THE EFFECT OF ENZYMES AND STARCH DAMAGE ON WHEAT FLOUR

TORTILLA QUALITY

A Thesis

by

SAPNA ARORA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2003

Major Subject: Food Science and Technology

THE EFFECT OF ENZYMES AND STARCH DAMAGE ON WHEAT FLOUR

TORTILLA QUALITY

A Thesis

by

SAPNA ARORA

Submitted to Texas A&M University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Approved as to style and content by:

_____
Ralph D. Waniska
(Chair of Committee)

_____
Lloyd W. Rooney
(Member)

_____
Luis Cisneros
(Member)

_____
Rhonda Miller
(Chair of Food Science and Technology)

_____
Mark Hussey
(Head of Department)

December 2003
Major Subject: Food Science and Technology

ABSTRACT

The Effect of Enzymes and Starch Damage on Wheat Flour Tortilla Quality.

(December 2003)

Sapna Arora, B.Sc., Delhi University;

M.Sc., Mysore University, India

Chair of Advisory Committee: Dr. Ralph D.Waniska

Specific enzymes have been used to improve flour quality for bread but enzyme action in tortilla flour has not been investigated. Two different wheat flours were prepared into tortillas using laboratory-scale, commercial equipment with fixed processing parameters. Dough and tortilla properties were evaluated using subjective and objective methods. Tortillas were stored in plastic bags at 22°C for evaluation. The effects of nine enzymes (amyloglucosidase 1, amyloglucosidase 2, bacterial 1, bacterial 2, fungal, maltogenic 1, maltogenic 2, malted barley and xylanase) on quality of wheat flour tortillas were evaluated. Dough absorption was adjusted to attain uniform dough for tortillas. Enzyme addition to tortilla flour did not significantly affect tortilla weight, moisture and pH.

Bacterial 2 amylase extended shelf stability while maltogenic 1 and xylanase exhibited smaller improvements in shelf stability and other tortilla properties. Addition of 0.05 activity unit bacterial 2 amylase improved tortilla diameter and improved tortilla shelf life from 12 to 28 days. Maltogenic 1 at 280 ppm improved tortilla diameter, opacity and shelf life. Addition of 100 ppm of xylanase effectively improved tortilla

diameter and shelf life. Bacterial 1 amylase at 60 ppm improved tortilla diameter but did not improve shelf stability. Amyloglucosidase 2, maltogenic 2 and malted barley amylase did not improve tortilla quality at any of the evaluated levels. Amyloglucosidase 1 and fungal amylase reduced overall tortilla quality at all the evaluated levels.

Bread-making quality of wheat flour is correlated with the damaged starch present in the flour. Damage was induced by grinding the samples for 0, 1, 4 and 8 hr to determine the effects of starch damage on tortilla quality. Processing increased starch damage of control tortilla flour from 5.4% to 12.6%. Damage starch increased dough water absorption, toughness and press rating and reduced diameter and opacity of tortillas. Damage starch improved tortilla rollability at higher levels but did not improve tortilla properties in combination with bacterial 2 amylase. Overall tortilla quality was not improved due to additional starch damage. Improved tortilla quality using bacterial 2 amylase at very low levels could be commercialized.

DEDICATION

This thesis is dedicated to my parents, my husband Ashish, my sister Radhika and my brother Saurabh.

With your support and help it became easier to face the challenges.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

TABLE   Page

CHAPTER I

INTRODUCTION

Traditionally flour tortillas have been consumed widely in Mexico but today tortillas have gone from an ethnic food to mainstream in the United States. Today majority of tortillas are made from either wheat or corn and are used for burritos, enchiladas and fajitas. Tortilla consumption (corn and wheat) is likely to outpace the combined consumption of all other types of ethnic and specialty bread products based on TIA figures. Tortilla industry is estimated to become a $6 billion industry by the end of 2004 (TIA 2002, 2003).

Consumers prefer tortillas that are flexible, opaque and large in diameter and have long shelf life (Bello et al 1991, Cepeda et al 2000). However, during storage there are changes that cause the firming of these products. To provide the desired quality, functional ingredients and additives are added to flour during processing. Chemical leavening agents, acidulants, preservatives, reducing agents and emulsifiers (Saldivar 1988, Waniska 1999) are usually added to improve the appearance and extend the shelf stability.

Enzymes have been added to bread for more than 100 years. The most extensively used enzymes are amylases, lipases, hemicellulases, pentosanases, proteases and oxidases (Martinez et al 1998, Gray and Bemiller 2003).

_____

This thesis follows the style and format of Cereal Chemistry.

Besides generating fermentable compounds, α-amylases have an anti-staling effect on bread and improve the softness retention of baked goods (De Stefanis and Turner 1981, Cole 1982).

The use of enzymes to overcome the loss of freshness in corn tortillas has been reported. Amylases have shown improvement in the texture of corn tortillas over storage (Aida 1996). Addition of amylase improves the shelf life of corn tortillas but needs CMC to improve texture (Bueso 2003, Silva 2003).

The optimum enzyme source, enzyme activity and starch damage in flour tortillas will be investigated. Different enzymes and levels of starch damage will be evaluated to determine which improves wheat tortilla properties during storage. There is a potential to improve tortilla flour quality by adding specific enzymes since no such studies have been conducted in wheat flour tortillas.

Specific Objectives

The goal of this project is to improve wheat flour tortillas using carbohydrate hydrolyases. Both the enzyme activity and substrate level need to be established to compare several enzymes and wheat flours. Thus the objectives were to:

1. Determine the relationship between enzyme activities from different sources using the falling number apparatus.

2. To evaluate the effects of different enzymes on tortilla properties and determine the optimum enzyme activity for improved tortilla quality.

3. Determine the effect of starch damage and enzyme levels on tortilla properties.

CHAPTER II

LITERATURE REVIEW

Wheat and Wheat Products

Wheat is the earliest food crop used for human food processing. World wheat (*Triticum aestivum*) production totaled 1.62 billion bushels in 2002. Wheat is the third largest cereal grain crop produced in the world and the second leading crop grown in US (Fig.1). Wheat flour is the main ingredient in most breads, bakery products, biscuits, cookies, crackers, noodles and processed foods such as prepared breakfast cereals, sauces, gravies, soups, confectionery products and flour tortillas.
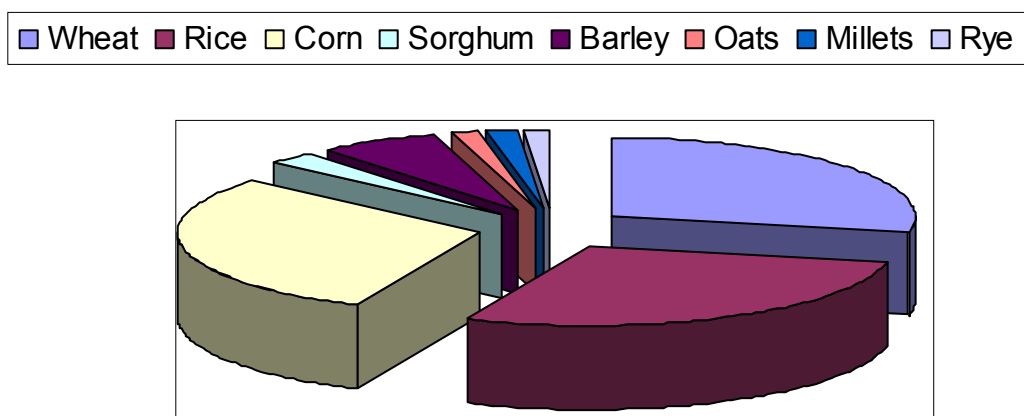
Fig. 1 Crop production in the world (FAO, 2002).

Tortilla History

Tortillas are an important part of the diet in Central America and Mexico. Traditionally grilled on earthenware utensils, they are used as bread and often filled or stuffed. The original Indian method of preparation consisted of kneading the cornmeal dough (masa) on a stone and then shaping into circles. The dishes made using tortillas are tacos, enchiladas and quesadillas. Fillings include guacamole, cheese, salsa, chopped raw onions and meat.

Tortillas are unleavened flat, circular, light colored breads made from either wheat or corn. The term tortilla was coined by Spaniards in Mexico among the Aztec in the sixteenth century. The word tortilla comes from the Spanish word "torta" which means round cake. The first tortillas were made of native corn with dried kernel (TIA 2002). It was the principal food of the Aztecs who were the pre-dominant people in Meso-America. When Spaniards brought wheat to the new world in 1519, wheat tortillas were created.

Tortilla Structure

Flour tortillas are a unique baked product made from wheat and other ingredients. Tortilla is a flat, circular product which usually varies in diameter from 100-700 mm and in thickness from 1-5 mm. Tortilla dough is gluten structured and most of the tortillas have chemical leavening agents. Small air bubbles are formed and distributed throughout the dough during mixing (McDonough et al 1996). The hydrated proteins form gluten, which surround hydrated starch granules and air bubbles in the dough.

U.S. Tortilla Market

In the United States, tortillas were once considered an ethnic food, but now they are used as a substitute for bread from hot dog buns, sandwiches, pizzas to casseroles. Americans consume tortillas instead of a variety of baked products. In the year 2000, U.S. citizens consumed a total of seven billion pounds of corn and wheat tortillas. This was equivalent to approximately 84 billion tortillas (not including tortilla chips) or almost one tortilla per U.S. citizen each day (TIA 2002). With the growing culture of Tex-Mex cuisine, tortillas are becoming an ideal accompaniment to many dishes. A survey conducted by the Aspex research confirmed tortilla segment as the fastest growing sector in the US baking industry. Today tortillas are manufactured under the quality guidelines. More than 2500 US companies make tortillas. Tortilla sales in the US reached $4.4 billion in 2001, $5.7 billion in 2002 and are expected to reach $6 billion by the end of 2004. Reports from Tortilla Industry Association (TIA) state that NASA STS-86 crewmembers consumed tortillas with at least one meal each day in space (TIA 2003).

Wheat Flour Tortilla Production

During the last two decades, large–scale commercial production of tortilla has become widespread. Commercially wheat flour tortillas are prepared by hand-stretch, die-cut, and hot-press methods (Bello et al 1991). In the hand-stretch method, tortillas are stretched into a round disk on a hot plate. This method yields tortillas which are larger, thinner and stronger than die-cut or hot-press method. This method requires more labor, time, sanitation and maintenance. Hand-stretch tortillas are irregular in shape and

have intermediate quality. They are used in the table- tortilla and burrito markets (Dally and Navarro 1999).

Die-cut tortillas require a higher water-absorption to enable proper sheeting. Die-cut tortillas have less elasticity, increased density, reduced resistance to cracking and/or baking.   Die-cut is an efficient method and yields a low cost product. This method yields less soft, pasty tortillas, which loose flexibility faster than hot-press tortillas. Die-cut tortillas are mainly used for frozen Mexican foods and the production of burritos (Saldivar et al 1988).

The most popular and the fastest growing procedure in the United States is the hot-press method (Waniska 1999). The resulting tortillas are slightly off-round, elastic, resistant to tearing, have a smooth surface texture and retain their flexibility longer during storage. Hot-press tortillas bakes at lower temperatures but have longer oven dwell times than those produced by other operations (Saldivar et al 1988).

Stages in Wheat Flour Tortilla Production

The important steps of tortilla production are: mixing, rounding and dividing, dough resting, formation of disks, baking, cooling and packaging (Fig.2). Each step in processing is critical in influencing the final tortilla quality. During mixing, flour absorbs water, gluten develops and gains strength. During this stage enzymes were added to flour with water to ensure proper mixing and sufficient mixing time. Water temperature is adjusted to attain the dough temperature of 30-32°C (Saldivar et al 1988).

MIX	Dry ingredients (2 min – low speed) Shortening (6 min – low speed) Water warm to 35°C
↓	
REST	5 min, 40 °C, 70% RH
↓	
DIVIDE/ ROUND	36, 43 g dough balls Time = 50 sec
↓	
REST	Time = 10 min, 40 °C, 70% RH
↓	
PRESS	Top platen = 395 °C Bottom platen = 395 °C Time = 1.3 sec, pressure = 1150 psi
↓	
BAKE	Time = 30 sec Oven temperature = 350-360 °F
↓	
COOLING	Three tier cooling chain Time on table = 3 min
↓	
PACKAGING	Package in low density polyethylene bags

Fig. 2 Flow chart for hot-press wheat flour tortilla (Bello et al 1991) as modified by Srinivasan et al (2000).

Flour type, flour treatment, dough weight, dough moisture content and pressing conditions determine tortilla diameter and thickness. Dough ball rounding and dividing is one of the most critical operations. Dough resting time and temperature influence tortilla shape. Platen temperature affects surface texture and chewiness (Saldivar et al 1988).

Tortillas are baked in three-tier commercial ovens at a temperature range from 250-270ºC with baking times from 18 to 40 sec (Waniska 1999). Tortillas can be baked by commercial ovens (three-tier, gas-fired oven), traditional (hot griddle) and by infrared radiation (IR). Commercial ovens can be generally heated by gas, electricity or hot air and have some drawbacks such as moisture loss, energy transfer, which result in a slow and inefficient process. Tortillas prepared using infrared radiations (IR) were baked for about 18 sec using black-body radiation at 549 or 584ºC. The IR baked tortillas showed good characteristics of rollability, puffing and texture compared to tortillas baked in a three-tier oven (Martinez et al 1999).

Wheat Flour Tortilla Ingredients

A traditional wheat flour tortilla is made of wheat flour, water, shortening and salt. To preserve the quality over extended period of time, most commercial formulations contain anti-microbial agents, emulsifiers, acidulants, leavening agents, reducing agents and hydrocolloids to improve the nutritional value, taste, shelf-life and stability (Friend et al 1995).

1. *Flour*: The type of wheat flour has the most important effect on the quality of tortilla. Weak gluten flours produce tortillas with broken outer margins, bad shape and

unacceptable rollability score. Strong gluten flours, yield tortillas with thicker diameter and tougher structure. Typical commercial tortilla flour is milled from hard red winter wheat with moderate to strong dough strength.

2. *Water*: Water acts as a medium for the distribution of all other ingredients in the formulation. Water is added as a plasticizer to hydrate the wheat proteins and facilitates the formation of gluten matrix. Water temperature is generally adjusted to provide dough at 32° C, which is optimum for dough resting.

3. *Shortening*: Shortening reduces the strength of gluten network, improves the rollability of tortillas and has an anti-staling effect in tortillas. Shortening decreases staling by modifying the interaction of starch components, especially amylose (Saldivar et al 1988, Dollack 1993).

4. *Salt*: Salt strengthens and toughens the gluten network, reduces the stickiness of the dough and increases the shelf life by reducing the water activity of the product.

5. *Preservatives and acidulants*: Preservatives like sodium and calcium propionates, potassium sorbates and sorbic acid are added to extend shelf-life by inhibiting mold growth. These additives are effective at low pH in the dough system. Acetic, citric and phosphoric acids are added to lower the pH of tortilla dough (Saldivar et al 1988). Encapsulated acids with high melting point edible coatings offer delayed release until baking. Fumaric acid has low solubility in the dough, has less interference with the leavening reaction and is commonly used commercially (Waniska 1999, Dally and Navarro 1999).

6. *Leavening agents*: Leavening agents improve internal structure of the tortilla allowing the formation of air bubbles during baking. Leavening agents form a product which is less dense, spongy, whiter and has an opaque appearance. During mixing CO_2 is formed by the soluble chemical leavening agents and bubbles are formed. Additional leavening reactions occur during hot pressing and during baking resulting in a foamy structure. The texture of dough and tortilla are affected by the type of leavening acid than the amount used in the formula (Cepeda et al 2000, Garza 2003). Commonly used chemical leavening agents used in tortilla production are the mixtures of sodium bicarbonate, mono-calcium phosphate and sodium aluminum sulfate.

7. *Reducing agents*: Reducing agents like L-cysteine, various salts of bisulfites and glutathione improve dough machinability by increasing extensibility and improving elasticity. These compounds break the disulfide bonds between proteins and decrease the protein molecular weight by reducing the degree of polymerization. (Saldivar et al 1988).

8. *Emulsifiers*: Emulsifiers are added to dough to improve the softness and extensibility of tortillas. Emulsifiers such as sodium stearoyl lactylate (SSL) improve dough mixing and machinability by interacting with gluten, gliadin and starch. Emulsifiers with amylose complexing properties such as mono-glycerides are added to reduce the stickiness and the staling of tortillas (Saldivar et al 1988, Adams 2001).

Evaluation of Tortilla Texture by Subjective Methods

Subjective rollability is used to evaluate changes in tortilla texture (Friend et al 1995). Extent of cracking and breaking is evaluated subjectively on 1 to 5 basis after wrapping the tortilla around a 1 cm diameter dowel.

Sensory evaluation is another subjective method, which is used to monitor the textural changes in different food products. Sensory methods can be divided into two main categories: effective and analytical (Joseph 1999). Effective tests measure responses like acceptance, preference, and degree of liking and disliking. Paired preference, ranking and hedonic ratings are commonly used. Analytical test are more discriminative and descriptive.

Evaluation of Tortilla Texture by Rheological Methods

Rheological methods used to measure tortilla texture are objective, reliable and sensitive which can differentiate the changes in flexibility and rollability of tortillas. Suhendro et al (1999) evaluated different objective methods to characterize changes in corn tortilla staling. Such methods are extensibility and bending tests, which can be used to evaluate textural changes. Joseph (1999) performed a similar study on wheat tortillas. The most effective method to objectively evaluate tortilla texture is extensibility. A tortilla strip is pulled apart by a tensile force. Force and distance required to rupture, work and modulus are measured. This test was used by Waniska (1976) to measure the tensile characteristic of sorghum chapatti, Joseph (1999) to measure the extensibility of wheat tortillas and Suhendro et al (1999) to measure the extensibility of corn tortillas.

Effect of Wheat Flour on Tortilla Texture

Mostly the type of wheat flour and its protein level determine tortilla quality. Many researchers have investigated the effects of starch and protein on the baking quality of bread. Harris and Sibitt (1941) fractionated wheat flour into gluten and starch and studied effects of the reconstituted flour for quality of breads. They showed that starch properties were affected by environmental factors as well as inherited factors. Hoseney et al (1969) also showed that starches from different wheat cultivars had similar baking quality, however starches from different grains behaved differently because of the particle size and gelatinization temperature.

Flour being the major ingredient in tortilla production influences the quality and shelf stability of tortillas (Wang and Flores 1999a, b, Waniska 1999, Guo et al 2003). Since starch and protein are the two major components of wheat flour, both of them influence the quality. Particle size, damaged starch and protein content of flour also influence dough rheology and baking properties. Sprout damage adversely affects wheat quality and results in a high level of alpha amylase activity in flour. Such damage changes the water holding capacity and dispersion in the dough. This affects many processing properties of grain meals such as dough handling and finished product texture (Finney et al 1988).

The effects of wheat starch and gluten has been sparsely investigated in wheat flour tortillas in contrast to bread. Suhendro et al (1995) reported that the tortillas made from 10.2% protein content were less rollable during storage as compared to tortillas made with 11.0% protein. Better quality tortillas have been prepared from hard wheat

flour of intermediate protein content as compared to tortillas made from high and low protein flours (Qoorani et al 1996, Wang and Flores 1998a). Poor handling dough properties result when too little protein is present in the flour (Adams 2001) but protein content by itself does not determine its suitability for tortillas (Waniska 1999). Flour protein correlates to tougher dough's, smaller diameter tortillas and longer shelf stability. Flours with intermediate levels of flour protein (amount and quality) are recommended for wheat flour tortilla quality (Waniska et al 2003).

Microscopic observations of wheat tortilla dough (McDonough et al 1996) revealed that starch granules are enveloped in a thin continuous film of protein on the surface of the dough ball. Previous studies have suggested that changes in starch during storage play a major role in case of bread firmness (Appolonia and Morad 1981, Zobel and Kulp1996) Amylopectin recrystallization can be delayed by the addition of enzymes. A reduced amount of amylose in wheat starch decreases tortilla opacity and thickness (Waniska et al 2002, Guo et al 2003). The amount of damaged starch in wheat flour adversely influenced the machinability and characteristics of baked tortillas (Wang and Flores 1999b, Mao and Flores 2001). However flour with intermediate levels of damaged starch was recommended for wheat flour tortillas (Waniska et al 2003).

