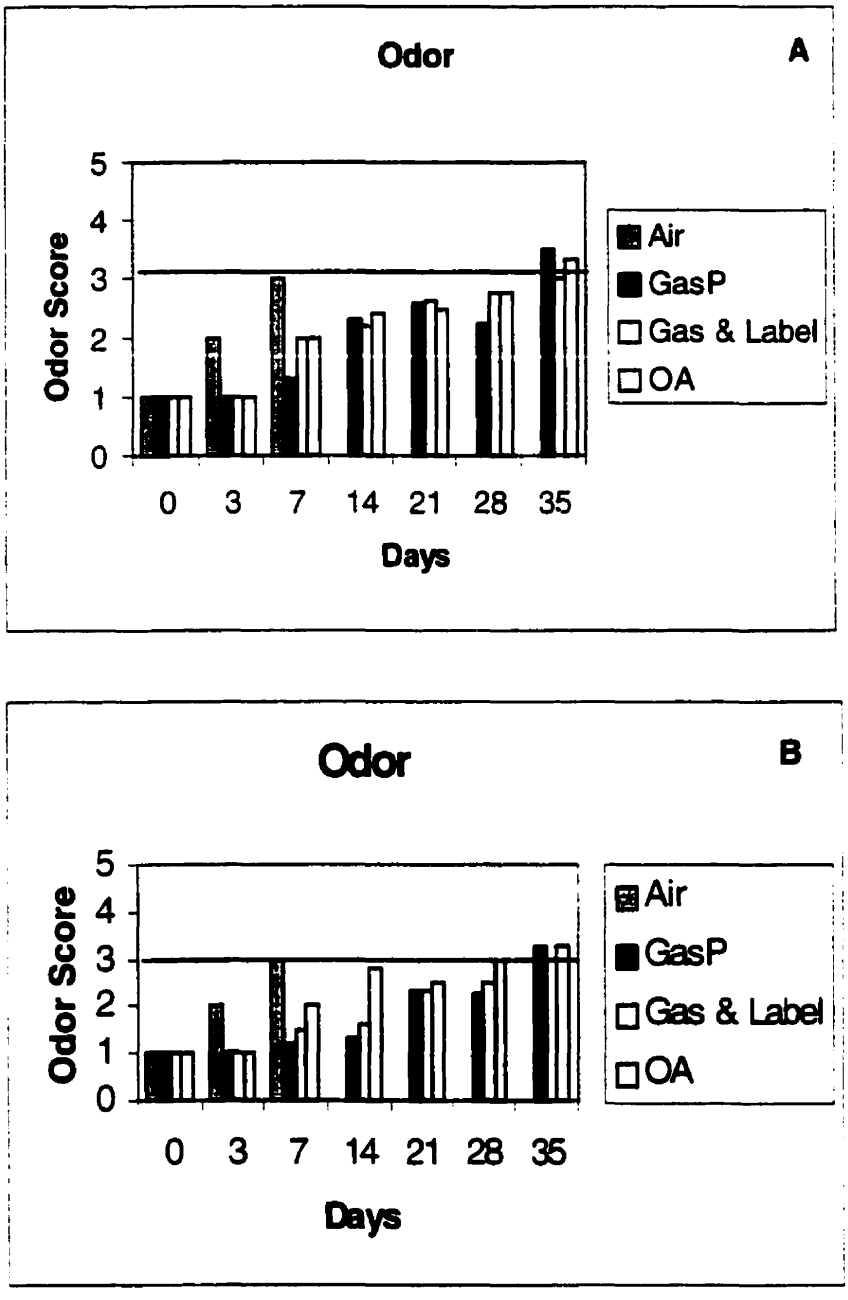


Figure 13. Changes in odor of pita bread without enzyme (A) and with enzyme (B) packaged under various gaseous atmospheres and stored at 25°C.

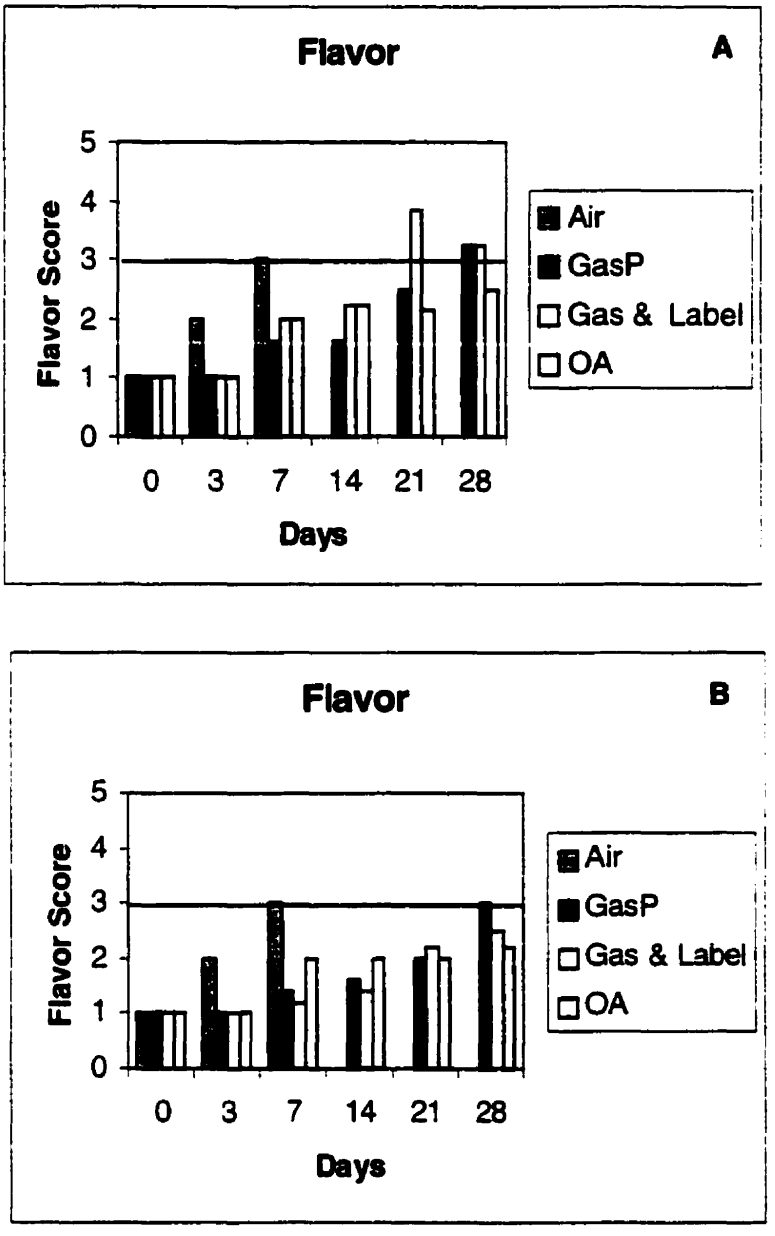


Figure 14. Changes in flavor of pita bread without enzyme (A) and with enzyme (B) packaged under various gaseous atmospheres and stored at 25°C.

2.3.5 Shelf-life

The shelf-life of pita bread, with and without enzyme, was based on days to visible mold growth and sensory scores. Shelf-life was associated with the days to visible mold growth, i.e, when mold appeared on the surface of the bread. The sensory shelf-life of the bread was based on the time to reach a score of 3 out of 5 on a hedonic scale and hence termination of shelf-life under the various packaging conditions. The overall shelf-life of pita bread was directly proportional to storage time and packaging treatment. Air packaged (AP) pita bread had a shelf-life of 3-4 days due to the visible mold growth and sensory scores. For gas packaged pita bread, with and without enzyme, a shelf-life of 14-30 days was attained. This limited extension in shelf-life was attributed to residual O_2 in the package headspace enhancing mold growth and the sharp odor and flavor produced from the possible production of by-products from the lactic acid bacteria in the packaged products. However, a mold free shelf-life of > 42days was possible in pita bread packaged with an Ageless FX 100 oxygen absorber. However, shelf-life was limited after 28days due to changes in texture and flavor. After day 28, sensory analysis showed that the bread was slightly stale and had a slight acidic flavor and odor. The overall shelf-life of pita bread, with and and without enzyme, packaged under the various gas packaging conditions and stored at ambient temperature (25°C) is shown in Table 16.

Table 16: Overall shelf-life of pita bread packaged under various gas packaging conditions and stored at ambient temperature (25°C).

Treatment	Mold free shelf-life (days)	Average sensory shelf-life (days)*
Air	3-4	3
60% CO_2	15	14
60% CO_2 + L	30	28
Oxygen Absorbent FX 100	NG[a]	28

*: Based on time(days) to reach a score of 3 out of 5

a: NG : No growth

2.4 CONCLUSION

In conclusion, the mold free shelf-life of pita bread packaged under modified atmospheres could be extended to a maximum of 28 days at ambient temperature. Pita bread, at this time, had minimal changes in texture, odor, color, and flavor especially in pita bread stored with an Ageless oxygen absorbent (FX 100). Therefore, oxygen absorbent technology offers the baking industry an inexpensive and viable method to extend the mold free shelf-life of pita bread.

Chapter 3

EFFECT OF ETHANOL VAPOR GENERATORS ON THE MOLD FREE SHELF-LIFE OF PITA BREAD

3.1 Introduction & Objectives

Ethanol has long been used as a disinfectant in the medical field for the storage of syringes and thermometers, as well as the sterilization of the skin before injection. Ethanol or ethyl alcohol (ETOH) has been used as an antimicrobial agent since the first alcoholic fermentation was developed to preserve fruits (Davidson and Branen, 1993). Ethanol is obtained by the fermentation of sugars. It is a colorless liquid with a molecular weight of 46 and a boiling point of 78°C. Ethanol is bactericidal at high concentrations (60-75%) ; it has a poor penetrating power and acts on microorganisms by denaturing proteins in their cytoplasm (Davidson and Branen, 1993). Recently, Seiler and Russell (1993) showed that the addition of low concentrations of ethanol to a packaged food product could extend its shelf-life. Shapero et al. (1978) reported that the effectiveness of ethanol against *Staphylococcus aureus* was a function of water activity (a_w). They showed that in broth media, adjusted to a_w values of 0.99, 0.95, 0.90 with glycerol, inhibition of *St. aureus* occurred at 9, 7, and 4% (w/w) ethanol, respectively. Since few studies have been done on the use of ethanol vapor on bakery products, the objectives of this study were:

1. To determine the effect of ethanol vapor on the growth of *Aspergillus niger* and *Penicillium notatum* in model agar systems and in packaged pita bread.

2. To determine the effect of ethanol vapor on the sensory characteristics of pita bread (texture, color, odor, taste).

3. To determine the effect of ethanol vapor on the permeability characteristics Oxygen Transmission Rate (OTR) used to package pita bread.

