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Reformulation/Packaging Studies to Delay Staling in a Bakery Product

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March, 1996

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Master's of Science

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Canada

To Colette, Elias, Philippe, Pierre and Carine.

ABSTRACT

M.Sc. Marie-Christine Assouad Food Science

Reformulation/Packaging Studies to Delay Staling in a Bakery Product

Bakery products are important sources of nutrients in our diet. However, spoilage occurs shortly after baking. After microbial spoilage, the main spoilage problem is staling.

Therefore, methods to control staling are of great importance to the bakery industry since staling results in millions of dollars annually in lost revenues.

Initial studies using a one variable at a time approach showed that enzymes, guar, algin and pectin gums and high fructose corn syrup could delay staling and resulted in an organoleptically acceptable product. Subsequent optimization studies using a Response Surface Methodology (RSM) approach show the appropriate levels of enzyme (Novamyl), guar gum and HFCS resulted in bagels with a textural and sensorial shelf life of 6 weeks at ambient temperature.

Furthermore, the cost of reformulating (~0.5 cent/ bagel) is minimal and could easily be recovered through reduced production costs, reduced losses due to staling and additional sales and market areas.

RESUMÉ

M.Sc. Marie-Christine Assouad Food Science

Études de Formulation/d'Emballage des Produits de Boulangerie pour Retader le Rassissement

Les produits de boulangerie sont une source importante d'éléments nutritifs. Mais leur altération, microbienne et de rassissement, se manifeste rapidement aprés la cuisson.

Les méthodes de contrôle du rassissement sont, donc, d'une grande importance pour l'industrie du pain car il en résulte des pertes en millions de dollars.

Des méthodes de changement d'une variable à la fois ont montré que les enzymes, certaines gommes et le sirop de maïs à haute concentration de fructose peuvent retarder le rassissement et garantir une bonne qualité organoleptique du produit.

Des études ulterieures d'optimisation utilisant une méthodologie de response de surface (RSM) ont determiné les concentrations d'enzymes, de gommes et de sirop qui ont permis d'obtenir des bagels d'une bonne qualité organoleptique et de texture pour une conservation de six semaines à température ambiante.

De plus, le coût de la nouvelle formule (+ 0.5 cent/bagel) est minime et peut être facilement amorti.

PREFACE

Claim of Original Research

1) Study on the effect of different enzymes, gums, high fructose corn syrups, low protein flours and surfactants on staling of bagels.

2) Optimization study: Effect of combining (i) enzyme (Novamyl), guar gum and high fructose corn syrup, and (ii) enzyme (Superfresh), algin and pectin gums on staling of bagels using a Response Surface Methodology (RSM) approach.

3) Comparative study of bagels baked commercially, (i) non-reformulated and reformulated with (ii) enzyme (Novamyl), (iii) enzyme (Novamyl), guar gum and high fructose corn syrup and (iv) enzyme (Superfresh), algin and pectin gums regarding texture and consumer acceptability.

4) Additional studies on the effect of carbon dioxide, retarding and high temperature on staling.

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I also would like to thank our research group for their help and support; Isle-Marie Tarte, Andre Lyver, Frances Taylor, Norma Borja, Sameer Al-Zenki, Salah Hasan and Sam Choucha. And all the panelists (especially Veronique Barthet) who tasted bagels almost everyday for 8 months! THANK YOU!

Thanks are also due to the many companies that supplied us with the ingredients for this research work, These include R.E.A.L. bagels, Amcan Ingredients, Novo Nordisk, Enzyme Biosystems, Soca Floc, Dow Ingredients, ADM Corn Processing, Inovative Grain Technology, ICI Surfactants and Multisac Packaging.

Last but not least, I would like to thank my family. Without my parents, brothers and sister's support, I wouldn't have even started this work. They always encouraged me to do further studies and to work hard to realize my potential.

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CHAPTER I: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction:

Baking is not a 20th century technology. Indeed, it is one of the most ancient of human arts (Kent-Jones and Price, 1951). Some of the earliest records on baking can be found in Egyptian tombs. Many of these tombs depict scenes of bread making, and the remains of bread were found buried with corpses (Kent-Jones and Price, 1951). Leavened bread was reported in Egypt in 800 B.C., and commercial bakeries were in existence by 500 B.C. From Egypt, bread making spread to the Mediterranean region. The Greeks made an unleavened flat bread to which they paid homage while Italians had large commercial bakeries by 200 B.C. In Asia, bread making can be traced in China more than 5000 years ago.

The importance of the bakery industry is well known worldwide. Bakery products are an important source of nutrients in our diet. Consumption of bakery products in North America is estimated at $23 billion dollars annually with roughly 50% being spent on bread and rolls (Peat Marwick Group, 1991).

However, spoilage problems are of major economical importance. These include microbial, chemical and physical spoilage. While mold problems are of concern, staling is also a major factor limiting the shelf life of baked products.

Staling has been extensively studied in the past century, and many theories have evolved. However, despite these theories, research is still ongoing in an attempt to extend the shelf life of the bakery products with respect to staling.

1.2 - Classification According to Product Type:

Bakery products can be grouped according to product type, the method of leavening or water activity (a_w). In this section, products will be classified according to product types found commercially.

1.2.1. BREAD AND ROLLS: Bread and rolls are by far the most important type of bakery products. Many varieties of bread and rolls are found according to country of origin, differing only in their composition and in their methods of production. However, they all consist of the same basic ingredients: flour, water, salt, yeast or sour dough. Other ingredients can be added e.g., fat or shortening, sugar, milk powder, eggs, honey, syrup, fruits, spices and flavors.

Production steps carried out to transform these raw ingredients into a loaf of bread include mixing, fermentation, scaling, rounding, proofing, molding, panning, baking, cooling, slicing and packaging.

Some of the main types of commercial bread and rolls are shown in Table 1.1.

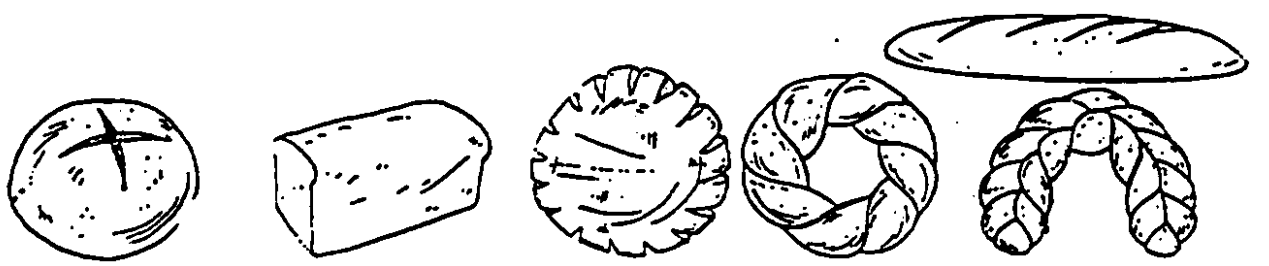


Figure 1.1: Examples of bread products.

1.2.2. OTHER BAKERY PRODUCTS:

Examples and a brief description of other bakery products are shown in Table 1.2.

Table 1.1: Types of bread and their characteristics.

Type of bread	Shape and popularity	Processing Characteristics
White pan	Most popular in the U.S. and Canada.	Fermented dough.
White mountain	Round or loaf form.	Surface is sprinkled with water and dusted with flour.
Pullman	Similar to white pan.	Baked in a pan closed on four sides.
French bread and rolls	Baguettes are the most popular variety.	Lean dough with small amount of malt and sugar.
Italian bread, rolls and bread sticks.	Large variety of shapes.	Similar dough to the French one. Bran added sometimes gives a brown color
Vienna bread	Austrian bread.	Rich dough.
Bolillos	Oval-shaped, most popular in Mexico.	
Pan de agua	Popular in the Dominican Republic.	Rich dough.
Egg bread and rolls	Many shapes: oval, snail, three, four, five or six twist.	High percentage of eggs: increased richness, volume and shelf life.
Hard rolls	Most popular: Kaiser roll. Others: salt sticks, crescent, onion, club, water rolls.	Different dough but all have a firm and crisp crust.
Soft rolls	Hamburger, frankfurter, twisted, butterflake and cloverleaf.	Rich dough with more sugar and fat as compared to hard rolls.
Pan de sal	Popular in the Philippines, small oval shape rolls.	Yeast-raised bread.
Rye bread and rolls	Many shapes.	Lean dough: rye flour, salt, water, yeast or sour dough.
Pumpernickel bread and rolls	Similar to rye bread except in color.	More sugar and pumpernickel flour.
Bagels and Bialys	Originally from Russia. Very popular in the U.S. and Canada.	Stiff dough.
Armenian cracker or biscocho.	Cracker shape, with a denser consistency.	
Pita	Used in the Middle-East. Pocket-like thin bread.	Lean dough, low in fat.

Adapted from Sultan (1990).

Table 1.2: Types of Bakery Products.

Types	Description	Examples
Sweet Yeast and Dough Products	Dough rich in sugar, fat eggs, milk and may be filled with topping	Buns, sweet rolls, babka, croissant, frozen dough, Danish pastries
Biscuits	Various recipes, usually with sugar, salt, milk, shortening, flour and leavening agents	Flavored biscuits
Muffins	Same as above, with commeal, whole wheat, bran flour.. added to the mix	Flavored muffins
Scones	Biscuit dough with added raisins	Raisin scone
Donuts	Formed, fried dough, dusted with sugar, chocolate or icing.	Yeast-raised, whole wheat donuts...
Crullers	Donut chemically leavened, no fermentation nor proofing	Twists, French, Cake type cruller....
Pies	Very popular, frozen fruits, instant gel thickeners, frozen pies, stabilizers are being extensively used	Apple, pumpkin, cheese, cream, chiffon pies...
Pastries	Made from a variety of dough; short, puff pastry, piecrust dough...	Eclairs
Cakes and Cake Specialties	Basic ingredients are sugar, butter or fat, eggs and flour.	Chocolate, spice, whipped cakes...
Cookies	Basic ingredients are sugar, fat, eggs and flour.	Sugar, bagged, butter, chocolate chips...
Dietetic Baking	Meets consumer dietary needs, Applied to all the types mentioned above	Cakes, cookies, muffins..
Passover Bakery Products	Products from the ones mentioned above with modifications to their formula	Cakes, cookies, muffins..
Natural Bakery Products	Use of "natural" ingredients: whole wheat, natural grain...	Bread, rolls, buns, donuts...
Pizzas	Basic bread dough with many different toppings. Used as a snack or as a meal	Vegetarian, all dressed..

Adapted from Sultan (1990)

1.3- Classification of Product According to Water Activity (a_w):

Water activity is one of the main factors affecting the shelf life of the bakery products. It is directly related to microbial spoilage, as discussed in subsequent chapters.

Bakery products can be divided into three major groups on the basis of their water activity (a_w) :

- low moisture bakery products. i.e., products with an a_w of less than 0.6 ($a_w < 0.6$). This group is the least affected by microbial growth.
- intermediate moisture bakery products having an a_w between 0.6 and 0.85 (a_w 0.6-0.85)
- high moisture products with an a_w higher than 0.85, usually between 0.95 and 0.99 (a_w 0.95-0.99). This group is most affected by microbial growth. (Smith and Simpson, 1995)

Examples of bakery products and their water activity are shown in Table 1.3.

Table 1.3 : Water activity of some bakery products.

PRODUCT TYPE	WATER ACTIVITY
LOW MOISTURE CONTENT	
Cookies	0.20 - 0.30
Crackers	0.20 - 0.30
INTERMEDIATE MOISTURE CONTENT	
Cake donut	0.85 - 0.87
Chocolate coated donuts	0.82 - 0.83
Danish pastries	0.82 - 0.83
Cream-filled snack cakes	0.78 - 0.81
Pound cake	0.84 - 0.86
Banana cake	0.84 - 0.86
Soft cookies	0.50 - 0.78
Bean/Jam cake	0.85
HIGH MOISTURE CONTENT	
Bread	0.96 - 0.98
Egg bread	0.90
Pumpernickel bread	0.90
Pita bread	0.90
Yeast raised donuts	0.96 - 0.98
Fruit pies	0.95 - 0.98
Soy bean pie	0.93
Carrot cake	0.94 - 0.96
Custard cake	0.92 - 0.94
Cheese cake	0.91 - 0.95
Butter cake	0.90
Pizza crust	0.94 - 0.95
Pizza	0.99

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

1.4 - Economic Importance of Bakery Products:

Bakery products are an important part of the average weekly food expenditure. According to Hunt and Robbins (1989), bakery products account for approximately 9% of total food expenditure. Bread was the most important product accounting for 27 cents for each dollar spent. However, in a recent study by Science and Technology Canada and International Trade Canada on the Industry Profile of the Bakery Products in 1991, the consumption of white bread per capita has decreased from 42 kg in 1961 to 28 kg in 1986. Furthermore, this decline has continued at an estimated 2% per year. On the other hand, sales of specialty packaged products such as croissants, cakes, pies and muffins have increased. The interest in these new types of fresh and frozen products is influenced by the nature of the product, the regional character of the Canadian market and related transportation and distribution costs and changing consumer tastes. The international market for Canada starts with the United States. Sales of bakery products in the United States are ~$23 billion per year with imports accounting for 9% of sales, i.e., ~$350 million (US). The major foreign exporters to the U.S. market are Canada, Denmark, West Germany, United Kingdom, Japan and Italy (Figure 1.2).

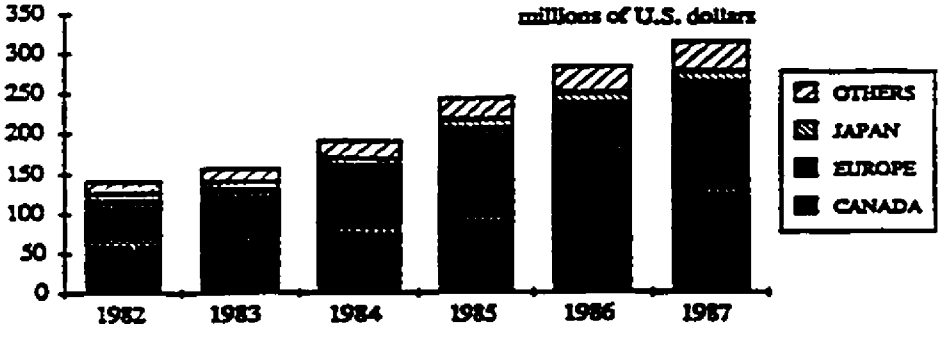


Figure 1.2: Total U.S. imports of bakery products.

Source: U.S. General Imports and Imports for Consumption, U.S. Department of Commerce in "Export Opportunities in Japan, the Bakery Mix Market". (1991)

In the bread category, 75% of all imports come from Canada, while biscuits account for 33% of total imports. As the result of higher prices for European and Japanese products, Canada could increase its market share further in the U.S.

Japan is also a good export market for Canada for bakery products. According to Japanese statistics, the volume of bakery products imported in 1988 reached approximately 78,750 metric tons, valued at $50 million (Can), of which about 8,300 metric tons were imported from Canada.

1.5 Ingredients:

There are many variations in bakery product formulations. However, formulations contain four basic ingredients: flour, yeast, salt and water.

Other ingredients can include fat or shortening, sugar, milk powder, eggs, honey, syrup, fruits, spices and flavors.

Flour as a major ingredient will be discussed in more details while the role of yeast, salt and water are summarized in Table 1.6.

1.5.1. FLOUR:

Flour is the major ingredient found in dough and is composed of five components: moisture, protein, starch, fat, and mineral matter. Table 1. 4. shows the chemical composition of flour.

Table 1.4: Chemical Composition of Flour.

	Moisture	Protein	CHO	Fat	Minerals
Flour (%)	14.5	11-14	69-72	2	1.8

Adapted from Sultan (1990).

The *moisture* content of flour varies with the tempering of wheat. This is the moistening of the wheat under controlled conditions to a standard moisture content which facilitates bran removal and flaking of the germ particles.

The endosperm *protein* consist of two general types: the water soluble albumins and globulins and the water insoluble gliadins and glutenins.

Water soluble proteins are present in small quantities. However, they can influence flour properties significantly. Water soluble proteins include alpha-amylases, beta-amylases, lipoxygenases, lipases. Other enzymes also found in minute quantities include catalase, phytase, polyphenoloxidase, peroxidase, and ascorbic acid oxidase (Bowers, 1992). When water is added during dough mixing, the water insoluble protein hydrates and forms gluten, a complex coherent mass in which starch, added yeast , and other dough components are imbedded (Figure 1.3). The total water-imbibing capacity of gluten is nearly three times its dry weight. Gluten is the skeleton or framework of wheat flour dough and is responsible for gas retention, and the production of light leavened products. Proper dough development is essential for optimum performance in bread making (Pomeranz, 1973). Gluten is formed of two proteins: gliadin and glutenin. They constitute 85% of flour protein, and are present in almost equal amounts. Gliadins are a large group of proteins with similar properties. They have an average molecular weight of about 40,000. The intramolecular disulfide bonds contribute to the compact globular nature of this protein. They are single chained and extremely sticky when hydrated. They have little or no resistance to extension and appear to be responsible for dough cohesiveness (Hoseney, 1986). The gliadin fraction controls loaf volume and varies in flours that differ in bread making potential. The glutenin proteins also are a heterogeneous group of proteins. They are multichained and vary in molecular weight from 100,000 to several million with intermolecular disulfide bonds being responsible for the large size of the molecules. Glutenin proteins are resilient but not cohesive and give a dough its property of resistance to extension (Hoseney, 1986). They are responsible for the mixing time and dough development (Pomeranz, 1973).

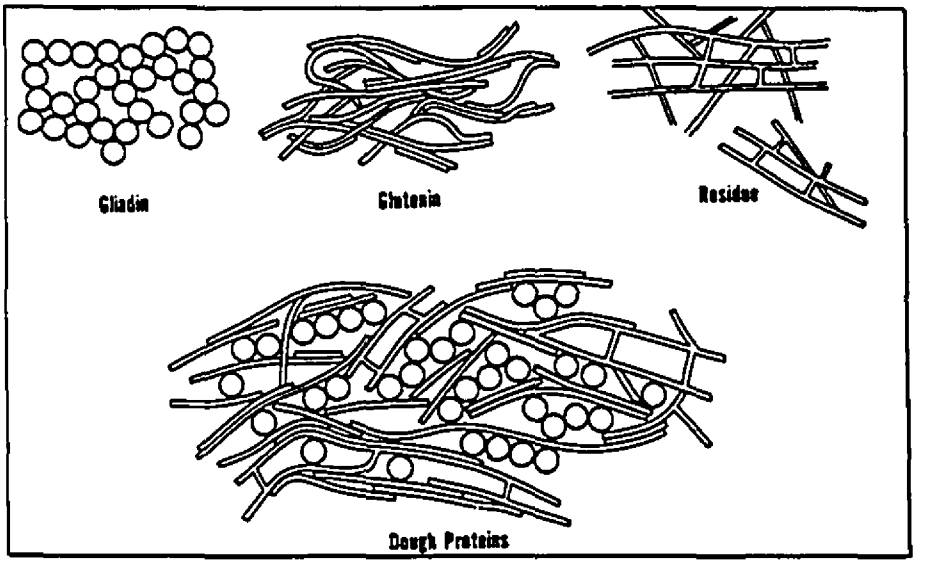


Figure 1.3: Glutenin, gliadin interaction in a dough. Adapted from Bowers (1992).

Starch represents 69-72% of the flour. Damaged starch particles are converted by enzymes into fermentable sugars leading to a more rapid fermentation, while fine starch content is the basis for improved grain and texture.

Fat represents 2% or less of flour. It can cause rancidity problems especially with whole wheat flour.

The *ash* portion is present in the bran and comprises mainly of mineral elements.

1.5.2. YEAST, SALT AND WATER:

Table 1.5. summarizes the effect of yeast, salt and water in baking.

Table 1.5: Functions of Yeast, Salt and Water in Baking.

Product	Type	Function
Yeast: Yeast cake, bulk yeast, Active dry baker's yeast, Instant active dry yeast.	Invertase, Maltase, Zymase.	Converts sucrose to invert sugars, Converts maltose to dextrose, Converts invert sugars and dextroses to CO_2 and H_2O
Salt	Purified salt, Cooking salt.	Flavor, Regulates yeast action, Strengthens gluten, Prevents bacteria growth, Affects crust color, Counteracts water softness.
Water	Potable, Cold.	Increases yield, Affects crumb softness, Counteracts temperature build up.

Adapted from Sultan (1990)

1.5.3. OTHER INGREDIENTS:

Other ingredients such as shortening, eggs, milk and sugar also have important roles in bakery formulations.. Their main functions are summarized in Table 1.6.

Other additives such as enzymes, emulsifiers, oxidizing agents, reducing agents and chemical leavening agents are used to compensate for varieties in the processing characteristics of the flour. Example of such ingredients are baking powder, baking soda, ammonium bicarbonate, potassium iodate, potassium bromate (oxidizing agents), L-cysteine (reducing agent), monoglycerides(crumb softening agent) calcium stearoyl-2-lactylate (emulsifier) and mineral buffers.

Table 1.6: Ingredients used in bakery products.

Product	Type	Function
Shortening	Solid Liquid Mixture of both	Shortness, richness and tenderness, Flavor, Desirable texture, Lubricates gluten, Acts as emulsifier.
Sugar	Disaccharides Monosaccharides	Flavor, Source of fermentable food for yeast, Desirable texture, Water absorption, Crust color.
Eggs	Fresh, Liquid, Frozen, Dried	Leavening agent, Emulsifier, Color, Flavor, Richness of the dough, Retain moisture.
Milk	Homogenized, Pasteurized, Evaporated, Condensed, Dried, Sour cream.	Water absorption, Decreases dough pH, Richness of the dough, Aids in creaming, Good texture and keeping qualities.

Adapted from Sultan (1990)

1.6 - Spoilage Problems:

Bakery products, like all processed food products, are subject to spoilage.

Spoilage of bakery products can be divided into:

- microbial spoilage
- chemical spoilage
- physical spoilage (Smith and Simpson, 1995)

Each of these methods of spoilage will be briefly discussed.

1.6.1. CHEMICAL SPOILAGE:

Chemical spoilage involves both oxidative and hydrolytic rancidity problems.

1.6.1.1. *Oxidative rancidity*:

A rancid product has a musty, rank taste or smell due to fats that have oxidized and decomposed with the liberation of short-chain fatty acids, aldehydes and ketones through an autolytic free radical mechanism. The free radicals and peroxides can bleach pigments, destroy vitamins A and E, breakdown proteins and cause darkening of fat (Smith and Simpson, 1995). They also have a disagreeable odor and flavor and are toxic in large amounts. Fortification of fats with antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), extends storage time and protects essential nutrients (Mahan and Arlin, 1992). The most susceptible bakery products to this kind of rancidity have an a_w lower than 0.3.

1.6.1.2. *Hydrolytic rancidity*:

This kind of rancidity occurs in the absence of oxygen. It results in the hydrolysis of triglycerides and the release of glycerol and short chain fatty acids.

1.6.2. MICROBIAL SPOILAGE:

Microbial spoilage comprises of bacterial, yeast and mold spoilage (Smith and Simpson, 1995). All microorganisms require three basic elements: food, temperature and moisture. Pre-packaged bakery products provide conditions conducive to microbial growth (Jenkins, 1975). The specific water activity (a_w) requirements for each spoilage microorganism are shown in Table 1.7. Mold spoilage is responsible for the majority of losses in the bakery industry in the United States. It accounts for 1 to 3% of spoiled products or over 90 million kg of product per year (Ooraikul, 1982).

Table 1.7: Major causes of spoilage based on water activity.

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

Adapted from Doerry (1990); Smith (1994).

Various bacteria, wild yeast and fungi are found in bread dough (Sugihara, 1985). Bacterial spoilage is mainly caused by *Bacillus subtilis* leading to a defect known as "rope" bread (Jenkins, 1975). Wild yeasts include the genera *Saccharomyces*, *Pichia*, *Zygosaccharomyces* (Graves *et al.*, 1967). The mycelial fungi are present in very low concentration in the dough but are important spoilage organisms in the final product (Lues *et al.*, 1993).

The most important microbial problem limiting the shelf life of high and intermediate bakery products is mold growth (Smith and Simpson, 1995). The majority of the molds found in white bread belong to the genus *Aspergillus* and *Penicillium* (Hartung, *et al.*, 1973). Other mold species e.g., *Rhizopus*, *Monilia*, and *Mucor* species have also been implicated (Jenkins, 1975). According to Bullerman and Hartung (1973), aflatoxin-producing molds have never been detected in either flour or bread. They also stated that flour contained more toxic molds than bread, due to the fact that mold spores are not very heat resistant. Thus, mold spoilage results from post-processing contamination. This occurs during cooling and packaging from contamination by air-borne spores or contact with contaminated surfaces (Black *et al.*, 1993). Contamination also results from food handlers and raw ingredients such as glazes, nuts, spices and sugars (Smith, 1994). Under warm humid conditions, mold problems are even more

troublesome and mold growth is visible within 48 hours after baking and packaging (Black *et al.*, 1993).

Several methods have been investigated to increase the mold free shelf life of bakery products. These include:

- Good manufacturing practices, (Jenkins, 1975).
- U.V. light and microwave heating, (Black *et al.*, 1993).
- Incorporation of preservatives such as sorbic and proprionic acid, or calcium salts either directly into the product or sprayed on the product surface, (Seiler, 1989).
- Freezing, (Matz, 1992).
- Modified atmosphere packaging, (MAP), involving gas packaging with mixtures of CO_2 and N_2, oxygen absorbents and ethanol vapor generators (Smith and Simpson, 1996).

1.6.3. PHYSICAL SPOILAGE:

Physical spoilage usually involves moisture loss or gain and staling.

1.6.3.1. *Moisture loss/gain:*

Moisture loss or gain is a problem in both high and low moisture products. Loss of moisture in high moisture bakery products results in a loss of texture and firmness. A gain of moisture in low moisture products also results in textural changes and may promote enzymatic and microbial spoilage problems. Both moisture loss and gain can be prevented by packaging products in a film which is a high barrier to moisture e.g., low density polyethylene (LDPE).

1.6.3.2. *Staling:*

By definition, bread staling refers to all the changes that occur in bread after baking. Staling can be divided into crumb and crust staling. However, most of the studies have focused on crumb staling (D' Appolonia and Morad, 1981).

Staling is a major problem limiting the shelf life of many bakery products. Annual losses to the industry from stale bread have been estimated at between 3 to 5% of total production (Pirrie, 1940). The percentage of stale returns could even reach 6 to 9% (Jackel, 1989). Also, there is the loss to the consumer as bread becomes stale and unpalatable following purchase (Bechtel and Meisnier, 1951).

The consumer perceives staling of bread by changes in the aroma, toughening of the crust and, most importantly, firming of the crumb (Bechtel *et al.*, 1953). Based on market studies, the wholesale baking industry believes that consumers equate "squeeze" softness with freshness and make their choice at the supermarket bread rack accordingly (Jackel, 1989). Thus, the bakery industry attempts to produce the most "squeezable" bread (Jackel, 1989). Objective measurements of staling are complicated since "staleness of bread is a subjective quality which is ultimately assessed by the senses" (Toufeili *et al.*, 1994)

While starch retrogradation is believed to be the most important factor causing crumb firmness, the importance of other contributing factors, and the means of retarding staling, remains open to further research (D' Appolonia and Morad, 1981).

In the following sections, the staling mechanism as well as the methods commonly used to retard the staling process, will be reviewed in great details.

1.7 - Staling Mechanism:

Under optimal storage conditions, bread "stales" after 2-3 days on supermarket shelves (Jackel, 1989). Ponte (1971) reported that returns due to staling in the United States are ~8% accounting for almost 50 million kg. of product returns annually. To overcome this major spoilage problem, staling of bakery products has been the subject of extensive investigation.

Several definitions of staling have been proposed. Bechtel *et al.* (1953) defined staling as a " decreasing consumer acceptance of bakery products

caused by changes in the crumb and the crust other than those resulting from the action of spoilage microorganisms". According to Bechtel and Meisner (1951) consumers view staling as "hardening of the crumb which has a dry mouth feel, an increase in crumbliness, a loss of flavor and aroma, and a softening and toughening of the crust". Kulp (1979) stated that staling was "the gross changes and the various underlying reactions, as well as other physical or chemical phenomena which contribute to the subjective estimate known as staling".

Staling can be divided into crust staling and crumb staling. The majority of research has focused on crumb staling as crust staling seems impossible to prevent.