Starch Changes during Baking

The starch present in the wheat endosperm represents approximately 80-85% of the whole kernel, and is the primary carbohydrate in wheat flour. Starch granules contain semi-crystalline (amylose), crystalline and non-crystalline (amylopectin) molecules, and polar lipids. Numerous studies have been conducted to study the changes in starch

texture and properties of baked products. Heating of starch granules in the presence of water causes hydration, swelling, melting of crystals (T_m) and dispersion of amylose and amylopectin molecules. Thermal processing conditions have significant effects on the gelatinization and dispersion of starches which affects the staling behavior of food products (Seetharaman et al 2002).

Use of Additives

Several approaches have been employed in the past to improve the quality and these involve use of hydrocolloids, emulsifiers and enzymes (Armero and Collar 1996, and Martinez et al 1999).

1. *Hydrocolloids***:** The effect of different hydrocolloids (sodium alginate, κ-carrageenan, xanthan gum and hydroxypropylmethylcellulose) on the rheological properties of wheat flour dough and bread has been studied. Hydrocolloids modify starch gelatinization and extend the overall quality of product over time and used as improvers in bread making.

2. *Emulsifiers***:** Some emulsifiers like sodium stearoyl lactylate (SSL) are used as dough conditioners and improve the bread loaf volume and produce longer crumb freshness (Krog 1984). Emulsifiers improve the ability of the gluten to form a film around the gas bubbles (Krog 1981). Emulsifiers reduce the crumb-firming rate (Joensson and Toernaes 1987, Krog et al 1989, Mettler and Seibel 1993).

3. *Enzymes***:** Enzymes are used in baking for optimizing baking properties and for improving the quality of baked products. Improvements have been achieved with both α-amylases and xylanolytic enzymes. Enzymes are proteins characterized by their catalytic

activity, high selectivity and specificity. Enzyme activity depends on pH, temperature, water activity, ionic strength and the presence of different molecules in the medium. Differences in enzyme activity also occur depending upon the origin. Fungal, cereal and bacterial amylase have different pH and thermal stabilities. Fungal alpha-amylase is inactivated in 2-3 minutes at 65 -75° C. Cereal alpha amylases are slightly more thermo-stable and remain active during early stages of starch gelatinization. Bacterial amylases have even higher thermal stability and survive the baking temperatures (Dragsdorf and Varriano 1980).

Many enzymes with expanded functionality are used as processing aids. Amylases retard the firming of bread (Martin and Hoseney 1991) and inhibit the retrogradation of amylopectin. Enzymes have been used to extend shelf life of bread. Amylases were used in bread originally to generate fermentable sugars from starch to improve bread loaf volume, crumb grain improvement crust and crumb color and flavor enhancement (Armero and Collar 1996, Qi Si 1996, Sahlstrom and Brathen 1997, Martinez et al 1999). There are four groups of starch converting enzymes: endoamylases, exoamylases, debranching and transferases.

1. *Endoamylases*: cleave α-1, 4 glycosidic bonds present in the inner part (endo) of the amylase or amylopectin chain. The end products of action of α-amylase (EC 3.2.1.1) are oligosaccharides with 2-8DP and α-limit dextrins.

2. *Exoamylases*: cleave α-1,4 glycosidic bonds of amylose and amylopectin. Exoamylases such as β-amylases and amyloglucosidases, shorten the external side chains of amylopectin by cleaving maltose or glucose molecules respectively. These

enzymes delay bread staling by decreasing retrogradation of amylopectin (Wursch and Gumy 1994). The synergistic use of α and β amylase is also claimed to increase the shelf life of baked goods (Van Eijk 1991).

3. *Debranching*: These enzymes exclusively hydrolyze α-1,6-glycosidic bonds. Pullulanases hydrolyze α-1,6-glycosidic bonds (pullulan or amylopectin) while isoamylase hydrolyzes the α-1,6 bond in amylopectin. (Israilides et al 1999).

4. *Transferases*: These cleave α-1,4-glycosidic bond of the donor molecule and transfer part of the donor molecule and transfer part of the donor to a glycosidic acceptor with the formation of a new glycosidic bond. Transglutaminase (EC2.3.2.13) is an acyl transferase that catalyzes inter- intramolecular cross-linking through the formation of peptide bonds between glutamine and lysine residues (Takaha and Smith 1999).

Industrial Applications of Enzymes

Numerous enzymes are used in the baking industry to improve dough handling properties and enhance bread quality. The use of enzymes is the best alternative to chemical compounds since they are generally recognized as safe (GRAS). α-amylases and proteases have been used in the bread industry for over more than hundred years. Proteases are added to reduce the dough mixing time. Pentosanases such as hemicellulases are used in baking due to their ability to reduce water absorption of the dough by hydrolyzing the pentosans (Mc Cleary 1986, Rouau 1994). Glucose-oxidase has an effect on the formation of tyrosine cross-links (Tilley et al 2001). Glucose oxidase resulted in wheat dough's with higher tenacity and lower extensibility. Transglutaminase also modified wheat storage proteins resulting in lower dough tenacity

and less extensibility (Rosell et al 2003). Cell wall degrading enzymes have also been used to increase the fiber content of wheat dough's and breads (Laurikainen et al 1998). Amylases hydrolyze α-1-4 linkages within the amorphous regions of the starch matrix during baking (Zobel and Senti 1959). The influence of β-amylase has been of minor importance in the bread quality as β-amylase cannot attack undamaged or non-gelatinized starch granules but α-amylase has been of major importance.

Flour Supplementation with Amylases

Grains contain a large number of specific enzymes, and the variation in their activity levels affects the quality of cereal raw materials. This variation is due to the factors mentioned below:

1. Cultivar variation

2. Environmental Conditions during cultivation

3. Pre-harvest sprouting

4. Storage Conditions

5. Milling fractionation

6. Processing conditions

In many baking applications, the activity of naturally occurring starch splitting enzymes (amylases) is insufficient. Consequently external source of amylase are used to

supplement wheat flour. Alpha-amylase supplementation to flour, dough, dough–conditioners and improvers is commonly done in the industry. Malted cereals and fungal amylases are used as supplements. The benefits includes improvement in machining and handling of the dough, ensuring active fermentation, enhanced product volume, better moisture retention and improved shelf life of products. Some of the properties of various amylases are listed in Table I.

Table I

Properties of amylases

Source	*A .oryzae*	*B. subtilis*	Barley Malt	Barley malt
Amylase type	Alpha-amylase	Alpha-amylase	Alpha-amylase	Beta-amylase
Optimum pH	4.8-5.8	5.0-7.0	4.0-5.8	5.0-5.5
Temperature	45– 55°C	60-70°C	50-65°C	40-50°C

Maltogenic Amylases

Maltogenic amylases, exhibit unique characteristics that are different from other α-amylases in that they exhibit (i) a dual activity of α-D-1,4 and α-D-1,6-glycosidic bond cleavages that yield maltose; (ii) an activity of α-D-1,4- to α-D-1,3-, α-D-1,4-, or α-D-1,6-transglycosylation that generates oligosaccharides of DP 3-6; and (iii) an activity of cleaving acarbose, a pseudo-tetra-saccharide competitive inhibitor of α-

amylases (Kim et al 1999). Some of these properties of maltogenic amylases, if not all, are shared by two other amylolytic enzymes with different names, including neopullulanases and cyclomaltodextrinases, both of which are homologous to maltogenic amylases with sequence identity of 40-86%. These three groups of amylases have intra-cellular activity in bacteria (*Bacillus sp*. and *Thermus sp*) and fungi (A. oryzae and P. expansum), unlike typical commercial α-amylases and pullulanases from *Bacillus subtilis* (Fresh-N® from EDC) and *Aspergillus* (Enzeco® from EDC) which have extra-cellular activity (Park et al 2000). The three groups of versatile amylases are high molecular weight (62-90 kDa for the monomers) amylases because of a unique addition of 130 residues at the N terminus compared with the conventional α-amylases containing the single activity of hydrolyzing α-D-1,4-glucosidic bonds. This addition is the binding site for cyclodextrins and branched oligosaccharides, and the host for transglycosylation (Kim et al 1999).

Maltogenic amylases prefer cyclodextrins (CDs) to starch or pullulan as substrates in that the hydrolysis of CDs (six to eight glucose units) is 100 times faster than that of starch and pullulan (Kim et al 1999). Large substrates, like amylopectin or starch, are assumed to be accessible only for a wide and shallow active site as found in conventional α-amylases or maltogenic amylase monomers, while the small compact substrates malto-oligosaccharides (DP 2-7) or CDs fit into the catalytic site of dimeric maltogenic amylases (Kim et al 1999). Therefore, maltogenic amylases specific for cleavage of amylopectin should be produced with a higher proportion of the monomeric form. Amylase activity is expressed in activity units (AU), defined as the amount of

enzyme (g or mg) necessary to release 1 g or mg of glucose equivalents from the substrate per unit of time(Doyle et al 1999).

Glycosyl hydrolases (amylases) can act as antistaling agents (Kulp and Ponte 1981). The addition of amylases retards the firming of bread (Martin and Hoseney 1991) and inhibits the retrogradation of amylopectin as measured by DSC (Defloor and Delcour 1999). Amylases hydrolyze α-1,4 linkages within the amorphous region of the starch matrix during baking (Zobel and Senti 1959). Conventional α-amylases derived from bacterial (*Bacillus subtilis*) or fungal (*Aspergillus oryzae*) sources are not well suited for this purpose due to excessive or insufficient thermo stability, respectively (Hebeda et al 1990).

Three different theories may explain why enzymes extend shelf-stability in baked products: 1. the shortening of amylopectin chain length by enzymes, reduce retrogradation tendencies of amylopectin (Boyle and Hebeda 1990) 2. the oligosaccharides (DP 2-7) produced by the enzymes are anti-staling agents (Martin and Hoseney 1991) and 3. production of low, molecular weight dextrins that interfere with the retrogradation of starch. Retrogradation of maize amylopectin was directly proportional to the amount of chains of DP 16-30 and inversely proportional to the amount of chains of DP 6-11 (Shi and Seib 1995). Treatment of starch with β-amylase (an exo-acting enzyme) shortened amylopectin chains and reduced the rate of retrogradation (Wursh and Gumy 1994). Zobel and Senti (1959) proposed that dextrins disrupted the continuity of the starch network and reduced its rigidity. However, Gerrard (1997) reported that staling rate was not related to the presence of dextrins in a

specific size class and that these dextrins are just symptomatic of a modification to the starch that retards staling.

Effect of Enzymes in Bread

Enzymes have been used as dough softeners, which lead to improved machining properties, higher loaf volume and softer bread crumb (Mc Donald 1969, Cauvain and Chamberlain 1988, Ranum and De-Stefanis 1990). Alpha amylase supplementation increases both the rate and quantity of fermentable sugar production via hydrolysis of soluble starch. Bread produced from wheat flour containing insufficient alpha amylase activity possesses a reduced volume and a dry, rough crumb and lacks a golden brown crust color. Amylase hydrolysis products improve the moisture retention of the crumb, enhancing softness, shelf life and freshness.

Addition of α-amylase to bread decreases staling by changing the structure of starch. The anti-staling effect of the branched-chain products is caused by a decrease or an interference with the crystallization of amylopectin or from interference with the formation of other interactions (Duedahl-Olesen et al 1999). The bacterial α-amylases of intermediate heat stability have been confirmed to be useful anti-staling additives by delaying the starch retrogradation (Hebeda 1991).

The anti-staling effect of α-amylases is due to their ability to retard amylopectin retrogradation during storage (Dragsdorf 1980, Akers 1994, Leon 1997) but when used in excessive amounts these can cause stickiness of baked goods. Stickiness can be solved using an exo-amylase since they do not produce the branched malto-oligosaccharides of DP 20-100. Such enzymes called maltogenic amylases produce linear oligosaccharides

of 2-6 glucose residues. α-amylase induces dextrinization of starch granules reducing its ability to immobilize water, and free water increases dough mobility (Miranda-Lopez 1999).

Martin and Hoseney (1991) studied the role of starch hydrolyzing enzymes using the theory of bread firming; they concluded that fungal or bacterial α-amylase produced low molecular weight dextrins and maltose may diffuse away, reducing starch-protein interactions. Maltodextrins of degree of polymerization (DP) between 3 and 7 are effective in retarding amylopectin retrogradation. A bacterial maltogenic amylase has been found to have anti-staling effect (Outtrup and Norman 1984).

Effect of Enzymes in Corn Tortillas

A limited hydrolysis of amylopectin chains can inhibit retrogradation with the concomitant extension of the shelf life (Iturbe-Chiňas et al 1996). Barley malt (Suhendro 1997), bacterial (Suhendro 1997, Quintero-Fuentes 1999), and fungal α-amylases (Aida et al 1996, Suhendro 1997) have been evaluated as anti-staling agents in corn tortillas. Aida et al (1996) found that addition of a conventional fungal α-amylase blend (10 AU /g) extended shelf life of corn tortillas according to 75 panelists. However, Suhendro (1997) reported that low levels (0.0005%) of either bacterial or fungal amylases had a detrimental effect on masa characteristics and machinability, as well as tortilla rollability. Therefore, additives that can increase viscosity and create a new network of viscoelastic structure to compensate for the weakened structure affected by the enzymes were needed. Suhendro (1997) found that a combination of 0.25-1% CMC and 0.005-0.01% barley malt produced masa with improved machinability and tortillas with better

rollability than control after 12 days of storage. Intermediate temperature stability (ITS) maltogenic enzymes, which have an optimum temperature range of 65-80°C, were effective as anti-staling agents for wheat dough systems (Hebeda et al 1991).

Maltogenic amylase type of enzyme would be adequate for the tortilla system, since the rest period of masa and baking time are very short (10 min and 1 min, respectively) compared to bread. Therefore, the enzyme should hydrolyze amylopectin during the rest period at a higher rate than regular enzymes and could be inactivated before the tortilla comes out of the oven. Novamyl$^{\circledR}$ 1500 MG removes oligosaccharides in the DP 2-7 range from amylopectin and amylose; it does not cause gumminess as other bacterial amylases. Fungal amylases when added at low levels to dry masa flour may be a valuable tool to reduce starch retrogradation and retard accelerated staling in tortillas, increasing their shelf life (Iturbe-Chiňas et al 1996).

Corn tortillas containing bacterial, fungal and malt amylases were softer to the touch but were more brittle as measured by bending technique, and more breakable when rolled on a dowel. Bacterial amylase enhanced softness of the tortillas; but the tortillas were more brittle than those with fungal amylase or malt (Suhendro 1997). Bacterial amylases which retain activity after baking due to high temperature stability have shown potential for increasing shelf life. Miranda (1999) used Novamyl$^{\circledR}$ 1500 MG at levels of 0.04% (600 maltogenic amylase units, MAU, per kg of NCF) in corn tortillas. Novamyl$^{\circledR}$ activity was optimum at pH 5 and tortillas stored under refrigeration were more rollable and pliable than control; but they required more extension force to break. Miranda (1999) suggested that combinations of amylase and other additives, such

as CMC and wheat gluten should reduce tortilla staling to a larger extent than using amylase alone. Suhendro (1997) suggested that interaction effects between potential additives (gums, amylase, shortening, emulsifiers and gluten) that can improve corn tortilla texture need evaluation.

Staling of Bread and Flour Tortilla

Staling is defined as chemical and physical changes that occur after baking of almost all cereal products, which decreases "fresh" characteristics. Increased firmness, dryness and a loss of product freshness are prevalent in staled products. Staling is caused by the gradual transition of amorphous starch to a partially crystalline, retrograded state. The rate of staling of bread depends on the wheat flour, the formulation and the processing and storage conditions (Hoseney 1996).

Staling in wheat tortillas is accompanied by gradual decrease in rollability, an increase in firmness and formation of brittle structure (Friend et al 1993). Kim and D'Appolonia (1977) studied the effect of protein content on staling rate and bread crumb pasting properties. Kinetic studies at two temperatures indicated that regardless of the protein content in flour, the basic mechanism of bread staling involves changes analogous to crystallization of the starch fraction of the crumb. Higher storage temperature and greater flour protein content decreased the staling rate of bread. Martin et al (1991) has suggested that starch retrogradation and bread firming are not related. Firming is caused by relatively weak cross-links (hydrogen bonds) between protein

fibrils and starch remnants caused firming. This implies that protein is essential to the staling process.

Every et al (1998) has performed reconstitution studies on starch bread made from protein-free synthetic flour and starch-gluten breads made from synthetic flours containing 1-15% gluten. The starch breads increase in firmness up to six days, indicating that gluten is not essential to the firming process. The starch-10% gluten breads and starch-15% gluten breads had similar specific loaf volumes, moisture contents and firming rates compared to the starch breads. They proposed that increasing bread firmness results from glucan chains of partially leached amylose and amylopectin attached to swollen starch granules forming hydrogen bonds with other starch granules and, to a smaller extent, with gluten fibrils.

Wheat tortilla dough is exposed to sufficient temperature, moisture and time to gelatinize starch. Retrogradation of amylopectin is believed to primarily involve associations of its outer branches and requires more time than amylose. Therefore, the amylopectin retrogradation that occurs during storage appears to correspond to the staling process (Seetharaman et al 2002).

Enzyme Activity

No work has been reported on use of enzymes in flour tortillas hence enzyme addition was looked into. Since enzyme activity is an important factor during the baking process, it is important to standardize the activity of enzymes incorporated in flour. High level of α-amylase activity occurs during sprout damage which adversely affects wheat

quality and reduces the trade value of wheat. Such damage changes the starch structure and affects many processing properties of grain meals such as dough handling and finished product texture (Finney et al 1988).

The principle of viscometry in determining the amylolytic activity of wheat flour is applied in the Falling Number test (AACC 2000) which was developed by Hagberg (1960). This method is used world-wide by millers and bakers to determine the enzyme activity of wheat flour. The Falling number system utilizes the principle of the rapid gelatinization of flour suspension with subsequent measurement of the liquefaction of the starch by α-amylase. This test is based on measuring the time required to stir and to allow a specified viscometer stirrer to fall a standard distance. The total time in seconds from the immersion of the viscometer tubes in the water bath until the viscometer stirrer dropped to the bottom was counted as the Falling number.

This system actually does not measure the amount of α-amylase present in the sample but rather the effect of activity of the enzyme to reduce starch viscosity. Falling number method is an internationally standardized method for the determination of α − amylase in grain, flour starch and other products.

α -amylase activity of the flour can be adjusted to an optimum by the addition of malt or by blending flours with different activities. Falling number values allow bakers to monitor their supply and set specifications for incoming products depending upon the end use of flours. Since the effect of α −amylase activity depends not only on the properties of the enzyme but also on the properties of the starch, it becomes important to evaluate the effect of starch structure on enzyme activity. Table II shows the typical

numbers, which are indicative of the resulting breads when flours with various falling number values are used. Flours are blended to achieve the desired falling number value.

Table II

Effect of falling number values on bread quality

Falling number values	Typical Results
<150	Sprouted wheat; high enzyme activity; sticky bread crumb
200-300	Unsprouted wheat, normal enzyme activity, moist crumb
>300	Unsprouted wheat, low enzyme activity, dry crumbly bread crumb

Damaged Starch

Undamaged starch granules are relatively resistant to amylases. However, damaged starch is rapidly hydrolyzed by amylases. When wheat is milled, a portion of the starch granules in the flour are damaged. The damaged starch absorbs more water than intact starch granules. Various factors that influence the production of starch damage are listed below (Evers and Stevens 1985).