3.2 MATERIALS & METHODS

3.2.1 Effect of ethanol vapor generators on mold growth in model agar systems

To determine the size of sachet required to extend the mold free shelf-life of pita bread, initial model agar studies were done. Duplicate Potato Dextrose Agar plates (PDA, Difco, Michigan, USA) were spotted at random with 100μl of a 10^3 spores /ml suspension of *Aspergillus niger* and *Penicillium notatum* on the surface of the plate. The mold suspensions were prepared as described in Chapter 2, section 2.2.2. After surface drying under the laminar flow hood, the plates were packaged (two plates per bag) in 210 x 210 mm high barrier Cryovac bags (Oxygen Transmission Rate 3-6cc/m^2/day at 4°C and a 0% relative humidity). Ethicap sachets (1, 2, 3, 4, 6, 8, 10)G and Negamold sachets (100, 200, 400, 600, 800,1000S) were taped inside the appropriate packages and the bags were sealed using an Impulse heat sealer. Two control plates for each mold were also prepared. Packaged plates were left at room temperature (25°C) and observed for visible signs of mold growth, recorded in days.

3.2.2 Pita bread processing

Pita bread was formulated according to a commercial recipe using all purpose flour, water, sugar, salt, yeast, and enzyme to delay staling as outlined in Chapter 2, section 2.2.1.

3.2.3 Inoculation and packaging

After cooling, the pita bread was inoculated with two mold species, *A. niger* and *P. notatum*, prepared as described previously. Pita bread was inoculated with 100μl of each mold suspension at random spots on the bread surface to give a final inoculum of 10^3 spores/g. Control pita bread was inoculated in a similar manner with 100μl of 0.1% peptone water. All pita bread was inoculated aseptically under a laminar flow safety cabinet (Labconco

Corporation, Kansas City, Missouri 64132, purifier™class II Safety Cabinet). The pita bread was then packaged in 210 x 210 mm Cryovac bags with the following ethanol vapor generators: Ethicap (2 and 4G) and Negamold (100 and 200S). Triplicate samples/ treatment /sampling time were performed on each sampling day, i.e., 0, 3, 7, 14, 21, 28, 35 and 42. The packaged and treated pita bread treatments was placed in large buckets at room temperature (25°C) and analyzed on each sampling day.

3.2.4 Preparation of standard curve for headspace ethanol

An 80% ethanol solution was used to prepare the following standards of alcohol solutions: 0.25, 0.5 , 0.75, 1.0, 1.5, 2, 3, 4, 6, 8 and 10% (v/v). These solutions were placed in volumetric flasks wrapped with parafilm to prevent the escape of ethanol vapor. After storage at refrigerated temperature, 1µl of each standard solution was injected into a Varian Gas Chromatograph equipped with a flame ionization detector (FID). The injector temperature set at 150°C and the column temperature at 60°C. Headspace ethanol samples for analysis were taken using an air tight syringe (Precision Sampling Corp., Baton Rouge, LA) through an adhesive septum placed on the parafilm of the volumetric flask. An average of 3 injections was done for each standard solution. The results were used to generate a standard curve of areas versus each ethanol concentration (Figure 15).

3.2.5 Days to visible mold growth

Plates and pita bread were examined on a daily basis for visible signs of mold growth, recorded in days.

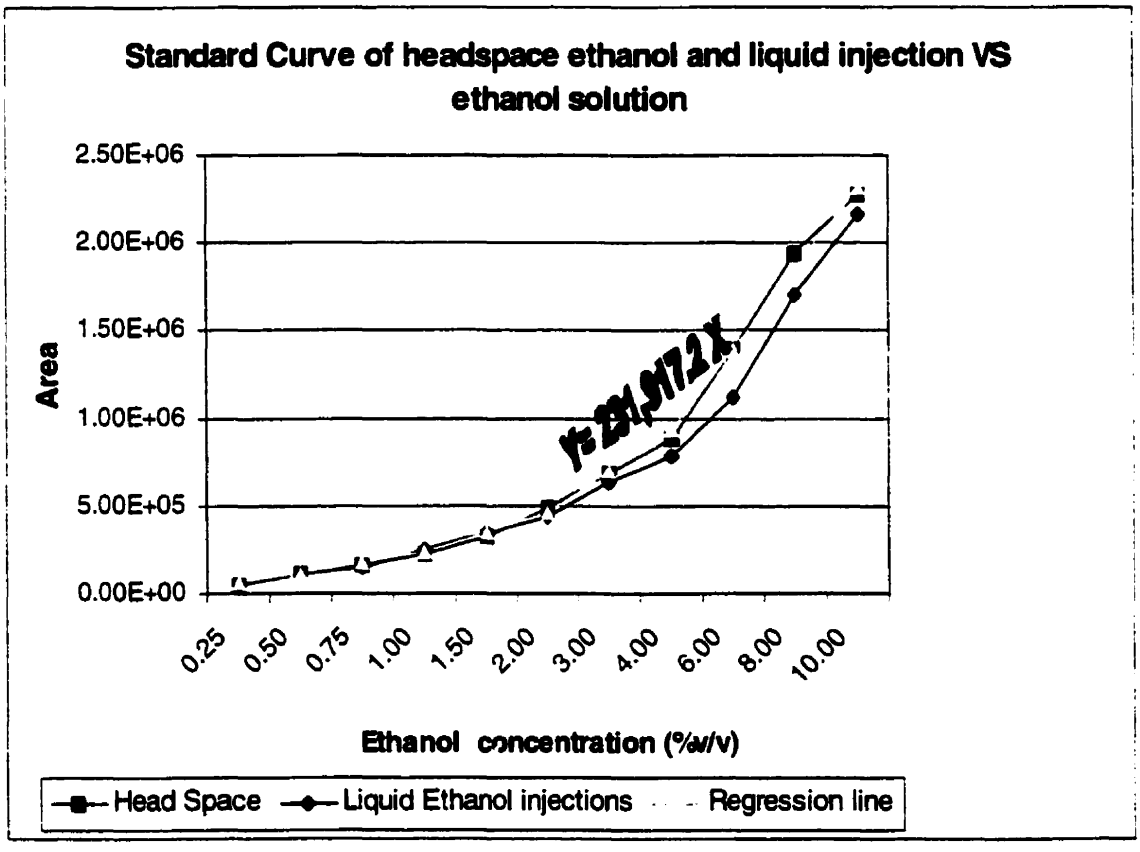


Figure 15. Standard curve of headspace ethanol vapor and solution

3.2.6 pH and a_w analysis

The pH and a_w of pita bread were measured using the methods outlined in Chapter 2, sections 2.2.4.3 and 2.2.4.4 respectively.

3.2.7 Headspace ethanol

Headspace ethanol was analyzed on each sampling day. Analysis of headspace ethanol was achieved through an adhesive septum placed on the surface of the package using an air tight syringe (Precision Sampling Corp., Baton Rouge, LA). Samples of 1μl were injected into a Varian Gas Chromatograph (Model 3400, Varian Canada Inc.) equipped with a flame ionization detector (FID) and using a Nukol column(30M X 0.53mm)(Supelco, Canada Ltd.). Helium was used as a carrier gas with a flow rate of 30ml/minute. The column temperature was set at 60°C and an injector port at 100°C. Resultant peaks of ethanol were recorded on a Hewlett Packard Integrator (Model 3390 A, Hewlett Packard Co., Avondale, PA). Headspace ethanol (%v/v) was determined from the standard curve (Figure 15) prepared as described in section 3.2.4. Results were the mean values of six measurements of headspace ethanol from duplicate treatments.

3.2.8 Sensory analysis

Sensory evaluation of the pita bread was done as outlined in Chapter 2, section 2.2.4.5. Pita bread was evaluated for changes in flavor, color, odor, and texture on each sampling day. When pita bread reached a score of >3 out of 5 for every sensory attribute, its shelf-life was terminated.

3.2.9 Analysis of headspace oxygen

Headspace oxygen was measured using an Oxygen Analyzer (Servomex, Food Package Analyzer Series1400, Minneapolis, U.S.A.).A sticky septum was placed on the package, and the headspace drawn in via the sampling syringe connected to the instrument. The analyzer had been previously calibrated using 80% CO_2 as well as 100% N_2 .

3.2.10 Analysis of film permeability: Oxygen Transmission Rate (OTR)

For the measurement of the oxygen transmission rate (OTR), a standard method (ASTMD 3985-81) was used, with 100% O_2 as the permeant gas. The OTR of the film B541 was measured by cutting a film of uniform size and placing it directly in the test cell. A 25µm thick piece of Mylar (polyester) was used as a standard to measure any leakage and to determine any correction factor. All films were conditioned at the test temperature and a 100% relative humidity for 24 to 48 hours prior to testing. For the OTR test, an Oxtran 2/20 Master (Mocon, Minnesota, USA) was used with an Oxtran 2/20 software package to monitor all the phases of testing, including entering test conditions (parameters), monitoring tests, and printing reports. Once the parameters were set, the computer controlled the components, gathered and logged data, printed all the data in the form of tables and bar charts. Both cells of the Oxtran 2/20 are divided into two chambers separated by the test film. Oxygen was passed through the upper chamber and humidified carrier gas (98% Nitrogen and 2% Hydrogen) passed through the bottom chamber to sweep the permeant gas to the sensor. A scanning automatic valve sent the sample gas, oxygen, to a specific coulometric sensor detector. The detector gave a current output directly proportional to the rate of oxygen arrival at the sensor. Therefore, the oxygen flux across the film was dynamically measured, the OTR expressed as $cc/m^2/day$ at 0% RH.

3.2.11 Preparation of standard curve for ethanol content in pita bread

A set of ethanol standards was prepared from an 80% ethanol solution. One gram of each of these solutions was then added to 9g of pita bread to give the following ethanol concentrations in pita bread: 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 %. Twenty ml of distilled water was added and the mixture macerated using a stomacher (Labblender 400) for 3 minutes to make a slurry. These slurries were then transferred into centrifuge tubes (Pittsburgh PA 15219, USA) and centrifuged (Damon, IEC, No· hern Heights, Massachusets 02941) at 6000 r.p.m. for 20 minutes to obtain a clear supernatant. One μl of this supernatant was then injected into the gas chromatograph with the settings being the same as previously described for liquid ethanol injections. The areas under each peak were recorded and used to generate the standard curve as shown Figure 16.

3.2.12 Ethanol content of pita bread

On each sampling day, pita bread slurries were prepared by weighing 15g of pita bread into a stomacher bag and adding 30ml of water giving a bread to water ratio of 1:2. The samples were then stomached for 3 minutes and centrifuged as described previously. One μl of the supernatant was then injected into the gas chromatograph with all the settings being the same as the liquid injections. The areas under the peaks were recorded and compared to the standard curve to give the ethanol content (%w/w) of pita bread. All results were the mean values of 3 injections from each packaging treatment.