Crust staling is due to moisture migration from the crumb to the crust and from absorption of moisture from the atmosphere if the relative humidity (RH) is high i.e., RH>80% (Kulp, 1979). If the bread is left unpacked, it dries out completely. If packaged, the crust soon stales (Kulp, 1979). Crust staling is enhanced by high moisture barrier packaging materials which do not permit moisture to pass from the crumb to the atmosphere. Thus, it remains in the crust (Maga, 1975). The effect of the crust on staling was studied by Bechtel *et al.* (1953). They observed that crustless bread maintained its freshness while intact loaves staled rapidly. In intact bread there was moisture migration from crumb to crust throughout the four day test period. However, crumb without a crust maintained a constant moisture level. Therefore, it was concluded that the presence of a crust is the major cause of crumb staling (Bechtel and Meisner, 1951; Bechtel *et al.*, 1953). They concluded that staling may be due to loss of moisture by the crumb or to undesirable flavors or odors being absorbed by the crumb from the crust, or by a combination of both. Boutroux, in 1897, did some similar work, observed that, as the crust cooled, its vapor pressure (VP) decreased while the VP of the warm crumb was still relatively high. According to Baker and Mize (1939), there is also a difference in the flavor between bread baked with and without a crust. They reported that bread baked with a crust

resulted in flavor migration to the interior which promoted a stale flavor which wasn't observed in crustless bread (Maga, 1975).

Crumb staling is an even more complex phenomenon. The crumb becomes firmer, less elastic, crumblier, harsh textured, and it has a dry mouth feel (Kulp, 1979).

1.7.1. MECHANISM OF CRUMB STALING:

The mechanism of staling has been studied since the 19th century. In 1852, Boussingault stated that staling wasn't due to an overall moisture loss. Since then many theories have been postulated for the crumb staling mechanism, some of which are summarized in Table 1.8.

Table 1.8: Proposed theories for staling mechanism.

AUTHOR /YEAR	THEORY
Boussingault (1852)	Staling isn't due to moisture loss. Stale bread can be refreshed by heating at 60°C.
Von Bibra (1861)	Fresh bread: water free form. Stale bread: chemically bound. Refreshed: free again (min. of 30% moisture needed).
Horsford (1876)	Exchange of water from starch to gluten.
Boutroux (1897)	Staling forms a derivative of starch which is hard.
Lindet (1902)	Change in the form of starch during staling, "retrogradation". Moisture migration from starch to other components.
Katz (1913)	Physicochemical equilibrium between the fresh and stale state. Temperature dependent.
Ostwald (1915)	Staling is due to extrusion of water by syneresis as in any gel system
Katz (1928)	Starch changes in bread similar to those in gels. Starch is the main reason for bread staling
Platt (1930)	Confirmation of the effect of temperature on bread staling.
Katz (1930)	Starch is important but other components are involved in the staling process.

Alsberg (1936)	Staling is a physical change in the starch and gluten complex. Water is lost from both fractions.
Katz (1937)	Fresh bread: starch in an amorphous and crystalline form Stale bread: only crystalline form.
Fuller (1938)	Reduction in hydration capacity. Staling is due to a shift in the proportion of α and β amylose.
Schoch and French (1947)	Amylopectin aggregation, more than amylose, is responsible for staling.
Bechtel and Meisner (1954)	Staling occurs in 2 stages: Increase in rigidity of the starch gel and then loss of water from gluten.
Cluskey *et al.* (1959)	Starch gel gradually loses moisture sorption capacity, gluten gels have a constant moisture sorption capacity.
Cluskey *et al.* (1959)	Heating restores elasticity to the starch and flour gel but not to gluten gel.
Bechtel / Zobel and Senti (1959)	Addition of heat stable bacterial α amylase decreases staling rate.
Senti and Dimler (1960)	During starch crystallization, the interlinking amylose chain are responsible for bread firming.
Schoch (1965)	Staling is due to the physical changes in the amylopectin fraction within swollen starch granules.
Knyaginichev (1965)	During cooling, starch chains associate to form a firm network, a structured gel. Water and gluten are also involved.
Erlander and Erlander (1969)	The ratio of starch to gluten is important Ethanol has an anti-staling effect: its loss during baking can result in staling.
Willhoft (1971)	Refutes the concept of moisture migration Developed theoretical equations for staling Considered the effect of temperature.
Pyler (1973)	Moisture is transferred from gluten to starch Usually 2% loss is a minor change.
Ghiasi *et al.* (1984)	Starch recrystallization and bread firming occurred at different rates after day 3 of storage.
Martin *et al.* (1991)	Model proposed showing that staling results from cross-links (hydrogen bonds) between the continuous protein matrix and the discontinuous remnants of starch.

Adapted from Maga (1975).

To understand the complex mechanism of staling, the major factors influencing staling and firming will be discussed.

1.7.1.1. *Moisture:*

Moisture loss was considered to be a major factor in bread staling. However, Maga (1975) reported that the moisture content of both stale and fresh bread was similar. The problem of staling is not moisture loss but redistribution of moisture between the different bread constituents. The moisture content of the crust after baking is ~12%. After four days at 70ºF, this increased to ~28% while the overall moisture content remained constant (Kulp, 1979). Moisture migration from the crumb to the crust is one of the major reasons of crust staling (Bechtel and Meisner, 1951). Martin *et al.* (1991) proposed a special type of bread called ERO (Electric Resistance Oven) bread which has a higher moisture content in the crust (47%) compared to conventional bread. This resulted in more water being available to starch granules in the interior of the loaf permitting more starch swelling. The end result was a greater hydration capacity of bread baked in ERO compared to the conventional oven method of baking.

In the dough, water hydrates gluten, pentosans and damaged starch during dough preparation. During baking, starch absorbs moisture during starch gelatinization while gluten releases water. Upon cooling, the swollen starch molecules release water to the gluten phase (Kulp, 1979). However, since starch only loses ~2% of its water to the gluten, it is considered a minor change (Maga, 1975). Conversely, Cross *et al.* (1971) reported that during storage the gluten phase loses ~15% water to starch. They concluded that since the gluten phase is continuous in the bread crumb, moisture migration contributes to bread staling. These results were confirmed by Willhoft (1971a, b, 1977) and Kulp and Ponte (1981).

Although moisture loss is not the major reason of staling, a minimum moisture content (30%) should be present in bread to have adequate refreshening by heat (Willhoft, 1971a). Bread containing the maximum

permissible moisture content of 38% will give better results than those containing lower levels of moisture i.e., 35-36% (Kulp, 1979).

1.7.1.2. *Starch:*

Most of the studies to determine the causes of bread staling have agreed that changes in the starch component are of major significance. The major factor in these changes has been attributed to increasing starch crystallization (Zobel, 1973). Starch crystallization has been defined as "a physical process with molecules coming into a more ordered arrangement" (Cornford *et al.*, 1964). This term is synonymous with the term retrogradation to describe the changes occurring in the starch portion of the bread after baking (Maga, 1975).

The first change occurring in starch during baking is gelatinization. Starch granules absorb water and swell (Martin *et al.*, 1991). Gelatinization occurs in wheat starch between 52 and 62ºC (Kulp, 1979). Kulp (1979) cited Sandstedt *et al.* who examined crumb starch granules microscopically and reported that the granules retained some identity and were separated from each other by a continuous protein phase. Each individual granule was held together by an internal network of interlacing molecules which rendered them insoluble in cold water and resistant to the action of enzymes (Kulp, 1979). During gelatinization, a portion of the amylose is solubilized and appears in the intergranular space and the rest is partly leached and extends as hair-like projections from the surface of the starch granules remnants (Toufeili *et al.*, 1994). In bread, not all the water required for starch to swell is present. A portion of the water is associated with proteins, pentosans and sugars and has to be released by heat during baking and thus made available for the gelatinization process (Kulp, 1979). After baking, starch appears fibrous and seems to be linked to protein although no cross linking of starch and protein is apparent in the dough. Martin *et al.* (1991) reported that starch-gluten interactions occur during baking.

Crumb from the center, middle, and outside of bread slices show increasing degrees of starch gelatinization (Zobel, 1973). In bread staling, the extent of gelatinization is particularly important because of its role in determining the distribution of moisture in the baked loaf (Yasunaga *et al.*, 1968).

After gelatinization, starch undergoes crystallization. In fully baked bread, starch is partially swollen and surrounded by solubilized amylose, lipids, pentosans, and gluten protein which has given most of its water to starch. Starch is in an amorphous, highly disorganized and unstable state. Upon cooling, the starch gel forms a more orderly structure (Kulp, 1979). Many factors are involved in this complex system. However, the main changes are due to the degree and rate of recrystallization of amylose and amylopectin. Amylose, due to its linearity, associates rapidly shortly after cooling. However, the more branched amylopectin fraction crystallizes at a much slower rate. Schoch and French (1947) believed that amylopectin was primarily responsible for bread staling since the water soluble starch leached from the crumb of fresh bread at 30ºC was primarily amylopectin. They believed that the spontaneous aggregation of amylopectin, and not amylose, contributed to bread firming. These results were later confirmed by Prentice *et al.* (1954) and Kim and D' Appolonia (1977b).

However, Ghiasi *et al.* (1979), observed that it was a degraded amylopectin, due to the action of amylases during baking, which was responsible for bread staling.

Kim and D' Appolonia (1977a) reported a strong correlation between firming rate of bread and changes of the amylopectin content at different storage temperatures, as the amylose content remained constant at all temperatures. They concluded that differences in staling rate can be attributed to a variation in the amylopectin fraction of starch in the crumb.

Most amylose retrogradation takes place during baking and subsequent cooling of the loaf (Schoch and French, 1947; D' Appolonia and Morad, 1981). Amylose was first thought to be independent of the process of bread firming (Schoch and French, 1947). However, considerable information is available to

show that retrogradation of amylose is also involved in staling even though the amylose content in the soluble starch of fresh bread crumb is initially small (Kim and D' Appolonia, 1977a).

Starch retrogradation is heat-reversible. When stale bread is reheated, provided enough moisture is available, it becomes soft and the fresh bread flavor and aroma appears. This reaction is explained by reversion of the crystalline amylopectin to the amorphous state and the release of the flavor compounds from a complex with amylose. While the changes in the amylopectin occurs at 60ºC, the amylose remains in the crystalline state (Kulp, 1979). Ghiasi *et al.* (1982) showed that starch recrystallization and bread firming occurred at different rates after the third day of storage (Martin *et al.*, 1991). Rogers *et al.* (1988) showed that low moisture bread firmed at a very rapid rate, whereas starch recrystallization remained essentially unchanged. These observations led Martin *et al.* (1991) to consider the possibility that the two processes were not synonymous. Furthermore, they might not even be related even if both occur after baking and during bread storage. They proposed a **new model** (Figure 1.4) showing that bread firming results from cross-links (hydrogen bonds) between the continuous protein matrix and the discontinuous remnants of starch (Martin and Hoseney, 1991). The ability of mild heating to reverse the effects of aging on firmness led to the presumption that hydrogen bonding, rather than more thermo-dynamically stable covalent bonding, is involved in bread firming (Martin and Hoseney, 1991).

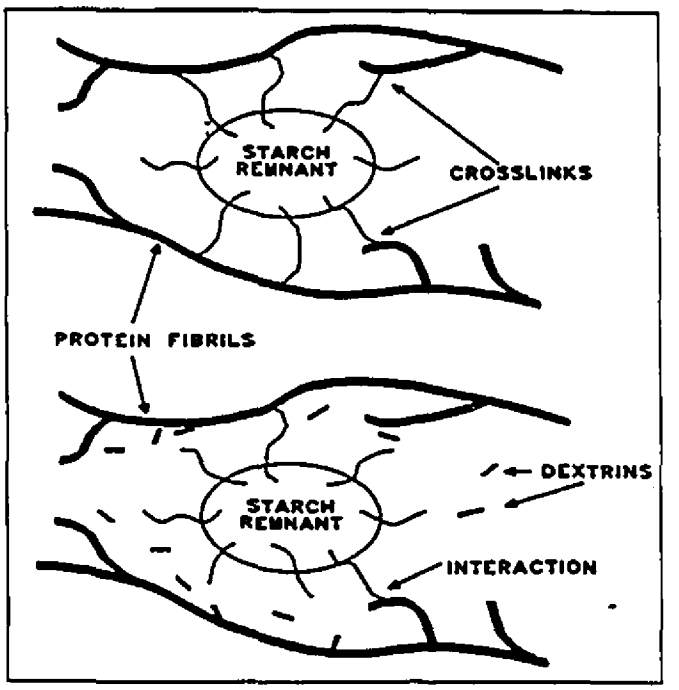


Figure 1.4: Model of a mechanism of bread firming (Martin and Hoseney, 1991).

1.7.1.3. *Protein:*

When flour proteins are hydrated and then stretched by mechanical action the gluten forms a viscoelastic dough. Upon heating, the gluten coagulates and forms a semi-rigid structure giving baked goods its cellular structure. In baked bread, gluten is in the continuous phase and remnants of starch granules are the discontinuous phase. As mentioned, cross-links between gluten and starch that contribute to bread firming are possibly hydrogen bonds (Martin and Hoseney, 1991).

The role of bread protein in bread firming has not been studied as extensively as starch retrogradation. However, Bechtel and Meisner (1954b), using a synthetic dough system of gluten and wheat starch, reported that changes in starch were responsible only for the staling during the first three days and thereafter gluten affected the staling properties of bread (Kim and D' Appolonia, 1977b).

Bread made from different flours has different gluten content and firm at different rates. Strong flour, with its high gluten content, is usually used in bread making. He and Hoseney (1991) demonstrated that bread from poor quality flour firm at a faster rate (Martin *et al.*, 1991). Kim and D' Appolonia (1977) also found that increasing the protein content increased the time required for bread to stale. They indicated that the effect of increased protein content was to dilute the starch which decreased the contribution of amylose towards staling. Maleki *et al.* (1980) also showed that protein quality contributed to staling. According to Martin *et al.* (1991) the effect of protein quality on bread firming may be explained in terms of interactions among swollen starch granules, partial solubilization of starch molecules and proteins.

1.7.1.4. *Pentosans:*

Pentosans are polysaccharides present in small amounts in wheat flour (2 to 3%). They are usually found in equal amounts as water soluble and water insoluble pentosans (D' Appolonia and Morad, 1981). The effect of pentosans on retarding starch retrogradation have been studied during the past decade. Pentosans seem to decrease the bread staling rate. The effect of the water-insoluble pentosans is more pronounced than the water-soluble portion. The water-soluble pentosans slowed down the rate of amylopectin retrogradation, while the water-insoluble portion affect both amylose and amylopectin retrogradation (Kim and D' Appolonia, 1977a). Pentosans reduce the amount of starch components available for crystallization, thus decreasing the bread staling rate (Kim and D' Appolonia, 1977c).

1.7.1.5. *Lipids:*

Bread varies in its fat content. The main function of lipids is to help entrap the gases during mixing and then allow a more uniform release of these gases during baking, On the other hand, lipids have been observed to have an effect in retarding bread firming which will be discussed in a later section.

1.7.2. METHODS OF MEASURING STALING:

1.7.2.1. *Objective measurement of staling:*

Various methods can be used objectively to measure staling. The main objective methods and the principles behind each method to measure staling are shown in Table 1.9.

1.7.2.2. *Subjective measurement of staling:*

A subjective assessment of staling is also very important. If bread is judged stale by the consumer, even if instrumental methods do not confirm the results, the consumer would not buy the product (Bechtel and Meisner, 1951a). Trained, or untrained taste panelists, evaluate staling by rating the flavor, odor, squeezability and appearance of the product. It has been reported that organoleptic tests can be standardized and are a valid predictor of staling (Kulp and Ponte, 1981).

Flavor changes are also significant during the staling process. Bread flavor disappears during storage but can be restored by reheating. It may be that bread flavor compounds form complexes with amylose which are released upon reheating (Kulp, 1979). Stale flavors also occur from absorption of volatiles from crust to crumb upon cooling and to evaporation of the flavor compounds from the bread surface. The stale flavor of bread is also due to oxidation of aldehydes to volatile organic acids (Kulp and Ponte, 1981).

1.7.3. FACTORS AFFECTING STALING:

Many factors affect staling. These are summarized in Table 1.10.

Table 1.9: Objective methods used to measure bread staling.

METHOD	PRINCIPLE
Photometric measurements **Glabou and Goldman (1938)**	The degree of opalescence is followed using a photoelectric cell. Gel becomes opaque with time.
Swelling power **Cathcart and Luber (1939)**	Crumb is crumbled through a sieve, suspended in water, centrifuged and sedimentation rate measured.
Compressibility **Platt and Powers (1940)**	Weight is applied on the bread piece for a specific time and the compression measured.
Farinograph **Bice and Geddes (1949)**	Crumb is mixed with distilled water in a Farinograph unit and recorded in Brabender units.
Soluble starch measurements **Bice and Geddes (1949)**	Precipitation of soluble starch with alcohol. Limited application.
Crumb / Crust moisture level **Bradley and Thompson (1950)**	Moisture content measured by air and vacuum drying.
Crumbliness determination **Bechtel and Meisner (1951)**	Percent of crumb passing through a sieve shaken at a constant rate for 15 minutes.
Sedimentation rate **Banasik and Harris (1953)**	Correlation between crumb sedimentation and age of bread.
Crumb hydration **Banasik and Harris (1953)**	Hydration calculated as gram of water per gram of dry matter.
Iodine absorption **Pelshenke and Hampel (1962)**	Iodine binds to starch in different amounts depending on the degree of polymerization. Intensity measured in a colorimeter.
Differential thermal analysis **Axford and Colwell (1967)**	Measure of starch retrogradation as related to the extend of the appearance of an endothermic peak.
Hand-squeeze test **Waldt (1968)**	Consumer evaluation of staleness. Results influenced by many factors. Important in side-by-side comparison.
Cell wall firmness measurements **Guy and Wren (1968)**	Firming effect of bread cell wall material measured by ultracentifugation prior to evaluation of firmness.
Measurement of stress strained curves **Conford *et al.* (1969)**	Measurements done by an Instron. Equation developed relating pressure to the deformation of bread i.e., staling.

Hydrostatic compression **Willhoft (1971)**	Bread in polyethylene bag submerged in water. Change in loaf volume recorded.
Gaseous compression **Willhoft (1971)**	Record the volume deformation calculated from the pressure drop between compressed air (2 atm) and then air.
Capacitance / conductance measurements **Kay and Willhoft (1972)**	Electrical measurements on crumb during storage.

Adapted from Maga (1975).

Table 1.10: Factors affecting staling.

FACTORS AFFECTING STALING	MAIN EFFECTS
Time of storage	Staling occurs during the first few days of storage.
Temperature of storage	Refrigeration temperatures enhance staling.
Flour protein	High protein flours yield bread with better keeping qualities.
Flour pentosans	Water-soluble pentosans are used as anti-staling agents.
Shortening	Decrease initial firmness and maintain it throughout storage.
Carbohydrates	Mono- and disaccharides were found to have an anti-staling effect.
Syrups	Due to their hygroscopic properties, could have an anti-staling effect.
Salt	Could have an anti-staling effect. Not common to modify salt levels to prevent staling
Eggs	Increase bread volume and give a finer, uniform structure, retard staling
Other ingredients	Milk solids, soy protein, yeast levels also showed anti-staling properties.
Mixing conditions	Under/over-mixing can increase the firming since it is related to the rate of moisture absorption.
Fermentation time	Under and over-fermentation have a firming effect.
Baking time	Baking time affects moisture content and rate of staling

Adapted from Maga (1975).

1.8 - Methods to Prevent Staling:

Several methods have been developed to retard staling. These include: the addition of shortenings, the use of mono- and diglycerides, surfactants, enzymes, gums, gluten free flour, storage under CO_2 atmosphere. Each of these preventive methods will be reviewed in this chapter.

1.8.1. SHORTENING:

Shortening can be defined as a "an edible fat used to shorten baked goods" (Merriam-Webster's Collegiate Dictionary, 1995).

Platt and Powers (1940) showed that shortening levels can influence the rate of bread firming. These results were confirmed by Carlin (1947). Edelmann *et al.* (1950) also demonstrated that lard had an important role in retarding staling. Maga (1975) cited Pelshenke and Hampel who noted that not all levels of shortening were effective and that high levels (such as 20%) resulted in bread as stale as the control bread. Elton (1969) confirmed previous observations that fat decreased initial firmness and maintained this decrease throughout storage. Maga (1975) stated that hard or semisolid fat acted better than liquid fats in retarding bread firming, and that lard was more effective then others due to the fact that during baking lard can be partially hydrolyzed into monoglycerides.

Since then, the use of shortening to delay staling has been the subject of extensive studies. Most researchers agree that shortening, fat, or a combination of vegetable oil and emulsifiers is an essential ingredient in bread making. In commercial bakeries these are added to facilitate dough handling and processing, to improve loaf volume and crumb grain, and to prolong shelf life (Pomeranz *et al.*, 1991). When shortening is used in the formulation an increase in loaf volume, an improvement in crumb grain and a retardation of crumb firming during storage was observed. Crumb color depends to a large extend on loaf volume and crumb grain (Pomeranz *et al.*, 1966). However, adding shortening had no significant effect on water absorption and mixing time.

Wheat flour lipids are important functional components in baking since shortening acts through these lipids (Rogers *et al.*, 1988). Shortening had no effect on firming rate of bread made with defatted flour (Rogers *et al.*, 1988). Defatting significantly reduced volume and softness of bread as well as impairing loaf volume and crumb grain of bread baked from the flour (Pomeranz *et al.*, 1991). This effect is related to the amount of polar lipids removed from the flour during defatting. However, the effects of shortening or of polar lipids on bread quality were independent of wheat class or variety. Many flours were tested and, in all cases, shortening resulted in improved products with poor flour quality being improved the most (Pomeranz *et al.*, 1966).

The influence of shortening on firming rate is concentration dependent (Rogers *et al.*, 1988). Usually 2- 3% shortening has shown to be effective in providing the highest volume and softness of bread (Rogers *et al.*, 1988; Pomeranz *et al.*, 1991). Higher levels had no additional improving effect (Pomeranz *et al.*, 1966). It appears that wheat flour protein governs bread-making properties. Lipids, however, seem to provide certain functional properties. Once those requirements are met, no additional benefits can be derived by adding more lipid (Pomeranz *et al.*, 1966). This lipid-protein interaction affects both the firming rate and the loaf volume (Rogers *et al.*, 1988). It has also been shown that 1 or 2% soy or corn oil produced bread with a volume comparable to that with 2% shortening (Pomeranz *et al.*, 1991).

Polar lipids have proven to be more effective than non-polar lipids. Even in defatted flours, it has been shown that the addition of non-polar lipids further damaged the quality of flour, while polar lipids increased loaf volume and improved texture. Moreover, glycolipids were more beneficial than phospholipids (Pomeranz *et al.*, 1991).

1.8.2. SURFACTANTS:

The baking industry uses many additives to produce soft bread (Kulp and Ponte, 1981). Some of the most widely used emulsifiers and surface active agents are summarized in Table 1.11.

Table 1.11: Examples of major surfactants used in the bakery industry.

PRODUCT	MAXIMUM LEVEL PERMITTED (%)	FUNCTION
Calcium stearoyl-2-lactylate	0.5	Dough strengthener (exc.) Crumb softener (good)
Sodium Stearoyl-2-lactylate	0.5	Dough strengthener (exc.) Crumb softener (v.good)
DATA esters	No limit	Dough strengthener (exc.) Crumb softener (fair)
Mono- and diglycerides	No limit	Dough strengthener (none) Crumb softener (exc.)
Succinylated monoglycerides	0.5	Dough strengthener (good) Crumb softener (good)
Polysorbate 60	0.5	Dough strengthener (fair) Crumb softener (good)
Ethoxylated monoglycerides	0.5	Dough strengthener(good) Crumb softener (poor)

%, flour weight basis. Adapted from Kulp and Ponte (1981)

Surfactants aid in the development of less tacky, more extensible doughs which process through machinery without tearing or sticking, or which result in baked products with finer crumb structure and improved volume and shape. Since 1988, ~103 million kg of surfactants have been used as foods additives, a level which is expected to increase by ~5% annually (Kamel, 1993).

Surfactants are generally used for the following reasons:

1. To promote crumb softness;
2. To strengthen dough for good handling properties;
3. To aid in water retention; and
4. To improve loaf volume.

In aqueous systems, amylose adopts a helical conformation with the hydrogen atoms oriented to the inner side of the helix. This result in a lipophylic region ideally suited for complex formation with a long chain fatty acid (Osman *et al.*, 1961). Thus, the crumb softening effect was attributed to the surface active properties of surfactants and their ability to form complexes with the amylose fraction of starch (Figure 1.5). The formation of these complexes with amylose affects the transfer of water between crystallizing starch and gluten which takes place during aging of bread. The softener complexes not only with amylose but also with some of the outer chains of amylopectin (Kulp, 1979). The substantially low complex forming capacity of amylopectin has been attributed to its limited capacity to form a helix. It has been reported that when more than 1% monoglycerides are added and the free amylose is bound, interaction with the amylopectin fraction then occurs (Hani, 1992).

Surfactants have also been shown to act in a similar manner as flour polar lipids. These are bound to glutenin by hydrophobic bonding between the hydrocarbon chain of the lipid and the lipophylic region of the protein, and to gliadin by hydrogen bonding or electrostatic bonds between the polar groups of the lipid and polar regions of the proteins. The binding of surfactants to gliadin and glutenin enhances the gas retention capacity of gluten and results in a larger loaf volume (Hani, 1992).

The emulsifying properties of surfactants result in a more uniform distribution of water throughout the dough and allow for the development of gluten structures with optimum mechanical properties. However, Pisesookbunterng and D' Appolonia (1983) observed that the adsorption of surfactants onto the starch surface, as well as the complex formation between starch and surfactant, prevented starch from absorbing water released from gluten during bread aging. Consequently, the water released from the gluten was available to migrate from the crumb to the crust of the bread promoting crust staling.

A controversy still exists as to whether surfactants affect initial crumb firmness (Zobel, 1973) or if, as noted by Ghiasi *et al.* (1982), they only slow the rate of staling during storage or both effects occurs (Valjakka *et al.*, 1994).

Sodium stearoyl-2-lactylate, calcium stearoyl-2-lactylate, lard, monoglycerides and tartaric acid ester of sucrose are the most often employed to delay staling (Zobel, 1973). However, Krog and Jensen (1970) reported that distilled monoglycerides had the best complexing ability among non-ionic surfactants and that sodium stearoyl-2-lactylate (SSL) and calcium stearoyl lactylate were best among the ionic ones.

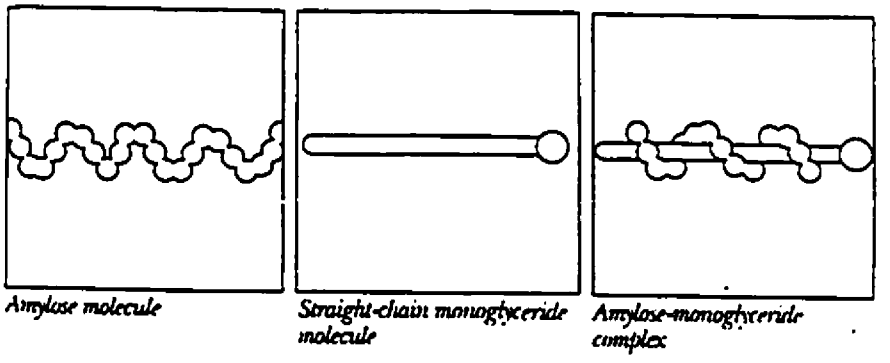


Figure 1.5: Amylose-monoglyceride complex (Hani, 1992).

1.8.3. ENZYMES:

Many enzymes can be added to dough to enhance its properties. Some of the enzymes commercially used in bread dough are listed in Table 1.12

Table 1.12: Major enzymes used in dough fermentation.

ENZYME	SOURCE	ACTS ON	PRODUCTS
α-amylase	Flour, Fungal preparation Malt Bacterial preparation	Starch	Soluble starch and dextrins
ß-amylase	Flour Malt	Dextrins	Maltose
Invertase	Yeast	Sucrose	Invert sugar
Maltase	Yeast	Maltose	Dextrose
Zymase	Yeast	Invert sugar Dextrose	Carbon dioxide Alcohol Flavors
Proteinase	Flour Fungal preparation Bacterial preparation	Gluten	Enables faster mixing and improved dough extensibility

Adapted from Baker's Digest (1986).

Other enzymes which could be used include lipoxygenases, pentosanases and others.

Since amylases and glucoamylases are most often used, their mode of action will be discussed in more detail.

Amylases are divided into α-amylases and β-amylases. These can be of different sources: bacterial, fungal or cereal. Different sources give enzymes with different properties.