Grain Hardness: The hardness of the grain affects the manner in which the endosperm and starch granules are fractured during the milling process. Grain hardness is a major factor affecting starch damage during milling. During milling, the fracture planes run between the starch granules and the protein matrix in soft wheat but within the starch

granules in the hard wheat. Thus starch from hard grains fractures more during milling and this leads to greater water absorption during dough mixing (Evers and Stevens 1985).

Feed Rate: Decreasing the feed rate gives an increase in flour release and in starch damage.

Roll Speed: Higher roll speeds increases starch damage, with little effect on flour release.

Roll Differential Speed: Increasing the differential speed ratio increases starch damage, especially for coarse feed material.

Roll Pressure: Higher pressure increases starch damage, especially for fine feed material.

Roll Surface: Matt surface produces more starch damage than do smooth surfaces.

The proportion of damaged starch granules in wheat flours varies with wheat variety and milling practices. Damaged starch in most of the U.S. wheat flour is 4.5-8.0 %. In general, soft wheat flours have less damaged starch content (2-3%) as compared to bread and durum wheat flours (Donelson and Yamazaki 1962). Hard wheat is more susceptible to starch damage than soft wheat because the starch granules in soft wheat are fairly loosely bound in the kernel and are easily released in milling. The starch granules in hard wheat kernels are firmly bound in a much stronger protein matrix, and are much more liable to damage when the endosperm fragments are reduced in size.

The phenomenon of birefringence and X-ray diffraction pattern demonstrates that in native starch, a part of the structure is crystalline. Gelatinization destroys the

ability of the starch to produce X-ray diffraction pattern and similar findings have been observed in case of damaged starch (Meuser et al 1978). The properties are listed (Table III). Starch is said to be 100% damaged when it absorbs its own weight in water at 30º C. Compact intact starch granules absorb 30% of their weight in water while damaged starch granules absorb 300% or more. In the dough stage, damaged starch avidly absorbs water in competition with the gluten forming proteins, and dough viscosity increases. Dough consistency, flow, development and forming are negatively impacted. An excess of damaged starch yields wet, sticky dough's that are difficult to machine and handle.

Physical Properties of Damaged Starch

Damaged starch shows many physical characteristics, which are similar to those shown by gelatinized granules. Farrand (1964) developed a procedure for the determination of damaged starch and alpha-amylase as they relate to flour water-absorption and bread quality. Numerous methods exist for the estimation of damage starch (Dadswell and Gardener 1947, Greer and Stewart 1959 and Sandstedt and Mattern 1960) but all these procedures are very tedious. Extraction of damaged starch with cold water preferentially leaches amylopectin of low molecular weight. Water absorption is highly correlated with enzyme digestibility and it is correlated with loss of birefringence and dye absorption (Craig and Stark 1984). Wheat and maize starches were ball milled to obtain various levels of damaged starch, and digested with fungal alpha amylase. The un-hydrolyzed portion was composed of native starch granules and birefringent remnants of larger granules that had been partially damaged (Morrison et al 1994).

Since grinding treatments can induce physical conversion of the starch granules causing a change of the properties of starch similar to gelatinization (Lelievre 1974), wheat flour was damaged at various levels. Methods currently used to measure starch damage are based on enzymatic and iodometric assays. The enzymatic methods use the increased susceptibility of the starch granules to degradation by amylolytic enzymes. These methods are based on the susceptibility of starch to α-amylases and or β-amylases. Undamaged starch granules are totally resistant to pure β-amylase, while damaged starch granules are attacked at a measurable rate. The hydrolysis products can be measured spectrophotometrically. The iodometric method or non-enzymatic method is based on the increased extractability of amylose from damaged starch granules. The extracted amylose reacts with iodine and is measured amperometrically or colorimetrically.

TABLE III

Characteristics of native, damaged and pre-gelatinized starch

(Evers and Stevens 1985)

Characteristic	Native	Damaged	Pregelatinized
Amylase Digestibility			
Alpha	Slow	Rapid	Rapid
Beta	None	Rapid	Rapid
Birefringence	Positive	Absent, partial	Absent
X-Ray Pattern	A type	Absent, partial	Absent
DSC gelatinization endo-therm	Present	Absent, partial	Absent
Fracture face	Concave	Flat with septum	Flat with septum
Paste Viscosity			
Cold	Low	Medium	High
Hot	High	Medium	Low
Water Capacity (ml/gm)	0.5	3-4	20
Solubility	Very low	High	High
Leached component	None	Amylopectin	Amylose
Surface imperfections	Absent	Present	Absent
Folding Pattern	Absent	Absent	Present

Importance of Damaged Starch in Bread

Damaged starch is an important flour specification for bread because it affects numerous properties such as water absorption and gas production in the fermenting dough (Tipples 1969). Up to the oven stage, the primary portion of the starch is in a

native, insoluble state. Consequently, the only starch susceptible to amylase hydrolysis is damaged starch. The enzymatic hydrolysis of damaged starch is significant during the dough stages, and most of the soluble starch is hydrolyzed and consumed with in 30 min of fermentation. At 60° C the insoluble starch granules begin to absorb water and lose their crystalline structure. Gelatinization continues over a range of $60\text{-}70^{\circ}$ C. When the natural sugars present in the flour have been utilized by yeast, fermentable sugars mainly maltose produced by the amylosis of damaged starch provide a further supply of substrate in the absence of which inadequate gassing occurs, giving bread a low loaf volume. Additionally, wheat starch is deficient in naturally occurring α-amylase; limiting the splitting of damaged, soluble starch to dextrins and subsequently the ability of β-amylase to liberate maltose. These limiting factors necessitate the cereal or fungal α–amylase supplementation. In case of bread the hydrolysis continues during proofing and for a few min in the oven stage. Endo hydrolysis of damaged starches rapidly reduces dough viscosity; liberating water and significantly reducing its ability to absorb and bind water in the dough and oven stages. The combined action of viscosity reduction and water release enhances dough mobility (softness and flexibility) and cohesiveness. In turn, the development of finely divided gas cells is promoted and gas retention capacity is enhanced.

Determination of damaged starch and α-amylase as they relate to flour water-absorption and tortilla quality hence becomes important as the disintegration or damaged starch determines the accessibility of amylose and amylopectin to starch degrading enzymes.

CHAPTER III

MATERIALS AND METHODS

Tortilla Production

Control tortillas were made according to the method of Bello et al (1991) (Fig.1). Hot-press wheat flour tortillas were prepared as a standard control (500g enriched, bleached and malted flour (13.4% moisture, 0.5% ash, 10.4% protein, falling number 254sec., 58.6% water absorption, ADM Arkaday, Olathe, KS). Untreated flour I (13.6% moisture, 0.4% ash, 10.8% protein, falling number 358sec., 58.0% water absorption, ADM Arkaday, Olathe, KS 500 g of flour/batch) and untreated flour II (13.1% moisture, 0.5% ash, 11.4% protein, falling number 371 sec., 57.0% water absorption, Morrison Milling Company, 500 g of flour/batch) was used for enzyme supplementation as it did not have malted barley flour.

Each batch contained 500 g flour, 7 g of salt (United Salt Corporation, Houston, TX), 3 g of sodium bicarbonate (Arm and Hammer, Church & Dwight Co., St. Louis, MO), 2.5 g sodium stearoyl lactylate (American Ingredients company, Grandview, MO), 2.0 g potassium sorbate (ADM Arkady, Olathe, KS), 2.5 g sodium propionate (ADM Arkady, Olathe, KS), 2.9 g sodium aluminum sulfate (Equisa, Cfb Budenheim Gallard Schlesinger Ind. Inc, Garden City, NY) and 2.4 g of fumaric acid (Balchem Corp. Stale Hill, NY). The dry ingredients were mixed for two min with a paddle at low speed; and then 60 g of shortening (Sysco Corporation, Houston, TX) were added and mixed for another 6 min at low speed. Distilled water containing 0.03 g of cysteine (Sigma-Aldrich

Chemie, Steinheimty, Germany) was heated to 35^0C and added to the mixture (Table IV). Tortilla water absorption was adjusted quantitatively to obtain smooth, soft and non-sticky dough's. Then it was mixed with a hook for 2 min at low speed. The dough was rested in a proof chamber (model 57638, National Manufacturing Co., Lincoln, NE) (70% relative humidity, 32-35^{0}C for 5 min. After proofing, the dough was divided and rounded into dough balls by dividing and rounding press (model RR 399, Dutchess Tool Company, Beacon, NY). A hot-press (Micro-Combo model 0P01004-02, Lawrence Equipment Company Inc., El Monte, CA) was used to press tortillas. The top and bottom platens were set at 395 F. Processing time was 1.35 sec and the pressure was 1100 psi. Then they were baked in a three-tier oven (Micro-Combo model 0P01004-02, Lawrence Equipment Company Inc., El Monte, CA) where the temperature was set at 350-360^{0}F. Dwell time was adjusted to 30 sec. Tortillas were cooled on a three-tier cooling chain (model 3106 INF, Food Machinery Inc. Pivo Machinery Inc. Pico Rivera, CA). Tortillas were packed in low-density polyethylene bags and stored at room temperature. The obtained tortillas were evaluated using objective and subjective methods.

TABLE IV

Formula for hot-press tortillas

Ingredient	Percentage (%Baker's)
Wheat Flour	97.0%
Wheat Protein Fraction	3.0%
Salt	1.5%
Sodium Stearoyl Lactylate (emulsifier)	0.5%
Sodium Propionate (preservative)	0.5%
Potassium Sorbate (preservative)	0.4%
Sodium Bicarbonate (leavening base)	0.6%
Fumaric Acid (pH, leavening acid)	0.24%
Sodium Aluminum Sulfate (leavening acid)	0.58%
Shortening All Purpose	6.0%
Cysteine	0.003%
Water	50-54%

Evaluation of Dough Properties

The dough properties were evaluated subjectively for smoothness, softness and toughness after the dough was mixed. Press rating was measured immediately before dividing and rounding.

1. *Smoothness*: It refers to the appearance and texture of the dough surface and was rated from 1 to 5, 1= smooth, 5= very rough. The "ideal" dough ratings were from 1.5-2.0.

2. *Softness*: It refers to the viscosity or firmness of the dough when is compressed by pressing the dough with the fingers in a fixed point and was rated from 1 to 5, 1=less viscous, 5= soft, more viscous.

3. *Toughness*: It refers to the elasticity of the dough when is pulled apart and was obtained by pulling the dough at the same point where softness was ranked. Toughness was rated from 1 to 5, 1= excessively elastic, 5= less tough, less elastic.

4. *Press rating*: It refers to the force required to press the dough on the stainless steel round plate before dividing and rounding and was rated from 1 to 5, 1= very easy to press, 5= very hard to press.

Stress relaxation of the dough balls were measured by compressing on a texture Analyzer (Model TA-XT2, Micro Systems, Scarsdale, NY) after10 min resting time. Texture analyzer settings for stress relaxation of dough are given in Table 2. A cylindrical probe with a diameter of 10 cm was attached to the texture analyzer arm and was calibrated to a distance of 35 mm from the texture analyzer platform. When the cylindrical probe was compressing the dough ball, force and distance values were recorded. Maximum force, modulus of deformation and adhesive force were calculated.

Evaluation of Tortilla Properties

Ten tortillas were selected randomly and weight, diameter, height, opacity, moisture and pH were determined the first day after processing (Bello et al 1991). Rollability and extensibility were done at 4, 8, 12, 16 and 20 days after production. The methods are described as follows:

1. *Moisture*: A two-stage moisture method consists of drying a tortilla for 24 hr in ambient conditions followed by three- hour moisture. The samples in duplicate were

placed in oven (model 16, Precision Scientific Co. PS, Chicago, IL) and dried for 1 hr at 100° C. Moisture was calculated by loss of weight.

2. *Tortilla pH*: Half of a tortilla was ground with a coffee grinder and mixed with 120 ml of distilled water. A Φ 10 – pH meter (Beckman Instruments, Fullerton, CA) was used to measure the pH of tortilla in the distilled water solution. An electrode probe (Corning, Inc., New York, NY) was dipped in water- tortilla solution and the pH recorded after 25 sec. Tortilla pH was measured 24 hr after processing.

3. *Diameter*: Diameter of ten tortillas was measured by using a ruler at two points across the tortilla: the large and the smaller diameter were recorded and averaged.

4. *Height*: Height of a stack of ten tortillas was recorded and averaged by using a digital caliper (Chicago Brand 12" Electronic Digital Caliper, Chicago, IL).

5. *Weight*: Ten, randomly selected tortillas were weighed using an analytical scale (Ohaus, Houston TX).

6. *Opacity*: Opacity (%) was evaluated subjectively on ten tortillas where opaque tortillas were rated as 100% and completely translucent tortillas were rated as 0%. The values were averaged.

7. *Specific Volume*: Tortilla specific volume was determined as follows:

$$\text{Specific Volume} = (height) * (\pi\, r^2)$$

Where height = height of a single tortilla (cm); weight = weight of a single tortilla (g), r=average radius of a tortilla (cm).

8. *Rollability*: Subjective rollability of tortillas, which measures the cracking and breakage of a tortilla, was used to evaluate tortilla stability. Two tortillas were evaluated

subjectively by rolling around a dowel (1.0 cm diameter) on one side of the tortilla after 4, 8, 12, 16 and 20 days of storage and were given a rollability score (RS). Cepeda et al (2000) used a continuous scale for rollability score: 5 = no cracking; 4 = signs of cracking, but no breaking; 3= cracking and breaking beginning on the surface; 2 = cracking and breaking imminent on both sides; and 1 = unrollable, breaks easily. Shelf stability was undesirable when the rollability score reached 3 (e.g. several, cracks and breaks on the surface) during storage.

9. *Extensibility*: Extensibility tests were conducted using the texture analyzer (model TA-XT2i, Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Survey, UK) using the method by Suhendro et al (1999). (Stress relaxation of dough was measured on two dough balls for each treatment by compressing the dough balls after they were proofed. Maximum force of compression, modulus of deformation (initial, middle and end), adhesive force and work values were measured. Extensibility test was measured on tortillas stored for 4, 8, 12, 16, 20 days of storage. Tortillas were evaluated by extending a strip (35 x 75 mm). Two tortilla strips from two tortillas were cut to avoid the puffed portions (to maintain sample uniformity) using an acrylic template (Joseph 1999). The extensibility test was conducted using the return to start option, in the tension mode and trigger force of 0.05 N. Pre and post test speeds were 10.0 mm/s and test speed of 1.0mm/s. The modulus of deformation (N/M), force (N) to extend tortilla strip 1.0 mm and force(N), distance (mm) and work to rupture (Nm, area under the curve) were recorded

10. *Quality Index*: Quality Index (QI) was calculated according to (Waniska 2002) as follows:

QI = Opacity * *Specific Volume* * *Rollability Score* (at 12 days of storage)

Quality Index values above 450 were considered to have good tortilla stability, opacity and diameter.

Statistical Analysis

Fischer least significant difference (LSD) values were calculated using the general linear model at a confidence level of 95% ($\alpha = 0.05$). LSD values were obtained for all the dough and tortilla properties. The statistical software SAS (version 8.0, SAS Institute, Cary NC) was used.

CHAPTER IV

STANDARDIZATION OF ENZYMES FOR TORTILLA QUALITY

Introduction

A variety of enzymes with different functionalities are being used as processing aids in bread and also in corn tortillas. The objective of their usage is improved dough handling and extended shelf life. Several enzymes were evaluated in production of wheat flour tortillas. The effects of increasing levels of eight commercial hydrolytic enzymes representing amyloglucosidases, bacterial amylases, fungal amylases, cereal amylases, maltogenic and xylanase on tortilla characteristics were studied. The falling number procedure was used to optimize the dosage and type of enzymes added to wheat flour for tortilla manufacture.

Objectives

The objectives were:

1. To determine falling number (FN) values for several amylases at different levels.

2. Use the FN information to select enzyme levels to optimize tortilla quality.

Materials and Methods

Untreated tortilla flour I (enriched, unbleached, without malt with 13.8% moisture content, 11.6% protein, 0.5% ash, 58.6% farinograph absorption, 42 Mixograph unit resistance, Source: ADM Arkaday, Enid OK) and untreated tortilla flour II (13.1%

moisture, 0.5% ash, 11.4% protein, falling number 371 sec., 57.0% water absorption, Morrison Milling Company, 500 g of flour/batch) were used.

Nine commercially available enzymes were used in the experiments. The two enzymes from Sigma Chemical Company had available activity levels and were used to establish standard curves between enzyme activity and falling number.

The bacterial α-amylase from *Bacillus amyloliquefaciens* was an endo amylase that rapidly hydrolyzed 1,4-α–glycosidic linkages in amylose and amylopectin. The breakdown products of this enzyme are oligosaccharides and dextrins of varying chain length. This enzyme was active at high temperature range of 70˚C-90˚C.

α-amylase from malted barley was used to create second standard curve for falling number and enzyme activity. This enzyme liberated maltose form starch. Amylase activity was expressed in activity units (AU). The optimum pH values were not available for most of the enzymes. The specifications, source and suggested usage for all the enzymes used in the experiments as supplied by the manufacturer are listed below (Table V).

Table V

Sources of enzymes and range of levels used in formulation

Enzyme Type	Suggested Levels	Enzyme Source	Enzyme Level (used)
amyloglucosidase1	60-120 ppm	Innovative Cereal Systems, Wilsonville, OR	40, 80, 150
amyloglucosidase 2	60-120 ppm	Innovative Cereal Systems, Wilsonville, OR	50, 100, 150, 200
bacterial 1	60 ppm	Innovative Cereal Systems, Wilsonville, OR	30, 60, 150, 500
bacterial 2	-	Sigma Chemical Company, MO	0.05, 0.1, 0.3, 1.0 (au)
fungal amylase	50-100 ppm	Innovative Cereal Systems, Wilsonville, OR	20, 40, 60, 100
maltogenic 1	150-300 ppm	Innovative Cereal Systems, Wilsonville, OR	140, 280, 450
maltogenic 2	-	Novozymes, Corp, NC	30,60, 100
malted barley	-	Sigma Chemical Company, MO	3, 5, 20, 50 (au)
xylanase	100-150 ppm	Enzyme Development Corporation, NY	30, 60, 100, 150

Falling Number Procedure

The Falling number procedure (AACC 2000) was used to measure the enzyme activity. Falling number apparatus (1800 Model, Perten Instruments) was used. Moisture content of the flour was determined using a two-stage moisture method (AACC 2000). A flour slurry containing 7 g of flour and 25 g water was added to the viscometer tube and the tube was immersed in water bath and stirred. The time taken in seconds for the viscometer-stirring rod to fall through the starch paste was the falling number value.

Tortilla Preparation

Hot press tortillas were prepared from control flour and untreated flour (specifications described earlier) according to the procedure described in chapter III. The bacterial 2 amylase was evaluated for tortilla properties at 0.05, 0.1, 0.3 and 1-activity units. Malted barley amylase was tested at 3, 5, 20 and 50 units. Choice of these levels was based on results from the calibration curves obtained from the falling number experiments. Tortillas were evaluated for all the attributes described in Chapter III.

Statistical Analysis

The Fischer's Least Significant Difference (LSD) level at $\alpha = 0.05$, was calculated for the subjective and objective tortilla properties as described in Chapter III.

Results

The falling numbers obtained by adding malted barley amylase at different levels are shown in Appendix (Table A.1). Control flour without added enzyme had a falling

number value of 371 sec. The effect of the enzymes on undamaged starch granules at normal room temperature is very small. During the procedure starch gelatinizes and is more easily attacked by amylases and is hydrolyzed rapidly. The time interval between gelatinization of starch and the final state of enzyme during baking influences product quality. In the falling number procedure it takes 30 sec to undergo this cycle which is similar to the time that is used in tortilla baking. This method is actually a measure of the viscosity of the gelatinized starch at nearly 100° C.

Falling number and α-amylase activity in malted barley flour were inversely related (R^2= 0.98) (Fig. 3). The falling number curve shows the effect of enzymatic decomposition during short heating cycle. Standard curve was established from 3 to 70 activity units. Falling number was 70 sec at the highest enzyme activity.