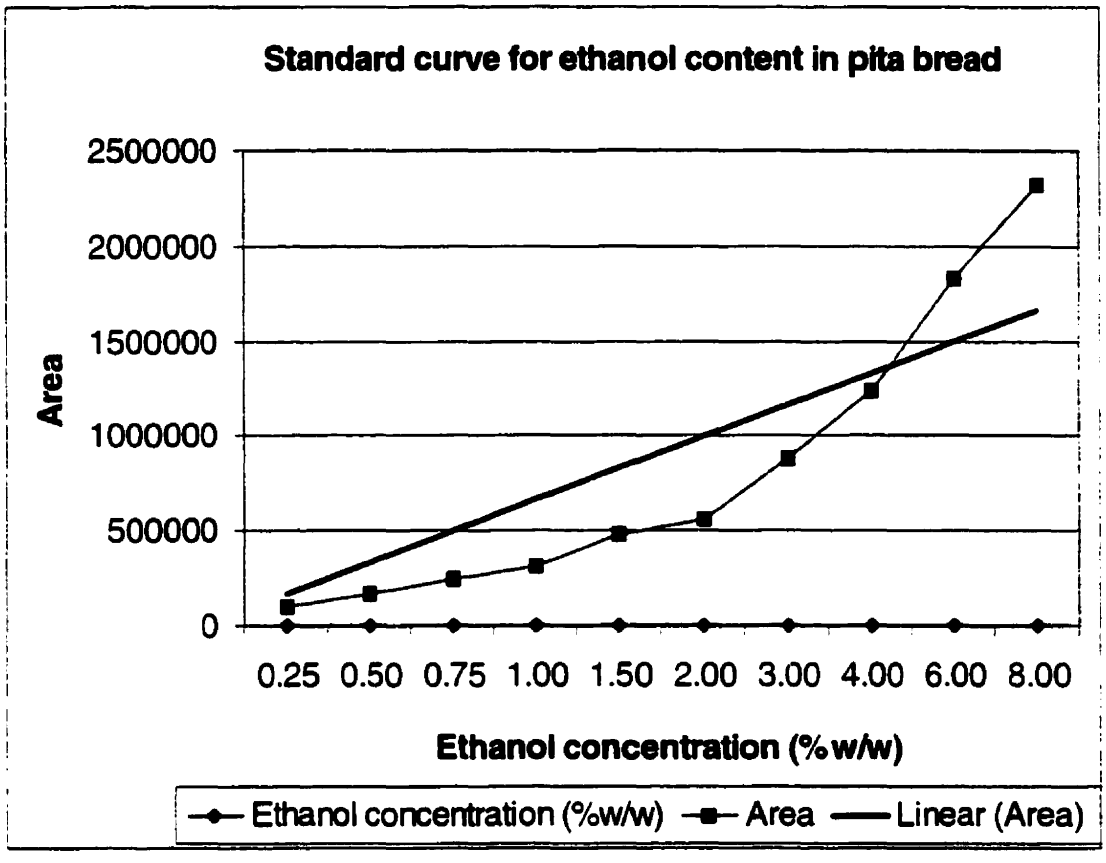


Figure 16. Standard curve for ethanol content in pita bread

3.3 Results & Discussions

3.3.1 Effect of Ethicap and Negamold on mold growth in model agar systems

Another interesting and relatively novel method to control mold spoilage in bakery products is through the use of ethanol vapor generators. To determine the effect of Ethicap and Negamold on mold growth, preliminary studies were done on agar plates. The effect of the various sizes of Ethicap and Negamold on mold growth in agar plates is shown in Tables 17 and 18 respectively. Mold growth was evident in control plates after 4-5 days (Tables 17 and 18). However, when plates were packaged with Ethicap (1G), *A. niger* appeared after 7 days while *P. notatum* appeared after 14 days. Again, *A. niger* was observed to be more resistant to low levels of ethanol vapor (Table 17). Mold growth did not appear on any other plates after 42 days at 25°C, clearly indicating that ethanol vapor had a powerful antimycotic effect.

The concentration of headspace ethanol vapor was obtained from the standard curve shown in Figure 15. There was an excellent correlation between headspace ethanol and liquid ethanol indicating that either curve could be used to calculate the headspace ethanol within the package as long as the sensitivity range was changed. Based on these standard curves, levels of ethanol vapor generated from the various sizes of Ethicap are shown in Table 17. It is evident that only 10% (v/v) ethanol was required to inhibit both *A. niger* and *P. notatum* and that higher levels of ethanol vapor (~16-38 % v/v) and, hence larger sachets of Ethicap, did not appear necessary to inhibit mold growth on agar plates. A similar trend was shown for the various sizes of Negamold (Table 18). However, the growth of both *A. niger* and *P. notatum* could be inhibited by 100S Negamold compared to 2G Ethicap. It is also evident that Negamold sachets generated ~50% less ethanol vapor than the corresponding size of Ethicap (Table 18).

However, Negamold sachets also absorbed oxygen and therefore less ethanol would be required to inhibit mold growth. In all cases, headspace O_2 decreased to <0.1% (v/v) while headspace ethanol ranged from ~ 6-25% (v/v). This initial study clearly demonstrated that both Ethicap and Negamold are powerful antimycotic agents and could be used as an alternative to gas packaging to extend the mold free shelf-life of pita bread.

3.3.2 Effect of Ethicap and Negamold on mold growth in pita bread

To determine the effect of Ethicap and Negamold on mold growth on pita bread, pita bread was inoculated with 10^3 spores/g of both test molds (*A. niger* and *P.notatum*). Control breads were inoculated in a similar manner with 0.1% sterile peptone water. All breads were packaged (1bread/package) in high barrier Cryovac bags (210x210mm) with either Ethicap (2 and 4G) and Negamold (100 and 200S) and monitored for visible signs of mold growth. The effect of Ethicap and Negamold on the mold free shelf-life of pita bread is shown in Table 19. Mold growth appeared in control pita bread after 4-5 days (Table 19). However, mold growth was inhibited for >42 days when pita bread was packaged with either Ethicap and Negamold, confirming agar plates studies.

Although it was shown that only 2G Ethicap or 100S Negamold would be sufficient to inhibit mold growth for 42 days in agar plates studies, higher levels were tested with pita bread. This study confirmed that ethanol vapor had a powerful antimycotic effect and could be used to extend the mold free shelf-life of pita bread.

The levels of headspace ethanol generated by the various sizes of Ethicap and Negamold are shown in Figure 18. Levels of ethanol generated from Ethicap ranged from ~18.5% (v/v) after 7 days to ~22.7% (v/v) after 28 days and then decreased to ~15.7% (v/v) at the end of the storage period.

This decrease can be attributed to the absorption of ethanol by pita bread. Negamold, as expected, generated ~50% less ethanol vapor than Ethicap. At the end of the storage period, pita bread packaged with Negamold had ~ 10% (v/v) in the package headspace. The advantage of Negamold as a dual functional absorbent /generator is that less ethanol vapor may result in less sensory rejection by consumers.

Changes in headspace O_2 for pita bread packaged with Negamold are shown in Figure 17. As expected, headspace O_2 decreased to <1 % after 3d and remained at this level throughout 42 days. These low levels of O_2, plus the headspace ethanol, delayed mold growth in pita bread for 42 d.

3.3.3 Effect of Ethicap and Negamold on the a_w and pH of pita bread

The effect of ethanol generated from Ethicap and Negamold on the water activity and pH of pita bread is shown in Figures 19 and 20 respectively. It is evident from Figure 19 that, with the exception of 200S Negamold, a_w of pita bread packaged with Ethicap and Negamold remained fairly constant throughout storage. Similar trends were observed for pH values, which changed were little throughout storage (Figure 20).

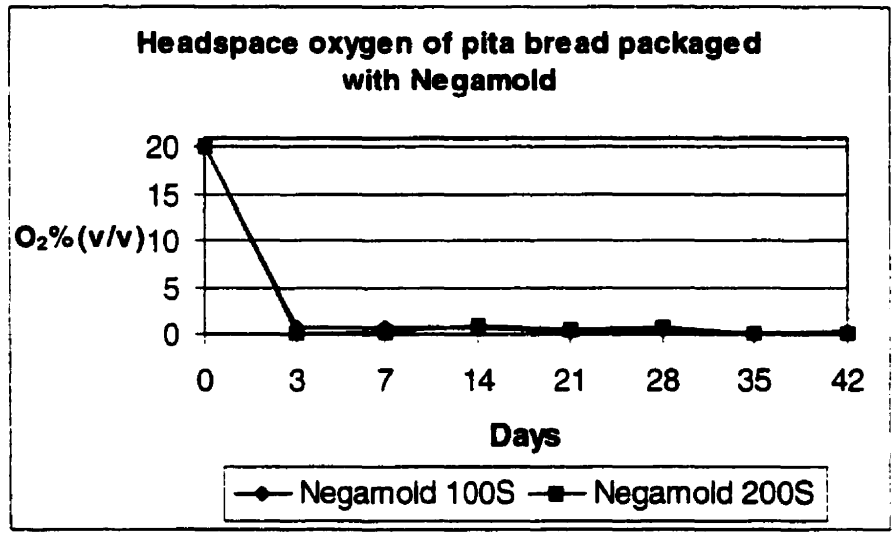


Figure 17. Headspace oxygen of pita bread packaged with Negamold

Table 17: Effect of Ethicap on mold growth of *A. niger* and *P. notatum* on PDA plates and stored at 25°C.

Size of Ethicap (G)	Days to visible mold growth		Headspace (% v/v)
	A. niger	*P. notatum*	Ethanol
0	4	5	N/D
1	7	14	10.00
2	N/G	N/G	16.80
3	N/G	N/G	20.05
4	N/G	N/G	25.40
6	N/G	N/G	30.75
8	N/G	N/G	32.46
10	N/G	N/G	38.75

N/G = no growth after 42 days, N/D = not detected

Table 18: Effect of Negamold on mold growth of *A. niger* and *P. notatum* on PDA plates and stored at 25°C.