Amylases are usually added to increase the level of fermentable sugars, to increase the production of simple sugars leading to a sweeter product and better color, since the reducing sugars produced react with other components in bread to give Maillard reaction products. They also improve gas and moisture retention properties of the dough. Furthermore, heat stable amylases retard bread staling.

Schultz *et al.* (1952) reported that small amounts of bacterial amylases had a beneficial softening effect in bread whereas high levels resulted in

unacceptable softness. They stated that the main advantage of bacterial α-amylase was its thermostability since its action occurs once starch has gelatinized. Miller *et al.* (1953) studied the effect of fungal, cereal and bacterial amylases and confirmed the results of Schultz *et al.* (1952) noting that all three types resulted in softer bread compared to the control bread. Bacterial amylases did not affect the initial bread firmness, but reduced the firming rate during storage. Conversely, fungal amylases, decreased the initial bread firming but did not affect the firming rate (Valjakka *et al.*, 1994).

α-amylases are the most widely used enzymes. Bacterial α-amylases, survive baking in contrast to cereal and fungal enzymes and are commercially used as anti-staling agents. However, excessive amounts can produce adverse effects during storage. Bread can turn gummy and lose desirable textural properties due to the thermostable property of the enzyme. New improved bacterial amylases with reduced thermostability have been introduced to prevent these problems occurring during storage.

Bacterial α-amylase cleaves linkages in the amorphous regions of starch where they are most accessible to enzyme attack. This gives the crystallites greater freedom to move and results in a decreased rigidity of the system (Valjakka *et al.*, 1994). The essential action of α-amylase is a random attack, with the enzyme having an equal affinity for all α-(1-4) glycosidic linkages except those near the two ends of the starch chain and at branch points. Once the enzyme complexes with the starch molecule and the initial cleavage has been made, the enzyme may remain with one fragment and produce one or more breaks before dissociating and moving to another substrate molecule (Martin and Hoseney, 1991). Prior to baking, they only digest the damaged starch (~5%). On the other hand, bacterial and fungal α-amylases produce small dextrins that interfere with hydrogen bonds formation in starch-protein interaction and, thus, retard bread firming (Valjakka *et al.*, 1994). Whether the α-amylases affects crust color is still a controversial issue.

β-amylase is an exoenzyme. It releases two joined glucose units (maltose) from starch. It attacks the penultimate α-(1-4) glycosidic bond from the non-reducing end of a starch molecule; α-(1-6) branch points block their action. β-amylase cleaves a starch molecule chain before dissociating (Martin and Hoseney, 1991). β-amylase is normally present in flour so that additional supplementation is not required. Still, the addition of α-amylase will enhance the action of β-amylases since it will produce small dextrins on which β-amylase can readily act.

Glucoamylase is an exoenzyme which works on the non-reducing end of a starch chain and releases glucose molecules in a step wise process. It is used in bread for glucose production since it results in a sweeter product compared to maltose produced by β-amylase.

Two other major groups of enzymes can also be used: non-starch polysaccharide degrading enzymes, and the lipid modifying enzymes.

The non-starch polysaccharide enzymes consist mainly of hemicellulases and pentosanases that have been shown to have some effect in retarding staling. The lipid modifying enzymes group include lipoxygenases, lipases and phospholipases. These have also been the subject of many studies and appear to have an effect on bread firming. The action of lipoxygenases, such as soy lipoxygenase, appear to be related with gluten development. It was proposed that the action of lipoxygenase involves modification of the hydrophobic areas of the gluten (Kulp and Ponte, 1981). It was assumed that the release of gluten bound lipids will provide additional free lipids for complexing with starch during baking leading to a softer bread.

1.8.4. GUMS:

Maga (1975) reported studies on gum carrageenan and gum karaya and reported that they could have an anti-staling effect. However, limited data was presented to confirm results. He also noted that some combinations of surfactants and gums were studied (Maga, 1975).

Christianson and Gardner (1974) studied the effect of xanthan gums in protein fortified starch bread. However, they found no effect on bread firming.

Mettler and Seibel (1993) worked with guar gum, carboxymethylcellulose, mono- and diglyceride and diacetyl tartaric ester of monoglycerides. Their results showed that gums had some effects in retarding the staling process.

The effect of gums on staling have not been investigated extensively. Further studies are required to determine if they play an important role as anti-staling agents.

1.8.5. COMBINATION TREATMENTS:

Most studies to date have examined the effect of softening agents individually. However, combination treatments with these agents could have a more pronounced effect on staling.

As mentioned previously, the combination of mono- and diglycerides did not appear to give favorable results. However, emulsifiers have been added with shortening to defatted flours to give better results. For example, 0.1% of ethoxylated monoglyceride (EMG) and 0.2% hydroxylated lecithin, alone or in combination with 2% shortening increased volume and improved softness of bread: each was superior to shortening alone. EMG primarily strengthened the dough and increased bread volume, and lecithin improved rheological properties of the dough and crumb grain texture (Pomeranz *et al.*, 1991).

The combination of an enzyme and an emulsifier resulted in a less firm bread than bread in which these additives were used separately (Pomeranz *et al.*, 1991). However, they did not have a synergistic interaction on bread firmness of white pan bread. Some reports indicated that enzymes alone have little effect on bread staling, and that emulsifiers alone increase bread softness. Others have reported that when bacterial α-amylase was added to the dough together with crumb softener emulsifiers, such as monoglycerides, firming rate was greatly reduced. However, Valjakka *et al.* (1994) did not find major interactions between enzymes and surfactants.

Martin and Hoseney (1991) reported that the amylose-lipid complex was shown to be an obstacle to starch hydrolysis with glucoamylase. The initial velocity of reducing end-groups released by the action of α-amylase was lower in the presence of 10% monoglycerides. Starch granules were less swollen in the presence of a monoglyceride, which may have decreased the rate of hydrolysis. However, their results showed that the monoglyceride did not function by affecting the thermal stability of the amylases. In a system with high levels of β-amylase, monoglycerides seemed to reduce β-amylase activity, while bacterial α-amylase overcame the effect of monoglycerides on β-amylase activity. Combination of many enzymes have been patented, such as debranching enzymes (acting on the α-(1-6) linkages) with α-amylases. Bacterial and fungal amylases in combination have a synergistic effect on softening. A glucoamylase-amylase preparation, able to digest native starch rapidly has been developed in Japan. It is an enzyme originating from *Aspergillus* K-27. It has 70% glucoamylase and 30% α-amylase activity and is able to hydrolyze native corn starch completely within 24 hours. Combination of raw starch digesting enzymes and amylases led to the conclusion that the degradation of raw starch granules is due mainly to the glucoamylase activity, while α-amylase exert a synergistic action (Valjakka *et al.*, 1994).

1.8.6. MODIFIED ATMOSPHERE PACKAGING (MAP):

MAP is a new packaging technique. Various methods can be used to modify the gas atmosphere surrounding a product including gas packaging, the use of oxygen absorbents or ethanol vapor generation.

MAP has been mostly used to increase the shelf life of many food products including bakery products where they were found to extend the mold free shelf life of products. However, MAP may also have some effect in delaying staling.

1.9 - Modified Atmosphere Packaging:

Under ambient storage conditions, baked products can develop visible mold and firming within 48 hours of baking and packaging. The main types of mold causing bread spoilage are *Monilia sitophilia* and members of the *Aspergillus*, *Rhizopus* and *Penicillium* families. Four methods are effective in retarding mold growth. These are modified atmosphere packaging (MAP), irradiation, preservatives and freezing (Black *et al.*, 1993). Only MAP will be discussed in this chapter.

Air is composed of ~78% nitrogen (N_2), 21% oxygen (O_2), and 1% carbon dioxide (CO_2). The principle of modified atmosphere packaging is that by changing the composition of the atmosphere around a food product, i.e., reducing the amount of O_2 and increasing the levels of CO_2, shelf life of the food is significantly increased (Doerry, 1985).

Smith and Simpson (1995) cited Young *et al.* who defined MAP as "the enclosure of food products in a high gas barrier film in which the gaseous environment has been changed or modified to slow respiration rates, reduce microbial growth and retard enzymatic spoilage with the intent of extending shelf life". 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 gas atmosphere surrounding bakery products. These include vacuum packaging (VP), gas packaging, the use of oxygen absorbents and ethanol vapor generators. Some of these methods of atmosphere modification will be discussed.

1.9.1. Vacuum Packaging (VP):

Vacuum packaging was the earliest form of MAP. In VP, products are placed in high gas barrier flexible bags or rigid thermoformed trays, the air is evacuated and the package sealed. Under good packaging conditions, oxygen in the head space could be as low as 1% or less. This low level of oxygen helps

extend the shelf life of some products by inhibiting oxidative rancidity and the growth of aerobic microorganisms (Smith and Simpson, 1995).

VP is not used for most bakery products since this process causes irreversible deformation of soft products (Parry, 1993). However, it is used to prevent rancidity problems in short bread (American Institute of Baking, Personal communication).

1.9.2. GAS PACKAGING:

Gas packaging consists of replacing the air with a gas or a mixture of gases within the package, which is usually an impermeable film. Gases commonly used in MAP are carbon dioxide, nitrogen and carbon monoxide. Other gases, such as chlorine, ethylene oxide, nitrogen oxide, ozone, propylene oxide and sulfur dioxide have been investigated but are not used commercially. The most commonly used gases are N_2 and CO_2 alone or in combination with each other. The reason for this is that they are neither toxic, nor dangerous and they are not considered as food additives (Smith and Simpson, 1995).

N_2 does not have a anti-microbial effect by itself since it is an inert gas. However, it is usually used as a filler gas to prevent the package collapsing in products that could absorb some CO_2 upon storage. It is also used to prevent rancidity problems in food of low a_w i.e., where microbial spoilage is not a problem.

CO_2 is the most important gas since it is both bacteriostatic, fungistatic and can prevent growth of insects in the package. However, it is highly soluble in water and fats, and forms carbonic acid, resulting in flavor changes when used in high concentrations. Moreover, the product can also absorb CO_2 causing the package to collapse.

The effect of CO_2 can be summarized as follows:

1. The exclusion of O_2 by replacement with CO_2 may contribute to the overall antimicrobial effect by slowing the growth of aerobic spoilage microorganisms,

2. The CO_2/HCO_3^- ion has an observed effect on the permeability of cell membranes,

3. CO_2 is able to produce a rapid acidification of the internal pH of the microbial cell with possible ramifications relating to metabolic activities,

4. CO_2 appears to exert an effect on certain enzyme systems (Smith and Simpson, 1995).

In bakery products, the mold free shelf life increases with increasing concentrations of CO_2 in the package headspace (Smith and Simpson, 1995). Extensive studies have shown that CO_2:N_2 (60:40) mixture is most suitable and that this concentration is an effective one to increase the chemical and microbial shelf life of bakery products.

However, problems, such as staling and discoloration still occurs in some products. Also, if food is eaten directly from an MAP pack, a bitter flavor of carbonic acid can noted. This usually appears in the product after four days of storage. The N_2 gas also produces a noticeable off-odor in bread within one day after baking, an odor which increases with time. The control (air atmosphere) produced a different "stale" odor after seven days at room temperature (Brody, 1989). However, these odors could be overcome by toasting products prior to consumption (Smith and Simpson, 1995).

1.9.3. OXYGEN ABSORBENTS:

Oxygen absorbents are composed of any substances, packaged in gas permeable materials in the form of small pouches, which react chemically with oxygen. Placed in sealed packed containers, they reduce the oxygen concentration to 100 parts per million or even lower and maintain this level, as long as the appropriate packaging film is used. Substances commonly used are iron powder and ascorbic acid (Smith and Simpson, 1995). The first oxygen absorbent was an iron powder based absorber developed by Mitsubishi Gas Chemical Company, under the trade name of Ageless in 1977. In 1989, almost

7000 million sachets were sold in Japan with sales of absorbents growing at a rate of 20% per year (Smith and Simpson, 1995).

The absorbing reaction is the following:

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

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

$Fe_2^{+} + 2OH^{-} \rightarrow Fe(OH)_2$

$Fe(OH)_2 + 1/4\ H_2O \rightarrow Fe(OH)_3$ (Brody, 1989).

Other types of absorbents are now available on the market. These are Freshilizer and Freshpax absorbents all of which act in a similar manner to Ageless (Smith and Simpson, 1995).

Oxygen absorbers should meet specific criteria. These are:

1. The ingredients should not be toxic,
2. They should absorb oxygen at an appropriate rate,
3. There should not be any unfavorable side reactions,
4. They should be of uniform quality,
5. They must be compact and uniform in size (Brody, 1989).

Many factors influence the choice of oxygen absorbents such as:

1. The nature of the food, i.e., size, shape, weight,
2. The a_w of the food,
3. The amount of dissolved oxygen in the food,
4. The desired shelf life of the product,
5. The initial level of oxygen in the package headspace,
6. The oxygen permeability of the packaging material (Smith and Simpson, 1995).

In Japan, oxygen absorbents are used extensively to prevent discoloration problems in pigmented products, and mold spoilage, especially in intermediate moisture and high moisture bakery products. Studies have shown that oxygen

absorbents to be three times more effective than gas packaging for increasing the mold free shelf life of some bakery products. Five to forty-five days for white bread at room temperature. fourteen days at 30°C for pizza crusts. In the United States, oxygen absorbents technology is still in its infancy.

Using oxygen absorbent technology, the shelf life of white pan bread could be increased 5 days to 45 days at room temperature while pizza crust had a mold free shelf life of 14 days at 30°C.

The main problems with oxygen absorbents are consumer resistance to their use in food. Two main consumer concerns are the fear of ingesting the absorbent and the spillage of sachet contents into the food thus adulterating the product (Smith and Simpson, 1995).

Table 1.13. summarizes the advantages and disadvantages of oxygen absorbents.

Table 1.13: Advantages and disadvantages of oxygen absorbents.

ADVANTAGES	DISADVANTAGES
Inexpensive and easy to use	Need to have a free flow of air surrounding the sachet to scavenge headspace oxygen
Non-toxic and fast to use	Consumer resistance or misuse of the sachet
Prevent aerobic microbial growth	
Arrest the development of rancid off flavors of fats and oils	
Product quality without additives	
Increased product shelf life and distribution	
Reduces distribution losses	

Adapted from Smith (1994).

1.10 - MAP and Bread Staling:

Most of the studies to date with MAP have focused on extension of the mold free shelf life of products. However, studies on the anti-staling effect of enriched CO_2 atmospheres produced conflicting results.

Doerry (1985) observed that the crumb of bread became firmer irrespective of the storage atmosphere i.e., storage in air, 100% CO_2 or 100% N_2. Brody (1989) reported that the staling rate of white and whole wheat bread was not significantly reduced when packaged in carbon dioxide or nitrogen as compared to air. Black *et al.* (1993) also reported no clear pattern of firming over time between packaging treatments for pita bread packaged under various atmospheres.

However, Knorr *et al.* (1985) showed that the compressibility of bread packed under CO_2 was lower than bread packed in air, suggesting that carbon dioxide delayed bread firming. Knorr (1987) reported that carbon dioxide significantly decreased compressibility of some baked goods compared to air-stored samples and that softer products were obtained when stored under 100% CO_2. While the initial compressibility of air and CO_2-stored bread was identical bread stored in CO_2 for 72 hours was significantly softer than the air-stored products (Knorr, 1987). Observed differences between water activity of the CO_2 stored samples and air-stored samples after 96 hours of storage suggests that CO_2 atmospheres may affect the water binding in bread (Knorr, 1987).

Avital *et al.* (1990) reported that CO_2 delayed bread staling. They proposed that changes in the sorption properties of MAP baked goods were responsible for this effect. Since amylose is in the crystalline state after one day, amylopectin is the main component with available water binding sites. CO_2 appears to block some of these sites, thus causing a reduction in hydrogen bonding between the amylopectin branches resulting in a reduced water sorption capacity. Since hydrogen bonding has been shown to result in bread staling, blockage of water binding regions may explain bread firming. The effect of CO_2 was found to exist when water was in "the solute state". The solubility of CO_2 in

water is 35 times higher than O_2. Thus, it is possible that when water is in the solute stage, CO_2 dissolved easily and bound strongly to amylopectin thus preventing hydrogen bonding.

Smith (1994, unpublished results) also reported that the staling rate of white and whole wheat bread and biscuits was significantly reduced when packaged in 100% CO_2 compared to packaging in 100% N_2 or air.

1.11. Research objectives:

Several methods have been investigated to decrease crumb hardness and staling. These include the use of shortening, surfactants or enzymes at different levels. On the other hand, the use of gums and packaging such as modified atmosphere packaging (MAP) involving carbon dioxide modified atmospheres have rarely been investigated. Furthermore, to date most research has been conducted on a one variable at a time basis. However, more meaningful data may be obtained through combination treatments of individual variables.

The combined use of several methods can be explained by the "hurdle concept", proposed by Leistner and Rodel in 1976. They stated that several hurdles (barriers) or "inhibitory factors", even if one of them individually can not have an important effect, could reduce or inhibit a process if the hurdles are incorporated into a substrate in sufficient number and height. With respect to staling, this implies the use of two, three, or more of the pre-existing treatments could be used to delay staling and to extend shelf life with respect to eating quality of baked products (Smith, 1994).

While many bakery products could be used in reformulation studies to delay staling, there has been a tremendous increase in the consumption of

bagels over the past twenty years. In 1993, the per capita consumption of bagels was 1.2kg, a 169% increase in consumption since 1984, making it the fastest growing bakery product in North America. In 1994, 59% of retail bakers, 79% of in-store bakers and 50% of food service bakers sold bagels on a daily basis (American Institute of Baking, Personal Communication).

Therefore, on the basis of the above comments, the specific objectives of this research are:

1. To determine the effect of formulation changes on the textural and sensorial quality of bagels, bagels were chosen since they are the fastest growing bakery product in North America;

2. To determine the optimum levels of selected ingredients to give a product with a textural shelf life of ~6 weeks at ambient temperature;

3. To determine the effect of ingredient combination through an optimization process termed Response Surface Methodology (RSM);

4. To monitor changes in staling at different storage temperatures and proofing times;

5. To study the effect of carbon dioxide on bagel staling.

CHAPTER 2: PRELIMINARY STUDIES

2.1. Introduction:

Staling begins immediately after the baking process is complete (Hebeda *et al.*, 1991). It leads to an increase in crumb firmness, a loss of product freshness and of consumer acceptance. However, the process can be delayed through appropriate formulation changes using surfactants, gums, enzymes, high fructose corn syrup and flours of different gluten content, either alone or in conjunction with each others. The objectives of the initial study were to reformulate bagels with the above mentioned ingredients and to monitor their effect on the textural and sensorial qualities of bagels over a 6 weeks period at ambient storage temperature (25°C).

2.2. Materials and Methods:

2.2.1. INGREDIENTS:

The ingredients used in the bagel recipe consisted of wheat flour, malt flour, sugar, eggs, yeast, oil, water, honey and sesame seeds. The ingredients and their suppliers are shown in Table 2.1.

Table 2.1: Ingredients for a standard bagel recipe.

Basic Ingredients	Supplier's Name
High Protein Flour (12.8% protein)	Rudolf Sales Inc., Montreal, QC
Malt Flour	Rudolf Sales Inc., Montreal, QC
Sugar (Lantinac granulated sugar)	Local Supermarket
Eggs	Local Supermarket
Baker's Yeast (Lallemend)	Rudolf Sales Inc., Montreal, QC
Oil (Crisco vegetable oil)	Local Supermarket
Honey (Doyon pure honey)	Local Supermarket
Sesame Seeds	Rudolf Sales Inc., Montreal, QC

2.2.2. BAGEL FORMULATION:

A standard bagel recipe, obtained from the R.E.A.L. Bagel Co., Montreal, was used to formulate bagels throughout this study. Ingredients, based on a percentage flour weight basis, are shown in Table 2.2. This formula produced 24 bagels per batch.

Table 2.2: Standard bagel recipe.

Ingredients	Percentage[1]	Weight (g)
Flour	100	1160
Water	51.7	600
Sugar	7	82
Egg (1)	4.8	~56
Malt	3.8	44
Oil	1.7	20
Yeast	0.8	10

[1] The percentage was on a flour weight basis.

2.2.3. STANDARD BAGEL PREPARATION:

All ingredients were added to a Hobart mixer (D-300, Hobart Canada Inc., Don Mills, Ontario) and mixed at a high speed, for 10 mins until the dough was formed and then at low speed for 5 mins until the dough was properly developed i.e., indicated by dough temperature (30°C) and by the feel of the dough. The dough was then removed from the mixer, kneaded, and proofed at room temperature for 10 mins. After proofing, the dough was cut into 75g pieces and shaped manually into a bagel form. The bagels were then proofed for an additional 5 mins prior to being boiled in a kettle filled with boiling water containing honey (4 tablespoons in 10L water) until they floated to the surface. Bagels were then removed from the kettle using a wire sieve and drained of excess water. The bagels were coated with sesame seeds on both sides, placed on wire racks and baked for 18 mins (9 mins on each side) in a convection oven at 400°F (Garland Convection Oven (T)TE-3,4-CH, Commercial Ranges Ltd., Mississauga, Ontario).

After baking, bagels were cooled to room temperature and packaged (2 per bag) in Cryovac barrier bags (size 210x210 mm, Cryovac, Mississauga, Ontario, Canada). An Ageless type FX-100 oxygen absorbent (Mitsubishi Gas Chemical Co., Tokyo, Japan) was added to each bag to prevent mold growth during storage. All packaged bagels were stored at 25° C for 6 weeks, and monitored for textural and sensorial qualities at regular intervals (days 0, 3, 7, 14, 28 and 42). A flow process of bagel preparation is shown in Figure 2.1.

2.2.4. REFORMULATION:

Bagels were reformulated using appropriate levels of enzymes, gums, high fructose corn syrups and surfactants. Reformulation was achieved using the standard recipe and baking procedure outlined in section 2.2.3. All ingredients were used at levels suggested in their commercial literature. The ingredients, and their levels of use, in the reformulated product are shown in Table 2.3.

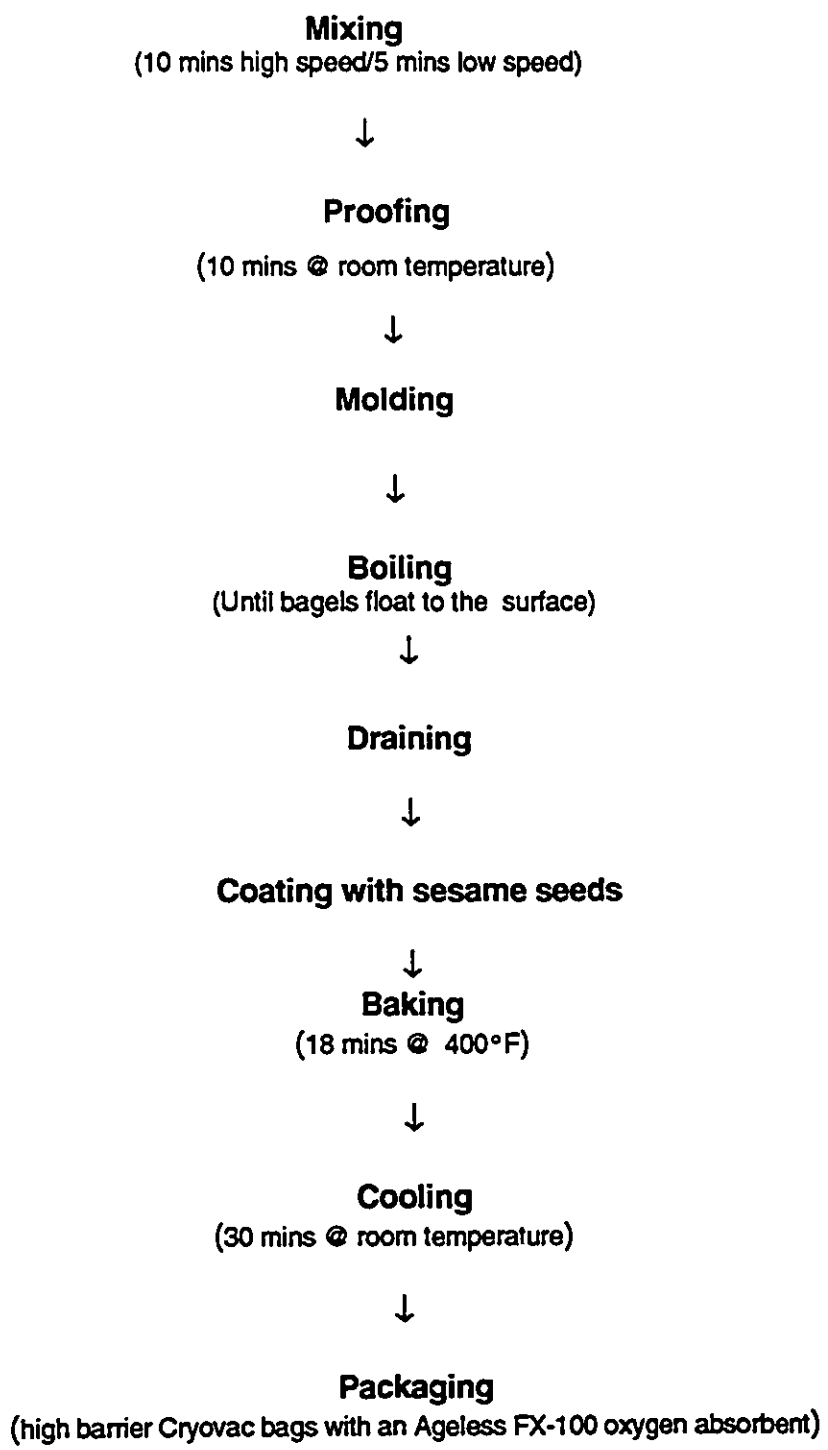


Figure 2.1: Bagel preparation.

Table 2.3.: Ingredients used in the reformulation and their percentages.

Ingredients	Trade Name	Supplier	Percentages used [1]
Enzymes:			
Genetically modified maltogenic α-amylase	Novamyl	Novo Nordisk (Danbury, CT)	0.031, 0.047
Fungal and bacterial α-amylases	Superfresh plus	Enzyme Biosystems (Beloit, WI)	0.1, 0.15, 0.2
Bacterial α-amylase and glucotransferase	Megafresh plus	Enzyme Biosystems (Beloit, WI)	0.1, 0.15, 0.2
Gums:			
Guar	Guar SG25	Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Xanthan	Xanthan 100	Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Locust bean	LBG SG14	Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Agar	Agar Agar	Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Cellulose	Cellulose 40	Soca Floc (Chicago, IL)	1
	Cellulose 300	Soca Floc (Chicago, IL)	1
	Cellulose 900	Soca Floc (Chicago, IL)	1
Methylcellulose	Methocel	Dow Ingredients (Midland, MI)	1
Algin	Kelvis	Kelco (Chicago, IL)	0.2, 0.6, 1
Pectin	Classic AB201	Herbstreith & Fox/ Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Syrups[2]*:*			
HFCS Liquid	HFCS Liquid	ADM Corn Processing (Decatur, AL)	50, 100
HFCS Granular	HFCS Granular	ADM Corn Processing (Decatur, AL)	50, 100
Flour:			
Rice	Instant Rice Flour	IGT (Lincoln, NE)	25
Barley	Instant Barley Flour	IGT (Lincoln, NE)	25
Corn	Instant Corn Flour	IGT (Lincoln, NE)	50
Surfactants:			
Sodium Stearoyl Lactylate	Atlas SSL	ICI Surfactants (Lachine, QC)	0.25, 0.375
SSL and amylase	Atlas p51	ICI Surfactants (Lachine, QC)	0.25, 0.375

1: Based on a flour weight basis.
2: Based on a sugar replacement basis.

2.2.5. TESTING:

2.2.5.1. *Textural Analysis:*

Textural analysis was done using an Instron Testing Machine (Instron Canada, Burlington, Ontario), on control and reformulated bagels. A compressibility test was done on a bagel cross section of approximately 15 mm thickness and 30 mm diameter. The crosshead speed was 25 mm/min. Two replicates were done on each of 4 bagels i.e. 8 measurements per formulation per test day. Textural shelf life was deemed unacceptable when a compression test of 0.01 MPa was reached. This level was determined based upon previous experiments done in our laboratory.

2.2.5.2. *Sensory Analysis:*

Sensory analysis, for odor, flavor, texture and overall acceptability, was done throughout the 6 weeks storage period using 5-10 untrained panelists. Bagels were ranked the bagels on days 3, 7, 14, 28 and 42 using a hedonic scale of 1 to 5 where 1= dislike extremely and 5= like extremely. A product was considered unacceptable, for each parameter when an average score of 3 was reached. The samples were numbered randomly with 3 numbers to prevent panelists from being biased. The test were conducted in a sensory evaluation room with separated desks, proper lighting and noise was reduced to a minimum.