In case of bacterial 2, enzyme activity was linearly related with R^2= 0.72 (Fig. 4). Lowest falling number values were observed at 1unit of enzyme activity and are shown in Appendix (Table A.2). With further increase in enzyme activity falling number stayed constant at 63.
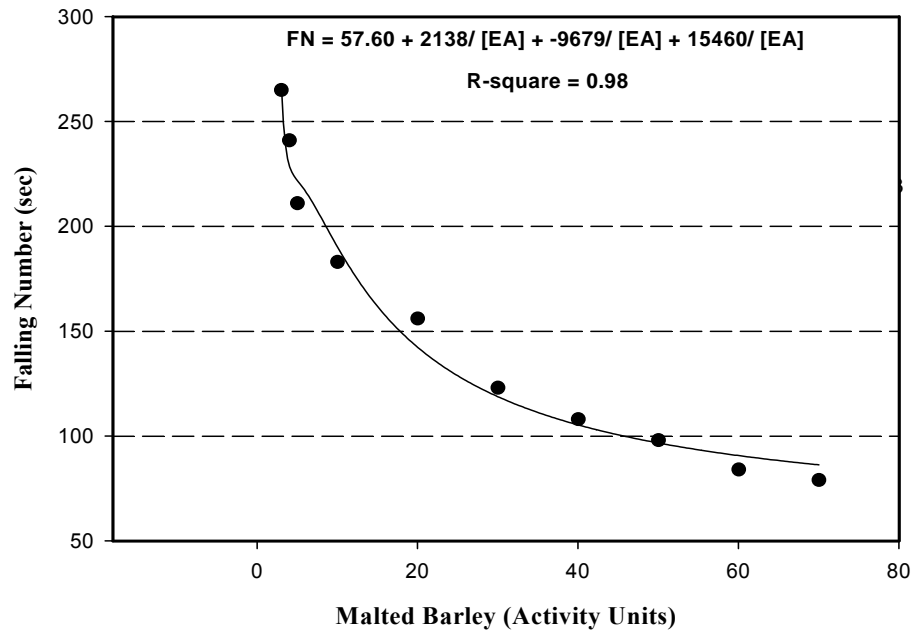
Fig. 3 Curve between falling number and enzyme activity for malted barley.

FN= Falling Number, EA= Enzyme Activity

The initial falling number value of the untreated flour was 370. Falling number values decreased from 316 to 79 with enzyme addition. Flours with FN values below 150 were obtained at activity levels greater than 20. Wheat flours with high amylase activity cause stickiness in bread. 3, 5, 20 and 50 activity levels showed potential for evaluating in tortillas, since at these levels the entire range of falling number values were covered.
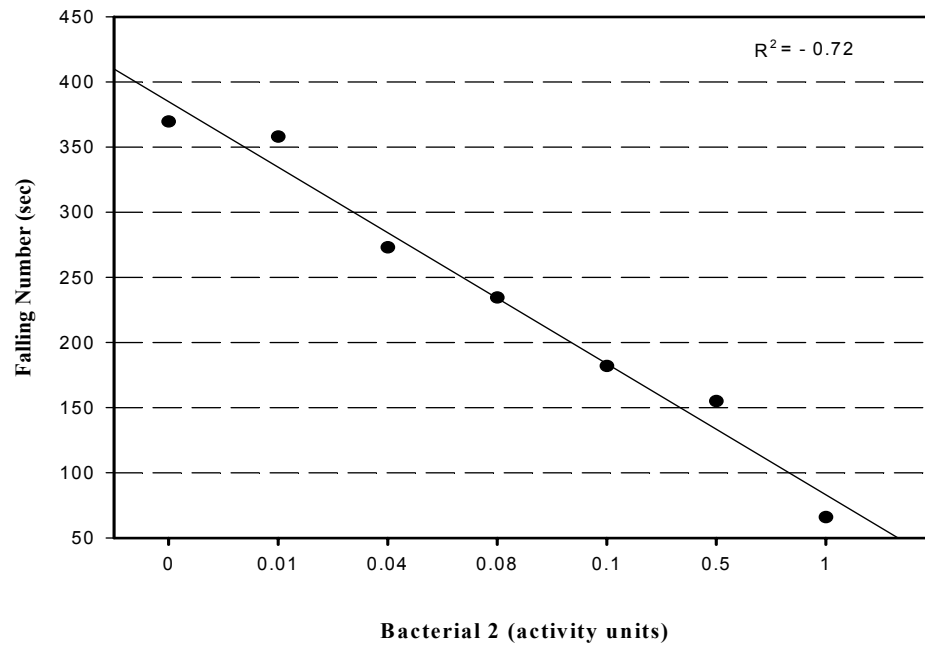
Fig. 4 Effect of bacterial 2 activity on falling number.

The bacterial 2 amylase was highly active at low concentrations. The falling number value was 63 at 2 activity units which indicated that this enzyme needed to be used at much lower levels. Falling number values in the range of 200-300 were obtained using 0.04 and 0.08 activity units respectively. Four concentrations were selected and further used to evaluate tortilla quality.

Subjective and Objective Analysis of Dough

Table VI

Effect of bacterial 2 and malted barley amylase on subjective dough properties

Enzyme Activity (activity units)	Water Absorption (%)	Smoothness Score	Softness Score	Toughness Score	Press rating score
bacterial 2					
0	51.5	1.9	1.9	2.0	1.9
0.05	51.4	1.8	1.6	1.6	1.7
0.1	51.4	1.7	1.7	1.7	1.7
0.3	50.0	1.8	1.6	1.6	1.7
1.0	50.0	1.8	1.6	1.7	1.7
malted barley					
3	51.4	2.0	2.0	2.0	2.2
5	51.4	2.0	2.0	2.0	2.0
20	51.4	1.8	1.8	1.8	1.8
50	51.4	1.8	1.7	1.6	1.8
LSD (α=0.05)	-	0.15	0.19	0.16	0.27

Water content was adjusted to attempt to have uniform comparison between dough properties. The effect of bacterial 2 amylase and malted barley amylase is shown (Table VI). The amount of water added to the dough was decreased at higher activity levels to attain similar dough properties. Both bacterial 2 and malted barley did not affect smoothness of dough. Dough softness and toughness were significantly decreased by all levels of bacterial 2 and at the highest level of malted barley addition.

Table VII

Effect of seven enzymes on subjective ratings of control flour dough

Enzyme Type	Water Abs. (%)	Smoothness	Softness	Toughness	Press Rating
amyloglucosidase 1	52.0	1.8	1.8	1.8	1.8
amyloglucosidase 2	52.0	2.0	1.7	1.5	1.5
bacterial 1	52.0	1.8	1.5	1.5	1.5
fungal	52.0	1.5	2.0	1.8	2.2
maltogenic 1	52.5	1.8	1.8	1.6	1.6
maltogenic 2	53.0	1.8	1.8	2.8	2.0
xylanase	53.0	2.0	1.8	1.8	2.2
Control	53.0	2.0	2.0	2.0	2.0
LSD (α =0.05)	**-**	**0.3**	**0.3**	**0.5**	**0.4**

All enzymes were evaluated at 150 ppm.

The effect of seven enzymes at 150 ppm on subjective dough properties is shown (Table VII). The properties of dough prepared with seven different enzymes are summarized in Appendix (Table A.3). Dough absorption was lowered for amyloglucosidase1, amyloglucosidase 2, bacterial 1, bacterial 2, fungal and maltogenic 2 enzyme to attain similar dough properties. Dough smoothness was significantly lower for fungal amylase and was not affected by any other enzyme. Dough softness was significantly reduced with amyloglucosidase 2 and bacterial 1 amylase. Maltogenic 2 significantly increased dough toughness whereas bacterial 1 and amyloglucosidase 2 decreased dough toughness. Press-rating was significantly lower for amyloglucosidase 2, bacterial 1 and maltogenic 2 amylase.

Table VIII

Effect of bacterial 2 and malted barley amylase on tortilla properties

Enzyme Activity (activity units)	Height (mm)	Diameter (mm)	Opacity (%)	Specific Volume (cm^3/g)	pH
bacterial 2					
0	3.0	170.5	90.7	1.65	5.3
0.05	2.4	176.2	87.0	1.45	5.3
0.1	2.9	174.3	88.3	1.73	5.2
0.3	2.7	177.0	88.5	1.64	5.2
1.0	2.4	180.8	84.0	1.53	5.4
malted barley					
3	2.5	171.0	85.3	1.40	5.3
5	3.0	170.6	91.3	1.72	5.2
20	2.9	174.5	84.3	1.72	5.5
50	2.8	169.5	84.0	1.65	5.3
LSD (α=0.05)	**0.26**	**3.8**	**4.5**	**0.16**	**0.27**

A good quality tortilla should have more than 90% opacity, 1.6-1.7 cm^3/g specific

volume, 2.5 mm height, more than170mm diameter and rollability score above 3 at 12

days of storage.
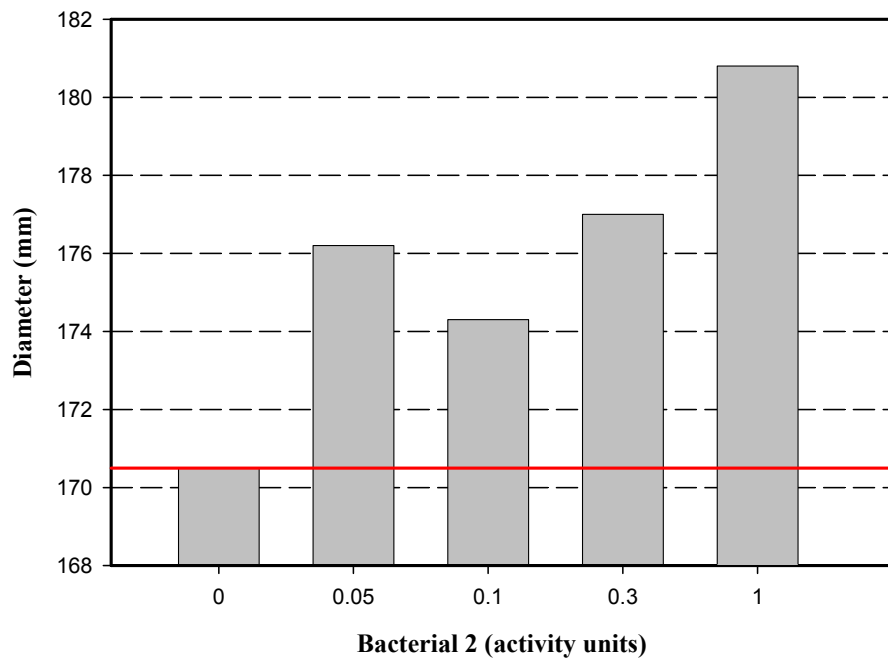
Fig. 5 Effect of bacterial 2 amylase on the diameter of flour tortillas.
Least significant difference (α =0.05) = 3.8
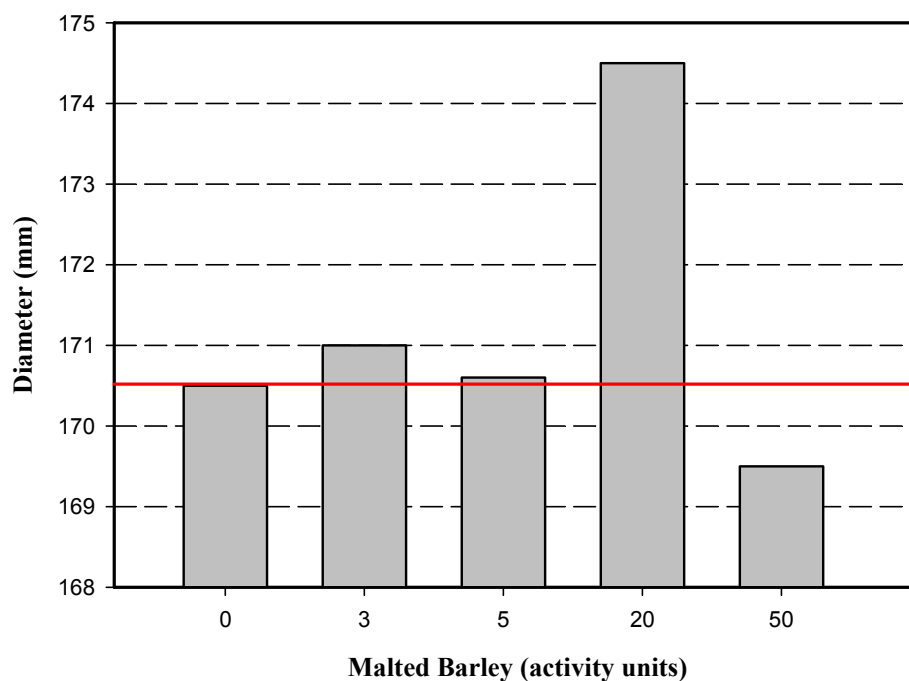
Fig. 6 Effect of malted barley amylase on the diameter of flour tortillas.
Least significant difference ($\alpha = 0.05$) = 3.8

The effect of bacterial 2 and malted barley amylase activity on tortilla properties is shown (Table VIII). Average tortilla moisture and tortilla pH was 33.7 % and 5.4 respectively. Tortilla moisture and pH were not affected by enzyme type or amount. Tortilla height was significantly lower at 3 units for malted barley and at 0.05 and 1.0 units for bacterial 2. Tortilla diameter increased significantly at higher levels of bacterial 2 (Fig. 5). Tortilla diameter was not affected but tortilla height decreased with malted barley addition and activity levels (Fig. 6). Opacity was compromised at the highest enzyme levels when tortilla became more translucent (Fig. 7). Specific volume at 0.05

units of bacterial 2 and 3.0 units of malted barley was significantly lower as compared to control (Table VIII).

There is a very narrow range of enzyme activity for malted barley which has potential to improve tortilla attributes. This suggested that enzyme activity between 5 and 20 units has potential to improve tortilla properties. Amylase from *Bacillus amyloliquefaciens* improved tortilla quality significantly at the lowest level. 1.0 unit improved tortilla diameter but reduced opacity and specific volume of tortilla.
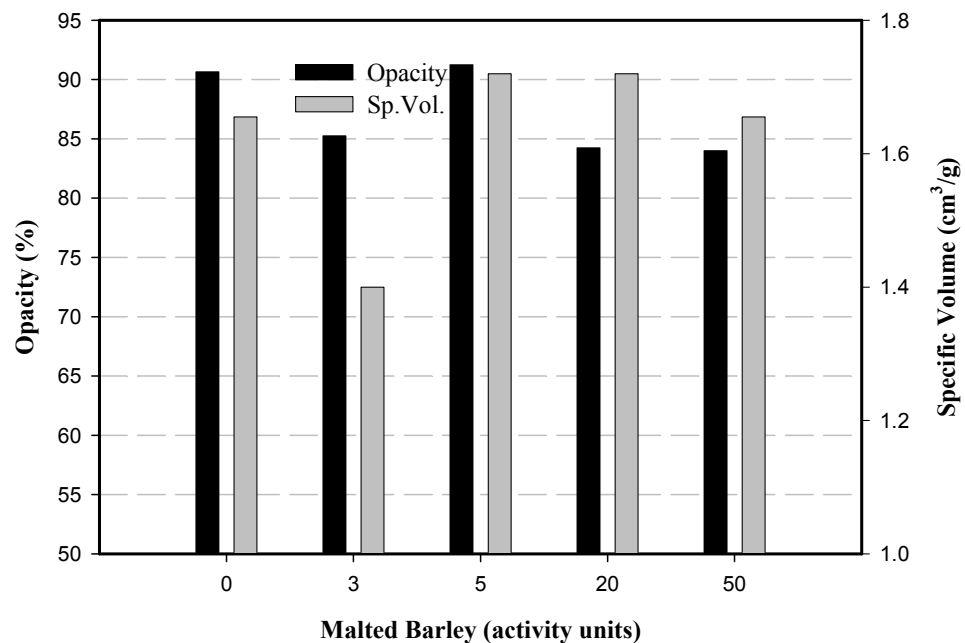
Fig. 7 Effect of malted barley amylase on tortilla opacity and specific volume.
Least significant difference (α =0.05) opacity = 4.5; Sp.Vol = 0.16

Good quality tortilla has

> 90% opacity
>1.6-1.7 cm^3/g specific volume

Table IX

Effect of malted barley and bacterial 2 amylase on tortilla rollability score (RS)

Enzyme Activity	RS (4 day)	RS (8 day)	RS (12 day)	RS (16 day)	RS (20 day)	RS (24 day)	RS (28 day)
Malted barley							
0	4.2	3.3	3.0	2.2	1.5	1.0	1.0
3	4.0	3.4	3.2	2.7	2.5	2.1	1.5
5	4.4	4.0	3.0	2.2	2.5	2.0	1.9
20	4.4	4.0	3.1	2.4	2.0	1.7	1.5
50	4.4	3.4	4.0	3.0	2.8	2.5	2.0
Bacterial 2							
0.05	4.4	4.0	4.0	4.0	3.9	3.7	3.0
0.1	4.3	3.9	3.9	3.5	3.5	2.9	2.7
0.3	4.4	4.0	4.0	3.7	3.5	3.0	2.8
1.0	4.3	4.0	3.4	2.8	2.5	2.0	1.8
LSD(α=0.05)	**0.3**	**0.2**	**0.3**	**0.3**	**0.1**	**0.3**	**0.2**

The shelf stability of flour tortillas is the duration in days they remain rollable. Another measure of overall quality is the "Quality Index", which involves opacity, specific volume and rollability scores. This is a good approach to evaluate overall tortilla quality. The effect of different enzymes at various levels in affecting overall tortilla quality index is shown (Table X). Tortilla quality index was affected by enzyme type and amount. Lower activities of amylase from malted barley were not effective in extending the shelf life of tortilla (Table IX).
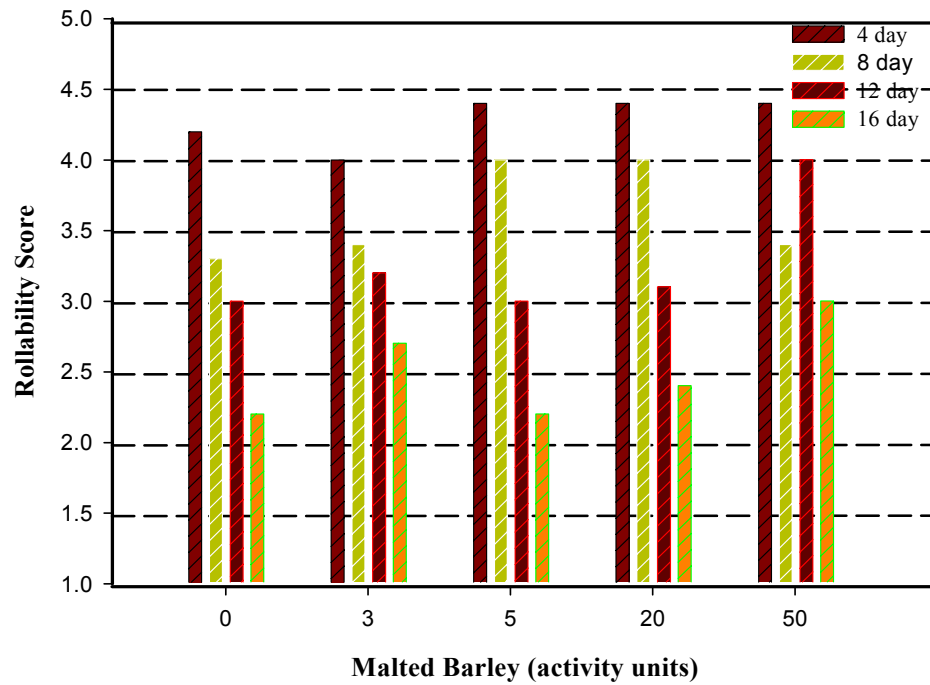
Fig. 8 Effect of malted barley amylase on tortilla rollability score at 4, 8, 12 and 16 days of storage.
Least significant difference (α =0.05) = 0.3, 0.2, 0.3, 0.3 for 4, 8, 12 and 16 days respectively
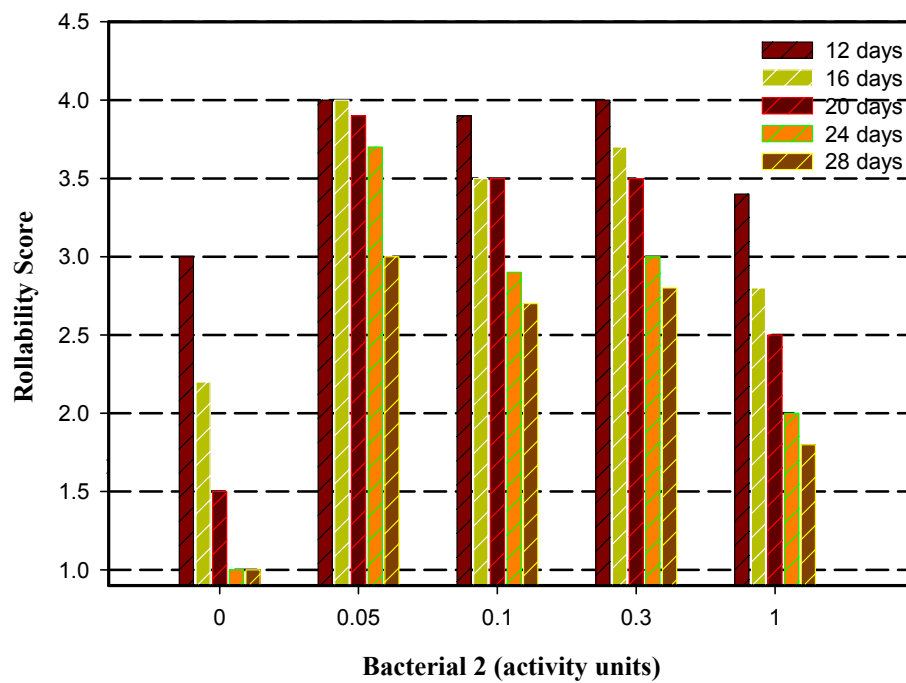
Fig. 9 Effect of bacterial 2 enzyme activity on tortilla rollability scores at 12, 16, 20, 24 and 28 days of storage.
Least significant difference (α =0.05) = 0.3, 0.3, 0.1, 0.3 and 0.2 at 12, 16, 20, 24 and 28 days respectively

Rollability of tortilla was better than control at 16 of storage at the highest level (Fig. 8). However at this level since tortilla diameter was smaller when compared to other activity levels. This enzyme concentration was not effective in improving the overall quality of tortilla.