Size of Negamold (S)	Days to visible mold growth		Headspace (% v/v)	
	A. niger	*P. notatum*	O_2	Ethanol
0	4	4	14	N/D
100	35	N/G	< 0.1	6.00
200	N/G	N/G	< 0.1	10.65
300	N/G	N/G	< 0.1	13.85
400	N/G	N/G	< 0.1	15.84
600	N/G	N/G	< 0.1	20.45
800	N/G	N/G	< 0.1	23.75
1000	N/G	N/G	< 0.1	25.35

N/G = no growth after 42 days, N/D = not detected

Table 19: Days to visible mold growth on pita bread packaged with Ethicap (2-4G) and Negamold (100-200S) and stored at ambient temperature (25°C)

Sachet Type	Sachet Size	Days to visible mold growth	
		A. niger	*P. notatum*
Control	-	5	4-5
Ethicap	2G	>42	>42
Ethicap	4G	>42	>42
Negamold	1G	>42	>42
Negamold	2G	>42	>42

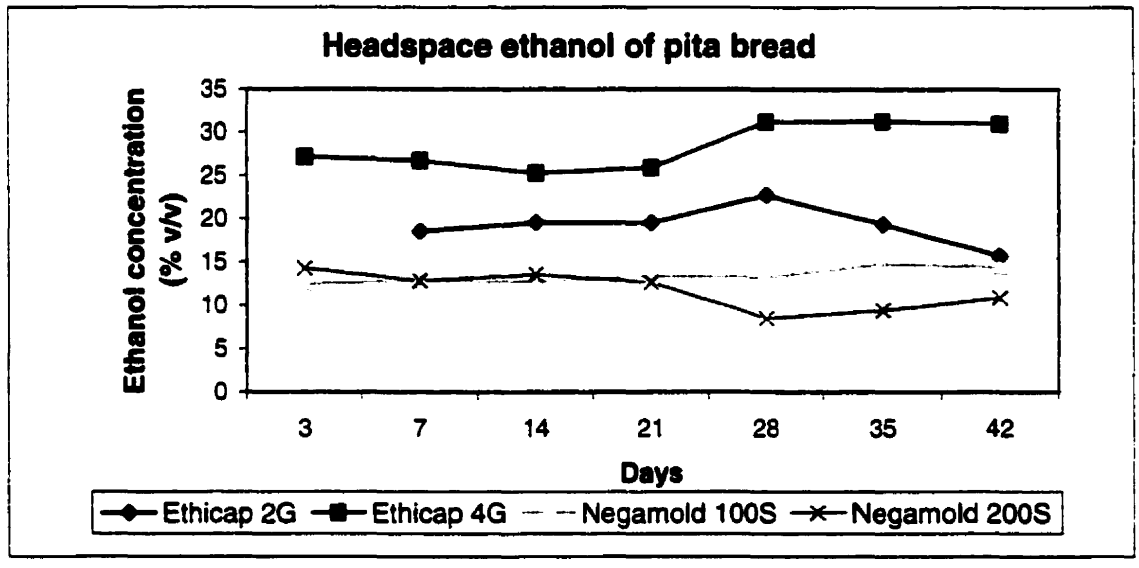


Figure 18. Headspace ethanol of pita bread packaged with ethanol vapor generators

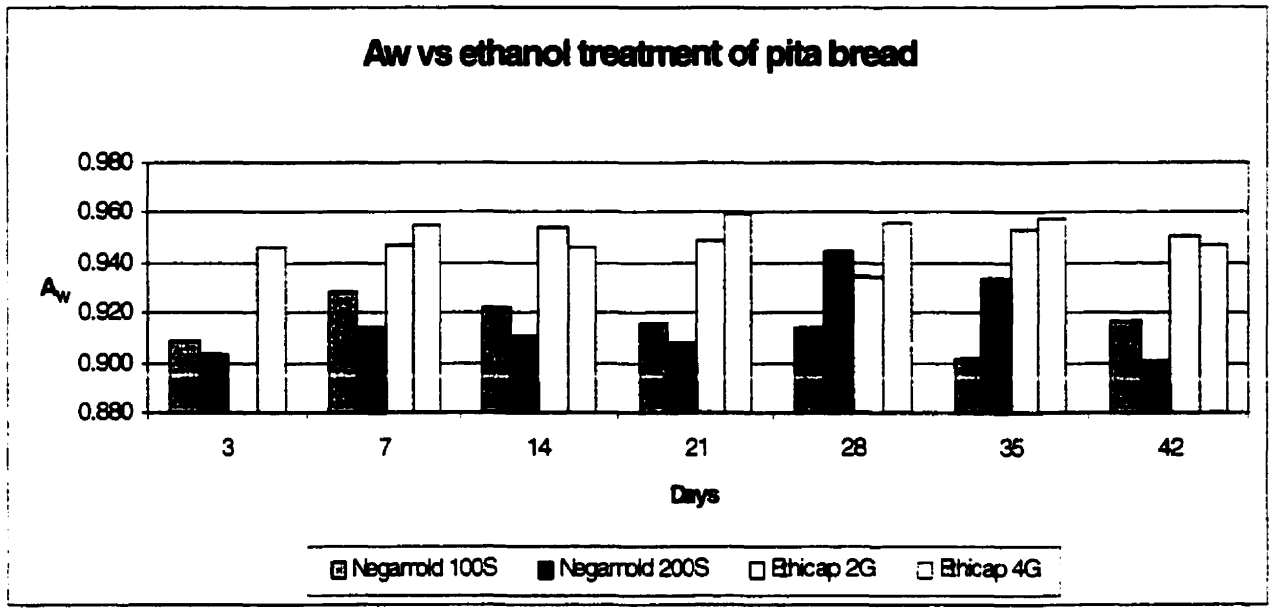


Figure 19: Water activity (aw) of pita bread packaged with Ethicap and Negamold

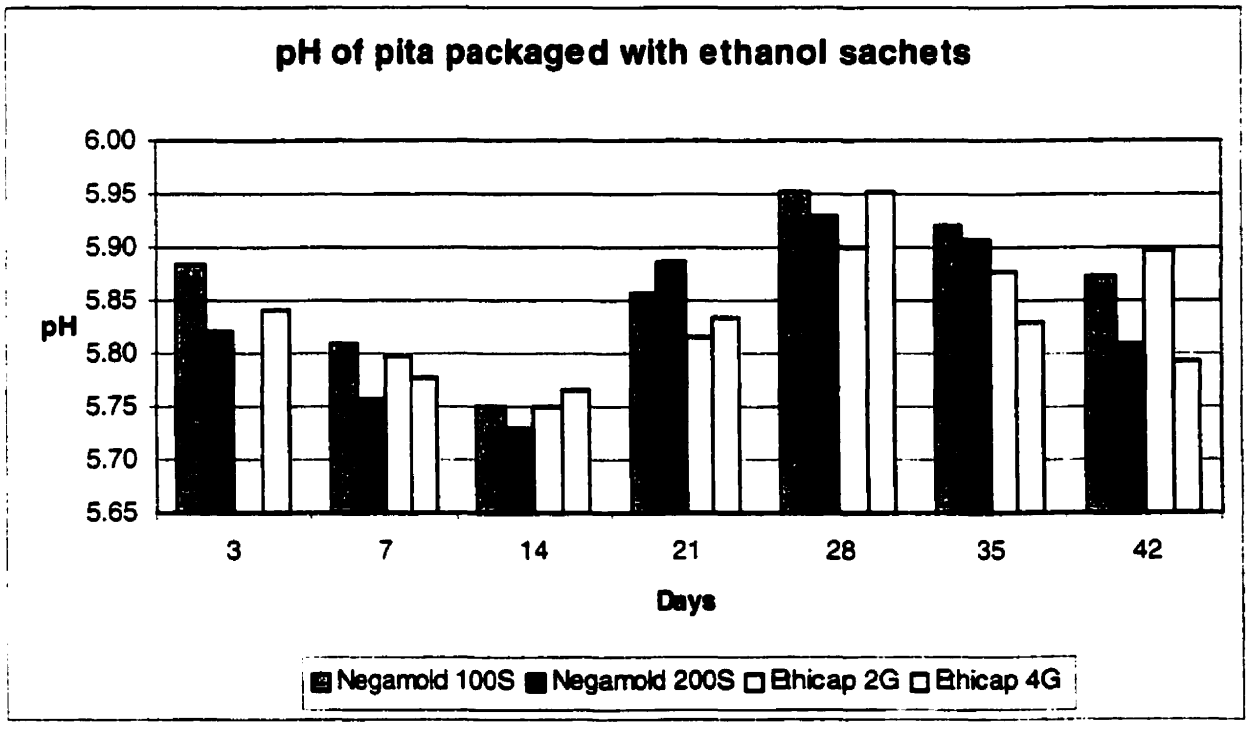


Figure 20: pH of pita bread packaged with Ethicap and Negamold

3.3.4 Effect of Ethicap and Negamold on the sensorial qualities and ethanol content of pita bread

The effect of both Ethicap and Negamold on the sensory qualities of pita bread are summarized in Tables 20 and 21 respectively. Pita bread was evaluated for texture, odor, color, and taste by 6 untrained panelists. All sensory attributes were assessed on a hedonic scale from 1-5 where 1= highly acceptable to 5= highly unacceptable. Pita bread was rejected when any attribute reached a score >3. All pita bread packaged with 2G Ethicap had an acceptable shelf-life in terms of odor and color after 42 days. Furthermore, their flavor was acceptable until day 35. However, pita bread was rejected on the basis of texture after 28 days (i.e., staleness). While the odor and color scores were similar for pita bread packaged with 4G Ethicap, these breads had a longer shelf-life in terms of texture (35d). However, most panelists rejected pita bread packaged with 4G Ethicap after only 14d due to the strong taste/flavor of ethanol (Table 20). Nevertheless, based on the overall acceptability, pita bread had a sensory shelf-life of 35days and 14 days when packaged with 2G and 4G Ethicap.

Similar trends were observed for pita bread packaged with Negamold (Table 21). While odor and color scores were similar to pita bread packaged in Ethicap, texture scores were lower and pita bread was rejected after 14 days, when packaged with 100-200S Negamold. These lower texture scores may be attributed to the lower levels of ethanol vapor generated by Negamold and hence the higher scores (and less rejection) for flavor. Again, the overall acceptability, the sensory shelf-life of pita bread could be extended to 35 days when packaged with 100-200S Negamold. These results would indicate the higher levels of ethanol in the package headspace may play a role in delaying staling and enhancing the texture of pita bread. Similar results were observed by Black et al. (1993). The actual mechanism through which this occurs is still unknown. However, since staling involves interaction between proteins and starch, one possible hypothesis is that ethanol may denature some flour proteins thereby preventing hydrogen bonding between protein and starch. In other words, ethanol is having a softening or "plasticizing" effect on the bread structure.

Ethanol content in pita bread is another parameter which can cause the rejection of pita bread by the consumer. According to Table 21, the sensory shelf-life of pita bread packaged with Ethicap 4G was terminated after 14 days due to a strong ethanol aftertaste in the bread. The ethanol content was recorded at 6% (Table 22) which is way above the 2% level accepted by CFR (1990). The ethanol content was taken at the beginning (day 3) and at the end (day 42) of the storage trial and recorded in % w/w basis. Table 22 summarizes the ethanol content of pita bread packaged with the various ethanol vapor generators. According to Table 22, the pita bread stored with Negamold (100 and 200S) was acceptable since the results basically complied with the recommended level of 2% in the product.