2.2.5.2. *Statistical Analysis:*

Statistical analysis (regression coefficients analysis of variance and correlation coefficients) were computed using the Statistical Analysis System (SAS) (1991).

2.3. Results and Discussion:

The textural and sensorial changes in non-reformulated (control) bagels are shown in Figure 2.2 and Table 2.4. All bagels had an initial compression test measurement of ~ 0.008 MPa at day 0. This value increased steadily throughout storage to ~0.015 -0.016 MPa as a result of crumb hardening i.e. staling. Based on these results, bagels were deemed stale when a compression test of 0.01 MPa was reached and this was used as the "staling standard" for all reformulated products.

However, staling does not just involve moisture migration and crumb hardening but also a loss of flavor components. It is evident that all control bagels had an unacceptable odor, flavor, texture and overall desirability scores (<3) after 3 days.

Therefore, while a 6 weeks mold free shelf life is possible using oxygen absorbent technology staling is still a major problem limiting the shelf life of bagels. This problem can be addressed through reformulation with enzymes, gums, high fructose corn syrups, flours of varying protein content and surfactants.

2.3.1. ENZYMES:

The effect of various levels of three enzymes, Novamyl, Superfresh and Megafresh on the textural and sensorial quality of bagels are shown in Figures 2.3 to 2.5 and Tables 2.5 to 2.7 respectively.

Novamyl is a genetically modified maltogenic α-amylase produced by a genetically modified strain of *Bacillus subtilis* (host) which has received the gene for maltogenic amylase from a strain of *Bacillus stearothermophilus* (donor) (Novo Nordisk Technical Information).

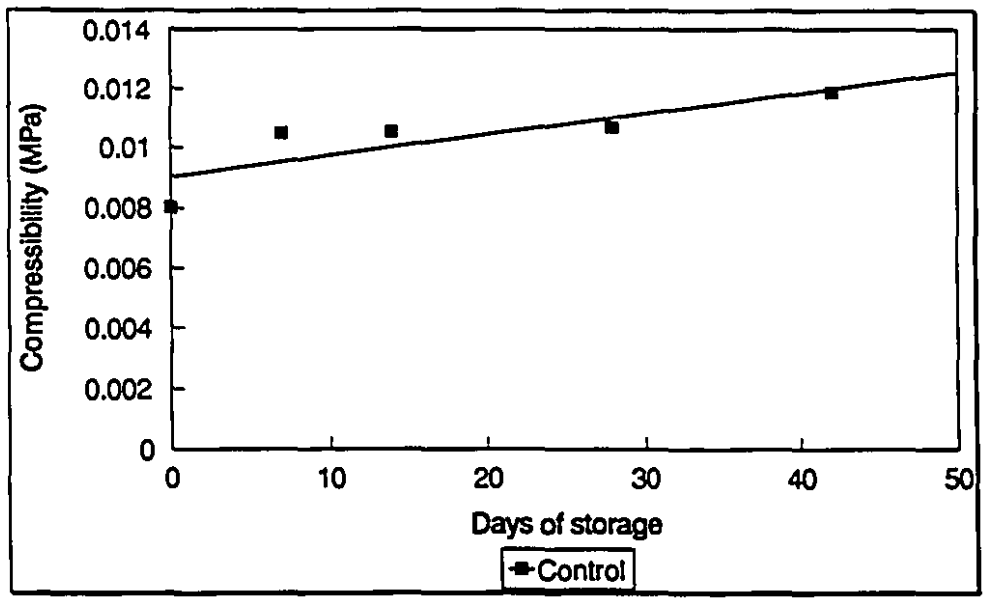


Figure 2.2: Compressibility results for control bagels.

Table 2.4: Sensory results for control bagels.

Control	Sensory Analysis															
	Odor				Flavor				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
Control	2.2	2.2	1.8	1.6	2.8	2.6	2.4	2.2	2.4	2.2	2.2	2	2.2	1.8	1.8	1.4
+/-	0.4	0.8	0.4	0.5	0.4	0.5	0.5	0.4	0.5	0.4	0.4	0.7	1	0.4	0.8	0.5

Average of 5 replicates followed (below) by its Standard Deviation.

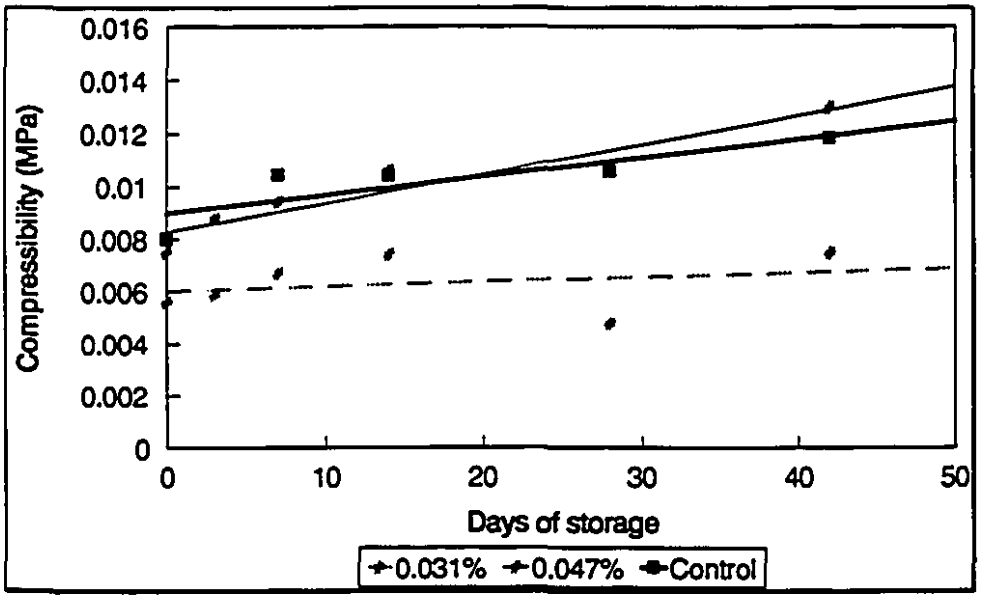


Figure 2.3: Compressibility results for Novamyl enzyme.

Table 2.5: Sensory results for Novamyl enzyme.

Novamyl	Sensory Analysis															
	Odor				Flavor				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.031%	3.6	3.7	3.8	3.5	3.8	3.8	3.8	2.2	3.8	3.7	3.8	2.7	3.8	3.7	3.7	2.8
+/-	0.9	1.2	0.7	0.8	1	0	0.7	0.9	1	0.5	0.7	1.5	0.9	0.5	0.8	1.1
0.047%	3.6	3.4	3.7	3.4	3.2	3.2	3.2	3.5	3.1	2.9	2.5	2.5	3.4	3.6	3.7	3.3
+/-	0.8	0.8	1	1	1	0.7	1.1	0.8	1	1	0.8	1.2	1	0.5	1	0.8

Average of 5 replicates followed (below) by its Standard Deviation.

When used at a level of 0.031% (flour weight basis) it had a pronounced effect on the textural shelf life of bagels (Figure 2.3). At the end of the 6 week storage period, bagel texture had changed very little (from 0.006 MPa to 0.007 MPa) over this time period. This was well below the textural standard of 0.01 MPa used as an indicator for staling. However, higher levels (0.047%) did not result in an improved textural shelf life. Indeed, product was regarded as stale after 14 days as indicated by a compressibility test of 0.01 MPa (Figure 2.3). The results for the sensory scores of bagels reformulated with Novamyl are shown in Table 2.5. Based on a "cut-off" acceptability score of 3, it is evident that bagels reformulated with 0.031% Novamyl, had a sensory shelf life of 28 days. Thus, while objective measurements resulted in a shelf life of >42 days, product had a stale flavor and odor after 28 days and was considered "stale" by panelists.

The compressibility results were highly significant with a p-value of <0.0005 (normally a p-value of <0.05 is considered significant). However, the sensory results were not significant and this is mainly due to the nature of the sensory analysis and the difficulty of the judging task. The p-value measures the relation between the variables and the outcome. When the p-value is < 0.05, the results are considered statistically significant, i.e., indicating that the results are not due to chance, but there is a real relation between the days of storage, the level used and compressibility outcome. Furthermore, as expected less than 25% correlation was observed between compressibility and the sensory results, showing once more that even if texture is an important cause of sample rejection, flavor and odor still influence panelist's perception of freshness.

Similar trends were observed for bagels reformulated with Superfresh and Megafresh enzymes.

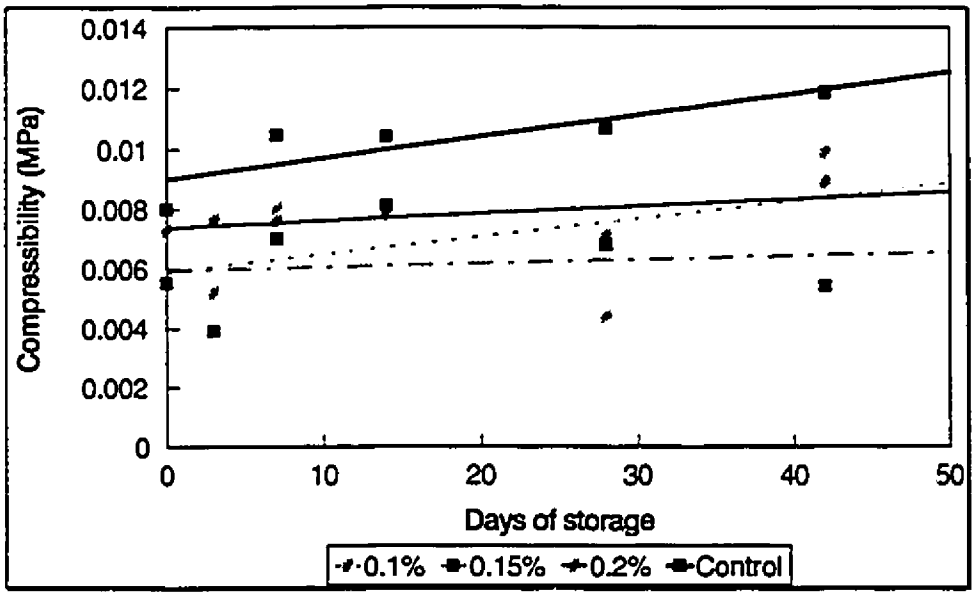


Figure 2.4: Compressibility results for Superfresh enzyme.

Table 2.6: Sensory results for Superfresh enzyme.

Superfresh	Sensory Analysis															
	Odor				Flavor				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.1%	3.9	3.7	3.6	3.4	3.8	3.7	3	2.6	3.4	3	3.2	2.6	3.9	3.5	3.2	2.8
+/-	0.6	0.4	1.1	0.5	0.5	0.4	1.5	0.8	1	0.8	1.3	1.1	0.6	0.5	1.3	0.8
0.15%	3.2	3	3.2	3.6	4	2.6	2.6	2.8	4	3.2	3.2	3.8	3.9	3.2	3.6	3.4
+/-	0.4	0.7	0.8	0.8	0.3	0.8	1.1	1.5	0.6	0.8	1	1.3	0.5	0.4	1.1	1.3
0.2%	3.6	3.6	3.6	3.4	3.7	3.6	3.6	3.2	3.4	3.2	3	3.2	3.6	3.4	3.2	3.4
+/-	0.9	1.1	1.1	0.5	1	1.1	1.1	1.3	1.1	0.8	1	1	1	1.1	0.8	0.5

Average of 5 replicates followed (below) by its Standard Deviation.

Superfresh is a mixture of fungal and bacterial amylases which act by hydrolyzing the α-1,4 glycosidic linkages of starch and by hydrolyzing maltose units into simple sugars. Its effect on the textural and sensorial shelf life of bagels at levels ranging from 0.1 to 0.2% (flour weight basis) are shown in Figure 2.4 and Table 2.6. At lower levels of use (0.1%) firmness was fairly constant over the storage period. At higher usage levels (0.15-0.2%), firmness measurements increased slightly from an initial level of 0.006 MPa but were well below the "staling standard" of 0.01 MPa after 42 days. Sensory results showed that optimum results could be achieved with 0.15 or 0.2% Superfresh in the formulation (Table 2.6), i.e. , a textural and sensorial shelf life of 6 weeks was possible using this level of enzyme in the reformulated product.

Statistically, Superfresh followed the same trend as Novamyl i.e., the compressibility results were highly significant with a p-value < 0.0005, while the sensory results were not significant. The correlation between the compressibility and the sensory was also less than 25%.

The effect of Megafresh, a bacterial α-amylase and glucotransferase enzyme system on staling are shown in Figure 2.5 and Table 2.7. At the lower level of use (0.1%) bagels had a compressibility measurements of ~0.006 MPa after 42 days.

At the 0.15% level, results were similar to those obtained with 0.15% Superfresh and 0.031% Novamyl i.e., products became slightly firmer throughout the 42 days storage period. At the higher level of use (0.2%) bagels reformulated with Megafresh reach their maximum firmness after 35 days (Figure 2.5). Sensory analysis showed that optimum results were obtained using 0.15% Megafresh i.e., a textural and sensorial shelf life of 6 weeks was possible using this level of enzyme in the reformulated product (Table 2.7).

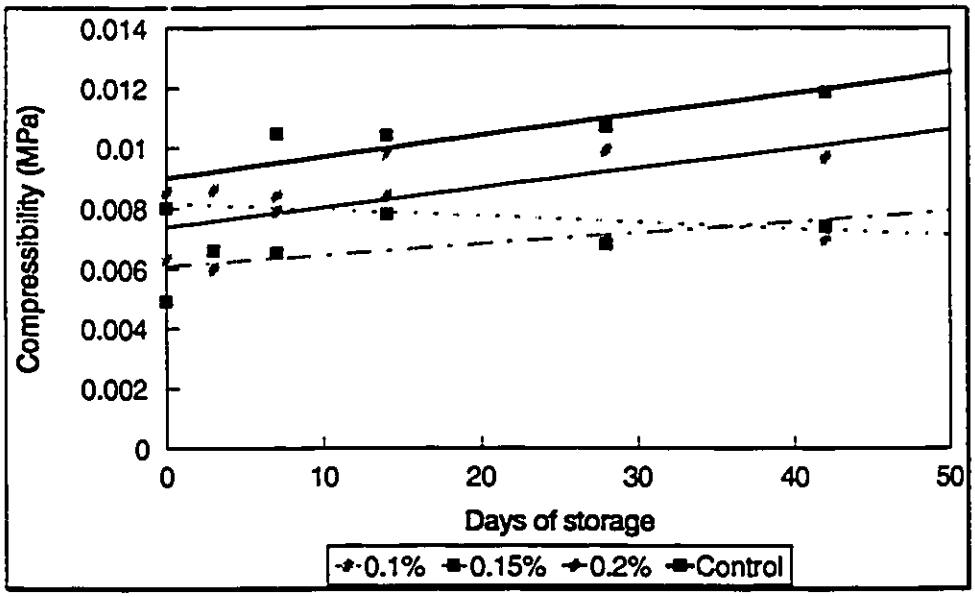


Figure 2.5: Compressibility results for Megafresh enzyme.

Table 2.7: Sensory results for Megafresh enzyme.

Megafresh	Sensory Analysis															
	Odor				Flavor				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.1%	3.6	3.6	3.6	--[1]	3.3	3	3	---	3.5	3.4	3.2	---	3.4	3	3	---
+/-	1	0.5	0.8	---	1.4	1	0.7	---	1.1	0.5	1	---	1.3	0.7	0.7	---
0.15%	3.7	3.8	3.8	3.6	3.9	3.4	3.4	3	4	3.4	3.4	3.4	3.8	3.4	3.4	3.4
+/-	0.6	0.8	0.8	0.5	0.8	1.1	0.5	0.7	1.1	0.5	0.5	0.8	0.7	0.5	0.5	0.5
0.2%	3.4	3.4	3.4	---	2.8	2.8	2.8	---	2.8	2.8	2.8	---	3	3	2.8	---
+/-	0.7	0.5	1.1	---	0.7	0.8	1.4	---	0.9	0.8	1.3	---	0.8	0.7	0.8	---

1. The sensory was interrupted due to mold growth.
Average of 5 replicates followed (below) by its Standard Deviation.

Compressibility results were again highly significant with a p-value < 0.0005, however the sensory results were also significant with a p-value <0.005. However, here again, statistically, no correlation was found between the compressibility and sensory results.

Thus, preliminary studies indicate that enzymes have a beneficial effect on crumb firmness, i.e., they delay the staling process. This can be attributed to the ability of these enzymes to "cut" the amylase and amylopectin branches of starch resulting in smaller branches which prevents starch-protein interaction. They also create low molecular weight sugars and dextrins improving the water retention capacity of the baked good. Furthermore, the three types of enzymes did not result in "stickiness" or "gumminess" in the end product.

The reduction in bread firmness due to enzymatic action has been discussed by Dragsdorf and Varriano-Marston (1980). They showed that bread supplemented with bacterial amylases to be the softest during storage. They noted that bread supplemented with barley, malt or fungal enzyme showed the same initial softness as the fresh product. Furthermore, they observed an order of decreasing degree of starch crystallinity from bacterial α-amylase, cereal α-amylase, fungal α-amylase and unsupplemented bread, postulating that the degree of crystallinity paralleled the heat stability of the enzyme, which produce lower molecular weight starch units. These will have more freedom of movement and can more easily arrange themselves into lattice position. Thus, they indicated that starch crystallinity and bread firming were not synonymous.

Martin and Hoseney (1991b) also observed that bacterial α-amylase and ß-amylase inhibited bread from firming during five days of storage. Bread supplemented with amylases contained great quantities of dextrins which appear to have an anti-firming effect.

Valjakka *et al.* (1994) showed that bacterial amylases reduced the firming rate of bread and that the rate of firming increased with increasing

concentration of enzyme confirming our observations. They noted that excessive amount of the enzymes could lead to keyholing (weakness of loaf side walls). However, this defect was not observed for bagels.

Finally, Akers and Hoseney (1994) recently reported the positive effect of enzymes on bread staling. They again concluded that the dextrins produced by amylases are important in controlling the rate of bread firming.

2.3.2. GUMS:

A variety of gums can be used to increase the keeping quality of bakery products. When incorporated into a baked good formulation, gums have the ability to bind water into a gel to reduce water migration and to control rheological properties resulting in an extended shelf life. This extension of freshness can be attributed to the ability of gums to immobilize and bind water as well as interfere with hydrogen bonding between starch and protein i.e., the "bound" water exerts a plasticizing effect.

Examples of gums include guar, xanthan, locust bean gum, agar gum, cellulose, methylcellulose, alginates and pectins.

As with other ingredients, they vary in their chemical structure and in their ability to bind water and to maintain freshness in a product.

Guar gum is a polysaccharide with a straight chain of D-mannopyranose units joined by β (1,4) linkages with a side branching unit of a single D-galactopyranose unit joined to every other mannose unit by a (1,6) linkages. It has a high hydration and water binding capacities, and forms a viscous colloidal solutions when hydrated in cold water systems.

Xanthan gum is a high molecular weight polysaccharide produced by the action of micro-organism on dextrose. It is very heat stable, it has a high moisture binding capacity and it contributes to the elasticity of the dough and shelf life extension of baked products.

Locust bean gum is a polysaccharide with a straight chain of D-mannopyranose units joined by ß (1,4) linkages with a side branching unit joined to every fourth mannose by an α (1,6) linkage. It has very good moisture binding capacity and it is used extensively in frozen deserts, soft cheese and composite meat products.

Agar gum is a complex polysaccharide mainly composed of agarose (which is the gelling agent) and another component which is very viscous and weak gelling. It is mainly used for its gelling and stabilizing properties.

Algin is a high molecular weight polymer of the salts of D-mannuronia and L-guluronic acids.

Pectin is a heteropolysaccharide which main component is the polygalacturonic acid partially esterified with methanol. Regular portions of pectin macromolecules join to form so-called adhesion zones. The resulting formation of a three dimensional network permits the trapping of large amounts of water. (Fennema, 1985).

The results for the various levels of gums included in the reformulated bagel recipes and their effect on freshness are shown in Figures 2.6 - 2.13 and Tables 2. 8 - 2.15.

The effect of two levels of guar gum (0.2 and 0.6%) on bagel softness is shown in Figure 2.6. Textural shelf life could be extended to ~20 days at the 0.2% level (flour weight basis) whereas at higher levels (0.6%) bagels were stale after ~ 30 days (shown by compressibility test of 0.01 MPa). However, for sensory analysis of products only bagels formulated with 0.6% guar gum were marginally acceptable after 28 days at ambient temperature.

Compressibility and sensory analysis test results for three levels of xanthan gum (0.2, 0.6 and 1%) are shown in Figure 2.7 and Table 2.9.

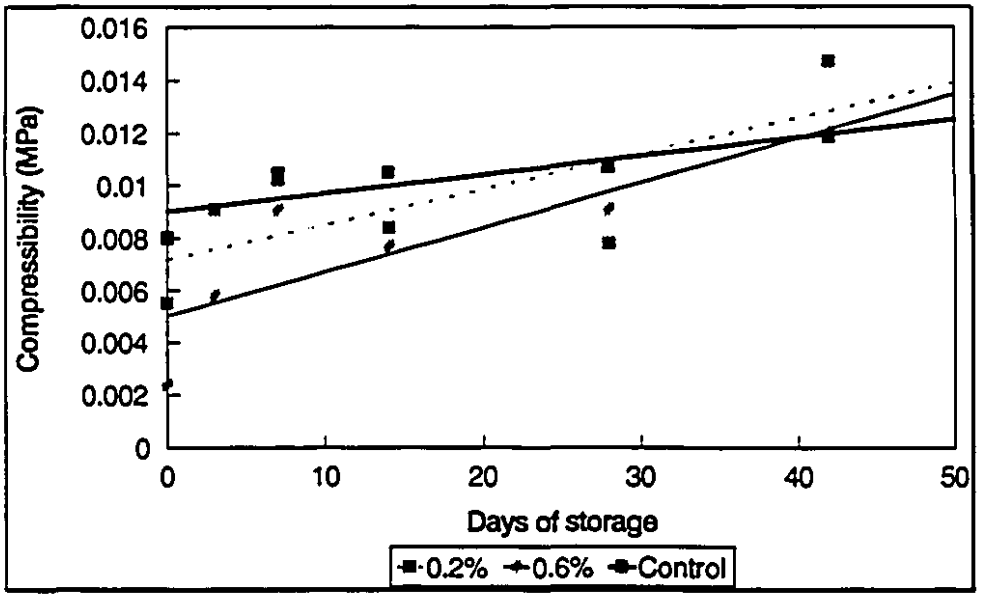


Figure 2.6: Compressibility results for guar gum.

Table 2.8: Sensory results for guar gum.

Guar	Sensory Analysis															
	Odor				Flavor				Texture*				Overall**			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.2%	3.2	3.2	3	--[1]	2.7	2.7	2.3	---	2	2	2.8	---	2.5	2.2	2.2	---
+/-	0.9	0.5	0.7	—	0.9	0.9	1.4	---	0.8	0.8	1.4	---	1	0.5	0.4	---
0.6%	3.2	3.2	3	---	3.2	3.2	3	—	3.2	3.5	3	—	3.2	3.2	3	—
+/-	0.5	0.5	0.8	---	0.5	0.5	0.8	—	0.9	0.5	0	---	0.5	0.9	0	

1. The sensory was interrupted due to mold growth.
*,**,*** significant with p<0.05, 0.005,0.0005.
Average of 5 replicates followed (below) by its Standard Deviation.

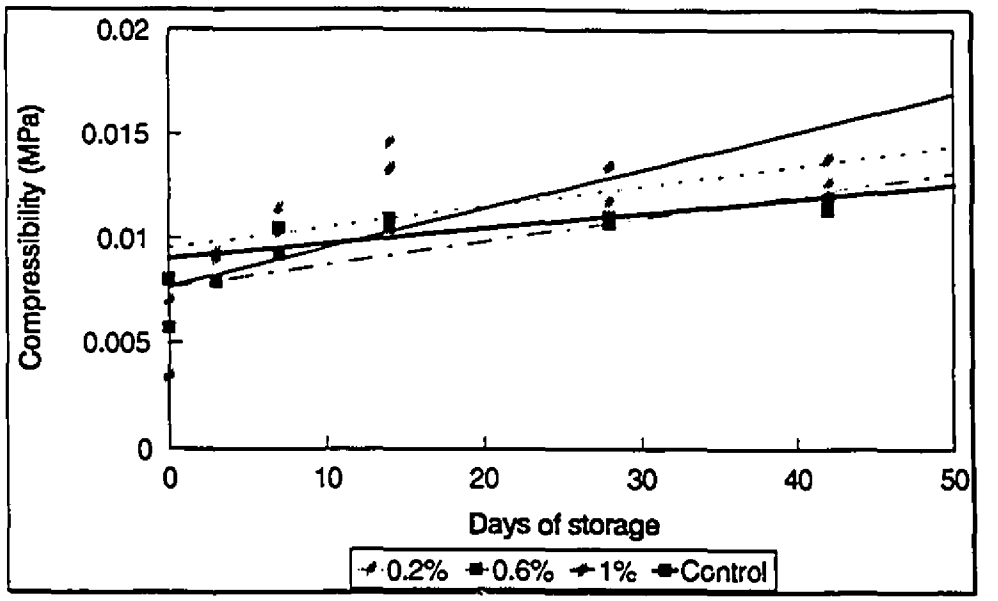


Figure 2.7: Compressibility results for xanthan gum.

Table 2.9: Sensory results for xanthan gum.

Xanthan	Sensory Analysis															
	Odor*				Flavor*				Texture***				Overall**			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.2%	3.7	3.8	3	3	3.4	3.2	3	2.5	3.1	3	2.8	2.6	3.2	3	2.6	2.4
+/-	0.9	0.4	1.3	1.3	0.7	0.7	1.5	1	1.3	1	1.6	1.3	0.8	0.6	1.2	1
0.6%	3.5	3	2.8	2.2	2.7	2.6	2.4	2	2.8	2.6	2	1.6	2.6	2.8	2.8	2
+/-	0.8	0.7	0.8	0.8	1	1.3	1.6	1	1.4	0.8	0.7	0.8	1.4	0.8	0.8	0.7
1%	2.5	2.5	2.5	2.6	2.5	2	1.6	2	1.7	1.5	1.6	1.6	2.2	2	2	2.2
+/-	0.8	1.2	1	1.2	1	0.8	0.5	0.8	1.2	0.7	0.5	0.8	0.4	1	0.9	0.8

*,**,*** significant with p<0.05, 0.005, 0.0005.
Average of 5 replicates followed (below) by its Standard Deviation.

Texturally, products were rejected after day 12 at lower and upper levels of xanthan gum i.e., 0.2 and 1% (Figure 2.7). However, at the 0.6% level, bagels had a textural shelf life of ~25 days. Sensorially, however, products were rejected after 7-14 days for all levels of xanthan gum used.

Favorable compressibility results were observed for bagels reformulated with locust bean gum (Figure 2.8) with the best results being obtained at the 0.6% level of use (Figure 2.8). However, with the exception of odor scores, products containing locust bean gums, were rejected by panelists after ~7 days of storage as shown by the sensory results in Table 2.10.

A similar trend in textural and sensorial shelf life was observed for bagels reformulated with agar gum (Figure 2.9 and Table 2.11). Thus, while gums appeared to inhibit staling due to their water binding capacity they fail to enhance the organoleptic quality of bagels as shown by the low sensory evaluation scores. These results confirm the controversy effect of gums to decrease bread firmness (Maga, 1975, Mettler and Seibel, 1993) while others found that gums had no effect on firmness (Christianson and Gardner, 1974).

The effect of three different types of cellulose (Type 40, 300 and 900) and methylcellulose, all used at the 1% level (flour weight basis) on the softness of bagels throughout storage are shown in Figures 2.10 and 2.11, and Table 2.12 and 2.13 respectively. The textural shelf life of bagels using cellulose 40 was terminated after ~14 days. However, the shelf life could be extended to ~ day 25 using cellulose 300 and 900 at the 1% level (Figure 2.10). Methylcellulose had an even greater effect on textural shelf life and bagels were still acceptable until day 30 using 1% methylcellulose in the formulation. Furthermore, methylcellulose also had a more pronounced effect on the sensory qualities of the reformulated bagel as compared to cellulose (Tables 2.12 and 2.13). With methylcellulose, products were still acceptable after 28 days at ambient temperature (Table 2.13).