Significant differences in tortilla rollability were obtained with bacterial 2 amylase (Fig. 9). For all treatments, rollability scores were significantly better than control at 12 days. Lower levels were effective in improving the shelf life of tortilla by 16 days as compared to the control. Rollability was significantly better than control after 24 days of storage at 0.05, 0.1 and 0.3 activity units. The best treatment at 0.05 activity units gave rollable tortillas at 28 days. Bacterial 2 amylase extended tortilla shelf stability. Lower levels than 0.05 were effective in improving the tortilla shelf life of tortilla by 16 days compared to control.

Bacterial amylase was evaluated in untreated II flour at the same levels. The effect of bacterial 2 amylase has been shown in Appendix (A.8 and A.9). The results indicated that the enzyme was effective in improving tortilla shelf life significantly irrespective of the flour type and source.
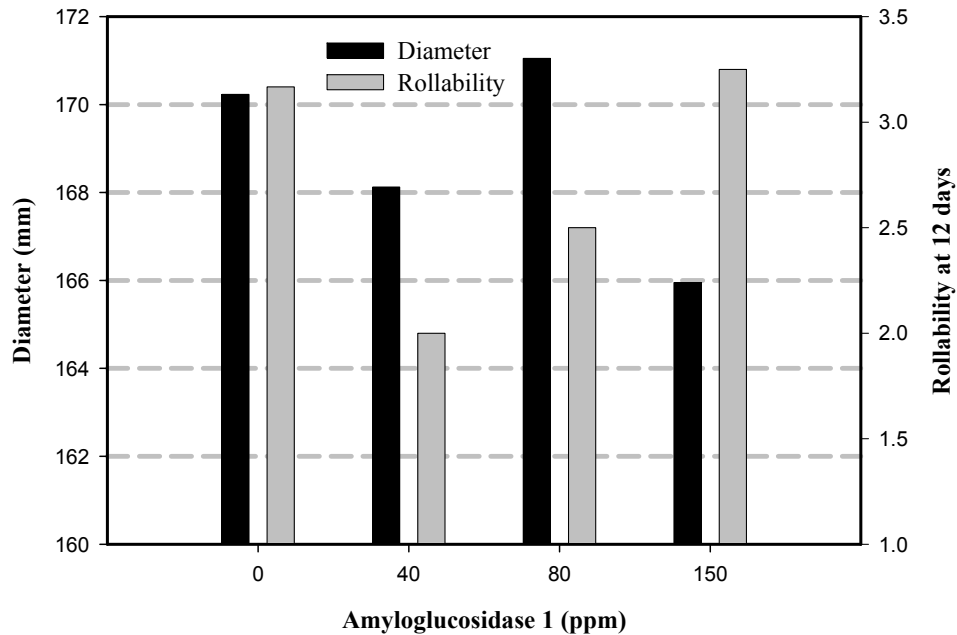
Fig. 10 Effect of amyloglucosidase 1 on tortilla diameter and rollability score at 12 days of storage.

Least significant difference (α =0.05) diameter = 1.87; rollability = 0.69

Amyloglucosidase 1 was evaluated at 40, 80 and 150 ppm. The effect of amyloglucosidase 1 on subjective and objective properties of dough and tortilla are summarized in Appendix (Table A.4 and A.5). Tortilla height was significantly lower than control with enzyme addition. Different levels of enzyme did not affect tortilla height. Tortilla diameter was significantly smaller using 150 ppm of amyloglucosidase 1 (Fig. 10). Addition of amyloglucosidase 1 reduced tortilla specific volume at all levels.

Fig. 11 Effect of amyloglucosidase 2 on tortilla diameter and rollability score at 16 days of storage.

Least significant difference (α =0.05) diameter =7.2; rollability = 0.8

Tortillas prepared using 40 and 80 ppm of amyloglucosidase 1 had lower rollability scores as compared to control at 12 days. Amyloglucosidase 1 was not effective in improving rollability at any of the levels. Tortillas prepared with amyloglucosidase 1 at all levels had lower quality index than control (Table X). None of the levels of amyloglucosidase 1 thus improved overall tortilla quality. Hence further optimization with this enzyme was not conducted.

The effect of amyloglucosidase 2 at different levels on dough and tortilla properties is summarized in Appendix (Table A4, A.5). Thicker tortillas were obtained as compared to control with 50 ppm of enzyme. Tortilla diameter was not affected by amyloglucosidase 2 additions (Fig. 11). Tortilla opacity was significantly lower than control at 50 ppm of amyloglucosidase 2. Tortilla specific volume was significantly lower than control at 200 ppm. Quality index of tortilla was not affected by amyloglucosidase 2 (Table X). There was no effect of ≤ 150 ppm of amyloglucosidase 2 on tortilla rollability scores at 16 days of storage; however 200 ppm of enzyme was effective in maintaining tortilla shelf life till 16 days of storage. Amyloglucosidase 2 did not improve tortilla properties. Hence further optimization with amyloglucosidase 2 was not done.

Table X

Effect of enzyme type and level on tortilla quality index

Enzyme Type	Level (ppm)	Quality Index (12 day)	Quality Index (16 day)
Control	0	506	359
amyloglucosidase 1	40	233	190
	80	323	274
	150	379	327
amyloglucosidase 2	50	399	367
	100	467	329
	150	497	386
	200	369	354
bacterial 1	30	445	397
	60	495	413
	150	421	347
	500	490	335
bacterial 2	0.05	504	504
	0.1	593	532
	0.3	580	544
	1.0	447	319
fungal	20	428	282
	40	446	327
	60	468	391
	100	322	201
maltogenic 1	140	461	343
	280	506	434
	450	348	322
maltogenic 2	30	360	304
	60	350	287
	100	439	380
malted barley	3	383	309
	5	549	431
	20	506	344
	50	485	451
xylanase	30	415	415
	60	352	264
	100	479	397
	150	421	421
LSD(α =0.05)	-	**60.2**	**55.0**

Good quality tortilla has quality index > 450 at 12 days of storage at 22º C
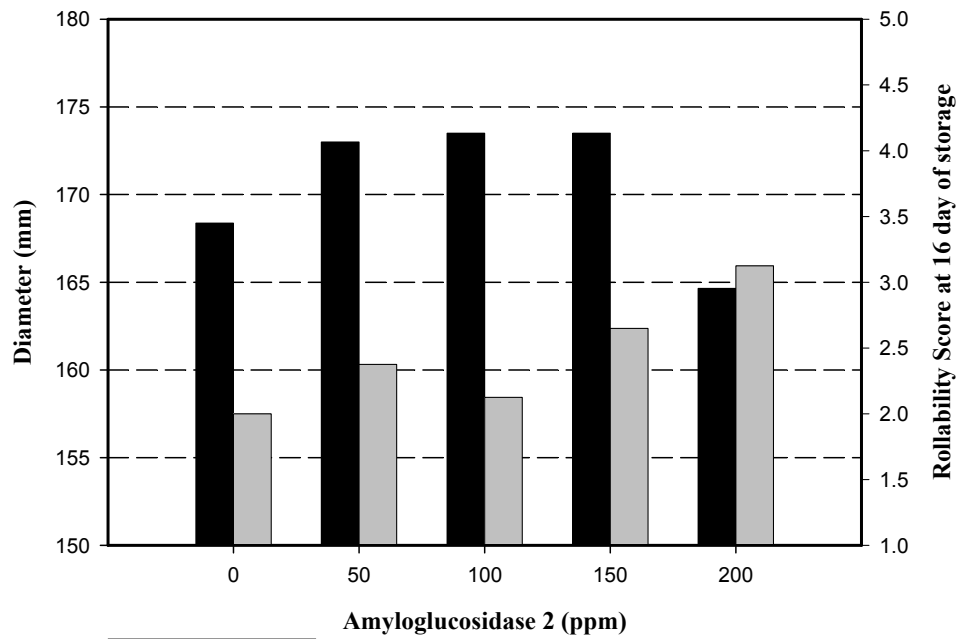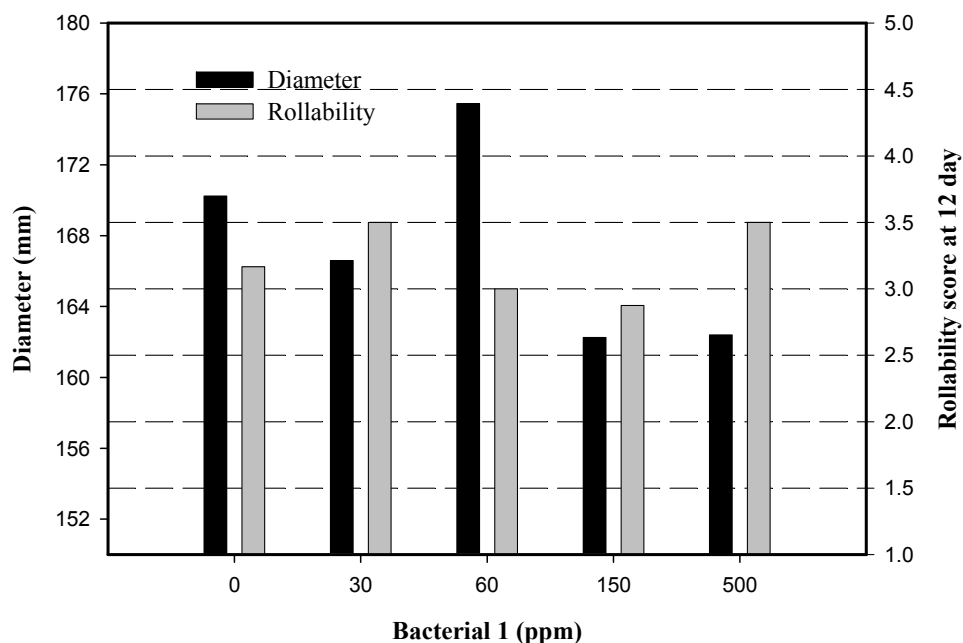
Fig. 12 Effect of bacterial 1 on tortilla diameter and rollability score at 12 days of storage at 22°C.

Least significant difference (α =0.05) diameter = 3.9; rollability = 0.2

The effect of bacterial 1 amylase on tortilla properties is shown in Appendix (Table A.4, A.5). Bacterial 1 was evaluated at 30, 60, 150 and 500 ppm levels. Tortillas containing 60 ppm of bacterial 1 had significantly larger diameter and specific volume than control (Fig. 12). Further enzyme addition reduced tortilla diameter. Rollability scores of tortillas containing 30 ppm of bacterial 1 amylase were significantly better than control at 16 days of storage. Bacterial 1 was not as effective as bacterial 2 in improving tortilla shelf life.
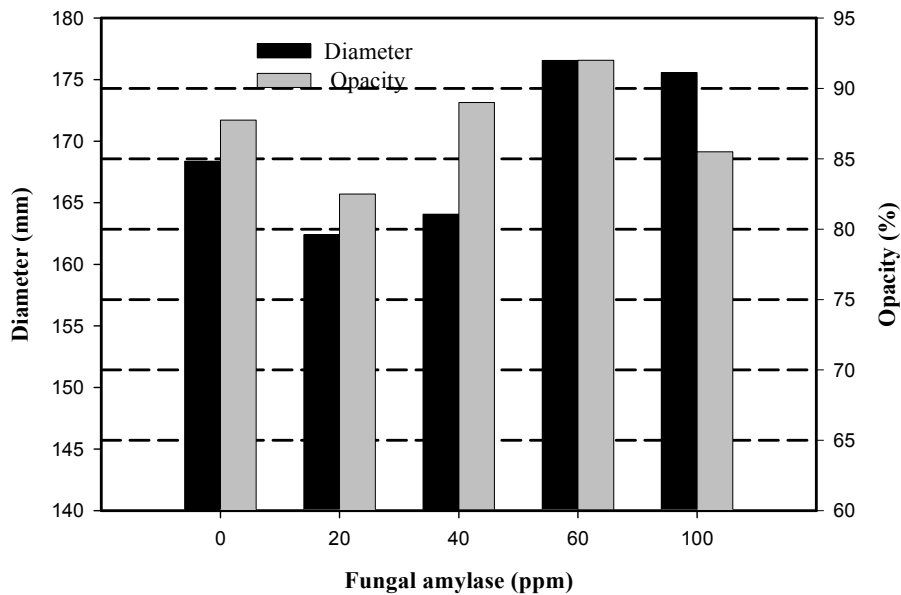
Fig. 13 Effect of fungal amylase on tortilla diameter and opacity.
Least significant difference (α =0.05) diameter = 5.1; opacity = 5.4

Fungal amylase was evaluated at 0, 20, 40, 60 and 100 ppm. There was no significant effect of fungal amylase on tortilla height. Diameter was significantly smaller at 20 ppm and larger at 60 and 100 ppm (Fig. 13). Tortilla opacity was significantly less at 20 ppm. Tortilla specific volume was significantly lower at 20 and 40 ppm. Tortilla quality index was significantly lower at 100 ppm of enzyme supplementation (Table X). Tortilla rollability scores were not affected by enzyme addition at any level. The enzyme was effective at 60 ppm in improving diameter and opacity but reduced overall tortilla quality. Further optimization of this enzyme was therefore not conducted.
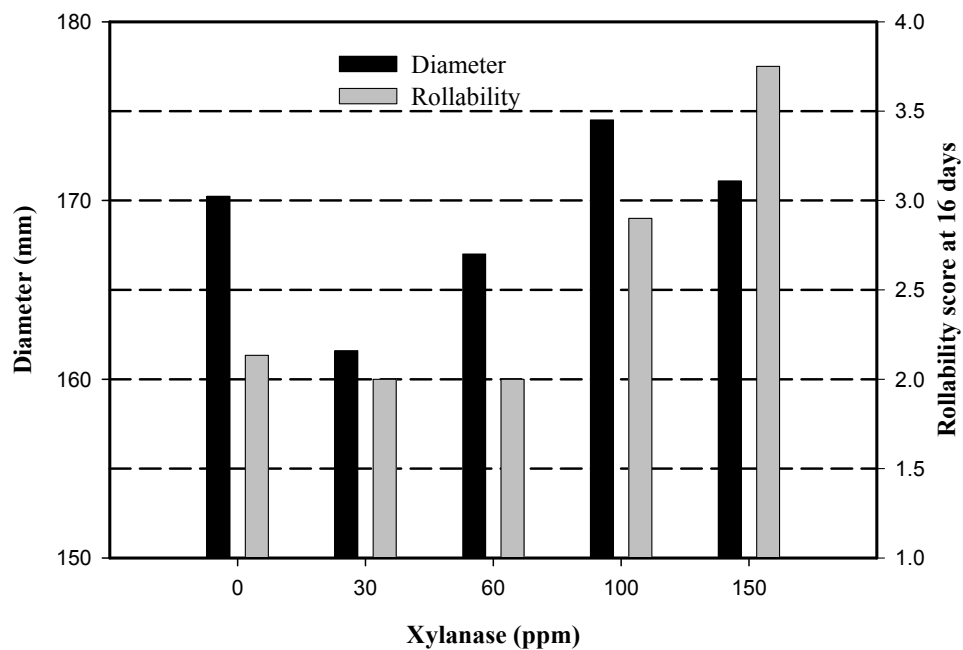
Fig. 14 Effect of xylanase on tortilla diameter and rollability score.
Least significant difference (α =0.05) diameter = 5.43; rollability = 0.8

Xylanase was evaluated at 30, 60, 100 and 150 ppm. Tortilla height was significantly smaller than control at the highest level of enzyme used (Fig. 14). At 100 ppm, tortilla diameter was significantly better than control (Fig. 14). Opacity was significantly lower than control at 30 and 150ppm. The effect of xylanase on tortilla properties is shown in Appendix (Table A.5). Tortilla specific volume was significantly lower than control at 30, 60 and 150ppm. Tortilla quality index was not affected significantly at any of the levels evaluated (Table X). Rollability score at 16 day was significantly better than control for 100 and 150 ppm. Stickiness was observed in tortillas at 150 ppm at 16 days of storage. Xylanase shows potential in improving tortilla

properties between 100 and 150 ppm. Further research is needed to standardize xylanase in tortillas.
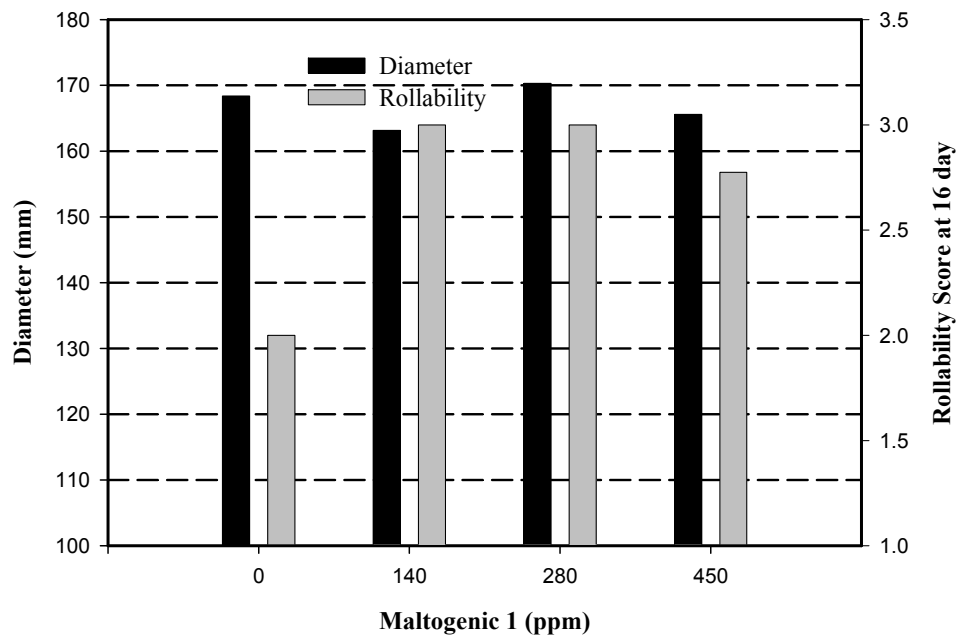
Fig. 15 Effect of maltogenic 1 amylase on tortilla diameter and rollability score at 16 days.
Least significant difference (α =0.05) diameter = 3.4; rollability at 16 day = 0.9

Tortilla height was significantly lower than control at all levels as shown in Appendix (Table A.4). Tortilla diameter was significantly smaller at 140 and 450 ppm (Fig. 15). Tortilla opacity was higher at 280 ppm. Specific volume of tortilla reduced significantly at 140 and 450 ppm. Tortilla quality index was reduced significantly with 450 ppm of enzyme (Table X). Tortilla rollability score was significantly better than control at 16 days of storage with 140 and 280 ppm of enzyme addition. Maltogenic 1 amylase at 280 ppm was effective in maintaining tortilla diameter, opacity and stability. Maltogenic 1, xylanase, malted barley and bacterial 1 showed better quality index than other enzymes at 16 days of storage.