3.3.5 Effects of ethanol vapor on the characteristics of the film

Since Ethicap generated higher levels of ethanol vapor, studies were done to determine if ethanol had a plasticizing effect on the film structure and would influence its oxygen transmission rate (OTR). The results, shown in Figure 21 clearly indicate that ethanol did have an effect on the OTR of the film. Furthermore, there was a very good correlation between the sizes of Ethicap and OTR (r^2=0.8464). The regression equation Y=12.76+0.08X (for 28d of storage) clearly showed that as the size of Ethicap increased (and hence level of ethanol vapor) the OTR of the film increased. However, this did not appear to effect the shelf-life of pita bread, as no mold growth was observed after 42 days. However, it may account for the observations of Black et al. (1993) who observed a slight increase in headspace O_2 in all gas packaged products stored with Ethicap. They hypothesized that the ethanol vapor may have dissolved in the film and acted as a plasticizer, thereby affecting the film's permeability to both O_2 and CO_2 and influencing mold free shelf-life. This initial study would appear to confirm this hypothesis.

Table 20: Sensory analysis of pita bread packaged with 2G and 4G Ethicap

Days	2G					4G				
	Texture	Odor	Color	Flavor	OA	Texture	Odor	Color	Flavor	OA
3						+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	-	-
21	+	+	+	+	+	+	+	+	-	-
28	-	+	+	+	+	+	+	+	-	-
35	-	+	+	+	+	+	+	+	-	-
42	-	+	+	-	-	-	+	+	-	-

+ : Pita accepted (score<3), -: Pita rejected (score>3), Average= average of the 4 characteristics, OA= overall acceptability asked during the analysis

Table 21: Sensory analysis of pita bread packaged with 100S and 200S Negamold

Days	100S					200S				
	Texture	Odor	Color	Flavor	OA	Texture	Odor	Color	Flavor	OA
3	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	-	+	+	+	+
14	-	+	+	+	+	-	+	+	+	+
21	-	+	+	+	+	-	+	+	+	+
28	-	+	+	+	+	-	+	+	+	+
35	-	+	+	+	+	-	+	+	+	+
42	-	+	+	-	-	-	+	+	-	-

+ : Pita accepted (score<3), -: Pita rejected (score>3), Average= average of the 4 characteristics, OA= overall acceptability asked during the analysis

Table 22: Ethanol content of pita bread packaged with the various ethanol vapor generators.

Treatment	Ethanol content (% w/w)	Ethanol content (% w/w)
	Initial Day 3	Initial Day 42
Ethicap 2G	~1	~3
Ethicap 4G	~2	~6
Negamold 100S	~0.5	~1
Negamold 200S	~1	~1.5

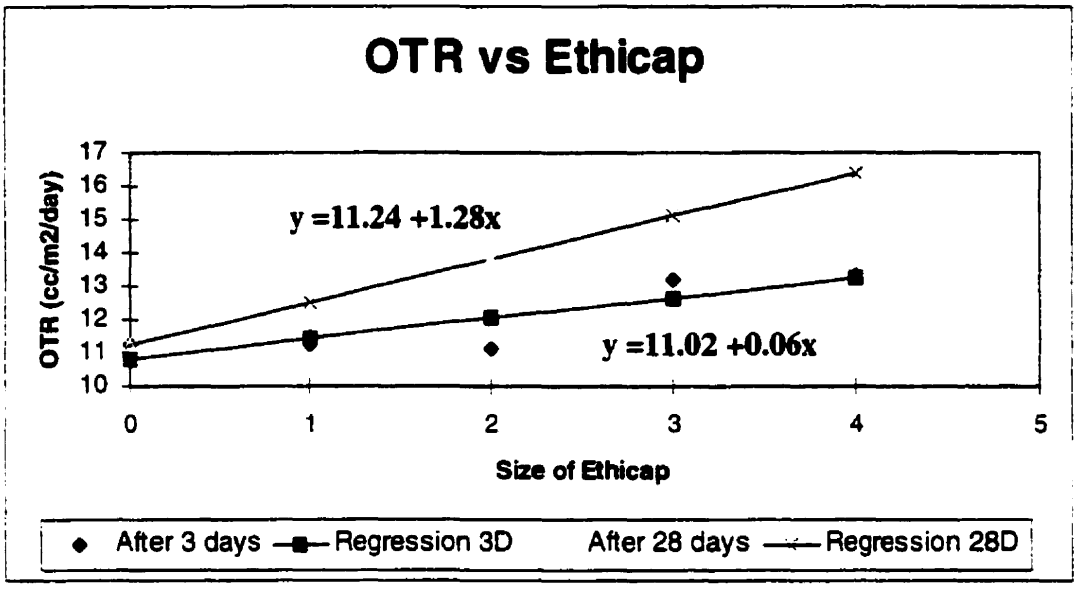


Figure 21. Oxygen Transmission Rate (OTR) of film B541 after 3 and 28 days of storage with Ethicap

3.4 Conclusion

In conclusion, ethanol vapor generators are a more commercially viable alternative to gas packaging to extend the mold free shelf-life of pita bread. Furthermore, they can give a longer extension of the mold free and textural shelf-life for pita bread than ultra high pressure. The main disadvantage of ethanol vapor generators is their effect on product taste, i.e, the absorption of ethanol by the bread rendering it bitter and hence being rejected by the panelists. Therefore, based on this study, Ethicap 2G and 4G would not be permissible. However, Negamold 100S and 200S would be acceptable since < 2%(w/w) of ethanol was detectable at the end of storage.

Another possible disadvantage of ethanol vapor generators may be the plasticizing effect on film permeability. This latter effect may, through time, change the headspace O_2 /ethanol ratio within the package to levels conducive to mold growth.

In conclusion, for optimum shelf-life, i.e., microbiological and sensory, packaging of pita bread with Negamold (100 and 200S) could be used (Tables 22 and 23). The days to visible mold growth and the sensory shelf-life of pita bread packaged with ethanol vapor generators is summarized in Table 23.

Table 23: Overall mold and sensory shelf-life of pita bread packaged with ethanol vapor generators.

Treatment	Visible Mold Growth (Days)	Average Sensory Shelf-life (Days)*
Ethicap 2G	>42	32
Ethicap 4G	>42	14
Negamold 100S	>42	32
Negamold 200S	>42	35

*: Based on time (days) when a score of 3 out of 5 is reached

Chapter 4

EFFECT OF HIGH PRESSURE ON THE MOLD FREE SHELF-LIFE OF PITA BREAD

4.1 Introduction & Objectives

High pressure processing is a novel method used to extend the shelf-life of food. High pressure processed foods, primarily acid foods, such as jams and juices, became available in Japan in the 1990's. Most studies with high pressure have been done on liquid foods, e.g., fruit juices (Butz et al., 1995). However, few other studies have been done on solid foods, e.g., fresh meat. El-Moueffak et al. (1995) examined the use of high pressures to extend the shelf-life of beef. He reported that a 50°C treatment associated with 400MPa pressure for 10 minutes substantially reduced microbiological contamination needed to extend the shelf-life of beef. Another potential application of UHP (ultra high pressure) is with bakery products as the shelf-life of most intermediate and high moisture bakery products is limited by mold growth, particularly *Aspergillus* and *Penicillium* species. While preservatives, such as sorbic and propionic acid, are most commonly used to extend the mold free shelf-life of bakery products, there is consumer resistance to the use of preservatives in food. Therefore, the bakery industry is seeking new methods to extend the shelf-life which are commercially viable and would eliminate the need for preservatives. One potential method which fulfills these objectives is ultra high pressure. The objectives of this study were:

1. To determine the effect of high pressure on the growth of *Aspergillus niger* and *Penicillium notatum* in packaged pita bread.

2. To determine the effect of high pressure on the sensory qualities (color, texture) of packaged pita bread by both objective and subjective methods.

3. To determine the effect of UHP on the permeability characteristics Oxygen Transmission Rate (OTR) and the Water Vapor Transmission Rate (WVTR) of a high barrier film B541,

as well as the tensile strength of the film, used to package pita bread.

4.2 Materials & Methods

4.2.1 Pita bread processing

Pita bread, an Arabic flat bread, was used throughout this study. Pita bread was chosen since its sales have increased ~50% worldwide due to changing consumer tastes and migration of societies and cultures. Pita bread was formulated according to a commercial recipe using all purpose flour, water, salt, sugar, yeast, and enzyme to delay staling as outlined in Chapter 2, section 2.2.1.

4.2.2 Inoculation and packaging

Two mold species, *Aspergillus niger* and *Penicillium notatum*, were used in this study. These molds were selected since they are the most common spoilage molds of bakery products. Both molds were grown on Potato Dextrose Agar (PDA) (Difco, Michigan, USA), the spores were harvested from plates by washing with 0.1% (v/v) peptone water, and enumerated using a Haemacytometer. Appropriate dilutions were made with 0.1% peptone water to give a stock suspension 10^5 spores /ml. Pita bread was inoculated with 100μl of each mold suspension at five spots to give a final inoculum level of 10^3 spores/g. Control pita bread was inoculated in a similar fashion with 100μl of 0.1% sterile peptone water. The control and inoculated pita bread was packaged (1 per bag) in a 210 x 210mm high barrier 541 Cryovac bags (O_2 transmission rate 3-6cc/m^2/day@ 4.0°C, 0%RH) obtained from Cryovac, Mississauga, Ontario. All bags were sealed with an Impulse heat sealer (A 300/42).

4.2.3. High Pressure Treatment

Pressure treatment was done using a UHP machine SA 723 (Autoclave Engineering Inc., Pennsylvania, USA). The working principle of the pilot plant machine is as follows: A single piston high pressure pump is connected to the vessel and a pressure sensor is used to control the pressure level. A pressure level release valve is used to remove the remaining air prior to pressurization and after decompression. A mixture of distilled water and mineral oil is required as the compression fluid at a ratio of 5 liters of distilled water to 2% of mineral oil. This mixture lubricates the chamber and prevents overrun of the pump. The press has a capacity of 1.4 liters and can operate up to a pressure of 500MPa. In the initial study, both the control and the inoculated packaged pita bread (in duplicate) were subjected to seven different UHP treatments ranging from 5- 400 MPa for 15 minutes as shown in Table 24. In the subsequent study, pita bread was subjected to only two pressures, i.e., 5-10MPa for 5, 15, 30 minutes respectively as shown in Table 25.

Table 24: Experimental plan for pressure treatment/shelf-life extension

Pressure (MPa)	Time (min)
0	15
5	15
10	15
30	15
50	15
70	15
200	15
400	15

Table 25 : Experimental plan for pressure treatment of pita bread.

Pressure (MPa)	Time (min)
0	
5	5
5	15
5	30
10	5
10	15
10	30

4.2.4 Mold growth

Pita bread was checked daily for visible signs of mold growth. Mold growth was recorded as days to visible mold growth.

4.2.5 Measurement of gas transmission rate

The Oxygen Transmission Rate (OTR) and Water Vapor Transmission Rate (WVTR) were measured at room temperature (25°C) and at 100% relative humidity. For the measurement of the OTR, a standard method (ASTMD 3985-81) was used, with 100% O_2 as the permeant gas. The OTR of the film B541was measured by cutting a film of uniform size and placing it directly in the test cell. A 25µm thick piece of Mylar (polyester) was used as a standard to measure any leakage and to determine any correction factor. All films were conditioned at the test temperature and a 100% relative humidity for 24 to 48 hours prior to testing.