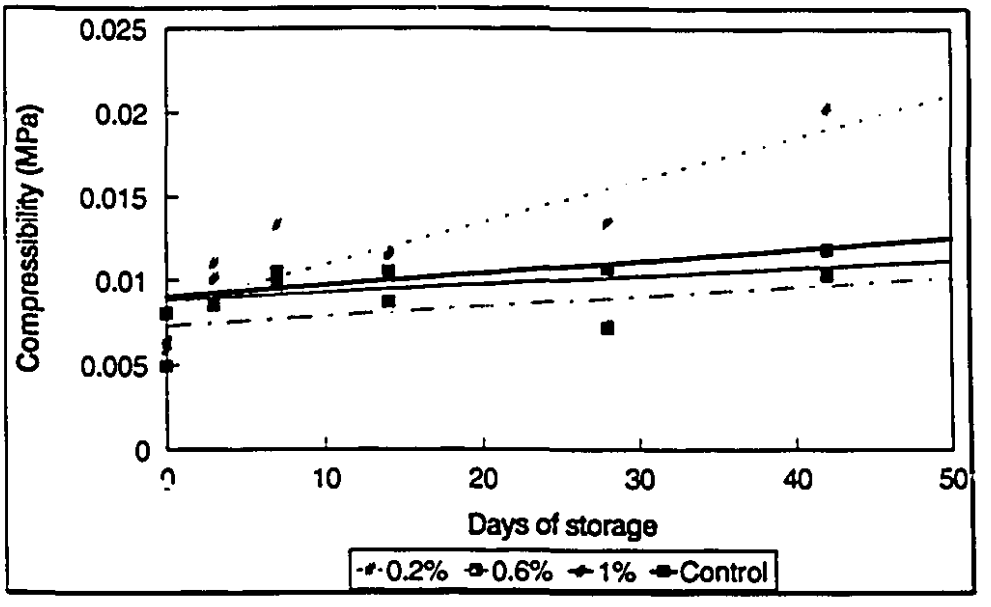


Figure 2.8: Compressibility results for locust bean gum.

Table 2.10: Sensory results for locust bean gum.

Locust bean	Sensory Analysis															
	Odor**				Flavor				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.2%	2.6	3	--[1]	—	2.4	2.2	—	—	2.2	2	—	—	2.4	2.2	—	—
+/-	0.8	0.8	—	—	0.5	0.9	—	—	0.8	0.8	—	—	0.5	0.9	—	—
0.6%	3.5	3.7	3.7	—	2.7	2.7	3	—	2.5	2.7	2.2	—	3.1	2.7	2.6	—
+/-	0.5	0.5	0.8	—	0.9	0.5	1.5	—	1.2	1.2	0.4	—	0.6	1.2	0.8	—
1%	3	3.2	3.5	—	3	3	3	—	2.7	2.5	2.8	—	2.4	2.7	2.8	—
+/-	0.7	0.5	0.8	—	0.8	0	1.2	—	0.8	0.5	0.9	—	0.5	0.5	0.9	—

1. The sensory was interrupted due to mold growth.
*,**,*** significant with p<0.05, 0.005, 0.0005.
Average of 5 replicates followed (below) by its Standard Deviation.

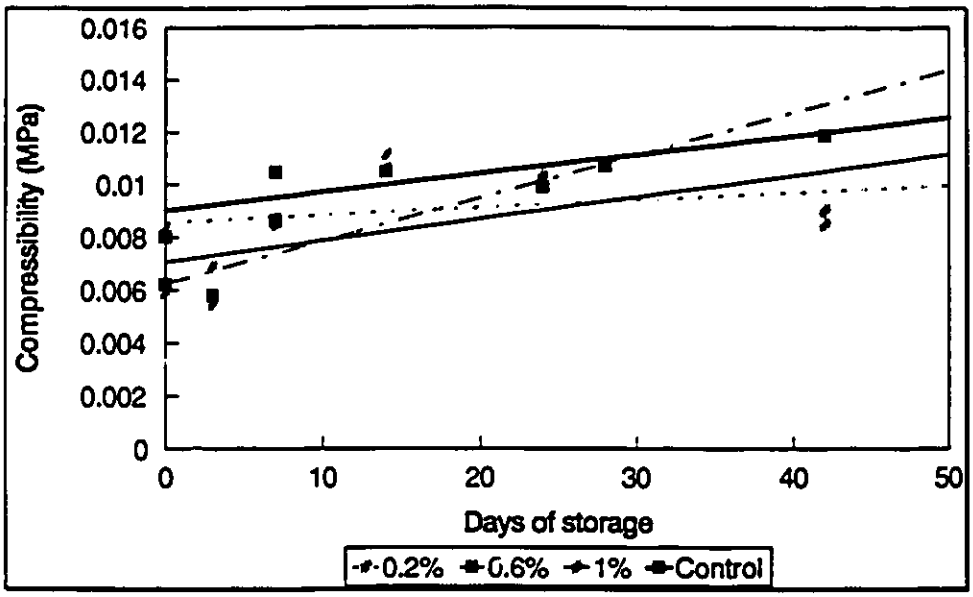


Figure 2.9: Compressibility results for agar gum.

Table 2.11: Sensory results for agar gum.

Agar	Sensory Analysis															
	Odor***				Flavor***				Texture*				Overall***			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.2%	3.7	3.5	3	--[1]	3.5	3	2.8	—	3.5	3.2	3	—	3.5	3	2.4	—
+/-	0.5	0.5	0	—	0.8	0.6	0.4	—	0.8	0.7	0.7	—	0.5	0.6	0.5	—
0.6%	2.8	2.8	2.8	—	3.3	2.3	2.2	—	3.1	1.8	1.5	—	3.4	2.2	2.1	—
+/-	0.7	0.4	0.4	—	1.1	0.8	0.7	—	1	1.1	0.5	—	0.9	0.4	0.4	—
1%	3.7	3.5	3.2	—	3	2.9	2.8	—	2.8	2.7	2.6	—	3	2.8	2.8	—
+/-	0.5	0.5	0.4	—	1	0.8	0.4	—	1.4	0.2	0.8	—	0.8	0.4	0.4	—

1. The sensory was interrupted due to mold growth.
*,**,*** significant with p<0.05, 0.005, 0.0005.
Average of 5 replicates followed (below) by its Standard Deviation.

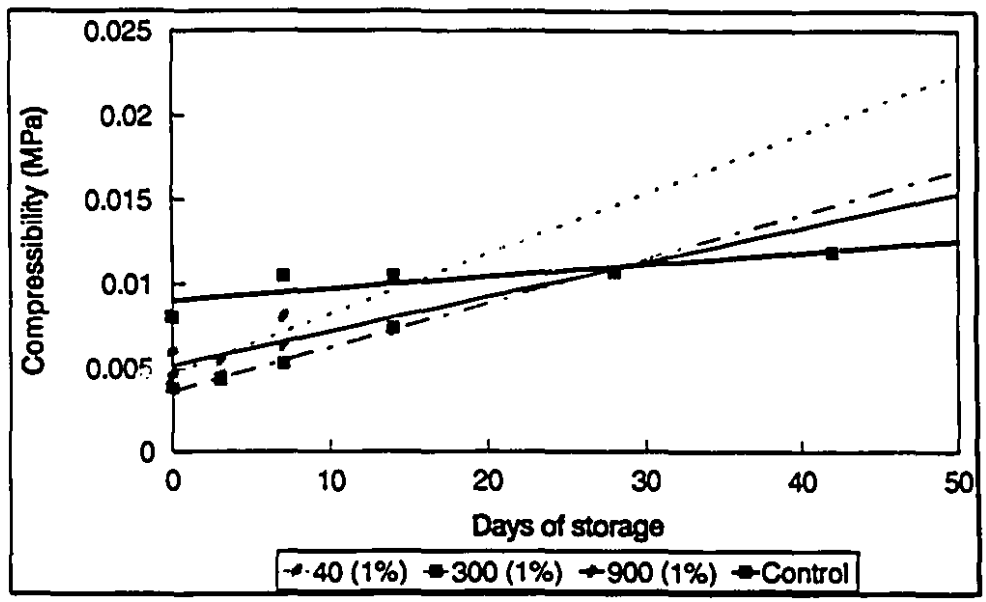


Figure 2.10: Compressibility results for cellulose.

Table 2.12: Sensory results for cellulose gum

Cellulose	Sensory Analysis															
	Odor				Flavor				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
Type 40 (1%)	2.8	2.8	—	—	2.7	2.7	—	—	2.3	1.7	—	—	2.6	2.2	—	—
+/-	1	0.5	—	—	0.7	0.4	—	—	1.1	0.9	—	—	0.8	0.5	—	—
Type 300 (1%)	3.4	3.1	—	—	3.2	2.6	—	—	3.2	2.3	—	—	3.3	2.1	—	—
+/-	0.9	0.8	—	—	0.9	0.8	—	—	1	1.2	—	—	1.2	1.2	—	—
Type 900 (1%)	3	3.1	—	—	3.1	2.8	—	—	2.6	2.1	—	—	3	2.1	—	—
+/-	1	0.8	—	—	1	0.7	—	—	1.5	1.2	—	—	1.1	1	—	—

1. The sensory was interrupted due to mold growth.
Average of 5 replicates followed (below) by its Standard Deviation.

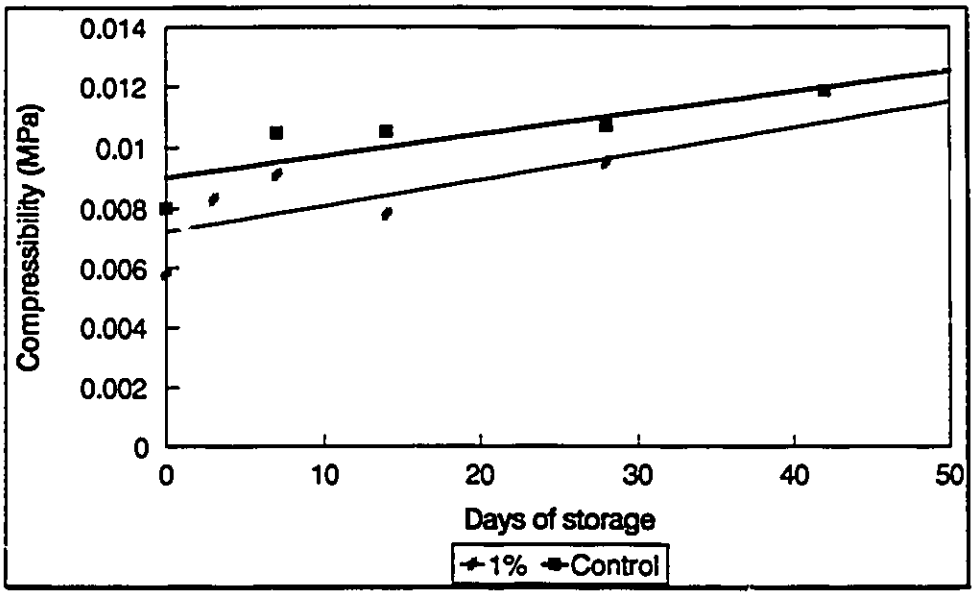


Figure 2.11: Compressibility results for methylcellulose.

Table 2.13: Sensory results for methylcellulose gum.

Methylcellulose	Sensory Analysis															
	Odor				Flavor				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
1%	3.4	4	3.6	3.6	3.6	3.8	3.5	3	3.3	3.5	3.1	2.9	3.3	3.5	3.1	2.9
+/-	0.9	1	0.5	0.5	0.7	0.7	0.5	0	1.1	0.8	1	0.4	0.7	1.1	0.5	0.2

Average of 5 replicates followed (below) by its Standard Deviation.

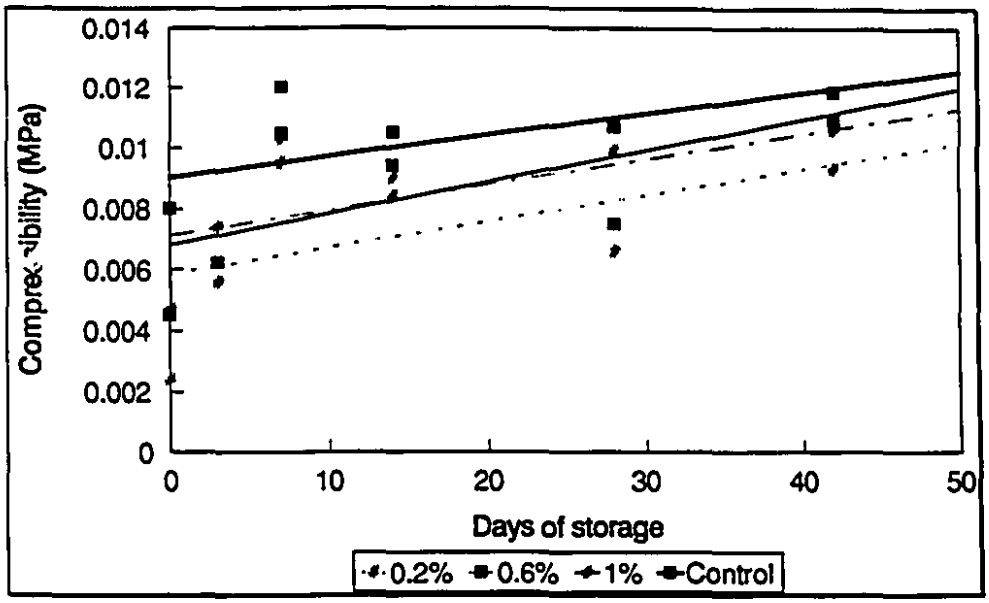


Figure 2.12: Compressibility results for algin gum

Table 2.14: Sensory results for algin gum.

Algin		Senscry Analysis															
		Odor				Flavor				Texture				Overall			
		Days of Storage															
		7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.2%		3.5	3.6	4	4	3.5	3.2	3.4	3	2.7	2.6	2.7	2.6	3	3.2	3.2	3
	+/-	0.5	0.5	1	0.6	0.5	1	0.8	0.6	1.5	0.8	0.7	0.4	1.1	0.8	0.8	0.7
0.6%		3.5	2.7	4.2	4	2.7	2.2	3.4	3	3.2	2	3.4	3.2	3	3.5	3.6	3.4
	+/-	0.5	0.9	0.8	0.6	0.5	0.9	1.1	0.6	0.9	0.8	0.5	0.4	0.8	0.5	0.5	0.4
1%		3.2	3.4	3.8	3.5	3.7	3.8	3.4	3	3.7	3.8	3.4	3	3.5	3	3	2.9
	+/-	0.5	0.8	0.9	0.5	0.5	1	1	0.6	0.2	0.7	0.6	0	0.8	1.1	1.1	0.4

Average of 5 replicates followed (below) by its Standard Deviation.

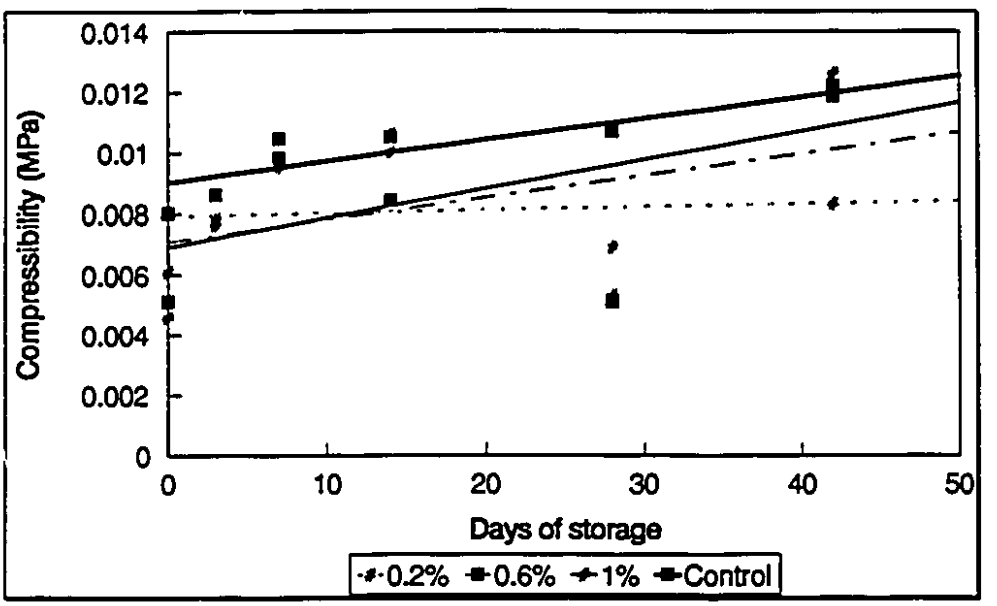


Figure 2.13: Compressibility results for pectin gum.

Table 2.15: Sensory results for pectin gum.

Pectin		Sensory Analysis															
		Odor				Flavor*				Texture				Overall			
		Days of Storage															
		7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
0.2%		3.3	3.3	3.7	3.5	3.6	3.5	3.3	3	3.4	3	2.7	2.7	3.4	3.2	2.9	2.7
	+/-	0.4	1.2	0.5	0.8	0.5	0.8	0.5	0	0.2	0.8	0.5	0.7	0.9	0.9	0.4	0.8
0.6%		3.1	3.8	3.7	3.4	2.7	4	3.3	3	3	3.3	3	2.7	3.8	3.5	3.2	2.9
	+/-	1	0.7	0.5	0.4	0.4	0.6	0.8	0	1	1	1.2	0.6	0.6	1	1.1	0.4
1%		3.4	3.8	3.5	3.2	3.6	3.7	2.8	2.5	3.8	3.3	2.8	2.5	3.6	3.7	3.2	2.9
	+/-	0.9	0.7	0.5	0.4	0.7	1	0.7	0.5	0.3	0.8	0.7	0.5	0.7	1	0.7	0.4

*,**,*** significant with p<0.05, 0.005, 0.0005.
Average of 5 replicates followed (below) by its Standard Deviation.

Both algin and pectin gums also gave favorable results from a textural viewpoint, particularly at the lower levels of use i.e., 0.2 and 0.6% (Figure 2.12 and 2.13). However, as with other gums, sensory shelf life was always less than the textural one as shown in Tables 2.14 and 2.15.

Thus, while gums have a beneficial effect on staling, its effect varies from gum to gum. This is expected since the chemical structure of each gum is different and hence the water binding capacity and plasticizing effect will vary. However, it is evident that gums appear to have a greater effect on the textural quality of bagels compared to their sensory effect as shown by the consistently lower sensory scores for bagels reformulated with gums.

Statistically, most gums followed the same pattern. The compressibility results were highly significant with p-values of less than 0.005 to 0.0005, while the sensory results were not significant with p-value of less than 0.5. However, for agar and xanthan gums, the compressibility results were not significant with p-values of 0.5 (xanthan) or less (agar), while their sensory results were significant with p-values of 0.0005 (agar) and 0.005 (xanthan). Very low correlations were found between compressibility and sensory results in most of the gums studied. However, correlations > 50% were observed for agar, guar and locust bean gums. This again shows that when the texture, measured objectively, is "acceptable", other subjective attributes such as flavor and odor influence shelf life.

2.3.3. SYRUP:

Humectants, such as high fructose corn syrup (HFCS) can provide shelf life extension by enhancing the water retention of baked goods. Thus, they retain moisture in the crumb resulting in a less firm, less stale fresher product.

HFCS is a bright, transparent liquid. It is produced by treating high conversion corn syrup with immobilized glucose isomerase, an enzyme that catalyzes the rearrangement of the sugar molecule from the aldose to the ketose form.

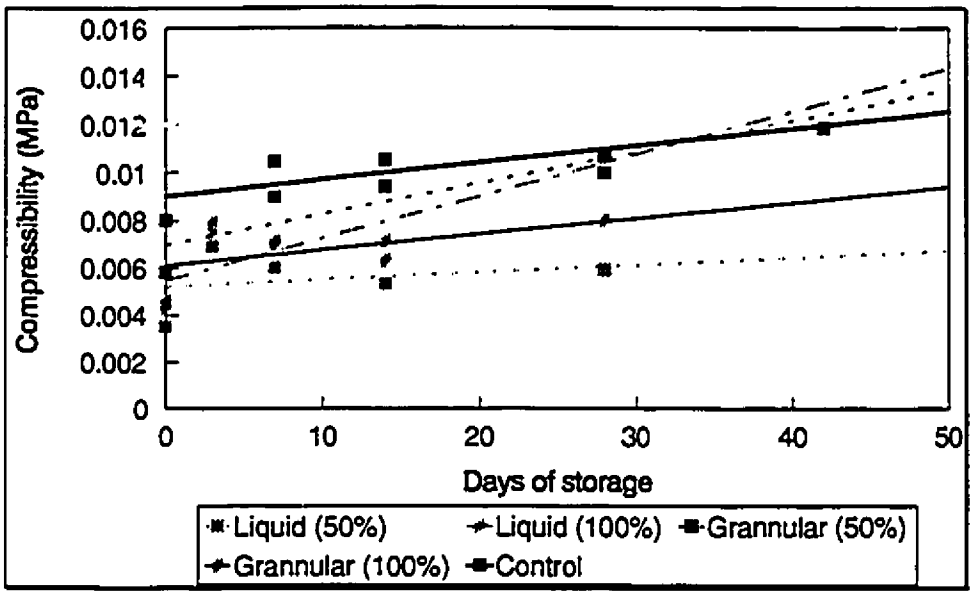


Figure 2.14: Compressibility results for high fructose corn syrup.

Table 2.16: Sensory results for high fructose corn syrup.

High Fructose Corn Syrup	Sensory Analysis															
	Odor				Flavor*				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
Liquid 50%[1]	3.5	3.6	3.5	3	2.8	3.6	3.4	3	3	3.4	3	3	2.5	3.2	3	3
+/-	0.4	0.5	0.7	0.7	0.5	0.5	0.5	0	0.8	0.8	2	0.7	2	0.8	0.7	1
Liquid 100%	3.5	3.5	3.2	3	3.3	3.5	3	2.5	3.8	3.2	3	2.5	3.5	3.2	3	2.6
+/-	1	1.2	1	0.5	0.9	1.5	1.5	1.4	0.5	1.5	1.2	0.7	0.7	1.5	1.1	0.7
Granular 50%	3.7	3	2.8	2.6	3.7	2.6	2.4	2	2.7	2.6	2	2	3	2.6	2.4	2
+/-	1	1.2	1	0.5	0.9	1.5	1.5	1.4	0.5	1.5	1.2	0.7	0.7	1.5	1.4	1.7
Granular 100%	3	3.3	3	2.5	3.2	2.8	2.4	2	2.6	2.5	2.2	2	3.2	3	2.6	2
+/-	1.1	0.5	0.7	0.5	0.9	0.8	0.8	0.7	1.2	0.5	0.8	0.7	1.2	0.8	0.5	1

1. Based on a sugar replacement basis.
The products with high fructose corn syrup were sweeter and stickier than the ones using sugar only.
*,**,*** significant with $p<0.05$, 0.005, 0.0005.
Average of 5 replicates followed (below) by its Standard Deviation.

The transformation involves an intermolecular transfer of hydrogen between adjacent carbon atoms to convert glucose to fructose. The high level of fructose gives it its hygroscopic and sweet properties. Thus, it could affect staling by binding the moisture and/or by interfering with the hydrogen bond formation between protein and starch. However, at higher levels of use, it can cause stickiness and may adhere to packaging materials upon storage.

The effect of HFCS (liquid or granular) as sugar replacement in the bagel formulation is shown in Figure 2.14 and Table 2.16. Both granular and liquid HFCS had a significant effect on crumb staling as shown by compressibility tests (Figure 2.14). After 42 days, products reformulated with liquid HFCS (50%) were almost as fresh as day 1 bagels while bagels containing granular HFCS (100%) were only slightly firmer than day 1 bagels. However, while higher levels also delayed firming, products were very sweet and sticky due to the hygroscopic nature of HFCS. From a sensory view point, only the 50% liquid HFCS gave acceptable scores with sensory shelf life being acceptable at the end of the 42 days storage period. Thus, HFCS at this level has the potential to delay staling and to produce an organoleptically acceptable product.

High fructose corn syrups compressibility results were highly significant with p-values of 0.0005, while the sensory results were not significant, i.e., similar results to enzymes and gums.

2.3.4. FLOURS:

Current theories on the staling process involves starch-protein interactions mainly through hydrogen bonding. Interference with this process through the use of enzymes or water binding ingredients such as HFCS can interfere with this hydrogen bonding. Since gluten is implicated in the staling process, another approach would be to replace high protein flour, either partially or completely with flour of lower protein content i.e. lower gluten content to delay staling.

However, while the use of low protein flour (rice, corn and barley) improved the textural shelf life of the product (Figure 2.15), the sensory shelf life of the product was of ~ 3 days (Table 2.17). In particular, bagel volume was low showing the importance of gluten for dough development and structure of the final product. Some studies have been done on the effect of different kinds of flours on bread firming. Boyacioglu and D' Appolonia (1994) showed that the incorporation of 25% durum wheat flour resulted in a less firm crumb bread structure without affecting any of the bread's characteristics. Torres *et al.* (1993) showed that the addition of up to 20% of sorghum flours, resulted in tortillas that were softer than the control without affecting their sensory qualities. These results disagree with our observations while the addition of barley, corn or rice flours resulted in softer products, panelists rejected the bagels based on their characteristics.

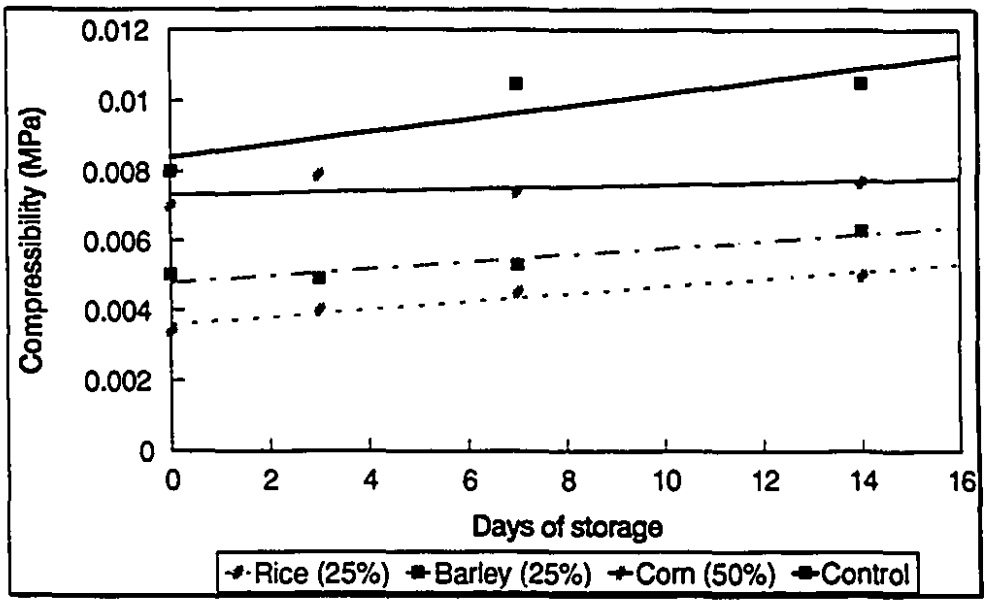


Figure 2.15: Compressibility results for rice, barley and corn flours.

Table 2.17: Sensory results for rice, barley and corn.

Flours	Sensory Analysis															
	Odor*[2]				Flavor				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
Barley (25%)[1]	3.1	3.4	-[2]	—	3	2.9	—	—	2.5	2.4	—	—	2.6	2.4	—	—
+/-	0.8	0.5	—	—	0.8	0.2	—	—	0.9	0.8	—	—	0.8	0.8	—	—
Corn (50%)	3	2.8	—	—	2.8	2.8	—	—	2.3	2.1	—	—	2.3	2.1	—	—
+/-	0	0.7	—	—	0.4	1.1	—	—	0.5	0.4	—	—	0.5	0.4	—	—
Rice (25%)	2.	3.1	—	—	2	3.1	—	—	2.1	2.5	—	—	2.3	2.5	—	—
+/-	1.2	0.6	—	—	0.4	0.8	—	—	0.5	0.4	—	—	0.5	0.4	—	—

1. Based on a wheat flour replacement.
2. The sensory was interrupted after 14 days due to panelists request. The bagels were mushy and sticky.

*,**,*** significant with p<0.05, 0.005, 0.0005.
*[2] only for rice.
Average of 5 replicates followed (below) by its Standard Deviation.