Fungal amylase and amyloglucosidase 1 had a detrimental effect on tortilla quality at the levels evaluated. Fungal amylase and amyloglucosidase action produced sugars that were not beneficial for tortilla. Amyloglucosidase 2 did not affect tortilla properties significantly. Xylanase was effective in maintaining tortilla quality at 30 and 150 ppm. Quality index of tortillas made with bacterial 2 and malted barley was significantly better than control at specific levels after 16 days of storage.
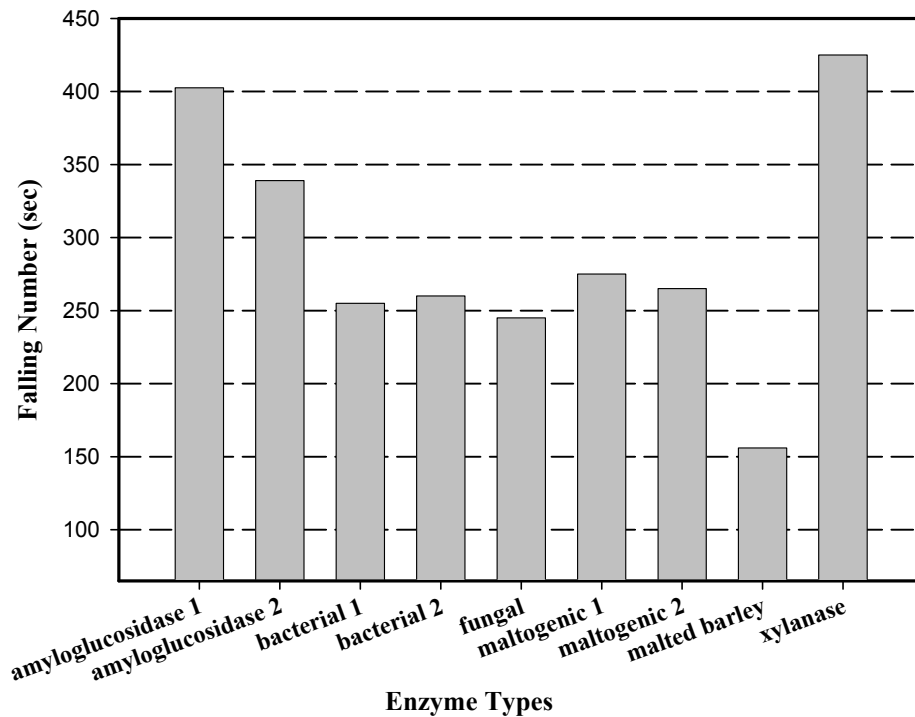
Fig. 16 Effect of different enzymes on falling number.

Control tortilla flour has falling number in the range of 250-350 sec. Optimization of enzyme activity for bacterial 2 and malted barley flour using falling number produced good quality tortillas. This indicated that enzyme activity was important in influencing quality. Hence falling number values were determined for all the above enzymes to see if the optimum level in tortilla flour (from Table IX) and enzyme activity were related. At the optimum level of each enzyme the falling numbers are shown (Fig. 16).

Falling number values for different enzymes were in three categories. >300, 200-300, <200. Amyloglucosidase 1 had higher falling number value as compared to other enzymes. Amyloglucosidase 2, bacterial 1, bacterial 2 and maltogenic 1 had falling number values in the range of 250–350 sec. This suggested that bacterial 2 amylase enzyme activity was related to tortilla properties. Fungal amylase was not effective at any level. Malted barley at the optimum level had low falling number values, suggesting that enzyme activity did not relate to tortilla quality for malted barley amylase.

Discussion

Amyloglucosidase 1 and amyloglucosidase 2 affected tortilla properties differently. Amyloglucosidase 1 reduced overall tortilla quality and was not useful in improving tortilla properties. Amyloglucosidase 1 had secondary protease side activity which might have produced a different effect on tortillas as compared to Amyloglucosidase 2 which was more effective than amyloglucosidase 1 in maintaining tortilla rollability.

Maltogenic amylase 1 was effective in maintaining tortilla quality at intermediate levels. It has been reported earlier that maltogenic amylase produce linear oligosaccharides of 2-6 glucose residues which might be interfering with amylopectin retrogradation. Negative effects on tortilla properties were evident at high levels of enzyme addition. Fungal amylase did not improve tortilla properties. The end products in case of fungal amylases are mostly sugars which are not effective in improving tortilla quality (Aida 1996 et al).

Maltogenic 2 was not effective in maintaining tortilla quality, though it has been effectively used in bread (Gray and Bemiller 2003). Maltogenic 2 effectively degrades amylopectin and amylose to maltose and oligosaccharides. This difference in the effect of maltogenic 2 in tortilla and bread is because of their different baking time and temperature conditions.

Bacterial 2 improved dough properties at certain levels. Intermediate levels of bacterial amylase extended tortilla shelf life from 12 to 28 days. Optimization and standardization of bacterial amylase significantly improves tortilla quality. The enzyme activity required to improve tortilla properties was very low. Fungal amylases have been found to be effective in case of bread but were not found to be effective in case of tortilla. This suggests and supports the fact that bread and tortilla functionality are governed by different mechanisms. Most of the intermediate temperature stability maltogenic enzymes have an activity in the temperature range of 65-80° C. However bacterial 2 was conventional endo-amylase with activity in the relatively higher range up to 90°C. It had maximum activity around 80°C with 50% activity at room temperature and was effective in improving tortilla quality.

The mechanism of action of α-amylase in bread has been investigated by many researchers for long time. Bacterial 2 affected the structure of starch and the anti-firming effect could be possibly due to the hydrolysis products that interfere with amylopectin retrogradation. Reaction products were formed as seen by HPLC due to this enzyme which might be possibly reducing gluten starch interactions shown in Appendix (Table A.7). This enzyme might be hydrolyzing amylopectin at a higher rate than other

enzymes. The bacterial 2 amylase was thus a useful anti-staling agent with special properties either due to specific isolation techniques or exceptional purity. This enzyme had unique properties in inhibiting staling. Further investigation of the carbohydrate profile is needed to understand the complexity of this enzyme and its mechanism in the tortilla system.

Xylanase at higher levels improved tortilla rollabilty scores without significantly lowering tortilla quality. Xylanases have been used to improve dough-handling properties in bread (Harada et al 2000). The effectiveness of xylanase could be due to the solublization of non-starch polysaccharides, especially flour's insoluble xylans, which otherwise have a tendency to bind water before it can be used by gluten and starch, thus contributing to firmness. The use of xylanase at higher levels might have solublized the insoluble xylans, resulting in improved rollability. Thus use of xylanase at higher levels might have potential to improve tortilla stability. Further research is needed to study the effect of combination of enzyme types on tortilla properties.

In summary, different enzymes exhibited a variety of hydrolysis reactions during processing in the tortilla system. Enzymes have specific mode of action and the differences might be because of different isolation procedures, different active sites, substrate inhibition effects, effect of cofactors and differences in enzyme kinetics. These mechanisms led to the various interactions in the dough and tortilla system which resulted in differences in tortilla quality.

CHAPTER V

EFFECT OF STARCH DAMAGE ON TORTILLA PROPERTIES

Introduction

The importance of mechanically damaged flour is because of its influence on high water absorption, dough mixing properties of the flour and enzyme activity. Damaged granules hydrate rapidly and are hydrolyzed by α and β-amylases. Consequently, the accurate measurement of the degree of starch damage is important for the assessments of quality of dough's and baked products. In a previous research on tortillas, diameter and shelf life were related to damage starch. The amount of damaged starch in wheat flour adversely influenced the machinability and characteristics of baked tortillas (Wang and Flores 1999b, Mao and Flores 2001). However flour with intermediate levels of damaged starch was recommended for wheat flour tortillas (Waniska et al 2003).

Materials and Methods

Generation of Samples: A rice grinder of 2-liter capacity (ELGI Ultra grind, Bangalore, India) was used to mechanically damage wheat flour. The conical shaped grinding stones inside the stainless steel drums caused mechanical damage due to the abrasion. Sample size was kept constant during the treatment. Untreated control flour (ADM Milling Company) was used for experiments. Samples (1 kg) were processed for 0.5, 1, 4 and 8 hr.

Estimation of Starch Damage: Starch damage induced by mechanical grinding was estimated using a modified colorimetric method (Williams and Fegol 1969). The method is based on the principle that amylose in mechanically damaged starch granules is more rapidly extracted by saturated sodium sulfate when compared to sound starch granules.

A 1.41 M stock solution of sodium sulfate was prepared by dissolving anhydrous sodium sulfate (400 g) in distilled water (1500 ml). An extracting solution was prepared by dissolving sulfo-salicylic acid (2 g) in stock solution (1 liter). Iodine stock solution was prepared by dissolving AR grade iodine crystals (5.5 g) and AR grade potassium iodide crystals (11.0 g) in water (25 ml) and diluted to 250 ml and stored in dark. Iodine reagent was prepared by diluting stock solution (10 ml) to 100 ml with distilled water. Gelatin (0.5 g) was dissolved in hot water (100 ml) and filtered through glass wool. Diluting solution was prepared by diluting gelatin solution (50 ml) and hydrogen peroxide (2.5 ml) to 500 ml with boiled distilled water.

Sample (1 g) was extracted with 25 ml of extraction solution for 15 min at 50ºC with thorough shaking at 5 minute intervals. Celite (0.25g) was added to the suspension followed by brief stirring. The mixture was allowed to stand for 1-2 min and later filtered through Whatman No.1 filter paper. To 10 ml of the aliquot, 10 ml of diluting solution and 0.5 ml of Iodine reagent were added. This mixture was kept in a water bath at 30º C for 15 min. Absorbance was measured at 560 mµ against a reagent blank.

Results

The figure contains the following text annotations:

- Y-axis label: Iodine Absorbance Value (Absorbance*100)
- X-axis label: Mechanical damage (hr)
- R-Square= 0.93
- $IAV = Y_O + 14.15 \text{ mech.damage (hr)} - 1.209 \text{ mech.damage}^2$
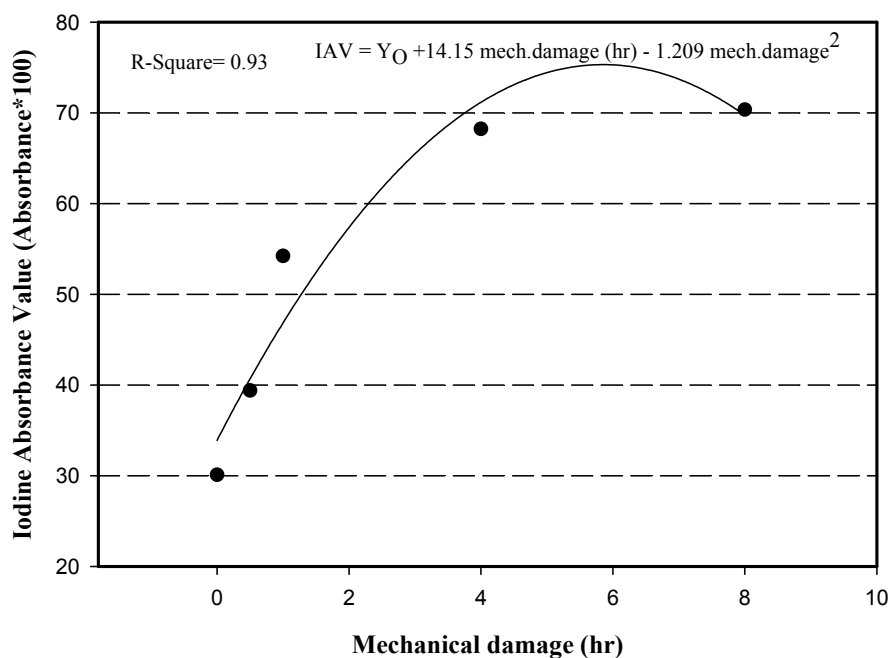
Fig. 17 Parabolic relationship showing the effect of grinding time on iodine absorbance of control tortilla flour.

Iodine absorption increased with mechanical damage of flour (Fig. 17). However the relationship was not linear for longer processing times. Previously it has been shown that mechanical damage by ball milling is linearly related to iodine absorbance (Donelson and Yamazaki 1962). This difference might be due to a different procedure and equipment used for processing. Processing for 4 hr induced sufficient starch damage in sample; longer processing times were unnecessary. Iodine absorbance values were used to calculate starch damage for the processed samples. The standard curve established between iodine absorbance value and starch damage created using enzymatic

method was used to calculate starch damage (Medcalf and Gilles 1965). Accordingly 50

units of iodine absorbance value corresponded to 9% starch damage.
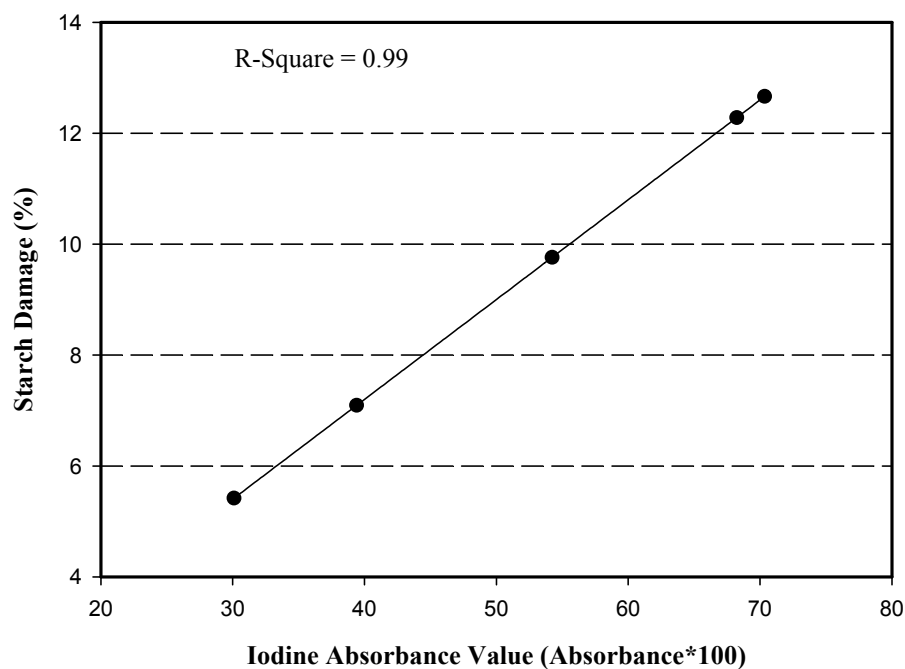
Fig. 18 Graph between iodine absorbance value and starch damage (%).

Iodine absorbance increased linearly with starch damage (R^2 = 0.99) (Fig. 18).

Processing increased the starch damage of control wheat flour from 5.4 % to 12.6%.

These results were similar to the previously reported results in literature. The technique

used to measure iodine absorbance was fast and gave reproducible results. The method

gave results similar to the previously used enzymatic methods.

Table XI

Effect of starch damage and bacterial 2 amylase on subjective dough properties

Grinding time (hr)	Bacterial 2 activity	Starch damage (%)	Smoothness	Softness	Toughness	Press Rating
0	0	5.4	2.0	2.0	2.2	2.2
0.5	0	7.1	2.0	2.0	2.2	2.0
1	0	9.7	2.0	2.0	2.2	2.0
4	0	12.2	2.0	2.5	3.0	2.6
8	0	12.6	2.5	2.0	2.8	2.5
0	0.1	5.4	2.0	2.0	2.0	2.0
0	1	5.4	2.2	2.4	2.0	2.0
0	0.05	9.7	1.5	2.5	2.4	2.4
1	0.1	9.7	1.5	2.2	2.5	2.2
4	0.1	12.2	1.8	2.3	2.5	2.4
4	0.5	12.2	1.5	1.5	1.6	1.8
8	0.1	12.6	2.5	2.2	2.4	2.2
8	1	12.6	2.5	2.0	2.1	2.0
LSD(α =0.05)	-	-	**0.4**	**0.5**	**0.5**	**0.4**

Note: 0 grinding time and 0 bacterial 2 represents control

Dough properties were significantly affected due to mechanical damage at some level (Table XI). Dough smoothness rating was higher at highest level of starch damage. Flour containing higher level of starch damage yielded dough with lower softness ratings. Enzyme addition significantly lowered dough softness ratings. Dough toughness rating and press-rating were significantly higher for higher levels of starch damage. Addition of enzyme to sample processed for 1 and 4 hr decreased dough smoothness and dough toughness. Press rating was not different from control when damaged samples were treated with enzyme.

Table XII
Effect of starch damage and bacterial 2 amylase on tortilla properties

Damage time (hr)	Enzyme Level	Starch damage (%)	Weight (g)	Height (mm)	Diameter (mm)	Opacity (%)	Sp. Vol. (cm³/g)	pH
0	0	5.4	41.9	3.0	168.4	87.8	1.65	5.4
0.5	0	7.1	40.4	2.7	167.1	81.0	1.41	5.4
1	0	9.7	41.0	2.7	167.5	78.0	1.41	5.4
4	0	12.2	41.1	3.1	161.6	78.0	1.50	5.3
8	0	12.6	41.2	2.9	165.9	78.0	1.57	5.3
0	0.1	5.4	41.5	2.9	174.3	88.3	1.73	5.3
0	1.0	5.4	41.3	2.4	180.8	84.0	1.53	5.4
1	0.05	9.7	41.2	2.5	165.8	69.0	1.27	5.3
1	0.1	9.7	40.5	2.4	172.5	72.5	1.38	5.3
4	0.1	12.2	41.4	2.8	167.3	72.5	1.34	5.3
4	0.5	12.2	40.0	2.5	172.0	70.0	1.41	5.3
8	0.1	12.6	41.6	2.7	167.4	81.0	1.38	5.4
8	1	12.6	40.6	2.7	170.8	71.3	1.49	5.4
LSD	-	-	1.9	0.2	4.3	5.5	0.15	1.3

(α=0.05)

Note: 0 grinding time and 0 bacterial 2 represents control

Tortilla weights were not significantly affected by treatments (Table XII). Tortillas were significantly thicker than control when flour contained more damaged starch. Tortilla diameter was significantly smaller than control at higher levels of starch damage. Enzyme addition to flour with starch damage resulted in tortillas with diameters similar to control tortillas. Tortilla opacity was significantly lower for all flours with > 1 hr processing. Specific volume was lower than control for lower levels of damaged starch and also for samples treated with bacterial amylase. The pH was not affected due to increasing mechanical damage or enzyme addition.