For the OTR test, an Oxtran 2/20 Master (Mocon, Minnesota, USA) was used with an Oxtran 2/20 software package to monitor all the phases of testing, including entering test conditions (parameters), monitoring tests, and printing reports. Once the parameters were set, the computer controlled the components, gathered and logged data, printed all the data

in the form of tables and bar charts. Both cells of the Oxtran 2/20 were divided in two chambers separated by the test film. Oxygen was passed through the upper chamber and carrier gas (98% Nitrogen and 2% Hydrogen) passed through the bottom chamber to sweep the permeant gas to the sensor. A scanning automatic valve sent the sample gas, oxygen, to a specific coulometric sensor detector. The detector gave a current output directly proportional to the rate of oxygen arrival at the sensor. Therefore, the oxygen flux across the film was dynamically measured, the OTR expressed as $cc/m^2/day$.

The WVTR was measured using a standard method ASTM D E96, with 100% relative humidity (RH) inside the cup and 30% RH in the atmosphere surrounding the cup. The 100% RH was maintained by means of distilled water while a 30% RH was achieved by means of a desiccant, Hydrated Calcium Chloride ($CaCl_2(H_2O)_5$). The weight difference of the cup after 24 hours was measured and expressed as $g/m^2/day$.

4.2.6 Tensile Strength

The tensile strength test was done using an Instron Universal Testing Machine (series 4502, Instron Canada Ltd., Laval, Canada). The machine was connected through a GIPB interface to a 386 IBM computer. An automated material testing software, series IX, was used to control operation of the machine. For each experimental setup, a test method was created for the series IX to control the machine. Test films were cut into 100mm x 20 mm strips in a vertical direction. Each sample was placed between the blocks and bolts were tightened to hold the sample firmly. A U test was performed.

A U test means that the film was placed in double layers. Therefore, the value of the load must be divided by two to obtain the tensile strength. Each test is replicated three times. When the film is broken at the level of the bolt, the experiment or the trial fails and have to be repeated. The Instron was programmed with a speed of 100mm/min and the load was 50 Newtons.

4.2.7 Color

For each sample, the effect of pressure on the color of pita bread was measured by a Spectrophotometer CM-508d or colorimeter (Minolta Inc., Mississauga, Ontario). This portable instrument is equipped with an integrating sphere and has a measuring area of 8mm in diameter and an illumination area of 11mm in diameter. During measurements, the instrument was placed on the surface of the bread. Light from a xenon lamp source was thoroughly diffused into the integrating sphere and reflectance measurements were collected from an eight degree viewing angle and spectral component excluded (SCE). Before data collection, the instrument was calibrated using white and zero calibrations. White calibration was achieved using a standard-white reflector plate. Zero calibration was achieved by aiming the spectrophotometer into the air to ensure that no object was within 1m of the measurement aperture (Choucha, 1997). This was done according to the manufacturer's recommendations to compensate for any effect of stray light due to flare characteristics of the spectrophotometer's optical system (Minolta, 1994). At each sampling day, slices of pita bread were placed on foil paper and packaged with 210x210mm cryovac bags. An average of 5 readings per sample were taken at five different spots as the pita bread color is not homogenous. The spectrophotometer averaged these five spots and the variations were standardized by the light source. Data were collected in CIELAB (CIE, 1978). This space is defined by the Commission Internationale de L'eclairage as the most widely used due to its uniformity. This space has the advantage over the Tristimulus (Yxy) method in that the distance between color points indicates the true perceived color. The three values measured indicate the lightness (L, separate bright and dark colors), and the chromaticity coordinates (a=yellowness and b=redness), the center of the a, b diagram is achromatic and when the point is moved out from the center the color saturation increases. In order to determine the degree of color difference between two samples, the following formula was used:

$$\Delta Eab=[(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^2$$

Where ΔL, Δa, Δb were the difference between the control sample values and the treated sample values. This indicates the size of the color difference but not the direction of this difference in the diagram. For each color sample, the values obtained are an average of 3x5 replicates.

4.3 Results & Discussions

4.3.1 Effect of high pressure on the mold free shelf-life of pita bread

In the initial study, the effect of high-pressure (ranging from 0- 400 MPa) on mold growth in pita bread, packaged in a high barrier film B541 (OTR= 10.56 cc/m^2/day at 25°C), was investigated. Mold growth appeared on all control samples after 4 days (Table 26) and after only 7 days pita bread treated with low pressures (5-10 MPa) for 15 minutes. At higher pressures (30-70 MPa), mold growth did not appear until day 14, while at even higher pressures (200-400 MPa), no mold growth was evident after 21 days at room temperature (Table 26). However, while high pressure did extend the mold free shelf-life of pita bread, it had an adverse effect on the packaging film structure. At low pressures (0-10 MPa), no delamination of the film was observed (Table 26). However, at higher pressures (30-400 MPa), slight to severe delamination of the film occurred, particularly at the heat sealant layers (Table 26). Thus, while longer extensions in shelf-life were possible at higher pressures, the packages were not aesthetically pleasing to the eye and hence, would be rejected by consumers.

Since high pressure affected film structure, it was believed that this may influence the OTR of the film. The effect of high pressure on the film's OTR is shown in Table 27. As pressure increased, the OTR also increased from an initial value of 10 cc/m^2/day to 16.5 cc/m^2/day at 400 MPa (estimated from the regression line) i.e., a 50% increase in permeability. The regression line of OTR vs UHP is shown in Figure 22. There was a very good correlation between pressure and OTR (r^2=0.9808), indicating that, as pressure increased, so did the OTR. It is surprising, that despite the increase in the OTR at higher pressures, mold growth did not occur. High pressures may have injured or inactivated any mold spores on the pita bread's surface. Therefore, based on these initial studies, all future studies were done at lower pressures, i.e., 5-10 MPa for 5, 15 and 30 minutes.

4.3.2 Effect of low pressure on mold growth

The effect of low pressures on the growth of *A. niger* and *P. notatum* on pita bread is shown in Table 28. As observed in Table 28, mold growth occured in control samples (0 MPa) after 3 days for *A. niger* and 4 days for *P. notatum.* At low pressures, mold growth was evident, after 5-6 days, irrespective of treatment times. These studies confirmed that low pressures have very little effect on mold growth and that *A. niger* was slightly more resistant to low pressures than *P. notatum.* These results also confirmed the observations of Butz et al. (1995) who observed that mold growth in physiological sodium chloride and grape juice occurred after treatment with low pressures. These authors concluded that low pressures, alone, could not be used to extend the mold free shelf-life of food products and that additional control measures, such as preservatives or low temperature, were necessary. It is also interesting to note that *A.niger* was consistently more resistant to high pressures than *P.notatum.* A similar effect has been observed with preservatives and high CO_2 levels.

4.3.3 Effect of pressure on the sensory qualities of pita bread

The effect of low pressure on the sensory quality of pita bread, particularly color, is shown in Figures 23-26. The effect of pressure (5-10MPa) on the lightness (L), redness (a), and yellowness (b) of pita bread is shown in Figures 23, 24, 25 respectively. The lightness of pita bread decreased at lower pressures (5 MPa) and then increased at slightly higher pressures. This may be explained by moisture being expressed from pita bread to its surface, thereby enhancing the light scattering effect as shown by an increase in (L) values. The effects of pressure on redness (a) and yellowness (b) are shown in Figures 24 and 25. At first, both these attributes increased and then decreased as pressure increased. Again, there was a good correlation between lightness, redness, and yellowness. As (L) values decreased both (a) and (b) values increased. Then, as (L) values increased, both (a) and (b) values decreased. Similar observations have been observed with packaged meat (Morris, 1996 ; Choucha, 1997).

The overall Eab (Figure 26) decreased when the time of pressure treatment increased. The color difference for treated pita bread became important as the pressurization time increased. Therefore, even low pressures influenced the color of pita bread.

4.3.4 Effect of pressure on film permeability and tensile strength

The effects of low pressure on both the tensile strength and the water vapor transmission rate (WVTR) of film B541 are shown in Figures 27 and 28 respectively. In both cases, low pressure had very little effect on the structure of the film, i.e., no delamination (Table 26). Therefore, it was expected that delamination would have little or no effect on the tensile strength and WVTR at lower pressures as shown in Figures 27and 28. However, these parameters may have changed at higher pressures in a similar fashion as the OTR of the film.

Table 26: Days to visible mold growth on pita bread treated with high pressure and stored at 25°C

Pressure (MPa)	Time (min)	Days to visible mold growth	Package delamination
0	15	4 to 5	0
5	15	7	0
10	15	7	0
30	15	14	+
50	15	14	+
70	15	14	+
200	15	N/G	+++
400	15	N/G	+++

+: slight delamination

+++: severe delamination

N/G: no growth

Table 27: Effect of high pressure on the OTR of B541 film

Pressure (MPa)	Time (min)	Permeability ($cc/m^2/day$)	Number of observations	Standard deviation
0	15	10.56	4	0.89
5	15	10.89	4	0.01
10	15	10.88	4	0.01
30	15	11.11	4	1.00
50	15	11.59	4	0.03
70	15	11.94	4	0.87
100	15	12.04	4	0.01
200	15	13.60	4	0.34
400	15	16.50	4	0.25

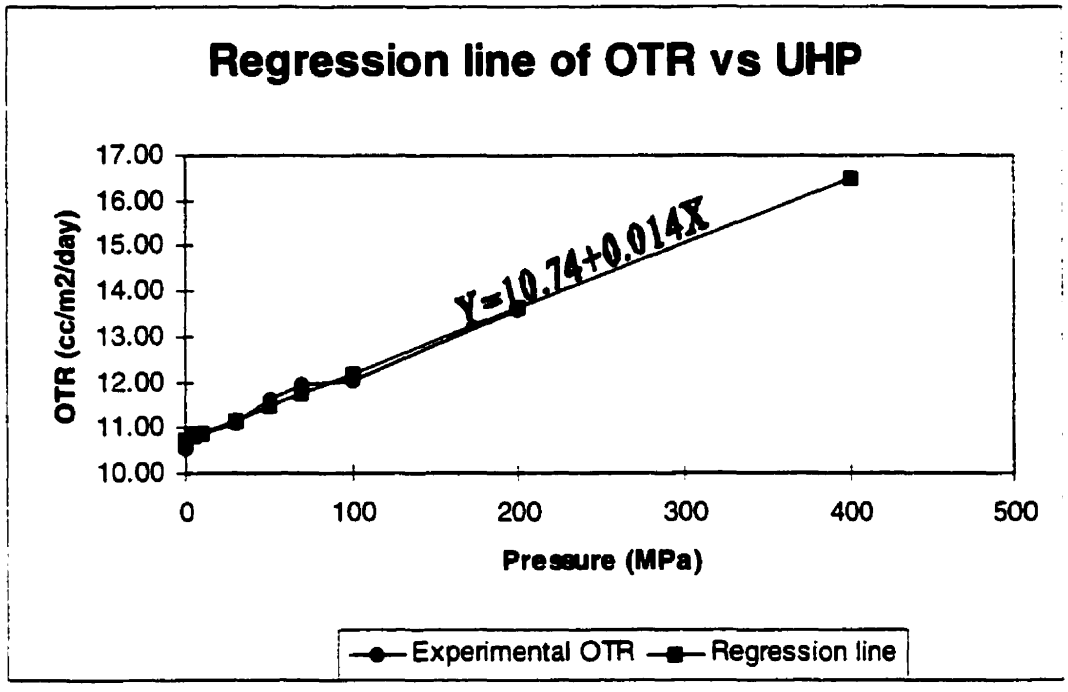


Figure 22. Regression line of OTR vs UHP

Table 28: **Effect of low pressure on the shelf-life of pita bread**

Pressure (MPa)	Time (min)	Days to visible mold growth	
		A. niger	*P. notatum*
Control		3	3-4
5	5	5	6
5	15	5	6
5	30	5	6
10	5	5	6
10	15	5	6
10	30	5	6