2.3.5. SURFACTANTS:

The incorporation of surfactants into the formulation resulted in the following (results not shown). Atlas MDA (mono- and diglyceride and α-amylase) at 0.25% level gave a stale free shelf life of 25 to 35 days, according to sensorial and compressibility tests, respectively. At the 0.375% level, it increased to 30 and 40 days respectively. However, the use of Atmul-500 (mono- and diglyceride, 2%), Atmul p-28 (mono- and diglyceride, sodium stearoyl-2-lactlylate and calcium sulfate, 0.375%) and Atlas SSL (0.375%) resulted in a shelf life of 10 days according to sensorial tests and 25 days as shown by compressibility tests. Thus, Atlas MDA appears to be more effective in delaying staling probably due to the presence of enzyme in its formulation. Furthermore, on comparing the results of Atlas MDA and enzyme alone, it was concluded that the addition of mono- and diglyceride were not necessary to delay staling and that enzymes alone could be used to extend the shelf life (Smith *et al.*, 1995, unpublished results). Previous studies with mono- and diglyceride (Maga, 1975) or with sodium stearoyl lactylate (De Stephanis *et al.*, 1977) showed that surfactants bind to starch thus delaying retrogradation. However, Pisesookbunterng and D' Appolonia (1983) observed that binding of surfactants to starch prevented the latter from absorbing moisture released from gluten. Thus, this moisture migrated to the crust leading to crust staling and mold growth. A controversy also existed on whether surfactants affect the initial crumb firmness (Zobel, 1973) or the rate of firming (Ghiasi *et al.*, 1982). Our results indicate a slight decrease in the firming rate.

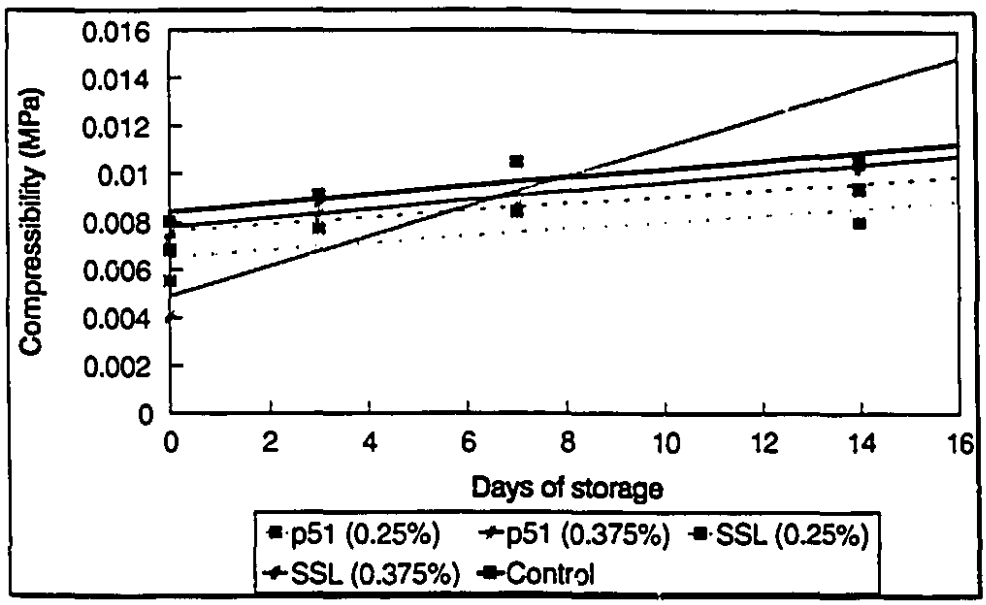


Figure 2.16: Compressibility results for surfactants.

2.4. Conclusion:

Based on this initial study, the estimated shelf life of bagels for all reformulated products stored at 25°C are shown in Table 2.18. The textural shelf life was based on the time (days) to reach a compressibility of 0.01 MPa, while sensory shelf life was based on time (days) to reach an overall acceptability score of <3. It is evident from these results that certain ingredients may result in a desired textural shelf life of 42 days, yet have a lower sensory shelf life, and vice versa.

However, certain formulation involving enzymes (Superfresh, and Megafresh at the 0.15-0.2% level) resulted in a 42 day extension in textural and sensorial shelf life of bagels. Algin gum at the 0.2% level also produced similar extensions in shelf life. While HFCS at the 50% level delayed staling for 42 days, sensory shelf life was regarded unacceptable after 28 days. Pectin also gave a favorable extension in both textural and sensory shelf life.

While these ingredients were examined on an individual basis, their combined use warrants further investigation, particularly to improve the sensory shelf life of bagels.

Table 2.18.: Summary of shelf lives from different formulations..

Formulation	Level of use[1]	Sensory[2] Days	Textural[3] Days
Enzymes:			
Novamyl	0.031	28	42***
	0.047	42	14***
Superfresh	0.1	28	42***
	0.15	42	42***
	0.2	42	42***
Megafresh	0.1	28*	42***
	0.15	42*	42***
	0.2	14*	28***
Gums:			
Guar [-b]	0.2	3*	20**
	0.6	28*	30**
Xanthan	0.2	14**	12*
	0.6	3**	25*
	1	3**	12*
Locust bean [-b]	0.2	3	7***
	0.6	7	42***
	1	3	25***
Agar [-b]	0.2	14***	40
	0.6	7***	20
	1	7***	35
Cellulose 40 [-c]	1	3	14
Cellulose 300	1	7	25
Cellulose 900	1	7	25
Methylcellulose	1	28	30
Algin	0.2	42	42**
	0.6	42	28**
	1	28	28**
Pectin	0.2	14	10***
	0.6	28	40***
	1	28	32***
Syrups:			
HFCS Liquid	50	42	42***
	100	28	35***
HFCS Granular [-c]	50	7	35***
	100	14	42***
Flours:			
Rice [-c]	25	3	42
Barley [-b]	25	3	42**
Corn	50	3	42
Surfactants:			
Atlas p51	0.25	-	14***
	0.375	-	14***
Atlas SSL	0.25	-	14***
	0.375	-	7***
Control		3	3

1: % flour basis except for high fructose syrup; % sugar replacement.
2: When a product score was of 3 or above.
3: When product score was of 0.01 or below.
*, **, *** significant with p<0.05, 0.005, 0.0005.
a, b, c when compressibility and sensory have a correlation >75%, >50%, >25%.

CHAPTER 3: OPTIMIZATION STUDIES ON BAGELS

3.1 Introduction:

Preliminary formulation studies showed that enzymes, guar, algin and pectin gums, and high fructose corn syrup (HFCS), all had a beneficial effect on the textural quality of bagels. However, certain ingredients, e.g., HFCS, had a more pronounced effect on sensory quality than others. Furthermore, the effect of these factors on textural and sensory quality of bagels was investigated using the traditional "one variable at a time approach". The disadvantages of this approach are: (i) it is laborious and time consuming, (ii) it generates large quantities of data which are difficult to interpret and (iii) it fails to measure interaction effects. To overcome the limitations of the one variable at a time approach and to adequately describe the effect of several factors, including their important interactions, a technique involving factorial designs and multiple regression analysis termed response surface methodology (RSM), was used in this study. RSM permits factors of interest to be examined, not simply one at a time, but simultaneously in a single set of experimental runs. Mathematical models, generally first order and second order polynomials are generated to define the optimal levels of the most significant factors to give the desired response (Box *et al.*, 1978). Developed initially for process optimization studies in chemical engineering, RSM has recently been applied to shelf life extensions studies in gas packaged bakery products (Smith *et al.*, 1990) and aflatoxin production in MAP peanuts (Ellis *et al.*, 1994).

This chapter describes the use of RSM in textural/sensory studies of bagels and the advantages of the technique is a research tool in food formulation studies.

3.2. Material and Methods:

To determine the effect of enzymes, guar, algin and pectin gums and HFCS on textural/ sensory quality of bagels, a 3 factor, 5 level central composite rotatable design (CCRD) of Box *et al.* (1978) was used for fitting second order response surfaces (Table 3.1). CCRDs have 2^k+2k+1 treatment combinations where k equals the number of variables under study. The experimental design is said to be rotatable since the variance of the predicted response Y, at designated points (X's), is a function only of this distance from the center, rather than a function of the direction. This implies that the variance contours of Y are concentric circles and a design with this property will leave the variance of Y unchanged with the design rotated about the center (0,0,0,0) leading to the term rotatable (Montgomery, 1984).

Table 3.1: Construction of a 3 factor CCRD ($2^k + 2k + 1$) (Box *et al.*, 1978).

Trials	Levels (%)		
	Variable 1	Variable 2	Variable 3
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-2	0	0
10	2	0	0
11	0	-2	0
12	0	2	0
13	0	0	-2
14	0	0	2
15	0	0	0

3.2.1. LEVELS OF FACTORS:

In the initial CCRD, (CCRD1), enzyme (Novamyl), guar gum and HFCS were investigated simultaneously to determine their effect on texture/ sensory quality of bagels. The range of levels of each factor used in the CCRD1 were enzyme (0.015-0.075%), guar gum (0.4-0.8%) and HFCS (15-75%). Variable levels were coded -2, -1, 0, +1, +2 to facilitate statistical analysis. Values of each level used were based on previous formulation studies. The coded and actual values of enzyme, guar gum and HFCS are shown in Table 3.2. In the second CCRD, (CCRD2), enzyme (Superfresh), algin and pectin gums were the variables investigated. The coded and actual values of enzyme, algin and pectin gums used again selected from previous formulation studies, are shown in Table 3.3. With the exception of HFCS, levels of ingredients were based on a flour weight basis while levels of HFCS were based on a percentage of sugar replacement. All experiments in both CCRD1 and CCRD2 were done in duplicate.

Table 3.2: Central Composite Rotatable Design 1: Levels of Novamyl, Guar Gum and High Fructose Corn Syrup.

Ingredients	Levels (%)[1]				
	-2	-1	0	1	2
Novamyl	0.015	0.030	0.045	0.060	0.075
Guar	0.4	0.5	0.6	0.7	0.8
HFCS	15	30	45	60	75

1- percentage based on flour weight basis except for high fructose corn syrup which was basis on a sugar replacement basis.

Table 3.3: Central Composite Rotatable Design 2: Levels of Superfresh, Algin and Pectin Gums.

Ingredients	Levels (%)[1]				
	-2	-1	0	1	2
Superfresh	0.05	0.1	0.15	0.2	0.25
Algin	0.1	0.2	0.3	0.4	0.5
Pectin	0.1	0.2	0.3	0.4	0.5

1- percentage based on flour weight basis except for high fructose corn syrup which was basis on a sugar replacement basis.

3.2.2. FORMULATION OF BAGELS:

All bagels were reformulated, baked and packaged as outlined in section 2.2.3. Bagels were stored at ambient temperature and examined for textural/sensory changes over a 42 day storage period as described previously. Bagels were rejected when a compressibility of 0.01 MPa and a sensory score of <3 was reached for each formulation

To test the accuracy of the second order polynomial fitted model to extend both the textural and sensory shelf life of bagels, bagels were reformulated with the desired level of enzyme, gum and HFCS observed at optimum response for each design. Reformulated bagels were again packaged with an Ageless FX-100 oxygen absorbent in high gas barrier Cryovac bags (2 bagels/bag) stored at 25°C and monitored for textural/ sensory changes over a 42 days storage period.

3.2.3. STATISTICAL ANALYSIS:

Statistical analysis (regression coefficients analysis of variance and correlation coefficients) were computed using the Statistical Analysis System (SAS) (1991). All three dimensional graphs and two dimensional contour plots were done using the SAS/Graph program on a McGill University mainframe.

3.3. Results and Discussion:

3.3.1. Central Composite Rotatable Design 1:

The combined effect of enzyme (Novamyl) guar gum and HFCS on the textural and sensory (overall acceptability) quality of reformulated bagels is shown in Table 3.4. (CCRD1). With respect to texture, texture scores ranged from a low of 0.002 MPa (runs # 11,14,15) to 0.0066 MPa (run # 6) (Table 3.4). Sensory evaluation scores (average score for overall acceptability) after 42 days are also shown in Table 3.4. It is evident that most scores (with the exception of runs 4, 9 and 10) were >3, i.e., the overall acceptability of products was acceptable after 42 days of storage at 25°C. There was a good correlation of 55% between texture and overall acceptability indicating that objective textural measurements were good indicators of product quality and consumer acceptance of the reformulated product. While there was a good correlation between texture and overall acceptability, some panelists commented that reformulated bagels in run # 11 (i.e. texture =0.002 MPa and sensory =3.2) were somewhat "mushy" or "soft and tacky". This can be attributed to the amyolytic activity of Novamyl enzyme resulting in a higher glucose concentration which, in conjunction with HFCS, would be very hygroscopic, bind water and have a plasticizing effect on texture by interfering with hydrogen bonding between protein-starch matrix i.e., the main cause of staling (Martin *et al.*, 1991).

To quantify the effect of enzyme (Novamyl), guar gum and HFCS on the texture/ sensory quality of bagels, a RSM approach was used. The second order models resulting from the multiple regression of the uncoded results for texture are:

$$Y_{texture} = -0.0078 + 0.13X_1 + 0.008X_2 + 0.0002X_3 + 1.06X_1^2 + 0.02X_2^2 - 0.000005X_3^2 - 0.33X_1X_2 + 0.000001X_1X_3 - 0.0001X_2X_3$$

Table 3.4: Central Composite Rotatable Design (CCRD1)

Trials	Levels (%)			Response	
	Novamyl	Guar	HFCS	Texture[1]	Sensory[2]
1	0.030	0.5	30	0.0030	3.6
2	0.060	0.5	30	0.0047	4.0
3	0.030	0.7	30	0.0033	4.2
4	0.060	0.7	30	0.0030	2.8
5	0.030	0.5	60	0.0044	4.0
6	0.060	0.5	60	0.0066	3.0
7	0.030	0.7	60	0.0040	3.6
8	0.060	0.7	60	0.0037	3.6
9	0.015	0.6	45	0.0045	2.6
10	0.075	0.6	45	0.0052	2.8
11	0.045	0.4	45	0.0020	3.2
12	0.045	0.8	45	0.0062	3.2
13	0.045	0.6	15	0.0030	3.2
14	0.045	0.6	75	0.0022	3.0
15	0.045	0.6	45	0.0022	4.2

All tests done in duplicates 1: Compressibility 2: Overall acceptability

Analysis of variance for the fitted model showed that the F-value and the overall correlation coefficient were significant ($P<0.05$) and that the model accounted for 45% of the total variation after being corrected for the mean.

Analysis of least square estimates of the second order polynomial model parameters are shown in Table 3.5.

Table 3.5: Analysis of least square estimates of second order polynomial model (parameters for texture).

Model	Estimate	T - Ratio
Intercept (b_0)	-0.008 (0.008)[a]	-0.96**
Enzyme (X_1)	0.126 (0.09)	1.32***
Guar Gum (X_2)	0.0079 (0.02)	0.42***
HFCS (X_3)	0.002 (0.0001)	2.2***
Enzyme^2 (X_1^2)	1.05 (0.64)	1.65*
Guar Gum^2 (X_2^2)	0.01 (0.01)	1.0*
HFCS^2 (X_3^2)	-0.0001 (0.0001)	-0.88*
Enzyme*Guar Gum (X_1X_2)	-0.033 (0.11)	-2.95***
Enzyme*HFCS (X_1X_3)	0.0001 (0.0001)	0.0001_{ns}
Guar Gum*HFCS (X_2X_3)	-0.0001 (0.0001)	-1.74*
R_2[b] (%)	45	

a: The number in parenthesis is the standard error
b: Coefficient of determination
Level of significance * P<0.05, ** P<0.005, *** P<0.0005, ns= non significant

Regression analysis of the model showed that the fitted model was highly significant ($P<0.0005$), yet only accounted for 45% of the total variation after being corrected for the means. Examination of the fitted model show that all the linear, quadratic and cross product terms (except Enzyme*HFCS, X_1X_3) had a significant effect. The significant effect of enzyme or HFCS alone was in agreement with earlier reformulation results. Thus, the combination of these ingredients had a significant effect on staling. This synergistic effect may be explained due to the high carbohydrate content in these treatment combinations and hence a higher water binding and plasticizing effect on crumb texture.

The second order model of the uncoded results for sensory for CCRD1 is:

$$Y_{sensory} = -11.58 + 196.66X_1 + 33.75X_2 + 0.08X_3 - 1814.81X_1^2 - 28.33X_2^2 - 0.001X_3^2 - 66.66X_1X_2 - 0.000001X_1X_3 + 0.06X_2X_3$$

Analysis of variance for the fitted model showed that the F-value and the overall correlation coefficient were significant ($P<0.05$) and that the model accounted for 24% of the total variation after being corrected for the mean.

Analysis of least square estimates of the second order polynomial model parameters are shown in Table 3.6.

It is evident from this table that most of the terms are significant. With the exception of the linear term HFCS (X3), and the cross product terms, all linear and quadratic terms were significant and had a pronounced effect on the overall acceptability of the product. Thus, while the cross products did not influence the overall acceptability of the product, (i.e. taste, odor and texture) they still had an effect on texture of bagels.

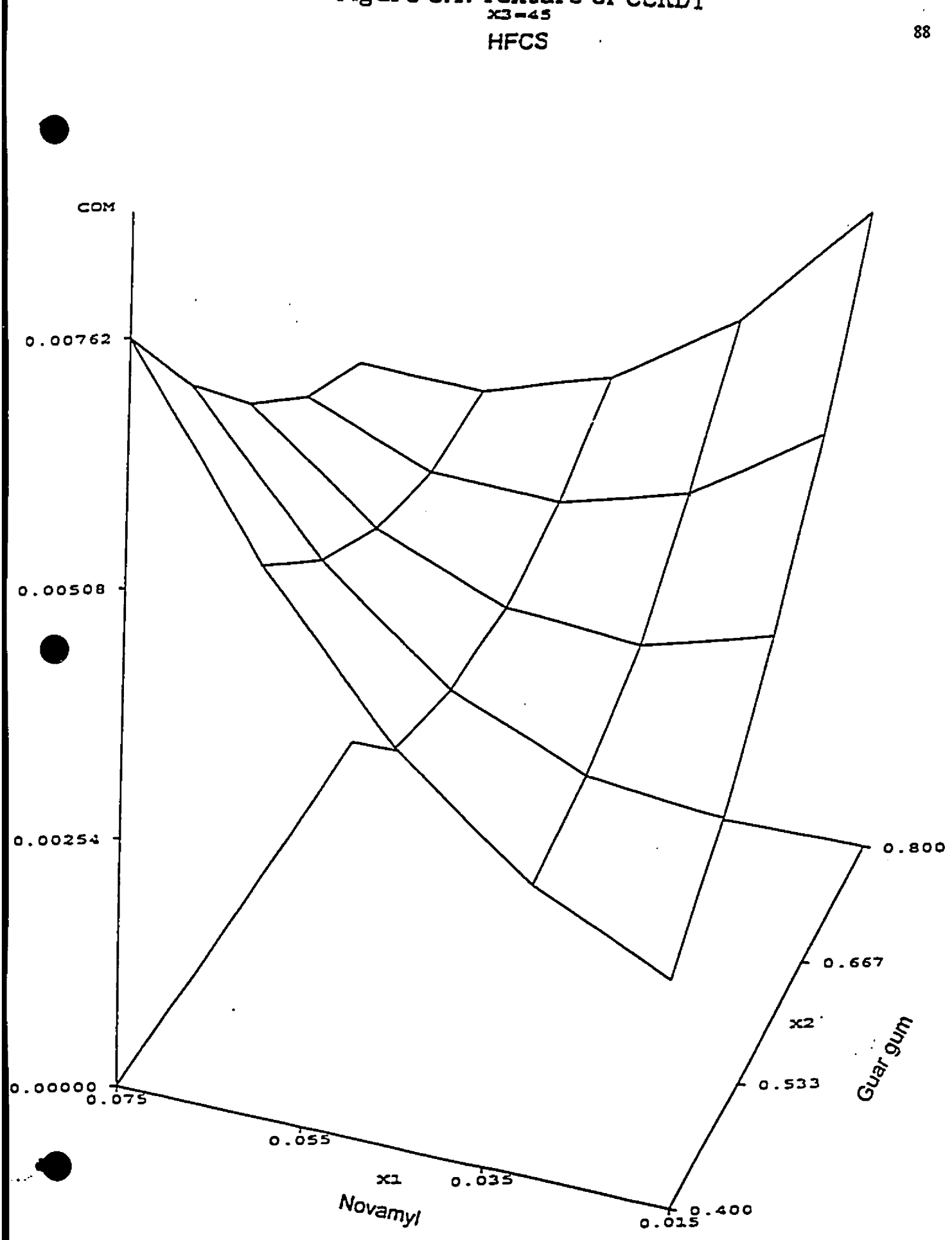
Table 3.6: Analysis of least square estimates of second order polynomial model (parameters for sensory).

Model	Estimate	T - Ratio
Intercept (b_0)	-11.58 (-2.08)[a]	0.04*
Enzyme (X_1)	196.66 (3.02)	0.003**
Guar Gum (X_2)	33.75 (2.64)	0.01*
HFCS (X_3)	0.08 (1.23)	0.22_{ns}
Enzyme^2 (X_1^2)	-1814.81 (-4.16)	0.0001***
Guar Gum^2 (X_2^2)	-28.33 (-2.88)	0.005**
HFCS^2 (X_3^2)	-0.001 (-3.14)	0.002**
Enzyme*Guar Gum (X_1X_2)	-66.66 (-0.86)	0.4_{ns}
Enzyme*HFCS (X_1X_3)	-0.0001 (-0.0001)	1_{ns}
Guar Gum*HFCS (X_2X_3)	0.06 (0.86)	0.39_{ns}
R_2[b] (%)	24	

a: The number in parenthesis is the standard error
b: Coefficient of determination
Level of significance * P<0.05, ** P<0.005, *** P<0.0005, ns= non significant

The significant linear, quadratic and cross product terms influencing both texture and overall acceptability were subsequently used to generate 3-dimension response surface graphs. These graphs graphically illustrate the important relationship between product variables and their effect on texture and overall acceptability. An example of response surface graphs of enzyme, guar gum and HFCS held constant at a 45% HFCS (sugar replacement basis) for texture and sensory evaluation are shown in Figures 3.1 and 3.2 respectively. The response surface graph for texture (Figure 3.1) is an example of a saddle point (Box *et al.*, 1978) where the optimum response is either along the sides or in one or more of the four corners. As Figure 3.1 illustrates the optimum result i.e., lowest compressibility and hence best texture and less stale product can be achieved by decreasing both the levels of the enzyme and guar gum in the formulation.

Figure 3.1. Texture of CCRD1



However, if the concentration of enzyme or guar gum increases, products will become harder, more stale as shown by increase in compressibility (Figure 3.1).

Canonical analysis of this set of experimental data indicated that stationary point i.e., point of optimum texture on the fitted surface is neither a minimum or maximum. Actual values of the variables at the stationary point (X_0) are shown in Table 3.7.

Table 3.7: Actual values of variables at stationary point X_0 (Point of optimum texture).

Variable	Actual Value (%)
Enzyme (X_1)	0.064
Guar Gum (X_2)	0.786
HFCS (X_3)	47.33

All the actual values are well within the experimental range. Furthermore, the predicted value at the stationary point is a compressibility of 0.0043 MPa. When these values are put in the sensory equation the result is a sensory score of 3, i.e. an acceptable score.

Similar trends between these two variables with HFCS held constant at 45% on sensory quality (overall acceptability) of product are shown in Figure 3.2. Low sensory scores are possible through formulation with low levels of enzyme-guar gum or increasing levels of enzyme and guar gum. However, optimum i.e. maximum sensory scores can be achieved by reformulating the product with levels of variables at stationary point shown in Table 3.8.

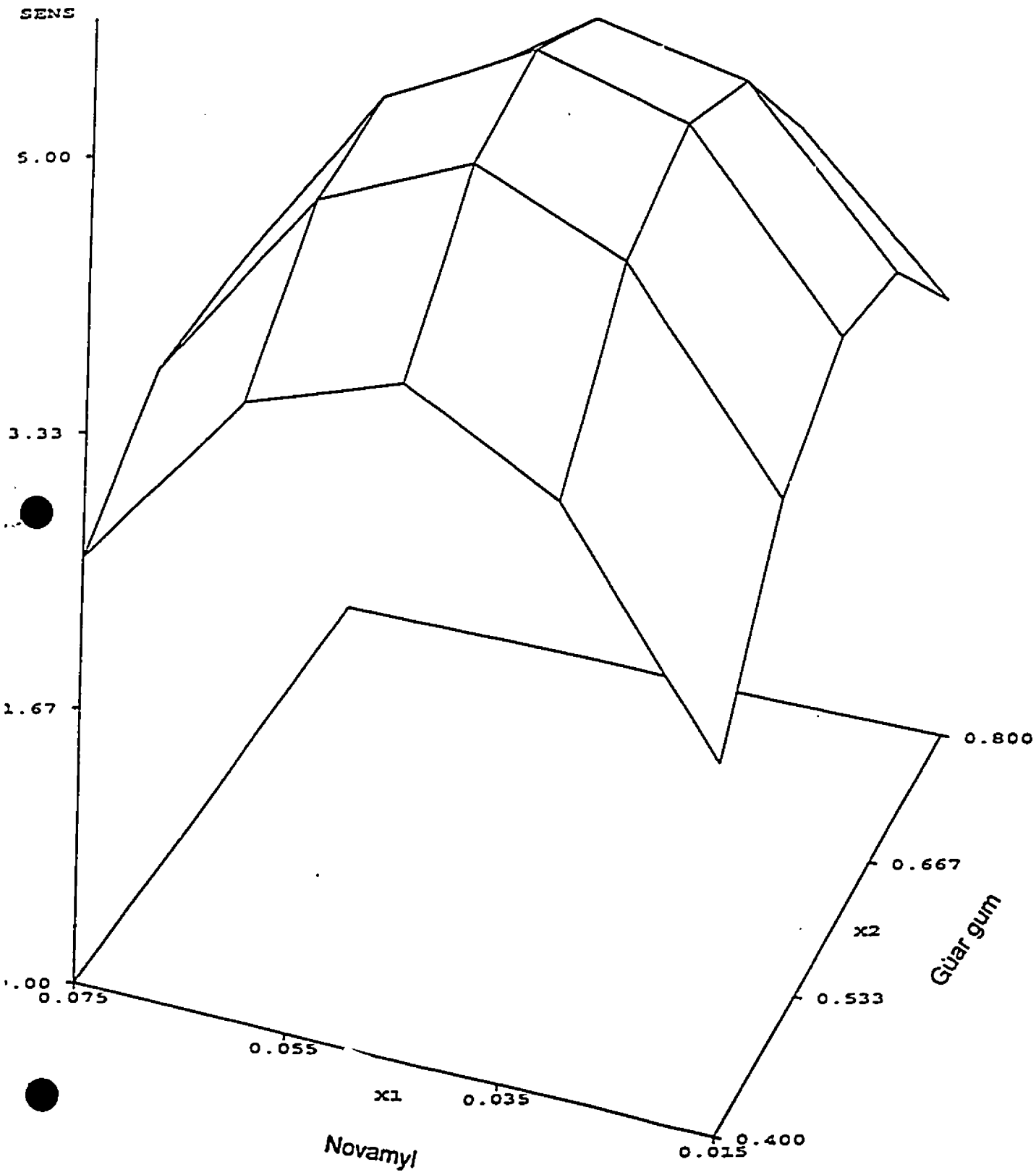
Table 3.8: Actual values of variables at stationary point X_0 (Point of optimum sensory quality).

Variable	Actual Value (%)
Enzyme (X_1)	0.045
Guar Gum (X_2)	0.597
HFCS (X_3)	43.69

Figure 3.2. Sensory of CCRD1

X3=45

HFCS



Again, all values are within the experimental range. The predicted maximum sensory value (overall acceptability) is 4.47 out of a possible 5 (highly acceptable). These values when plugged into the compressibility equation will result in a compressibility outcome of 0.0061 MPa, which is also highly acceptable.

Response graphs and canonical analysis for flavor and texture all had similiar maximum stationary points. Values of variables at the stationary points for both flavor and texture are shown in Table 3.9.

Table 3.9: Actual values of variables at stationary point X_0 (Point of optimum sensorial qualities).

Variable	Actual Value (%)	
	Flavor	Texture
Enzyme (X_1)	0.043	0.045
Guar Gum (X_2)	0.616	0.597
HFCS (X_3)	46.04	39.13

These variables levels resulted in flavor and texture scores of 4.2 and 4.4 respectively. Furthermore, there was a significant correlation between flavor, texture and overall acceptability test scores and values at the stationary points, indicating that either one of the tests could be used as an indicator of sensory quality of bagels.

3.3.2. Central Composite Rotatable Design 2:

In this design various levels of enzyme (Superfresh), algin and pectin gums were used to reformulate the product. The actual values used in the design are as shown in Table 3.10. and were again selected from previous reformulation studies.

Table 3.10: Coded and uncoded values used in design 2.