Table XIII

Effect of starch damage and bacterial 2 amylase on objective dough properties

Starch damage (%)	Enzyme Level	Force 1 (N)	Force2 (N)	Modulus (N/m)
5.4	0	11.0	3.7	-13.1
5.4	0.1	9.5	3.2	-10.6
5.4	1.0	11.8	3.9	-13.5
7.1	0	12.5	4.1	-13.5
9.7	0	16.6	6.0	-18.1
9.7	0.05	12.9	5.4	-12.7
9.7	0.1	10.8	4.5	-10.6
12.2	0	20.8	7.3	-22.8
12.2	0.1	14.9	5.8	-15.3
12.2	0.5	9.5	3.8	-9.5
12.6	0	22.3	8.3	-24.0
12.6	0.1	15.5	5.9	-16.1
12.6	1	15.2	6.3	-14.8
LSD (α=0.05)	**-**	**3.0**	**1.1**	**4.1**

Objective properties of the dough were significantly affected due to damaged starch and α-amylase addition (Table XIII). Force and modulus for the mechanically damaged samples was significantly higher than control and higher values were obtained for higher starch damage. Addition of amylase to flours with starch damage lowered the force and modulus values. These results confirmed the subjective ratings, which were also lower for enzyme addition to flours with starch damage.

Table XIV

Effect of starch damage and bacterial 2 amylase on rollability scores of tortillas during storage at 22º C

Starch damage (%)	Enzyme Level	Storage Time (Days)				
		4	**8**	**12**	**16**	**20**
5.4	0	4.5	3.5	3.3	2.3	1.3
5.4	0.1	4.3	3.9	3.9	3.5	3.5
5.4	1.0	4.3	3.7	3.3	2.5	2.3
7.1	0	3.8	3.8	2.8	2.1	2.0
9.7	0	4.0	3.5	2.5	2.3	1.3
9.7	0.05	4.5	4.5	3.5	2.8	2.0
9.7	0.1	4.5	4.0	3.5	3.0	2.3
12.2	0	4.0	3.3	3.0	3.0	2.8
12.2	0.1	4.3	3.8	3.8	3.6	3.5
12.2	0.5	4.5	4.5	4.0	3.3	2.8
12.6	0.1	4.5	4.0	4.0	4.0	3.3
12.6	1	4.3	4.0	3.5	3.5	3.0
12.6	0	4.0	4.0	3.8	3.5	3.3
LSD (α=0.05)	**-**	**0.4**	**0.7**	**0.7**	**0.6**	**0.5**

Rollability scores were significantly affected due to starch damage and α-amylase (Table XIV). Scores below 3.0 represent a poor rollable tortilla. Rollability scores were higher than 3.0 for flours with high levels of starch damage. Significantly higher rollability scores were shown after sixteen and twenty days of storage when starch damage and enzyme addition were tested for some of the levels. These results were similar to the previous findings (Mao and Flores 1999) where it was shown that moderate levels of starch damage improve tortilla rollability.

Table XV
**Effect of starch damage and bacterial 2 amylase on quality index (QI) of
tortillas during storage**

Starch damage (%)	Enzyme Level	Storage Time (Days)			
		4	8	12	16
5.4[a]	0[a]	513	398	371	256
5.4	0.1	494	450	453	407
5.4	1.0	506	431	387	297
7.1	0	415	413	305	234
9.7	0				
9.7	0.05	513	401	286	258
9.7	0.1	535	535	419	328
12.2	0	532	473	414	354
12.2	0.1	434	350	325	325
12.2	0.5	475	419	419	403
12.6	0	497	497	441	361
12.6	0.1	564	564	529	494
12.6	1	394	351	351	351
LSD (α=0.05)		423	401	346	350
		83.8	**97.3**	**106.6**	**98.1**

[a] represents control sample

Quality index is another measure of tortilla that involves the opacity, specific volume and rollability scores. The QI scores were calculated using rollability scores from 4, 8, 12 and 16 days (Table XV). Since there were significant differences in the rollable attribute of tortilla with starch damage and α-amylase, tortilla quality index was significantly higher than control at 8, 12 and 16 days of storage for flours with higher levels of starch damage. QI was similar to control with enzyme addition. The lower specific volume of tortillas with α-amylase contributed to these lower QI scores.

Discussion

Mechanical grinding of flour for different time intervals was directly related to starch damage. Iodine absorption of the mechanically damaged samples was linearly related to damage starch. Subjective and objective dough properties were negatively affected at higher levels of starch damage with or without α-amylase. Addition of enzymes to these samples improved dough properties but did not affect most tortilla properties. Starch damage resulting from mechanical grinding had an adverse effect on most tortilla properties except shelf stability. Shelf life of tortillas was significantly improved due to starch damage which is similar to the previously reported results (Mao and Flores 1999, Waniska et al 2003). Moderate to high level of starch damage improved tortilla rollabilty but lowered tortilla opacity. Higher levels of starch damage led to higher water absorption of the dough's with which resulted in insufficient gluten being available to cover the surface of starch (Farrand 1961). This improved tortilla rollability. Mechanical grinding using the rice-grinder might have generated finer flour particles. Damaged starch granules were not able to diffract light uniformly as compared to the control resulting in lower tortilla opacity. The change in protein fractions due to starch damage might be influencing tortilla rollability as shown in Appendix (Table A.6). Action of enzyme on substrate (damaged starch) did not improve tortilla properties as was expected. Thus starch damage did not improve any other tortilla properties except rollability. Further research is needed to understand the effect starch damage on enzyme action.

CHAPTER VI

CONCLUSIONS

The effect of nine enzymes (amyloglucosidase 1, amyloglucosidase 2, bacterial 1, bacterial 2, fungal, maltogenic1, maltogenic 2, malted barley and xylanase) on the subjective and objective dough and tortilla properties was studied. Certain tortilla properties were improved at specific levels with certain enzymes but no improvement in overall tortilla quality was achieved except using bacterial 2, xylanase and maltogenic 2.

Bacterial amylases have been evaluated in bread but are not used in bread because of their tendency to produce gumminess. No research has been published using bacterial amylase in tortillas. Significant differences were obtained in tortilla properties at 0.05, 0.1 0.3 and 1 activity units. Bacterial 2 at 0.5 and 0.1 activity unit was effective in maintaining tortilla diameter and improving rollability. Tortilla staling was significantly reduced using the lowest levels of bacterial 2 amylase. Bacterial 2 was more effective in improving tortilla properties than bacterial 1. This difference in enzyme action could be attributed to differences in isolation procedures of the enzymes. Bacterial 2 amylase was effective in the dough as well as after baking. Reaction products detected using HPLC indicated that 0.1% of bacterial 2 amylase produced oligosaccharides in tortillas after 1 week of storage. These fractions inhibited amylopectin retrogradation and extended tortilla shelf life. However at high levels of bacterial 2 amylase tortillas became sticky after 24 days of storage. This indicated that

enzyme was not completely inactivated during processing and continued to work during storage.

Xylanase at 100 ppm was effective in improving tortilla rollability. At very high levels xylanase caused stickiness in tortillas. More research is needed to standardize the xylanase level and understand its mechanism in limiting staling in flour tortillas.

The effect of damaged starch on tortilla properties was also evaluated. Dough and tortilla properties were affected due to damage starch. Damage starch significantly increased dough water absorption, toughness and press rating. Damage starch lowered tortilla diameter and opacity. Increased Dispersion of amylose and amylopectin reduced gel forming capacity of amylose, leading to less retention of air bubbles. This lowered tortilla opacity. Tortilla rollability improved significantly at higher levels of starch damage. This was because of the higher resistance of the dough which made proteins more flexible resulting in better rollability. Moderate to high level of starch damage is useful for improving tortilla shelf life but lowers tortilla diameter and opacity. Overall tortilla quality is not improved due to starch damage.

Further Research

Optimize the addition of bacterial 2 in combination with additives like TSPP (tetra sodium pyrophosphate) and encapsulated sodium bicarbonate to improve tortilla properties. The mode of action of bacterial 2 amylase in limiting tortilla staling needs to be studied. HPLC can be used to determine the types of oligosaccharides produced due to the hydrolysis of starch by bacterial 2 on amylose and amylopectin in flour tortillas.

Effect of dextrin and glucose addition on tortilla properties can be an approach to determine the mechanism of bacterial 2. Optimization of xylanase can be an alternative approach to improve tortilla properties. Use of xylanase inhibitors in controlling tortilla stickiness can be investigated. The role of damaged starch in limiting tortilla staling needs further research. Optimization of starch damage and enzyme in achieving optimum tortilla quality needs further investigation. Characterization of the protein fractions such as IPP and SPP needs to be done. The effect of storage temperature on the action of bacterial 2 amylase needs research.

LITERATURE CITED

Adams, J. G. 2001. Effects of the timing and amounts of leavening during processing of wheat flour tortillas. MS thesis. Texas A&M University: College Station, TX.

Adams, J. G., and Waniska, R. D. 2002. Effects of amount and solubility of leavening compounds on flour tortilla characteristics. Cereal Foods World 47:60-64.

Aida, I., Martha, L. and Agustin, L. 1996. Shelf life of tortilla extended with fungal amylases. International Journal of Food Science and Technology. 31(6):505-509.

Akers, A. and Hoseney, R. C. 1994. Water soluble dextrins from α – amylases treated bread and their relationships to bread firming. Cereal Chem. 68:570-572.

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Methods 02-52, 44-15, 44-19, 46-30 and 82-23. The Association: St. Paul, MN.

Appolonia, B. L. and Morad, M. 1981. Bread staling. Cereal Chem. 58:186-190.

Armero, E. and Collar, C. 1996. Antistaling additive effect on fresh wheat bread quality. Food Sci. Technol. Int. 2:323-333.

Bello, A. B., Serna- Saldivar, S. O., Waniska, R. D., and Rooney, L. W. 1991. Methods to prepare and evaluate wheat tortillas. Cereal Foods World. 36:315-322.

Boyle, P. J., and Hebeda, R. E. 1990. Antistaling enzyme for baked goods. Food Technology. 44:129.

Bueso- Ucles, F. J. 2003. Antistaling properties of amylases, wheat gluten and cmc on corn tortilla. PhD dissertation. Texas A&M University: College Station, TX.

Cauvain, S. P., and Chamberlain, N. 1988. The bread improving effect of fungal α-amylase. J. Cereal Sci. 8:239-248.

Cepeda, M., Waniska, R. D., Rooney, L. W., and Beujasano, F. P. 2000. Effects of leavening acids and dough temperature in wheat flour tortillas. Cereal Chem. 77:489-494.

Cole, M.S. 1982. Antistaling baking composition. Patent application US 4320151.
Collar, C., Andreu, P., Martinez, J. C. and Armero, E. 1999. Food Hydrocolloids 13: 467-475.

Craig, S. and Stark, J. 1984. The effect of physical damage on the molecular structure of wheat starch. Carbohydrate Research. 125:117-125.

Dadswell, W. and Gardner, F. 1947. The relation of alpha amylase and damage starch to diastatic activity. Cereal Chem. 24:79-99.

Dally, V. and Navarro, L. 1999. Flour tortillas. A growing sector of the U.S. food industry. Cereal Foods World 40:494-497.

De Stefanis, V. A., Turner, E. W. 1981. Modified enzyme system to inhibit bread firming method for preparing same and use of same in bread and other bakery products. Patent application US 4299848.

Defloor, I., and Delcour, J. A. 1999. Impact of maltodextrins and antistaling enzymes on the differential scanning calorimetry staling endotherm of baked bread doughs. J. Agri. Food Chem. 46:737-741.

Dollack, C. 1993. Development of a low fat wheat tortilla: optimization of lipids, emulsifiers and fat replacers. MS thesis. Texas A&M University: College Station, TX.

Donelson, J. R., and Yamajaki, W. T. 1962. Note on a rapid method for the estimation of damaged starch in soft wheat flours. Cereal Chem. 39:460-462.

Doyle, E. M., Noone, A. M., Kelly, C. T., and Fogarty, W. M. 1999. Comparison of the action pattern of two high maltose-forming α-amylases on linear maltooligosaccharides. Enzyme and Microbial Technology. 25:330-335.

Dragsdorf, R. D., and Varriano, M. E. 1980. Bread staling: X- ray diffraction studies on bread supplemented with α – amylases from different sources. Cereal Chem. 57:310-314.

Duedahl-Olesen, L., Zimmermann, W. and Delcour, J. A. 1999. Effects of low molecular weight carbohydrates on faringoraph characteristics and staling endotherms of wheat flour-water doughs. Cereal Chem. 76:227-230.

Duran, E., Leon, A., Barber, B., and Barber, C. 2001. Effect of low molecular weight dextrins on gelatinization and retrogradation of starch. Eur. Food Res Technol 212:203. Chem Abstr. 135:60349.

Evers, A. and Stevens, D. 1985. Starch damaged. Pages 321-349 in: Advances in Cereal Science and Technology, Vol.VII. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Every, D., Gerrard, J. A., Gilpin, M. J., Ross, M., and Newberry, M. P. 1998. Staling in starch bread: the effect of gluten additions on specific loaf volume and firming rate. Starch/Starke. 50:443-446.

Food and Agricultural Organization (FAO) 2002. Web page: http://www.fao.org/

Farrand, E. A. 1969. Starch damage and alpha amylase as bases for mathematical models relating to flour water absorption. Cereal Chem. 46:103-116.

Finney P. L., Kinney, J. E. and Donelson, J. R. 1988. Prediction of damaged starch in straight –grade flour by near infra red reflectance analysis of whole ground wheat. Cereal Chem. 65: 449- 454.

Friend, C. P., Ross, R. G., Waniska, R. D., and Rooney, L. W. 1995. Effects of additives in wheat flour tortillas. Cereal Foods World. 40:494-497.

Friend, C. P., Waniska, R. D., and Rooney, L. W. 1993. Effects of hydrocolloids on processing and qualities of wheat tortillas. Cereal Chem. 70:252-256.

Garza, Jessica. August 2003. Effects of amounts and types of sodium bicarbonate in wheat flour tortillas. MS thesis. Texas A&M University: College Station, TX.

Gerrard, J. A. 1997. The role of maltodextrins in the staling of bread. Journal of Cereal Science. 26:201-209.

Gray, J. A. and Bemiller, J. N. 2003. Bread staling: molecular basis and control. Comprehensive Reviews in Food Science and Food Safety. 2:1-21.

Greer, E. and Stewart, B. 1959.The water absorption of wheat flour: Relative effects on protein and starch. J. Sci. Food. Agri. 10:248-252.

Guo, G. S., Jackson, D. S., Graybosch, R. A., and Parkhurst, A. M. 2003. Wheat tortilla quality: impact of amylose content adjustments using waxy wheat flour. Cereal Chem. 80:427-436.

Hagberg, S. A rapid method for determining alpha amylase activity. 1960. Cereal Chem. 37:218-222.

Harada, O. Lysenko, E. D., and Preston, K. R. 2000. Effects of commercial hydrolytic enzyme additives on Canadian short process bread properties and processing characteristics. Cereal Chem. 77:70-76.

Harris, R. H. and Sibitt, L. D. 1941. The comparative baking quality of hard red spring wheat starches and glutens as prepared by the gluten starch blend baking method. Cereal Chem. 18:585-604.

Hebeda, R. E., Bowles, L. K., and Teague, W. M. 1990. Developments in enzymes for retarding staling of baked goods. Cereal Foods World. 35:453-457.

Hebeda, R. E., Bowles, L. K., and Teague, W. M. 1991. Use of intermediate temperature stability enzymes for retarding staling in baked goods. Cereal Food World. 38:619-624.

Hoseney, R. C. 1996. Starch. Pages 29-63 in: Principles of Cereal Science and Technology. American Association of Cereal Chem.: Inc. St. Paul, MN.

Hoseney, R. C., Finney, K. F., Shogren, M. D., and Pomeranz, Y. 1969. Functional (Breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem.46:126-135.

Israilides, C., Smith, A., Scanlon, B., Barnett, C., 1999. Pullulan from agro-industrial wastes. Biotechnol. Genet. Eng. Rev. 16:309-324.

Iturbe-Chiňas, F. A., Lucio-Aguerrebere, R. M., and Lopez-Munguia, A. 1996. Shelf life of tortilla extended with fungal amylases. International Journal of Food Science and Technology. 31:505-509.

Joensson, T., and Toernaes, H. 1987. The Effect of selected surfactants on bread crumb softness and its measurement. Cereal Foods World 32:482-485.

Joseph, S. 1999. Instrumental and sensory methods to evaluate texture of wheat flour tortillas during storage. MS thesis. Texas A&M University: College Station, TX.

Kim, J. S., Cha, S. S., and Kim, H. J. 1999. Crystal structure of a maltogenic amylase provides insights into a catalytic versatility. The Journal of Biological Chemistry. 274:26279-26286.

Kim, S. K., and D'Appolonia. 1977. Bread staling. II. Effect of protein content and storage temperature on the role of starch. Cereal Chem. 54:216-219.

Krog, N. 1984. Food grade emulsifiers contra wheat flour lipids: chemical composition and toxicological status. Proc. 22nd Nordic Cereal Congress, Lillehammer, Norge, 83-98.

Krog, N., Olsen, S. K., Toeranes, T., and Joensson, T. 1989. Retrogradation of starch fraction in wheat bread. Cereal Foods World. 34:281-285.

Krog, N.1981. Theoretical aspects of surfactants in relation to their bread making. Cereal Chem. 58:158-164.

Kulp, K., and Ponte, J. G. 1981. Staling white pan bread: fundamental causes. Crit. Rev. Food Sci. Nutr. 15:1-48.

Laurikainen, T., Harkonen, H., Autio, K., and Poutanen, K. 1998. Effects of enzymes in fiber-enriched baking. Journal of the Science of Food and Agriculture. 76:239-249.

Lelievere, J. 1974. Starch damage. Starch. 26:85-88.

Leon, A., Duran, E. and Benedito, C. 1997. Firming of starch gels and amylopectin retrogradation as related to dextrin production by α − amylase. Z. Lebensm. Unters. Forsch. 205:131-134.

Limanond, B., Castell-Perez, M. E., and Moreira, R. G. 2002. Modeling the kinetics of corn tortilla staling using stress relaxation data. Journal of Food Engineering. 53:237-247.

Maninder, K., and Bains, G. S. 1976. Effects of amylase supplements on the rheological baking quality of Indian wheats. J. Food Sci.Technol. 13:328.

Mao, Y. and Flores, R. A. 2001. Mechanical starch damage effects on wheat flour tortilla texture. Cereal Chem. 78: 286-293.

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

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

Martinez, J.C., and Andreu, P. and Collar, C. 1999. Storage of wheat breads with hydrocolloids, enzymes and surfactants: anti-staling effects. Leatherhead Food RA Food Ind. J. 2:133-149.

Martinez-Anaya, M. A., Devesa, A., Andreau, P., Escriva,C., Collar, C. 1998. Effects of combination of starters and enzymes in regulating bread quality and shelf life. Food Sci. Technol. Int. 4: 425-435.

Martinez-Bustos, F., Morales, S. E., Chang Y. K., Herrera-Gomez, A., Martinez, M. J. L., Banos, L., Rodriguez, M. E., and Flores, M. H. E. 1999. Effect of infrared baking on wheat flour tortilla characteristics. Cereal Chem. 76:491-195.

Mc Cleary, B. V. 1986. Enzymatic modification of plant polysaccharides. Int. J. Biol. Macromol. 8:349-354.

McDonald, E. C. 1969. Proteolytic enzymes of wheat and their relation to baking quality. Baker's Dig. 43:26-28, 30, 72.

McDonough, C. M., Seetharaman, K., Waniska, R. D., and Rooney, L. W. 1996. Microstructure changes in wheat flour tortillas during baking. J. Food Sci. 61:995-999.

Medcalf, D. G., and Gilles, K. A. 1965. Determination of starch damage by rate of iodine absorption. Cereal Chem. 42:546-557.