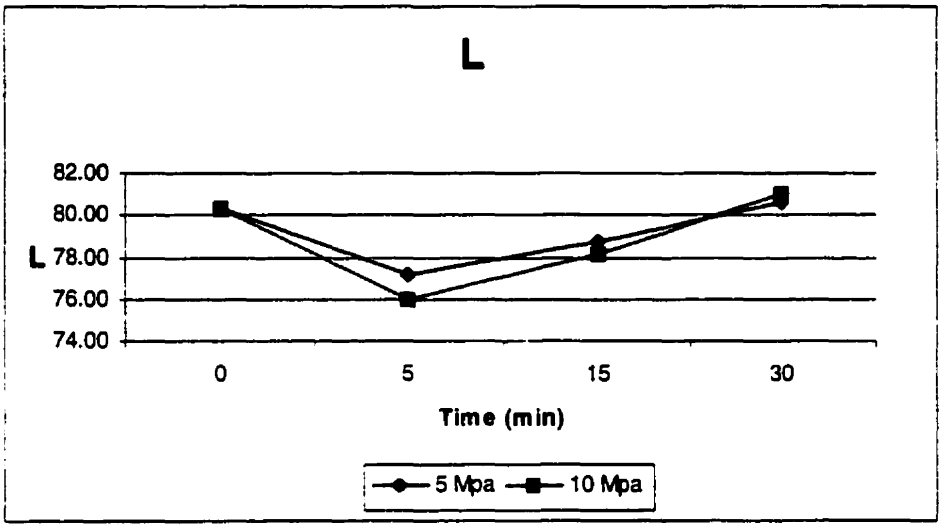


Figure 23. Effect of pressure on the lightness of pita bread

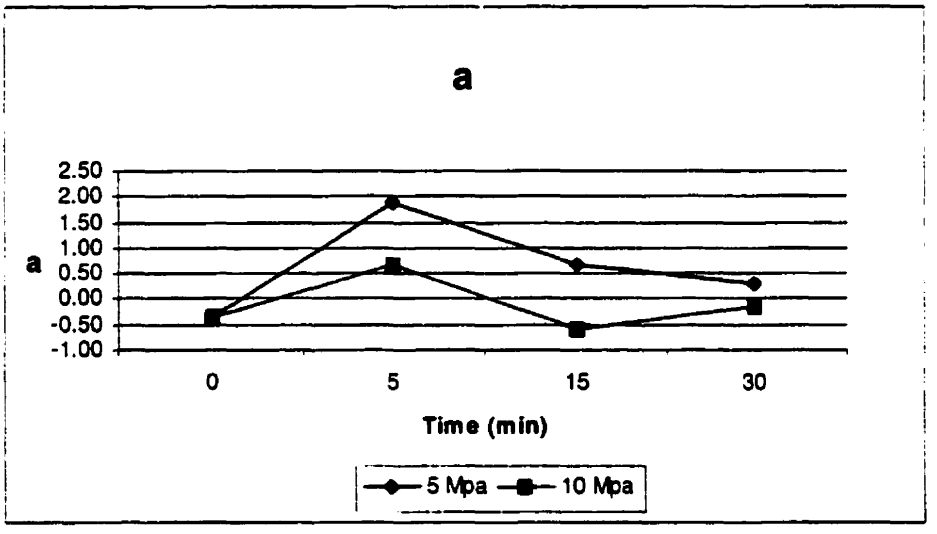


Figure 24. Effect of pressure on the redness of pita bread

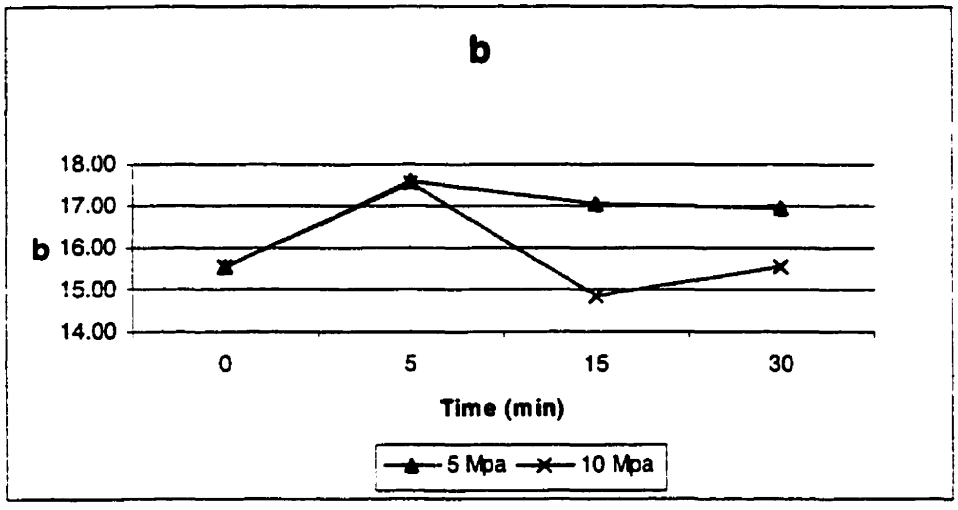


Figure 25. Effect of pressure on the yellowness of pita bread

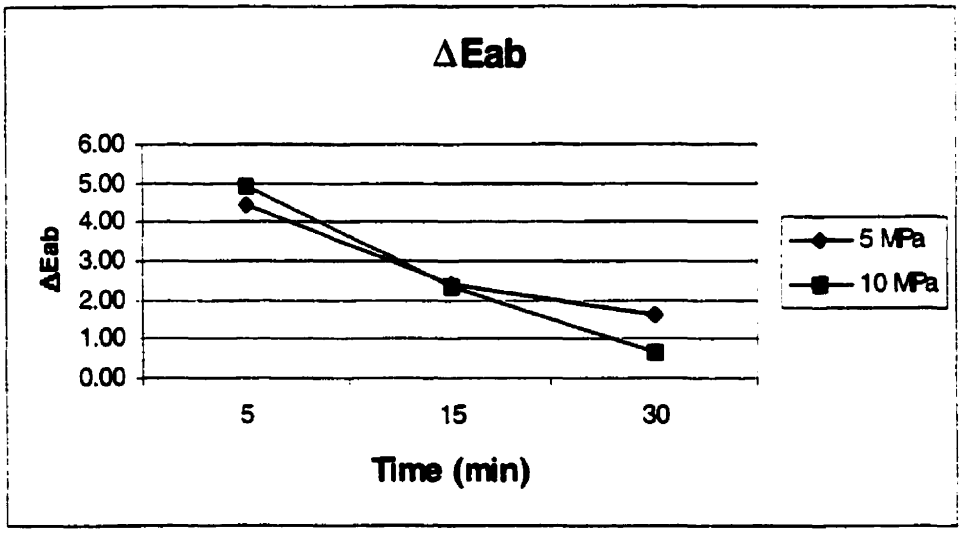


Figure 26. Effect of pressure on the color of pita bread

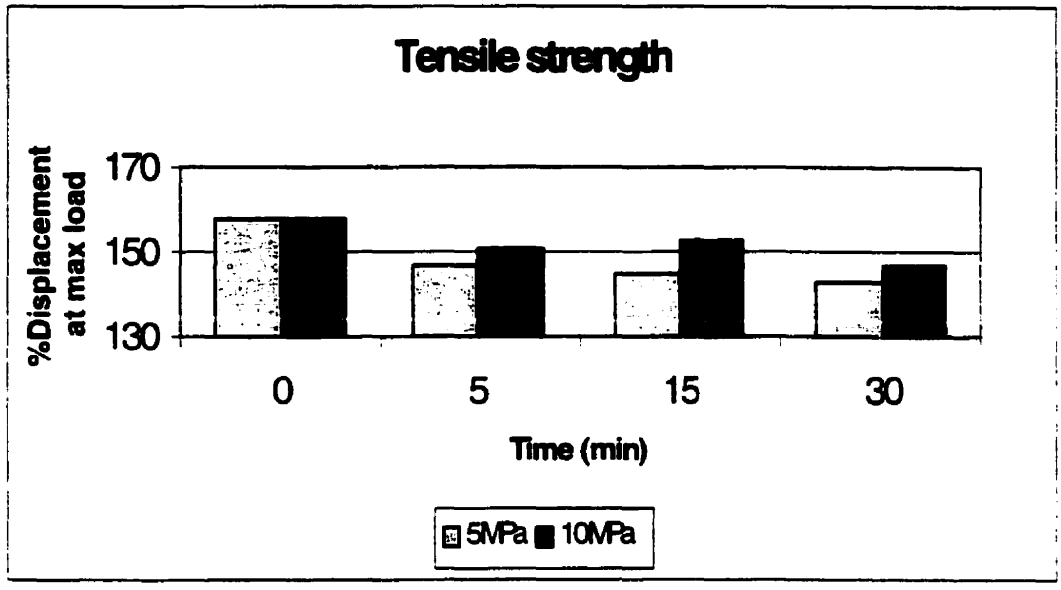


Figure 27. Effect of pressure on the tensile strength of B541 film

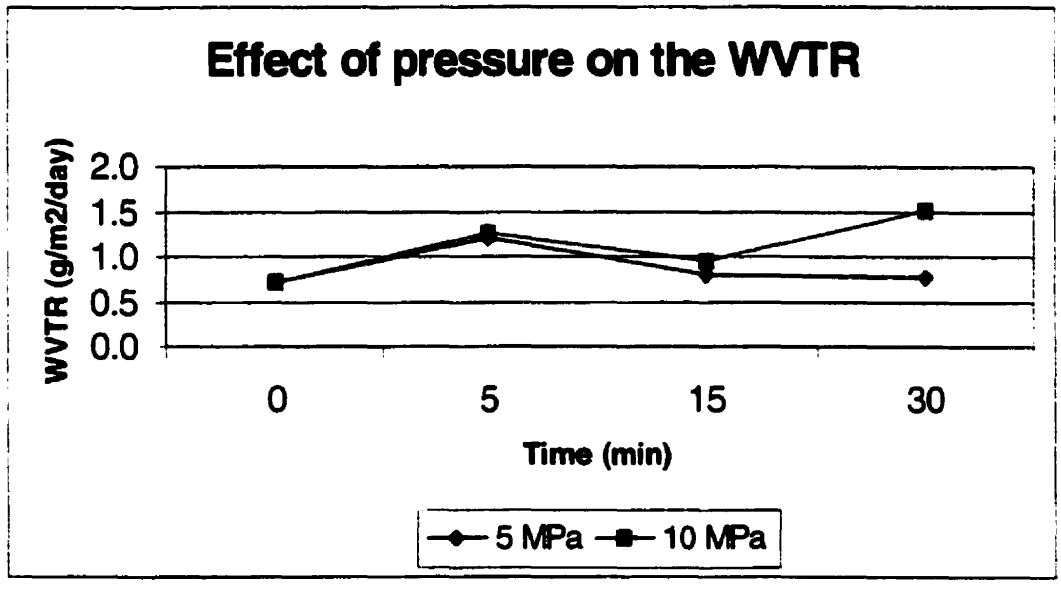


Figure 28. Effect of pressure on the Water Vapor Transmission Rate of B541 film

4.4 Conclusion

In conclusion, this study has shown that low pressures had little or no effect on film characteristics and on mold free shelf-life extension. For a longer extension in mold free shelf-life, UHP would need to be used in conjunction with additional barriers, such as preservatives and MAP (oxygen absorbents). While higher pressures could also be employed, these would have an adverse effect on film's strength and permeability as well as the sensory properties of pita bread.

Chapter 5

EFFECT OF DIRECT AND INDIRECT HEATING ON THE MOLD FREE SHELF-LIFE OF PITA BREAD

5.1 Introduction & Objectives

To date, MAP involving gas packaging, oxygen absorbents, and ethanol vapor generators have been shown to extend the mold free shelf-life of pita bread. However, these methods require gases, sachets, high barrier films, and in some cases, expensive packaging equipment, all of which will add to the cost of the packaged pita bread. Another, and less expensive method, to extend the shelf-life of pita bread could be direct and indirect heating. Direct heating involves the application of a hot flame directly to the surface of the bread. The heat source causes the bread to heat from the surface inwards so that the successive layers heat in turn. This produces a temperature gradient; however if the time-temperature is too long, the outside of the food will char (Potter and Hotchkiss, 1995). Indirect heating is any application that doesn't involve direct contact of the product with the heating source, e.g., microwave heating. Microwaves penetrate food uniformly and result in an increase in the kinetic energy of water and other polar molecules within the food. Unlike direct heating, heat is not passed by conduction from the surface inwards, but it is generated quickly and uniformly throughout the food (Potter and Hotchkiss, 1995). The objectives of this study were to evaluate the effect of direct and indirect heating on the mold free shelf-life of pita bread.

5.2 Materials & Methods

5.2.1 Pita bread processing

Pita bread was produced with enzyme as outlined in chapter 2, section 2.2.1.

5.2.2 Inoculation

Two mold species, *Aspergillus niger* and *Penicillium notatum*, were again used in this study. These molds were selected since they are the most common spoilage molds of bakery products. Cultivation, harvesting, and enumeration of each mold was described in chapter 2, section 2.2.3. Pita bread was inoculated with 100μl of each mold suspension at 5 random spots on the surface of the bread to give a final inoculum of 10^3 spores/g. Control pita bread was inoculated in a similar manner with 100μl of 0.1% peptone water. All pita bread was inoculated aseptically under a laminar flow cabinet (Labconco Corporation, Kansas City, Missouri 64132, purifier^{TM}class II Safety Cabinet).

5.2.3 Direct heating

After inoculation, pita bread was placed on sheets of aluminium foil in a sterile Laminar flow hood and heated directly with an inverted Bunsen burner (estimated temperature of flame ~800°C). The flame was held at a height of ~12"from the bread and passed over the entire surface of pita bread (in triplicate) for each of the following times: 3, 6, 9, 12 seconds. Non-inoculated samples were also heat treated and immediately after heating, the temperature of the bread was monitored using a thermocouple (Barnant Company Model 600-1020, Barrington, Illinois, USA 60010-2392). Upon cooling, the pita bread was packaged (1 per bag) in 210 x 210mm B541 high barrier (Oxygen Transmission Rate (OTR) of 3-6 cc/m^2/day @ 4°C & 0% RH) (Cryovac, Missusauga, Ontario). These bags were than heat sealed with an Impulse heat sealer (A 300/42), stored at room temperature (~25°C), and

monitored daily for visible signs of mold growth.

5.2.4 Microwave heating

Pita bread was placed directly into a domestic Microwave Oven with a power and frequency of 800watts and 2450Mhz respectively (Jutan International Limited JM 55481, Toronto, Canada) and microwaved for 3, 6, 9, 12 seconds. Triplicate samples per treatment were then packaged and stored at room temperature and monitored daily for visible mold growth. Temperature was also recorded on non-inoculated pita bread as described in section 5.2.3.

5.3 Results & Discussions

5.3.1 Direct heating