Variable	Levels (%)[1]				
	-2	-1	0	1	2
Enzyme (X_1)	0.05	0.10	0.15	0.20	0.25
Algin (X_2)	0.1	0.2	0.3	0.4	0.5
Guar Gum (X_3)	0.1	0.2	0.3	0.4	0.5

1 Flour weight basis.

The results are shown in Table 3.11. In general, products reformulated with enzyme (Superfresh) algin and pectin gums did not have as good a textural and sensorial shelf life as bagels reformulated with Novamyl, guar gum and HFCS (Table 3.4).

Table 3.11: Central Composite Rotatable Design (CCRD 2)

Trials	Levels (%)			Response	
	Superfresh	Algin	Pectin	Texture[1]	Sensory[2]
1	0.1	0.2	0.2	0.0057	2.4
2	0.2	0.2	0.2	0.0075	3.0
3	0.1	0.4	0.2	0.0071	3.6
4	0.2	0.4	0.2	0.0047	3.6
5	0.1	0.2	0.4	0.0076	2.8
6	0.2	0.2	0.4	0.0074	3.2
7	0.1	0.4	0.4	0.0070	3.8
8	0.2	0.4	0.4	0.0112	2.8
9	0.05	0.3	0.3	0.0089	3.4
10	0.25	0.3	0.3	0.0071	4.2
11	0.15	0.1	0.3	0.0098	3.4
12	0.15	0.5	0.3	0.0071	3.0
13	0.15	0.3	0.1	0.0069	4.0
14	0.15	0.3	0.5	0.0080	2.4
15	0.15	0.3	0.3	0.0123	2.6

All tests done in duplicates 1:Compressibility 2: Overall acceptability

Indeed, several products had a texture of >0.01 MPa and a sensory score of <3, at the end of the 42 days storage period indicating staleness and consumer rejection. Furthermore, many of these products were rejected after only 28 days based on their textural or sensorial scores. However, a shelf life of 42 days was possible for both parameters using ~0.2-0.25% enzyme, 0.3-0.4% algin gum and 0.2-0.3% pectin gum (runs 4 and 10).

The second order polynomial models for both texture and sensorial (overall acceptability) were:

$$Y_{texture} = 0.001 + 0.04X_1 + 0.01X_2 + 0.02X_3 - 0.35X_1^2 - 0.08X_2^2 - 0.103X_3^2 + 0.04X_1X_2 + 0.15X_1X_3 + 0.07X_2X_3$$

$Y_{sensory} = 7.33 - 26.25X_1 - 13.12X_2 - 3.87X_3 + 110.00X_1^2 + 22.50X_2^2 + 12.50X_3^2 + 5.00X_1X_2 - 25.00X_1X_3 - 2.50X_2X_3$

Regression analysis of both models indicated that they were significant ($P<0.05$) after being corrected for the means. Analysis of least squares estimates of second order polynomial models for both texture and sensory are shown in Table 3.12.

Table 3.12: Analysis of least square estimates of second order polynomial model (parameters for texture and sensory).

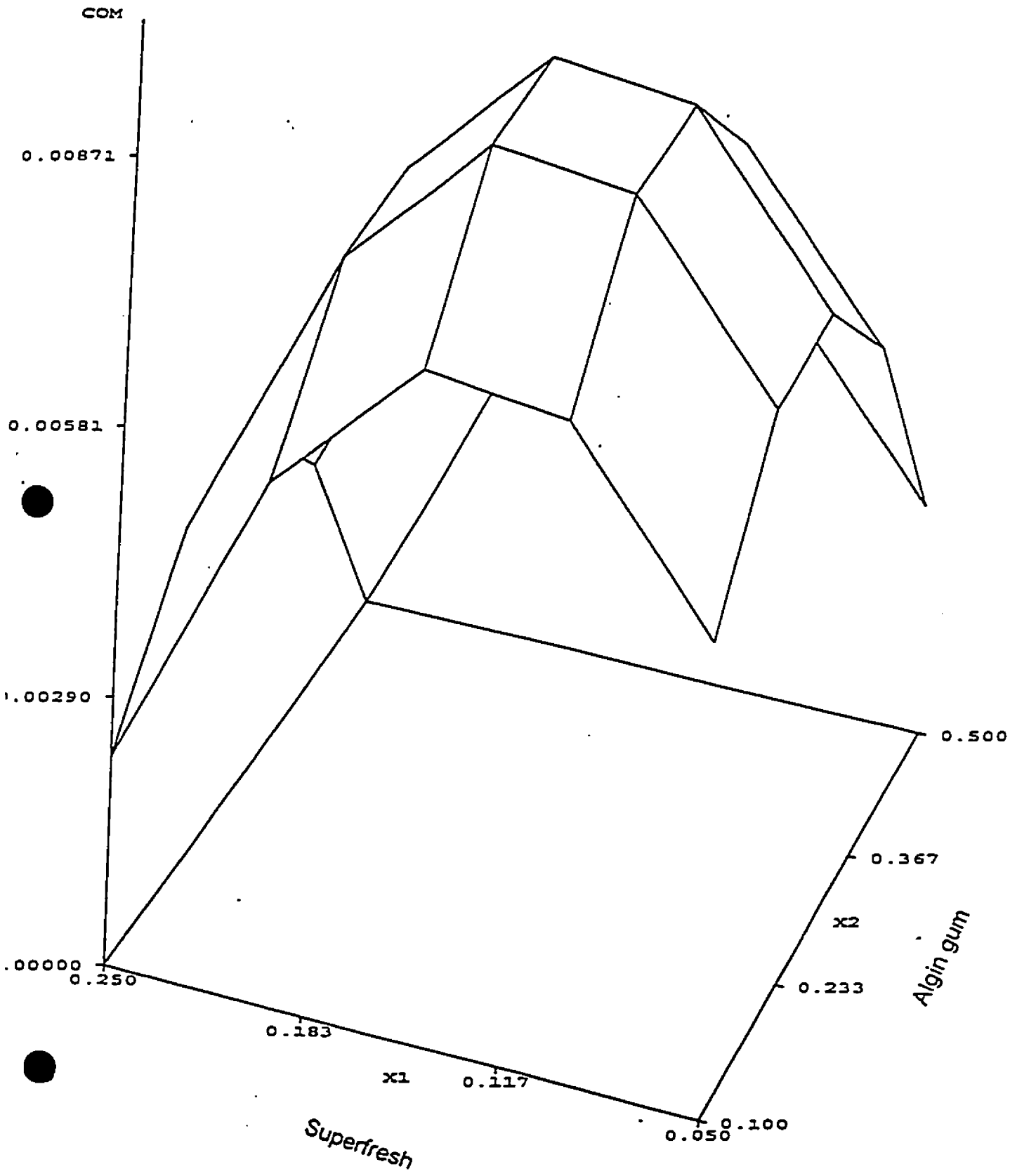
Model Term	Texture		Sensory	
	Estimate	T-ratio	Estimate	T-ratio
Intercept (b_0)	0.0015	0.81	7.33	0.01**
Enzyme (X_1)	0.046	0.22***	-26.25	0.11_{ns}
Algin (X_2)	0.015	0.43***	-13.12	0.11_{ns}
Pectin (X_3)	0.022	0.24***	-3.87	0.63**
Enzyme^2 (X_1^2)	-0.356	0.0005***	110.00	0.009*
Algin^2 (X_2^2)	-0.078	0.001***	22.50	0.03_{ns}
Pectin^2 (X_3^2)	-0.103	0.0001***	12.50	0.22_{ns}
Enzyme*Algin (X_1X_2)	-0.044	0.43_{ns}	5.00	0.83_{ns}
Enzyme*Pectin (X_1X_3)	0.152	0.009**	-25.00	0.30_{ns}
Algin*Pectin (X_2X_3)	0.074	0.01**	-2.50	0.83_{ns}
R_2^b (%)	61		14	

a: The number in parenthesis is the standard error
b: Coefficient of determination
Level of significance * P<0.05, ** P<0.005, *** P<0.0005, ns= non significant

Examination of the fitted model for texture showed that all factors were significant, while for sensory only a few were significant (Pectin (X_3) and Superfresh^2 (X_1^2). These significant factors were again used to compute response surface plots for texture and sensory qualities, example of which are shown in Figure 3.3 and 3.4 respectively.

Figure 3.3. shows that low compressibility (texture) scores are possible using either a high level of enzyme and low level of algin or high levels of enzyme and algin. At levels in between, texture scores increase and are even unacceptable (>0.01 MPa) after 42 days storage at ambient temperature.

Figure 3.3. Texture of CCRD2
x3=0.3
Pectin gum



Analysis of the stationary point on the fitted surface showed that it was a maximum and the values at the point for each variable are shown in Table 3.13.

Table 3.13: Actual values of variables at stationary point X_0 (Point of optimum texture).

Variable	Actual Value (%)
Enzyme (X_1)	0.152
Algin (X_2)	0.293
Pectin (X_3)	0.326

The predicted value of bagel texture for these levels of variables was 0.011 MPa which is slightly higher than the maximum staling "standard" of 0.01 MPa. These predicted values, in the sensory equation will lead to an outcome of 2.78, which is slightly lower than the "standard" of 3 for sensory acceptability and exactly the same as the outcome of the sensory stationary point as shown in Table 3.14.

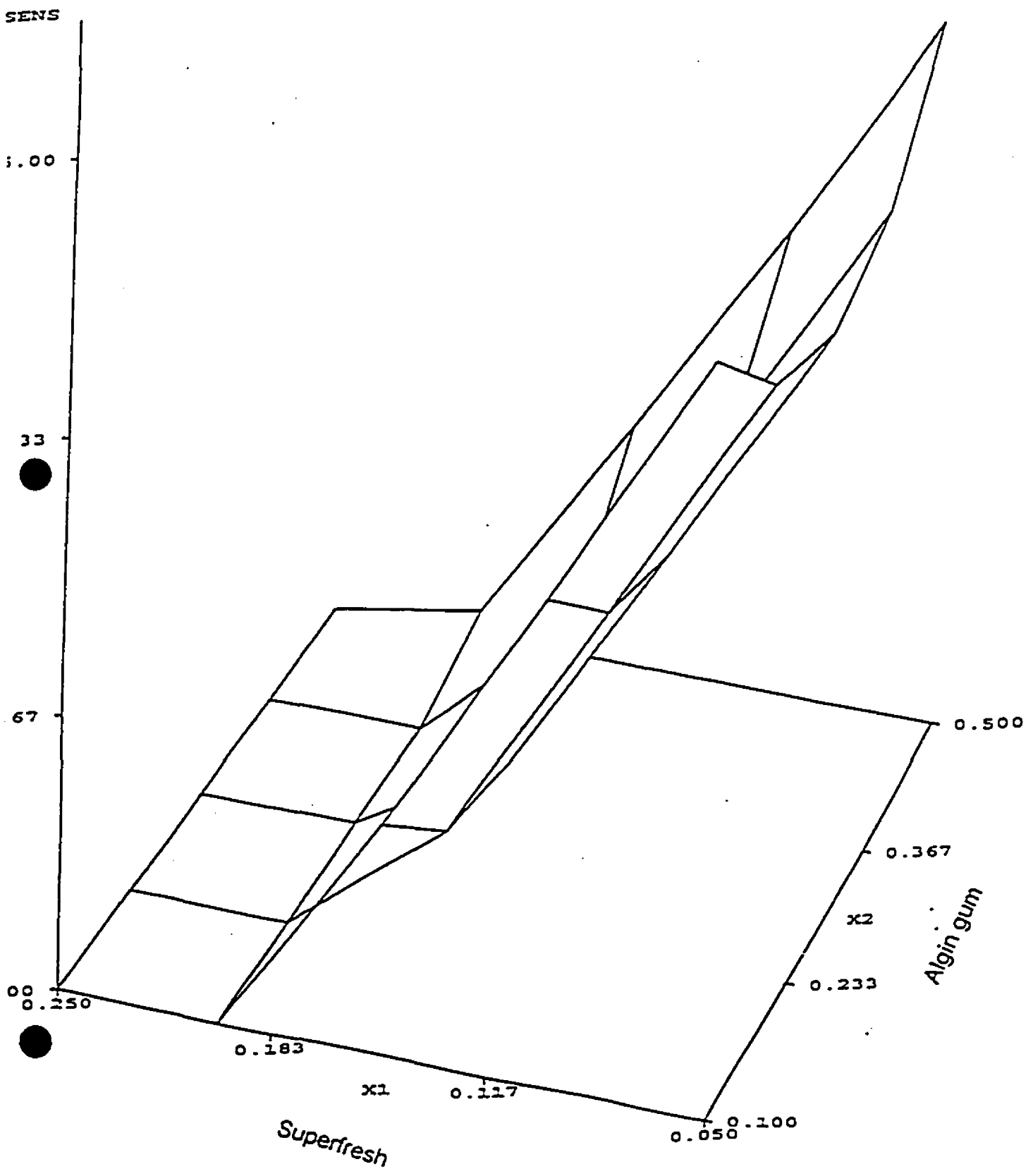
A response surface graph of enzyme versus algin with pectin held constant at 0.3% for sensory quality of bagels is shown in Figure 3.7. High sensory scores are possible through reformulation with low levels of both enzyme and algin gum or low levels of enzyme and high level of algin. However, canonical analysis of the fitted surface indicated that the stationary point was a minimum with the variable levels shown in Table 3.14.

Table 3.14: Actual values of variables at stationary point X_0 (Point of optimum sensory).

Variable	Actual Value (%)
Enzyme (X_1)	0.150
Algin (X_2)	0.293
Pectin (X_3)	0.335

These values gave a predicted sensory score of 2.78, well below the minimum sensory score of 3 for product acceptability. These values, used in the compressibility equation will lead to an outcome of 0.007, which is lower than expected since the sensory outcome is lower than the "standard" of 3.

Figure 3.4. Sensory of CCRD2
x3=0.3
Pectin gum



However, the values of the stationary points for texture and sensory are very close confirming the very high correlation (r=0.98) found between the two for texture and compressibility.

3.4. Conclusion:

In conclusion, this study has shown that using an RSM approach, several variables can be examined simultaneously to determine their combined effect on bagel texture and sensory qualities.

Based on these studies, Novamyl, guar gum and HFCS at all levels used, produced bagels with a 6 week textural shelf life and which were organoleptically acceptable on the basis of odor, flavor and texture at the end of this storage period. However, bagels reformulated with Superfresh, algin and pectin gums at most levels resulted in bagels which were unacceptable for both textural and sensorial standards of 0.01 MPa and sensory scores of <3, i.e., bagels were regarded as stale.

To test the validity of the predicted model, (CCRD1) to give a 6 week shelf life bagels were reformulated with levels of Novamyl, guar gum and HFCS shown at the stationary point for both texture and sensory quality of bagels (Tables 3.7, 3.8). Bagels were packaged with an oxygen absorbent and monitored for texture and sensory qualities over a 6 weeks period. At the end of this time the average scores for texture and sensory were 0.05 MPa and 4.2, i.e., reformulated bagels were highly acceptable from both a textural and sensory viewpoint. Furthermore, there was an excellent correlation (98%) between the predicted and actual values for both texture and sensory (overall acceptability) indicating the validity of the RSM approach to predict shelf life.

Further studies are now underway to determine the cost of the reformulated product and to produce the product under commercial conditions. Studies will also be done using other methods to delay staling.

CHAPTER 4: COMMERCIAL BAKING STUDIES

4.1. Introduction:

In previous studies, the effect of formulation changes to delay staling was first studied by using each ingredient alone ("one variable at a time approach") and then with selected ingredients in conjunction with each others (RSM approach). In these studies, all bagels were baked in a convection oven. The objectives of this study was to reformulate bagels, to bake them in a commercial wood oven and to compare the reformulated bagels with the commercial product.

4.2. Material and Method:

4.2.1. FORMULATION:

Four formulations (A-D) were made as shown in Table 4.1.

Table 4.1: Formulations used in commercial baking studies

Formula	Basic recipe	Novamyl	Guar Gum	HFCS	Superfresh	Algin Gum	Pectin Gum
A	+	+ (0.06%)	+ (0.7%)	+ (45%)	-	-	-
B	+	-	-	-	+ (0.2%)	+ (0.4%)	+ (0.2%)
C	+	+ (0.03%)	-	-	-	-	-
D	+	-	-	-	-	-	-

All basic recipes contained the same percentage of ingredients. With the exception of HFCS, all additional ingredients were added on a flour weight basis. HFCS was added as a percentage of sugar replacement. Bagels were formulated, then transported to R.E.A.L Bagel, Pointe-Claire, where they were mixed, proofed, boiled, dipped with sesame seeds and then baked in a wood oven under commercial conditions.

After baking, bagels were cooled and transported in boxes to the department of Food Science for packaging. All bagels were packaged in 20X20

cm high gas barrier Cryovac bags (2 bagels per bag) with an Ageless FX-100 oxygen absorbent to prevent mold growth throughout storage. Bagels were stored at ambient temperature and monitored for textural and sensorial changes throughout storage.

4.2.2. TESTING:

Sensory analysis was conducted with 30 untrained panelists (students and staff) under controlled conditions. Bagels (A, B, C and D) were evaluated using a hedonic scale of 1 to 5 (1- dislike extremely to 5- like extremely) on days 0, 7, 14, 28 and 42. Bagels were rejected when a score of <3 was noted. In addition, panelists were asked to rank samples in order of preferences (1-4).

Compressibility tests for texture were also carried out on bagels, on the same test days using an Instron Universal Testing Machine as described in section 2.2.5. A compressibility of 0.01 MPa was again used as the "staling standard".

4.3. Results and Discussion:

The results of compressibility (texture) tests on bagels formulations A-D are shown in Table 4.2.

As expected commercial, control bagels (D), had a score of >0.01 MPa after day 7 and were therefore stale. However, all other formulations remained stale free (from a textural viewpoint) until day 42. These results confirmed previous reformulation studies using enzymes alone or in conjunction with other ingredients. Statistically, compressibility results were very significant ($P<0.001$) with an R^2 of 71% for compressibility. Furthermore, the compressibility values obtained in this study were very close to the predicted levels found at the stationary point of CCRD1. For example, if the level of ingredients used in formulation A were computed in the multiple regression

equation $Y_{texture}$ (Equation 1, section 3.3.1) a compressibility of 0.008 would be predicted while an actual value of 0.0069 was observed for compressibility. This confirms earlier comments on the validity of the model from CCRD1 to predict the test results. For the CCRD2 design, a compressibility of 0.0068 would be predicted for the level of Superfresh, algin and pectin used in this study (Equation 1, section 3.3.2). This agrees favorably with an actual compressibility of 0.0064.

The results for sensory analysis were also very interesting. The results for overall acceptability are shown in Table 4.3. The commercial bagel (formulation D) was also rejected after day 7 as it had a stale flavor. Thus, the sensory results agree with textural results for commercial bagels. In general, most bagels were stale within 2-3 days or earlier depending on their formulation so these results are in general agreement with other reported results on staling of bakery products (Maga, 1975).

While the addition of Novamyl (Formulation C) to the basic recipe, improved the textural shelf life , the sensory shelf life was limited to 28 days, which is also in agreement with earlier studies (Table 2.5). Several panelists reported that these bagels were too sweet. This can be attributed to the amyolytic activity of the enzyme on starch and producing glucose. This problem could be overcome by reducing the level of enzyme or sugar in the initial recipe. However, sufficient glucose is needed to be left for yeast fermentation.

Formulations A and B gave the best sensory results with formulation B (Superfresh, algin and pectin) giving slightly better results. The results for formulation A agree with previous results for optimization studies (section 3.3.1). While this formulation was highly acceptable to most panelists, some also found the reformulated bagels too sweet due to the additional glucose being formed from enzyme activity and HFCS. However overall, panelists rated this product as very acceptable due to the moist mouth feel of the product caused by the hygroscopic ratio of glucose/HFCS.

The results of formulation B were almost identical to formulation A. Indeed, formulation B was preferred by most panelists from day 1. These results were slightly contradictory to optimization studies using these variables. However, this study clearly shows that, when used at appropriate levels, these ingredients can result in very acceptable bagels, which is in agreement with the results observed with formulations used in the CCRD2 design (Table 3.11).

Panelists ranking of bagels are shown in Figures 4.1-4.5. As shown, formulation D (control bagels) were ranked 4th by the majority of the panelists from day 1, while formulation B, A and C, were ranked 1st, 2nd and 3rd throughout the storage. After 6 weeks of storage, formulation B was ranked 1st by 15 panelists (out of 30) and second by 13 panelists. Formulation A, was ranked 1st by 9 panelists and 2nd by 10 panelists. Formulation C was ranked 3rd by 17 panelists. Finally, formulation D, was ranked 4th by 22 panelists (Figure 4.5).

4.4. Conclusion:

In conclusion, this study has shown that, with appropriate formulation changes and baked under commercial conditions, highly acceptable bagels can be produced from both a sensory and a textural viewpoint. With appropriate addition of enzyme and/or guar gum/HFCS/algin and pectin, bagels can remain stale free for at least 42 days and remain highly acceptable for consumers.

Table 4. 2: Compressibility results of formulations used in commerecial bbaking studies.

	Compressibility***				
Formulation	**Day 0**	**Day 7**	**Day 14**	**Day 28**	**Day 42**
A	0.00302	0.00404	0.00498	0.00494	0.00594
B	0.00414	0.00460	0.00450	0.00478	0.00536
C	0.00378	0.00444	0.00508	0.00514	0.00574
D	0.00732	0.01046	0.01204	0.01368	0.01486

*** Significant with p=0.0001
A: Novamyl + Guar Gum + High Fructose Corn Syrup
B: Superfresh + Pectin + Algin
C: Novamyl
D: Control

Table 4.3: Sensory results of formulations used in commercial baking studies.

	Sensory***				
Formulation	**Day 0**	**Day 7**	**Day 14**	**Day 28**	**Day 42**
A	3.00	3.56	3.26	3.36	3.36
B	3.43	3.86	3.53	3.73	3.70
C	3.00	3.43	3.23	2.73	2.80
D	3.04	2.46	2.00	1.83	2.20

*** Significant with p=0.0001
A: Novamyl + Guar Gum + High Fructose Corn Syrup
B: Superfresh + Pectin + Algin
C: Novamyl
D: Control

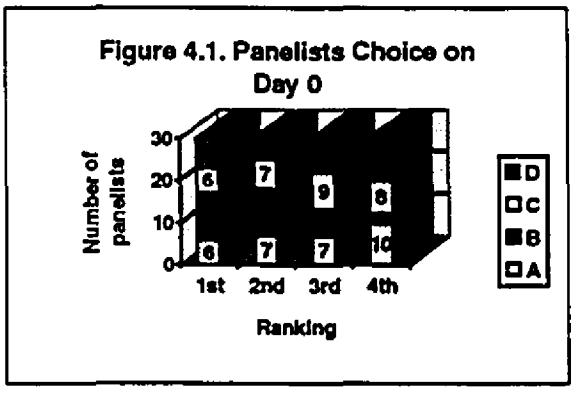


Figure 4.1. Panelists Choice on Day 0
Number of panelists
30
20
10
0
6
7
9
8
6
7
7
10
1st
2nd
3rd
4th
Ranking
D
C
B
A

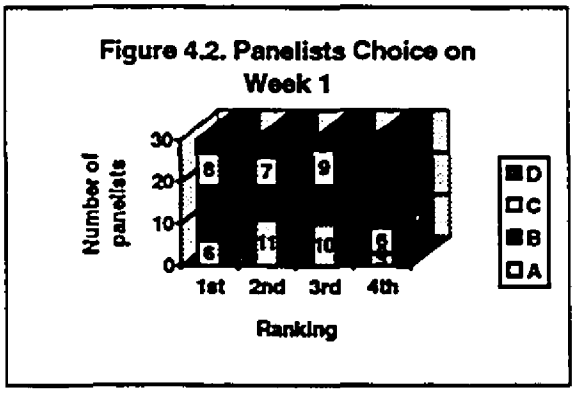


Figure 4.2. Panelists Choice on Week 1
Number of panelists
30
20
10
0
8
7
9
6
11
10
6
1st
2nd
3rd
4th
Ranking
D
C
B
A

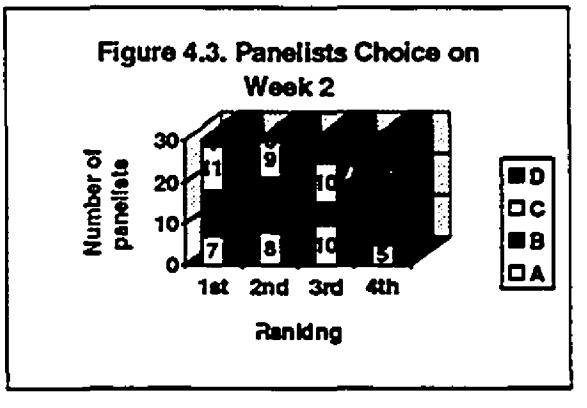


Figure 4.3. Panelists Choice on Week 2
Number of panelists
30
20
10
0
11
9
7
8
10
5
1st
2nd
3rd
4th
Ranking
D
C
B
A

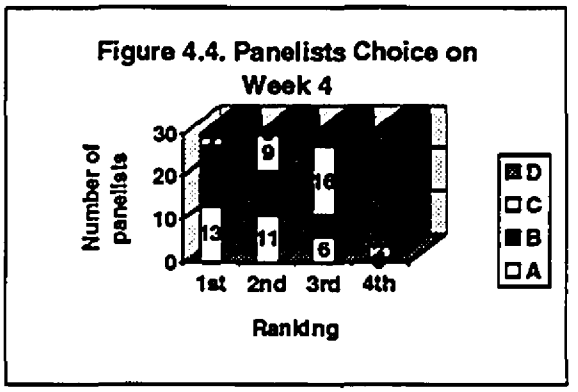


Figure 4.4. Panelists Choice on Week 4
Number of panelists
30
20
10
0
13
11
6
1st
2nd
3rd
4th
Ranking
D
C
B
A

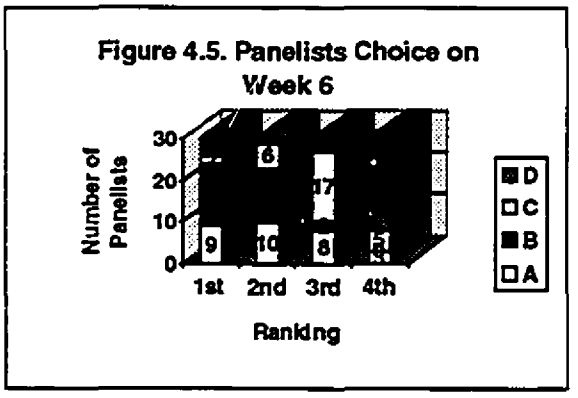


Figure 4.5. Panelists Choice on Week 6
Number of Panelists
30
20
10
0
9
8
1st
2nd
3rd
4th
Ranking
D
C
B
A

CHAPTER 5: ADDITIONAL ANTI-STALING STUDIES

5.1. Introduction:

Studies to date have focused on formulation changes to delay staling and enhance product shelf life. However, other factors such as storage atmosphere, storage temperature and method of production (i.e., retarding or non-retarding) may also influence the texture of the product. Therefore, additional studies were done to determine if these storage/ processing factors had any effect in delaying staling in bagels.

5.2. Effect of CO_2 on staling:

5.2.1. INTRODUCTION:

Several studies have shown that gas packaging in a CO_2 enriched atmosphere can be used to extend the mold free shelf life of baked products. Furthermore, some studies have shown that in addition to its antimycotic effect, CO_2 may also have an anti-staling effect, although results to date have been contradictory.

Therefore, the objective of this study was to confirm the antimycotic effect of CO_2 and to determine its effect, if any, on staling.

5.2.2. MATERIALS AND METHODS:

5.2.2.1. *Formulation:*

A standard bagel recipe, as outlined in section 2.2.3., was used through this study. To determine the effect of CO_2 on shelf life, 4 processing/ packaging conditions were investigated. These were:

A. Flushing dough with CO_2 during mixing/ packaging in 100% CO_2;

B. Flushing dough with CO_2 during mixing/ packaging with an Ageless FX-100 oxygen absorbent;

C. Packaging baked bagels in 100% CO_2;

D. Packaging bagels with an Ageless FX oxygen absorbent; and

E. Packaging bagels in air.

In A and B, CO_2 was flushed directly into the dough in the Hobart mixer for ~10 mins until dough was properly developed. The dough was then proofed at room temperature for ~10 mins, cut in 75g portions, formed, boiled, dipped in sesame seeds and baked as described in section 2.2.3.

In C, D and E, bagels were mixed, proofed, cut, formed, boiled, dipped in sesame seeds and baked as described in section 2.2.3.

5.2.2.2. *Packaging:*

All bagels were packaged in 20x20 cm Cryovac bags (2 per bags). Formulations A and C were packaged/ sealed with 100% CO_2 in a Multivac chamber type heat seal packaging machine (Model 4300/4s, Multivac Wolfertachwenden, Germany). A Smith proportional gas mixer, model 299-028 (Tescom Corporation, Minneapolis, Minnesota 55441, USA), was used to give the desired proportion of CO_2 in the package headspace. Gases (CO_2 and N_2) were obtained from Medigas Ltd (Quebec, Canada). Formulations B and D were packaged with an Ageless FX-100 oxygen absorbent taped inside the bag. All packages were sealed manually using an impulse heat sealer. Control bagels (E) were packaged in air as described above.

All packaged bagels were stored at 25°C and monitored for visible signs of mold growth. Textural and sensory analysis were done at day 0, 7, 14, 28 and 42 as described previously in section 2.2.5.

5.2.3. RESULTS AND DISCUSSION:

The antimycotic effect of various gas atmosphere on mold growth on bagels are shown in Table 5.1.

Table 5.1: Effect of packaging conditions on mold spoilage of bagels.

Formulation	Packaging conditions	Days to visible mold growth
A	100% CO_2	NG*
B	Ageless FX absorbent	NG
C	100% CO_2	NG
D	Ageless FX absorbent	NG
E	Air	5-6

* NG= No growth after 42 days.

Mold growth was visible in all air packaged bagels after 5-6 days at ambient storage temperature. However, by packaging bagels in either 100% CO_2 or with an Ageless type FX-100 oxygen absorbent mold growth could be inhibited throughout the 42 day storage period. These results are in agreement of previous studies by Smith et al. (1996) and confirm the antimycotic effect of high CO_2 levels and low O_2 levels on mold growth.

The results for textural and sensory changes throughout storage are summarized in Table 5.2. Shelf life in days was determined from graphical results when a compressibility of 0.01 MPa and a sensory score of <3 was reached (results not shown).

Table 5.2: Effect of packaging conditions on textural and sensorial shelf life of bagels.

Formulation	Dough flushed with CO_2	Packaging atmosphere	Shelf life	
			Texture	Sensory
A	+	100% CO_2	~14	~14
B	+	Ageless FX	~14	~14
C	-	100% CO_2	~42	~21
D	-	Ageless FX	<7	<7
E	-	Air	<7	<7

Air packaged bagels were stale in <7 days as observed previously. Flushing bagels with 100% CO_2 during mixing and subsequently packaging in 100% CO_2 or with oxygen absorbents had little effect on either the textural or sensory shelf life. Indeed, bagels were staler than non-flushed bagels packaged in either CO_2 or with an oxygen absorbent (C and D). Furthermore,

bagels were rejected after 14 days due to an acidic sharp taste which can be attributed to either the CO_2 in the dough or absorption of CO_2 from the packaging atmosphere (Formulation A). These results are contrary to the observations of Knorr (1987) who reported that flushing enriched white bread dough under a CO_2 atmosphere resulted in a softer bread. However, in these studies Knorr (1987), flushed CO_2 during the fermentation (proofing) stage and not during mixing as in our study. This latter route was taken as bagels formulated in our study had a limited proofing or fermentation time. However, our results agree with the observations of Knorr and Tomlins (1985) who reported that French bread and white bread packaged under 100% CO_2 were significantly softer than air stored samples. As shown in Figure 5.1. bagels packaged under 100% CO_2 had a compressibility of 0.009 after 42 days at room temperature i.e., within the "staling standard" of 0.01 MPa. However, while textural shelf life was acceptable, bagels were rejected after 21 days again due to sharp acidic taste probably caused in dissolution of headspace CO_2 in the aqueous phase of the product.

Finally, bagels packaged with an oxygen absorbent (Formulation D) had a textural and sensory shelf life of less than 7 days i.e., no better than control bagels.

5.2.4. CONCLUSION:

In conclusion, the results confirm earlier observation that flushing CO_2 into the dough during the mixing stage does not have a beneficial effect on crumb texture i.e., staling. It has also shown that packaging bagels in 100% CO_2 could be a useful alternative to reformulation to delay staling. While the exact anti-staling mechanism of CO_2 is not known, it may affect the hydrogen capacity of proteins which would have a plasticizing effect on starch-protein interactions. However, this warrants further investigation.

5.3. Effect of retarding on staling:

5.3.1. INTRODUCTION:

Bagels used in all studies to date were formulated and baked without a retardation stage i.e., similar to traditional, commercial Montreal bagels. However, many bagels are made with a controlled retardation step in their production, followed by proofing at room temperature prior to baking. During the retardation stage, gluten structure is more fully developed and results in a bagel with a slightly greater volume.

The objectives of this study was to determine if the retardation step had an effect on staling.

5.3.2. MATERIALS AND METHODS:

Bagels were formulated with and without 0.03% Novamyl (flour weight). One set of control bagels and one set of bagels containing enzyme were proofed, formed, boiled, baked and packaged as described in section 2.2.3. The other set were covered with plastic, retarded for 12 hours at refrigeration temperature. Upon removal from the retarder, they were then proofed to reach room temperature and treated as outlined above.

In this study, bagels were evaluated for change in texture only at days 7, 21, 35 and 42 using the Instron testing machine as described in section 2.2.5.

5.3.3. RESULTS AND DISCUSSION:

The results for control and enzyme treated bagels are shown in Table 5.3.

Table 5.3: Compressibility results of retarded versus non-retarded bagels.

Formulation	Days of Storage			
	7	21	35	42
Control (non retarded)	0.0101	0.0109	0.0120	0.0125*
Control (retarded)	0.0105	0.0112	0.0125	0.0129*
Novamyl (retarded)	0.0045	0.0074	0.0072	0.0079*
Novamyl (non-retarded)	0.0059	0.0067	0.0080	0.0081***

*,**,*** significant with p<0.05, 0.005, 0.0005.

Both non-retarded and retarded control bagels were stale after 7 days and became progressively staler with time agreeing with previous results. Enzyme treated bagels, as expected, remained stale free for the duration of the 42 day storage period. Furthermore, there was no significant differences between retarded and non-retarded, control and enzyme treated bagels, indicating that retardation had no effect on the staling process. All results were highly significant ($P<0.05$) with R^2 values of 77 and 92% respectively for retarded and non-retarded samples. Finally, there was a high correlation (83%) between non-retarded and retarded results confirming that retardation had no effect on staling.

5.3.4. Conclusion:

Retardation proved to have no effect on bagel texture. However, retarded bagels had a slightly larger volume than non-retarded bagels due to the longer time allowed for gluten development.

5.4. Effect of temperature on staling:

5.4.1. INTRODUCTION:

Temperature, particularly low temperature, has been shown to influence staling properties of bakery products. Freezing has been shown to delay staling in bread while refrigeration has been shown to increase the rate of staling (Maga, 1975). The beneficial effect of freezing is due to the decreased rate of starch recrystallization; however, at refrigeration temperature, starch recrystallization is enhanced resulting in a staler product. While most studies to date have focused on storage and staling of bakery products at low temperature, the effect of storage at high temperature has been less investigated. Since bagels, particularly reformulated bagels, are stored and shipped under ambient storage conditions, the objective of this study was to monitor the effect of high storage temperature on staling in bagels.

5.4.2. MATERIALS AND METHODS:

Bagels (control, enzyme treated (0.03% Novamyl), and bagels containing 1.5 glucose) were made as described in section 2.2.3. Bagels were in 20x20 cm Cryovac bags containing an Ageless FX-100 oxygen absorbent. Compressibility tests were done as described in section 2.2.5. One set (all treatments) of bagels was stored at ambient temperature, while the other set was stored at 60°C in an incubator.

5.4.3. RESULTS AND DISCUSSION:

The results for bagels stored at 25 and 60°C are shown in Table 5.4.

Table 5.4: Compressibility results of bagels stored at different temperature.

Formulation	Storage temp. (°C)	Days			
		7	14	28	42
Control	25	0.01	0.0105	0.0115	0.0130*
Enzyme	25	0.004	0.006	0.0065	0.0080***
Control	60	>0.01*	-	-	-
Enzyme	60	>0.01*	-	-	-

*,**,*** significant with p<0.05, 0.005, 0.0005.

At 25°C, control bagels were stale after 7 days while enzyme treated bagels were texturally acceptable after 42 days. At 60°C, both control bagels and enzyme treated bagels were "brick" hard and no further compressibility tests were done on these bagels (Table 5.4). Furthermore, enzyme treated bagels were very dark in color and unacceptable from a sensory viewpoint. The darker color can be attributed to the Maillard reaction between additional sugar in the enzyme treated bagels with flour protein. These results were unexpected as storage at high temperature is supposed to improve the freshness of bagels. One possible explanation for the observed results may be that the Maillard reaction resulted in a decrease in carbohydrates. Since carbohydrates are highly hygroscopic and have a plasticizing effect on staling, a decrease in concentration will result in less moisture being held and hence to result in a firmer or stale crumb.

To determine the effect of additional glucose on staling and color, bagels were reformulated with 1.5x glucose content and stored at 60°C. The compressibility results are shown in Table 5.5.

Table 5.5: Compressibility results of bagels stored at 60°C.

Formulation	Days*		
	1	3	7
Control	0.006	0.140	-
1.5x sugar	0.003	0.005	0.016
Enzyme	0.003	0.006	0.016

*,**,*** significant with p<0.05, 0.005, 0.0005.

All control bagels were stale after 3 days at 60°C. Furthermore, all control bagels containing 1.5x sugar concentration and enzyme treated bagels were again "brick" hard after 7 days at 60°C. All bagels were again dark brown in color, with the exception of control bagel, indicating that additional glucose would appear to exert a role, not only in color (Maillard reaction) but also in staling. These results confirm that storage at 60°C does not enhance bagel quality, particularly enzyme treated products. Indeed, staling in enzyme treated bagels was actually enhanced at 60°C Furthermore, packages with enzyme treated bagels and those containing additional sugar had a blown appearance. Suspecting microbial activity, headspace gas analysis was done on these samples. However, O_2 and N_2 (20:80) were the only gases present inferring no microbial activity due to the absence of CO_2.

5.4.4. Conclusion

These results show that storage at high temperature, does not improve the stale free shelf life of bagels. Further studies need to be done at lower temperatures to determine the effect of temperature on bagels

CHAPTER 6: GENERAL CONCLUSION

Staling is still a major problem limiting the shelf life of many high and intermediate moisture bakery products. Losses due to staling have been estimated at 3-5% of annual production, resulting in millions of dollars in lost revenue for the baking industry. Therefore, methods to control staling and to extend the marketable shelf life of bakery product is of paramount importance to the baking industry to ensure its economical survival in an increasingly global and competitive marketplace.

Two approaches to delay staling were investigated in this study. The first approach is through packaging under a modified atmosphere involving elevated CO_2 levels. While packaging under 100% CO_2 delayed both mold growth and staling for ~6 weeks, products were rejected by consumers after 4 weeks due to the sharp acidic taste of CO_2 dissolved in the aqueous phase of the product.

The other and more successful approach was through reformulation studies involving various gums, surfactants, high fructose corn syrup (HFCS), enzymes and low protein flours. Results showed that a highly acceptable product from both a textural and sensorial viewpoint could be produced commercially through appropriate levels of enzyme, gum and HFCS in the formulation. Thus, both staling and mold growth could be prevented/delayed in bagels for ~6 weeks through appropriate reformulation and modified atmosphere packaging (MAP) using an oxygen absorbent.

The estimated cost for this shelf life extension is ~20-30 cents/ dozen bagels i.e., ~5 cents for reformulation costs, ~10-15 cents for high gas barrier bags and ~5-10 cents for an oxygen absorbent. This would increase the retail price of bagels from ~$ 3.50 to $ 3.70- 3.80 per dozen. However, the increased

costs could be defrayed through less returns and downgrading of products to croutons, less production costs through bulk processing/packaging and most importantly extended shelf life, market growth and increased profitability. Moreover, costs could be drastically reduced if the baker decided to incorporate preservatives e.g., sorbic acid into the product to delay mold growth instead of MAP. Yet another cost cutting approach would be to use the new generation of oxygen scavenging films which are gradually appearing on the marketplace.

Whatever the approach, this study has shown that staling can be delayed for 6 weeks or longer through reformulation with enzymes, gums and HFCS alone or in combination with each other.

Furthermore, reformulation and packaging offers the baking industry a viable approach to inhibit two potential spoilage concerns - staling and mold growth. However, further studies are required to determine the public health safety of these reformulated bakery products, particularly with respect to the growth of *Clostridium botulinum* in bagels stored under reduced oxygen tensions and at room temperature.

REFERENCES

1. Akers, A. A. and Hoseney, R.C. 1994. Water-soluble dextrins from α-amylase-treated bread and their relationship to bread firming. *Cereal Chemistry. 71: 223-226.*
2. Avital, Y., Mannheim, C.H. and Miltz, J. 1990. Effect of carbon dioxide atmosphere on staling and water relations in bread. *Journal of Food Science. 55: 413-416, 461.*
3. Baker, J.C. and Mize, M.D. 1939. Some observations regarding the flavor of bread. *Cereal Chemistry. 16: 295-297.*
4. Bechtel, W.G. and Meisner, D.F. 1951. Present status of the theory of bread staling. *Food Technology. 5: 503-505.*
5. Bechtel, W.G., Meisner, D.F. and Bradley, W. B. 1953. The effect of the crust on the staling of bread. *Cereal Chemistry. 30: 160-168.*
6. Bechtel, W.G. and Meisner, D.F. 1954a. Staling studies of bread made with flour Fractions III. Effect of crumb moisture and of tailings of starch. *Cereal Chemistry. 31: 176-181.*
7. Bechtel, W.G. and Meisner, D.F. 1954b. Staling studies of bread made with flour Fractions IV. Effects of gluten and wheat starch. *Cereal Chemistry. 31: 182-187.*
8. Black, R.P., Quail, V., Kuzyk, M. and Ruddick, L. 1993. Shelf-life extension of pita bread by modified atmosphere packaging. *Food Australia. 45: 387-391.*
9. Bowers, J. 1992. In *Food Theory and Applications.* Second Edition. Macmillan Publishing Company. New York. USA. pp. 269-276.
10. Box, G.E.P., Hunter, W.G. and Hunter J.S. 1978. *Statistics for Experimenters. An Introduction to Design Data Analysis and Model Building.* New York, John Wiley and Sons.
11. Boyacioglu, M.H. and D'Appolonia, B.L. 1994. Characterization and utilization of durum wheat for breadmaking III. Staling properties of bread baked from bread wheat flours and durum wheat flours. *Cereal Chemistry. 71: 34-41.*

12. Brody, A.L. 1989. In *Controlled/ Modified Atmosphere/ Vacuum Packaging of Foods.* Food and Nutrition Press, Inc. Trumbull, USA. pp. 1-16, 119-134, 159-174.

13. Bullerman, L.B. and Hartung, T.E. 1973. Mycotoxin-producing potential of molds isolated from flour and bread. *Cereal Science Today. 18: 346-347.*

14. Carlin, G.T. 1947. The function of fat in bread dough. *Baker's Digest. 21: 22-27.*

15. Christianson, D.D. and Gardner, H.W. 1974. Xanthan gum in protein-fortified starch bread. *Food Technology. 28: 23-26, 28-29.*

16. Conford, S.J., Axford, D.W.E. and Elton, G.A.H. 1964. The elastic modulus of bread crumb in linear compression in relation to staling. *Cereal Chemistry. 41: 216-229.*

17. Cross, D.E., Willhoft, E.M.A. and Wren, J.J. 1971. Migration of water from gluten to starch during bread staling. *Chemistry and Industry. pp. 174-176.*

18. D'Appolonia, B.L. and Morad, M.M. 1981. Bread staling. *Cereal Chemistry. 58: 186-190.*

19. De Stefanis, V.A., Ponte, J.G., Chung, F.H. and Ruzza, N.A. 1977. Binding of crumb softness and dough strengtheners during breadmaking. *Cereal Chemistry. 54: 13-19.*

20. Doerry, W.T. 1985. Packaging bakery foods in controlled atmospheres. *American Institute of Baking. 7: 1-8.*

21. Dragsdorf, R.D. and Varriano-Marston, E. 1980. Bread staling: x-ray diffraction studies on bread supplemented with α-amylases from different sources. *Cereal Chemistry. 57: 310-314.*

22. Edelmann, E.C., Carthart, W.H., and Berquist, C.B. 1950. The effect of various ingredients on the rate of firming of bread crumb in the presence of polyoxyethylene monostearate and glyceryl monostearate. *Cereal Chemistry. 27: 1-14.*

23. Ellis, W.O., Smith, J.P., Simpson, B.K., Ramaswamy, H.S. and Doyon, G. 1994. Effect of gas barrier characteristics of films on aflatoxin production by

Aspergillus flavus in peanuts packaged under MAP conditions. *Food Research International. 27: 505-512.*

24. Elton, G.A.H. 1969. Some quantitative aspects of bread staling. *Baker's Digest. 43: 24-28.*
25. External affairs and international trade Canada. 1991. Export opportunities in Japan. *The Bakery Mix Market. pp. 7-9.*
26. Fennema, O.R. 1985. In *Food Chemistry.* Second edition. Food Science and Technology, Marcel Dekker, Inc. New York. pp. 108-130.
27. Ghiasi, K., Hoseney, R.C. and Lineback, D.R. 1979. Characterization of soluble starch from bread crumb. *Cereal Chemistry. 56: 485-490.*
28. Ghiasi, K., Varriano-Marston, E. and Hoseney, R.C. 1982. Gelatinization of wheat starch II. Starch-surfactants interaction. *Cereal Chemistry. 59: 86-88.*
29. Graves, R.R., Rogers, R.F., Lyons, A.J. and Hesseltine, C.W. 1967. Bacterial and actinomycete flora of Kansas-Nebraska and pacific northwest wheat and wheat flour. *Cereal Chemistry. 44: 288-299.*
30. Hani, I. 1992. Effects of shortening and surfactants on quality parameters of Arabic bread. *M.Sc. Thesis. Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon.*
31. Hartung, T.E., Bullerman, L.B., Arnold, R.G. and Heidelbaugh, N.D. 1973. Application of low dose irradiation to a fresh bread system for space flights. *Journal of Food Science. 38: 129-131.*
32. He, H. and Hoseney, R.C. 1991. Differences in gas retention. Protein solubility and rheological properties between different baking flours. *Cereal Chemistry. 68: 526-530.*
33. Hebeda, R.E., Bowles, L.K., Teague, W.M. 1991. Use of intermediate temperature stability enzymes for retarding staling in baked goods. *Cereal Foods World. 36: 619-620, 622, 624.*
34. Hoseney, R.C. 1986. In *Principles of Cereal Science and Technology.* American Association of Cereal Chemists. Minnesota. USA. pp. 69-88.

35. Hunt, L. and Robbins, L. 1989. Food expenditure patterns of Canadian consumers, 1986. *Food Market Commentary. 11: 42-51.*

36. Jackel, S.S. 1989. The control of bread staling. *Cereal Foods World. 34: 430-431.*

37. Jenkins, S.M. 1975. In *Bakery Technology.* Book 1. First Edition. Lester and Orpen Limited. Toronto. Canada. pp. 223-239.

38. Kamel, B.S. 1993. Surfactants in bakery foods. *American Institute of Baking. 15: 1-12.*

39. Kent-Jones, D.W. and Price, J. 1951. In *The Practice and Science of Bread Making.* Second edition. The Northern Publishing Company. Liverpool, England. pp. 1-7.

40. Kim, S.K. and D'Appolonia, B.L. 1977a. Effect of pentosans on the retrogradation of wheat starch gels. *Cereal Chemistry. 54: 150-160.*

41. Kim, S.K. and D'Appolonia, B.L. 1977b. Bread staling studies II. Effect of protein content and storage temperature on the role of starch. *Cereal Chemistry. 54: 216-224.*

42. Kim, S.K. and D'Appolonia, B.L. 1977c. Bread staling studies III. Effect of pentosans on dough, bread and bread staling rate. *Cereal Chemistry. 54: 225-229.*

43. Knorr, D. and Tomlins, R.I. 1985. Effect of carbon dioxide modified atmosphere on the compressibility of stored baked goods. *Journal of Food Science. 50: 1172-1173, 1176.*

44. Knorr, D. 1987. Compressibility of baked goods after carbon dicxide atmosphere processing and storage. *Cereal Chemistry. 64: 150-153.*

45. Krog, N. and Jensen, B.N. 1970. Interaction of monoglycerides in different physical states with amylose and their antifirming effect in bread. *Journal of Food Technology. 5: 77-87.*

46. Kulp, K. 1979. Staling of bread. *American Institute of Baking. 1: 1-7.*

47. Kulp, K. and Ponte, J.G.Jr. 1981. Staling of white pan bread: fundamental causes. *CRC Critical Reviews in Food Technology. 15: 1-48.*

48. Lues, J.F.R., Vilijoen, B.C., Miller, M. and Prior, B.A. 1993. Interaction of non-culture microbial flora on dough fermentation. *Food Microbiology. 10: 205-213.*

49. Maga, J.A. 1975. Bread staling. *CRC Critical Reviews in Food Technology. 5: 443-486.*

50. Mahan, L.K. and Arlin, M. 1992. In *Krause's Food Nutrition & Diet Therapy.* Eighth Edition. W.B. Saunders Company. Philadelphia. United States of America. pp. 45-56.

51. Maleki, M., Hoseney, R.C. and Mattern, P.J. 1980. Effects of loaf volume. moisture content, and protein quality on the staling rate of bread. *Cereal Chemistry. 57: 138-140.*

52. Martin, M.L., Zeleznack, K.J. and Hoseney, R.C. 1991. A mechanism of bread firming I. Role of starch swelling. *Cereal Chemistry. 68: 498-503.*

53. Martin, M.L. and Hoseney, R.C. 1991. A mechanism of bread firming II. Role of starch hydrolyzing enzymes. *Cereal Chemistry. 68: 503-507.*

54. Matz, S.A. 1992. In *Bakery Technology and Engineering.* Third Edition. Van Nostrand Reinhold. New York. United States. pp. 777-794.

55. Merriam-Webster's Collegiate Dictionary. 1995. 10th edition. Merriam-Webster's Incorporated. Springfield, Massachusetts, U.S.A. pp. 1084.

56. Mettler, E. and Seibel, W. 1993. Effects of emulsifiers and hydrocolloids on whole wheat bread quality: A response surface methodology study. *Cereal Chemistry. 70: 373-377.*

57. Miller, B.S., Johnson, J.A. and Palmer, D.L. 1953. A comparison of cereal, fungal and bacterial α-amylase as supplements for bread making. *Food Technology. 7: 38-41.*

58. Montgomery, D.C. 1984. Response surface methodology. *Design and Analysis of Experiments.* Second edition. New York, John Wiley and Sons.

59. Ooraikul, B. 1982. Gas packaging for a bakery product. *Canadian Institute Food Science and Technology Journal. 15: 313-317.*

60. Osman, E.M., Leith, S.J. and Fles, M. 1961. Complexes of amylose with surfactants. *Cereal Chemistry. 38: 449-463.*

61. Parry, R.T. 1993. In *Principles and applications of modified atmosphere packaging of food.* Blackie Academic and Professional. Glasgow, UK. pp. 1-19, 134-169.

62. Peat Marwick Group. Department of External Affairs. Canada. 1991. *Bakery products: Studies in Canadian export opportunities in the U.S market. pp. 4-10.*

63. Pirrie, P.G. 1940. Stale problems and salesman relationships among house-to-house bakers. *Baker's Weekly. 108: 37-39.*

64. Pisesookbunterng, W. and D'Appolonia, B.L. 1983. Bread staling studies I. effect of surfactants on moisture migration from crumb to crust and firmness values of bread crumb. *Cereal Chemistry. 60: 298-305.*

65. Platt, W. and Powers, R. 1940. Compressibility of bread crumb. *Cereal Chemistry. 17: 601-604.*

66. Pomeranz, Y., Rubenthaler, G.L., Daftary, R.D. and Finney, K.F. 1966. Effects of lipids on bread baked from flour varying widely in bread-making potentialities. *Food Technology. 20: 131-134.*

67. Pomeranz, Y. 1973. From wheat to bread: a biochemical study. *American Scientist. 61: 683-691.*

68. Pomeranz, Y., Huang, M. and Rubenthaler, G.L. 1991. Steamed bread III. Role of lipids. *Cereal Chemistry. 68: 353-356.*

69. Ponte, J.G. 1971. In *Wheat Chemistry and Technology.* Second edition. American Association of Cereal Chemists, St. Paul, Minn. pp. 675-679.

70. Prentice, N., Cuendet, L.S. and Gedees, W.F. 1954. Studies on bread staling V. Effect of flour extraction and various starches on the firming of bread crumb. *Cereal Chemistry. 31: 188-206.*

71. Rogers, P.E., Zeleznack, K.J., Lai, C.S. and Hoseney, R.C. 1988. Effect of native lipids, shortening and bread moisture on bread firming. *Cereal Chemistry. 65: 398-401.*

72. Schoch, T.J. and French, D. 1947. Studies on bread staling I. The role of starch. *Cereal Chemistry. 24: 231-249.*

73. Schultz, A.S., Schoonover, F.D., Fisher, R.A. and Jackel, S.S. 1952. Retardation of crumb starch staling by bacterial α-amylases. *Cereal Chemistry. 29: 200-206.*

74. Seiler, D.A.L. 1989. In *Controlled/Modified Atmosphere/Vacuum Packaging of Food.* Trumbell. CT: Food and Nutrition Press. pp. 119-133.

75. Smith, J.P., Simpson, B.K., Khanizadeh, S., Ooraikul, B. and Jackson, E.D. 1990. Control of a fermentation problem in a gas packaged bakery product using a Response Surface Methodology approach. *Food Microbiology. 7: 147-161.*

76. Smith, J.P. 1994. Modified atmosphere packaging for bakery products. *American Institute of Baking. 16: 1-9.*

77. Smith, J.P. and Simpson, B.K. 1995. MAP of Bakery and Pasta Products. In: *Principles of Modified Atmosphere Packaging and Sous-Vide Processing.* Eds. J. Farber and K.L. Dodds. Technomic Publishing Co., Lancaster, P.A., U.S.A. pp. 207-242.

78. Smith, J.P. and Simpson, B.K. 1996. Modified atmosphere packaging for shelf life extension of bakery products. In *Technology and techniques for freshness of baked products.* (Ed. R. Hebeda) Marcell Dekker Publishing Co., New York, N.Y. (In press).

79. Sugihara, T.F. 1985. In *Microbiology of Bread Making. Microbiology of Fermented Foods.* Wood. B.J.B. London. Elsevier Applied Science. pp. 249-261.

80. Sultan, W.J. 1990. In *Practical Baking.* Fifth Edition. Van Nostrand Reinhold. New York. United States. pp. 81-777.

81. Torres, P.I., Ramirez-Wong, B., Serena-Saldivar, S.O. and Rooney, L.W. 1993. Effect of sorghum flour addition on the characteristics of wheat flour tortillas. *Cereal Chemistry. 70: 8-13.*

82. Toufeili, I., Dager, S., Shadarevian, S., Noureddine, A., Sarakbi, M. and Farran, M. 1993. Formulation of gluten free pocket-type flat bread:

Optimization of methylcellulose, gum arabic and egg albumin level by response surface methodology. *Cereal Chemistry. 70: 373-377.*

83. Toufeili, I., Sleiman, G., Abu Salman, R. and Brockway, B. 1994. E[illegible] staling on viscoelastic properties of pastes prepared from Arabic bread. *Journal of the Food Science and Agriculture. 64: 271-273.*

84. Valjakka, T.T., Ponte, J.G.Jr. and Kulp, K. 1994. Studies on a raw-starch digesting enzyme I. Comparison to fungal and bacterial enzymes and an emulsifier in white pan bread. *Cereal Chemistry. 71: 139-144.*

85. Willhoft, E.M.A. 1971a. Bread staling I. Experimental study. *Journal of the Science of Food and Agriculture. 22: 175-180.*

86. Willhoft, E.M.A. 1971b. Bread staling II. Theoretical study. *Journal of the Science of Food and Agriculture. 58: 180-183.*

87. Wilhoft, E.M.A. 1977. Mechanism and theory of staling of bread and baked goods and associated changes in textural properties. *Journal of Textural Studies. 4: 292-295.*

88. Yasunaga, T., Bushuk, W. and Irvine, G.N. 1968. Gelatinization of starch during bread baking. *Cereal Chemistry. 45: 269-279.*

89. Zobel, H.E. 1973. A review of bread staling. *Baker's Digest. 44: 54-61.*