The effect of direct heating on the mold free shelf-life of pita bread is shown in Tables 29 and 30 respectively. Mold growth appeared on all non-heated pita bread (control) after 3-4 days. Mold growth was evident in all pita bread inoculated with *A. niger* and *P. notatum* spores after 7 days, irrespective of duration of heat treatment (Tables 29-30). This growth can be attributed to the fact that the temperature of pita bread only increased to 33.8°C after heat processing time of 12 seconds (Tables 29-30). This temperature was clearly inadequate to destroy all the mold spores as shown by a 3-4 day extension in shelf-life. One disadvantage of direct heating was that it created fogginess inside the package due to water vapor which could have condensed on the surface of the pita bread, thus, creating an environment conducive to mold growth (Dodds and Farber, 1995).

5.3.2 Microwave heating

The effect of microwave processing on the mold free shelf-life of pita bread is shown in Tables 31 and 32 respectively. Mold growth appeared on non-microwaved bread after 3-4 days (Tables 31-32). Microwaving bread for 3-12 seconds resulted in ~100% increase in the shelf-life before the growth of both *A. niger* and *P. notatum* was evident in ~7days (Table 31-32). The limited extension in the shelf-life again can be attributed to the fact that microwaving only increased the temperature of pita bread to 43.5°C after 12 seconds heating. While longer processing times resulted in a longer mold free shelf-life, texture was unacceptable, i.e, pita bread was hard due to moisture loss during microwaving.

Table 29: Effect of direct heating on pita bread inoculated with *A. niger* (10^3spores/g) and stored at 25°C.

Time (sec)	Temperature (°C)	Days to Visible Mold Growth
0	25.0	3-4
3	26.5	7
6	28.4	7
9	31.5	7
12	33.8	7

Table 30: Effect of direct heating on pita bread inoculated with *P. notatum* (10^3 spores/g) and stored at 25°C.

Time (sec)	Temperature (°C)	Days to Visible Mold Growth
0	25.0	3-4
3	26.5	7
6	28.4	7
9	31.5	7
12	33.8	7

Table 31: Effect of microwave processing on pita bread inoculated with *A. niger* (10^3spores/g) and stored at 25°C.

Time (sec)	Temperature (°C)	Days to Visible Mold Growth
0	25.0	3-4
3	28.4	7
6	30.2	7
9	37.3	7
12	43.5	7

Table 32: Effect of microwave processing on pita bread inoculated with *P. notatum* (10^3 spores/g) and stored at 25°C.

Time (sec)	Temperature (°C)	Days to Visible Mold Growth
0	25.0	3-4
3	28.4	7
6	30.2	7
9	37.3	7
12	43.5	7

5.4 Conclusion

In conclusion, the use of direct and indirect heating appears to have limited application to extend the mold free shelf-life of pita bread. It could be used if only a 3-4 day extension in shelf-life is required. However, a longer mold free shelf-life appears to be only possible using some form of modified atmosphere packaging.

GENERAL CONCLUSION

Pita bread, a flatbread, is an important bakery product in many Middle Eastern and international diets. A major factor limiting the shelf-life of pita bread is mold growth. While preservatives, such as calcium propionate or potassium sorbate can be used to extend the mold free shelf-life of bakery products, there is an increasing demand for preservative free products. In this research, three alternative approaches to chemical preservatives to extend the mold free shelf-life and quality of pita bread were investigated namely, Modified Atmosphere Packaging (MAP) involving gas packaging, oxygen absorbents technology and ethanol vapor generators, high pressures, and direct and indirect heating.

Gas packaging using 60% CO_2 (balance N_2) could extend the mold-free shelf-life from 3d (pita bread packaged in air) to 35d at ambient temperature (~ 25 °C). A longer extension in shelf-life (42d) was possible using an oxygen absorbent (Ageless type FX) inside the packages or a Freshmax oxygen absorbent label in conjunction with gas packaging (CO_2:N_2-60:40). In all MAP products, sensory shelf-life was terminated ~7d prior to microbiological shelf-life due to changes in odor, texture, and flavour.

Similar results were obtained with 2G-4G sachets of Ethicap, an ethanol vapor generator and 100-200S sachets of Negamold, a dual functional oxygen absorbent - ethanol vapor generator. While the mold free shelf-life could be extended to 42d, irrespective of sachet type or size, sensory shelf-life of pita bread ranged from 30-35d. Furthermore, ethanol vapor had a plasticizing effect on film permeability as shown by an increase in the OTR of the film throughout storage.

While high pressures (30-400MPa) could be used to inhibit mold growth, higher pressures resulted in delamination of the packaging film and textural changes to the pita bread. While lower pressures (5-20 MPa) prevented these defects, the mold free shelf-life of the product could only be extended ~ 3- 4d at these pressure treatments.

Even such low pressures increased the OTR of the high barrier laminated packaging film.

Other alternatives, such as direct heating, microwave processing had a minimal effect in increasing the shelf-life of pita bread due to the short processing time and low temperature within the product.

In conclusion, this study has shown that oxygen absorbent sachets or labels in conjunction with gas packaging were the most effective methods to increase the mold free shelf-life and quality of pita bread. These sachets/labels are relatively inexpensive (5-20 cents), simple to use and offer a viable alternative to chemical preservatives to extend the mold free shelf-life and sensory quality of pita bread. They could easily be used in developing countries, such as Lebanon, to extend the keeping quality of bakery products without the need for expensive packaging equipment.

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