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

Meuser, F., Klingler, R. W., and Niediek, E. A. 1978. Charcterization of mechanically modified starch. Staerke. 30:376-384.

Miller, B. S., Johnson, J. A., and Palmer, D. L. 1953. A comparison of cereal, fungal and bacterial α-amylase as supplements for bread baking. Food Technol. 7(1):38.

Miranda-Lopez, R. 1999. Effect of some anti-staling additives, pH and storage on the staling of corn tortillas. PhD dissertation. Texas A&M University: College Station, TX.

Morrison, W., Tester, R. and Gidley, J. 1994. Properties of damaged starch granules. 11. Crystallinity, molecular order and gelatinization of ball-milled starches. Journal of Cereal Science. 19:209—217.

Outtrup, H., and Norman, B. E. 1984. Properties and applications of a thermo-stable maltogenic amylase produced by strain of Bacillus modified by recombinant DNA techniques. Starch 36:405-412.

Park, K.H., Kim, T. J., Cheong, T.K., and Kim, J. W. 2000. Structure, specificity and function of cyclomatodextrinase, a multispecific enzyme of the α-amylase family. Biochimica et Biophysica Acta. 1478:165-168.

Pflugfelder, R. L., Rooney, R. W., and Waniska, R. D, 1988. Fractionation and composition of commercial corn masa. Cereal Chem. 65:262-266.

Qi Si, J. 1996. New enzymes for the baking industry. Food Technol. Europe. 3: 60-64.

Qi Si, J. 1997. Synergistic effect of enzymes for bread making. Cereal Foods World. 42(10):802-807.

Qoorani, C., Campos, D., and Steffe, J. 1996. Rheological behaviour of undeveloped and developed wheat dough. Cereal Chem. 74:489-494.

Quintero-Fuentes, X. 1999. Characterization of corn and sorghum tortillas during storage. Ph D dissertation. Texas A&M University: College Station, TX.

Rosell, C. M., Wang, J., Aja, S., Bean, S., and Lookhart, G. 2003. Wheat flour proteins as affected by transglutaminase and glucose oxidase. Cereal Chem. 80:52-55.

Rouau, X. 1994. Investigations into the effects of an enzyme preparation for baking on wheat flour dough pentosans. J. Cereal Sci.19:259-272.

Sahlstrom, S., and Brathen E. 1997. Effects of enzyme preparations for baking, mixing time and resting time on bread quality and bread staling. Food Chem. 58:75-80.

Saldivar, S. O., Rooney, L. W. and Waniska, R. D. 1988. Wheat flour tortilla production. Cereal Foods World. 33:855-864.

Sandstedt, R. and Mattern, P. 1960. Damaged starch. quantitative determination in flour. Cereal Chem. 57:379-390.

Seetharaman, K., Chinnapha, N., Waniska, R. D., and White, P. 2002. Changes in textural, pasting and thermal properties of wheat buns and tortillas during storage. J. Cereal Sci. 35:215-223.

Shi, Y. C., and Seib, P. A. 1995. Fine structure of maize starches 4 WX-containing genotypes of the W64A inbred line in relation to gelatinization and retrogradation. Carbohydrate Polymers. 26:141-147.

Silva, Laura. 2003. Effects of barley flour and β-glucans in corn tortilla. MS thesis. Texas A&M University: College Station, TX.

Srinivasan, M., Waniska, R. D., and Rooney, L. W. 2000. Note. Effects of ingredients and processing on dough rheology of wheat flour tortillas. Food Sci. Tech. Int. 6:331-338.

Suhendro, E. 1997. Instrumental methods for the evaluation of corn tortilla texture. Ph D dissertation. Texas A&M University: College Station, TX.

Suhendro, E. L. Waniska, R. D., and Rooney, L. W. 1993. Effects of added proteins in wheat tortillas. Cereal Chem. 70: 412-416.

Suhendro, E. L., Almeida- Dominguez, H. D., Rooney, L. W., Waniska, R. D., and Moreira, R.G. 1999. Use of extensibility to measure corn tortilla texture. Cereal Chem. 76:536-540.

Suhendro, E. L., Waniska, R. D., Rooney, L.W. and Gomez, M.H. 1995. Effects of polyols on the processing and qualities of wheat tortillas. Cereal Chem. 72:122-127.

Takaha, T., and Smith, S. M., 1999. The functions of 4-α glucanotranferases and their use for the production of cyclic glucans. Biotechnol. Genet. Eng. Rev. 16:257-280.

Tortilla Industry Association (TIA). 2002. Web page: http: www.tortilla-info.com/industry/tortillasbg.htm

Tortilla Industry Association (TIA). 2003. http: www.tortilla-info.com/industry/tortillasbg.htm

Tilley, K. A., Benjamin, R. E., Bagorogoza, K. E., Moses Okot-Kober, B., Prakash, O., and Kwen, H. 2001. Tyrosine cross-links: Molecular basis of gluten structure and function. J. Agric. Food Chem. 49:2627-2632.

Tipples, K.1969. The relation of starch damage to the Baking Performance of Flour. The Baker's Digest. 43:28-32, 44.

Van Eijk, J. H., 1991. Retarding the firming of bread crumb during storage. Patent application. US 5023094.

Wang, L. and Flores, R. A. 1999b. Effects of wheat starch and gluten on tortilla texture. Cereal Chem. 76:807-810.

Wang, L., and Flores, R. A. 1999a. Effect of different wheat classes and their flour milling streams on textural properties of flour tortillas. Cereal Chem. 76:496-502.

Waniska, R. D. 1976. Methods to assess quality of boiled sorghum, gruel and chapaties from sorghum with different kernel characteristics. MS thesis. Texas A&M University: College Station, TX.

Waniska, R. D. 1999. Perspectives on flour tortillas. Cereal Foods World. 44:471-473.

Waniska, R. D., Cepeda, M., King, B., Adams, J., Rooney, L. Torres, P., Lookhart, G., Bean, S., Wilson, J. and Betchel, D. 2003. Effects of Flour Properties on Tortilla Qualities. Cereal Foods World. Submitted.

Waniska, R. D., Graybosch, R. A. and Adams. J. L. 2002. Effects of Partial Waxy Wheat on Processing Quality of Wheat Flour Tortillas. Cereal Chem. 79(2):210-214.

Williams, P., and Fegol, K. 1969. Colorimetric determination of starch damage in flour. Cereal Chem. 47:56-62.

Wŭrsch, P., and Gumy, D. 1994. Inhibition of amylopectin retrogradation by partial beta- amylosis. Carbohydr. Res. 256:129-137.

Zobel, H. and Kulp, K. 1996. The staling mechanism. Pages 1-64 in: Baked Goods Freshness: Technology, Evaluation and Inhibition of Staling. R.E. Hebeda and H.F. Zobel, eds. Marcel Dekker: New York.

Zobel, H. and Senti, F. 1959. The bread staling problem. X-Ray diffraction studies on breads containing a cross linked starch and a heat stable amylase. Cereal Chem. 36:441-451.

APPENDIX A

TABLES AND FIGURES

Table A.1

Means and standard deviation of the different enzyme activities of barley malt amylase

Enzyme Activity	N	Mean	Std. Deviation
0	2	371	2.0
0.01	2	352	4.2
0.1	2	317	1.4
0.5	2	311	0.0
1	2	305	2.8
2	2	302	7.1
3	2	272	10.6
4	2	238	4.2
5	2	205	7.8
10	2	181	2.1
20	2	158	2.8
30	2	121	2.1
40	2	109	2.1
50	2	96	3.5
60	2	85	1.4
70	2	77	2.8

Table A.2

Means and standard deviation for different enzyme activities of Bacterial 2 amylase

Enzyme Activity	N	Mean	Std. Deviation
0	2	370	2.1
0.01	2	358	0.0
0.04	2	273	4.2
0.08	2	235	2.1
0.1	2	182	2.8
0.5	2	155	2.8
1	2	66	3.5

Table A.3
Effect of Enzyme Type and Level on Stress Relaxation of Dough

Enzyme Type	Level	Force 1 (N)	Force2 (N)	Modulus (N/m)
Control	0	12.2	4.5	-13.1
Amyloglucosidase 1	40	24.0	8.7	-25.9
	80	14.5	5.3	-15.8
	150	17.9	6.7	-19.1
LSD (α = 0.05)	-	**10.3**	**3.3**	**11.9**
Amyloglucosidase 2	50	15.8	5.3	-17.8
	100	19.8	7.1	-21.7
	150	23.2	8.3	-25.2
	200	16.2	7.0	-15.4
LSD (α = 0.05)	-	**7.1**	**2.5**	**7.7**
Bacterial 1	0.05	15.1	5.2	-16.8
	0.1	9.5	3.2	-10.6
	0.3	10.4	3.6	-11.6
	1.0	11.8	3.9	-13.5
LSD (α = 0.05)	-	**3.7**	**1.1**	**4.4**
Bacterial 2	30	15.8	6.7	-15.3
	60	15.0	5.1	-16.9
	150	24.4	8.9	-26.3
	500	22.1	8.0	-23.9
LSD (α = 0.05)	-	**5.7**	**2.0**	**7.0**
Fungal	20	27.0	9.8	-29.2
	40	20.6	7.8	-24.6
	60	20.0	6.0	-18.9
	100	10.0	4.2	-9.6
LSD (α = 0.05)	-	16.4	1.3	4.9
Maltogenic 1	140	12.0	3.6	-14.4
	280	12.5	4.9	-12.7
	450	15.6	5.2	-17.7
LSD (α = 0.05)	-	**2.6**	**2.1**	**5.1**
Maltogenic 2	30	20.2	6.9	-22.6
	60	10.8	5.2	-18.0
	100	10.2	3.4	-11.5
LSD (α = 0.05)	-	**13.0**	**1.5**	**5.1**
Malted barley flour	3	13.8	5.0	-14.9
	5	17.7	5.9	-20.1
	20	12.5	4.4	-13.7

	50	15.0	5.0	-17.1
LSD (α = 0.05)	-	**6.5**	**2.1**	**7.3**
Xylanase	30	14.9	5.9	-15.0
	60	27.7	9.4	7.1
	100	13.8	4.6	-15.7
	150	16.1	7.0	-14.1
LSD (α = 0.05)	-	**5.4**	**2.0**	**6.8**

Table A.4
Effect of Enzyme Type and Levels on Tortilla Properties of Control Flour

Control	0	3.0	168.4	87.8	1.65
Amyloglucosidase 1	40	2.7	168.2	89.5	1.45
	80	2.5	171.1	90.7	1.43
	150	2.5	165.9	90.0	1.30
LSD (α = 0.05)	-	0.3	1.87	2.8	0.18
Amyloglucosidase 2	50	3.4	173.0	82.8	2.0
	100	3.1	173.5	89.3	1.80
	150	2.9	173.5	89.3	1.64
	200	2.7	164.7	87.8	1.41
LSD (α = 0.05)	-	0.4	7.2	4.9	0.40
Bacterial 1	30	2.9	168.8	87.8	1.57
	60	3.1	175.5	90.6	1.80
	150	3.2	162.3	88.9	1.65
	500	3.2	162.4	89.3	1.60
LSD (α = 0.05)	-	2.7	3.9	3.9	0.08
Fungal	20	2.9	162.4	82.5	1.5
	40	2.9	164.1	89.0	1.50
	60	2.7	176.6	92.0	1.55
	100	2.8	175.6	85.5	1.55
LSD (α = 0.05)	-	0.3	5.1	5.4	0.11
Maltogenic 1	140	2.8	163.2	83.3	1.35
	280	2.8	170.3	92.8	1.55
	450	2.7	165.6	84.8	1.40
LSD (α = 0.05)	-	0.13	3.4	3.0	0.14
Maltogenic 2	30	2.7	164.0	82.5	1.35
	60	2.6	170.5	88.3	1.45
	100	2.9	175.2	87.0	1.70
LSD (α = 0.05)	-	0.4	7.4	9.2	0.24
Xylanase	30	2.8	163.2	84.0	1.40
	60	2.9	169.2	84.8	1.51
	100	2.7	174.5	87.3	1.57
	150	2.6	172.7	84.3	1.48
LSD (α = 0.05)	-	0.3	5.4	3.0	0.11

Table A. 5

Effect of Enzyme Types and Levels on Tortilla rollability at 4, 8, 12 and 16 days of storage at 22º C

Enzyme Type	Level (ppm)	Roll 4 days	Roll 8 days	Roll 12 days	Roll 16 days
Control	0	4.0	3.4	3.5	2.0
Amyloglucosidase 1	40	3.6	2.9	2.0	1.6
	80	4.5	3.4	2.5	2.1
	150	4.5	4.0	3.2	2.8
LSD α = 0.05	-	**0.5**	**0.2**	**0.6**	**0.4**
Amyloglucosidase 2	50	4.3	2.8	2.3	2.4
	100	4.4	3.9	3.0	2.2
	150	4.4	4.2	3.4	2.7
	200	4.3	3.7	3.3	3.2
LSD α = 0.05	-	0.6	0.6	0.8	0.8
Bacterial 1	30	4.5	3.7	3.5	3.2
	60	4.0	3.4	3.0	2.5
	150	4.5	4.4	2.9	2.4
	500	4.5	3.8	3.5	2.4
LSD α = 0.05	-	**2.6**	**0.4**	**0.2**	**0.3**
Fungal	20	3.9	3.9	3.4	2.3
	40	4.0	3.8	3.4	2.5
	60	4.5	3.8	3.3	2.8
	100	4.2	3.4	2.4	1.5
LSD α = 0.05	-	0.3	0.6	1.2	0.9
Maltogenic 1	140	4.8	4.0	4.0	3.0
	280	4.4	4.0	3.5	3.0
	450	4.0	3.5	3.0	2.8
LSD α = 0.05	-	**0.2**	**0.2**	**0.6**	**0.9**
Maltogenic 2	30	4.0	3.6	3.2	2.0
	60	3.6	2.1	2.0	1.2
	100	4.4	4.0	3.3	2.9
LSD α = 0.05	-	0.3	0.4	0.5	0.7
Xylanase	30	4.0	3.8	3.5	2.3
	60	4.2	3.5	2.8	1.75
	100	4.5	3.9	3.5	2.9
	150	4.3	4.3	3.8	3.4
LSD α = 0.05	-	**0.4**	**0.6**	**0.9**	**0.8**

Table A.6

Effect of damage on various protein fractions

Damage Time (hr)	SPP %	GLI%	IPP%
0	8.1	43.3	45.9
0.5	8.2	44.5	44.3
1	7.9	43.1	46.1
4	7.7	40.2	49.2
8	7.5	40.9	49.4

Table A.7

HPLC Analysis of Tortilla after 1 week of storage

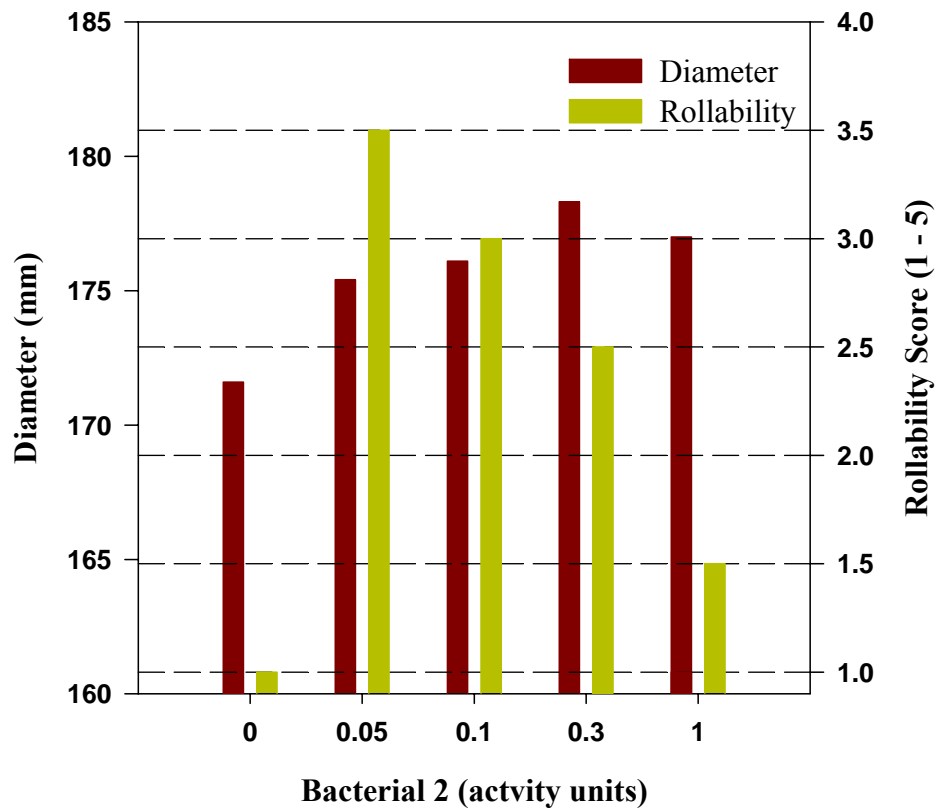| Treatment	Amounts (%)	Amounts (%)	Oligosaccharides (%)
Control	0.03	0.30	0.65
Bacterial 2	0.07	0.41	3.19

Fig. A.1 Effect of Bacterial 2 amylase on Tortilla properties of untreated flour I.
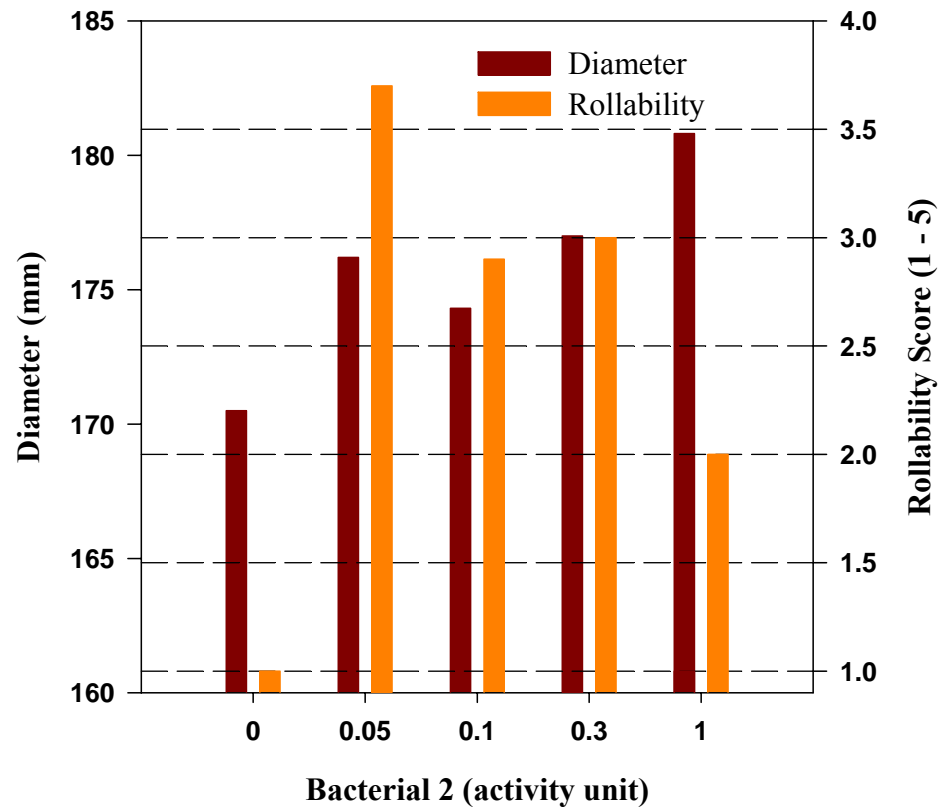
Fig. A.2 Effect of Bacterial 2 amylase on Tortilla properties of Untreated flour II.

VITA

Sapna Arora was born in Mumbai, India. She got her B.Sc. degree from Delhi University in Food Technology and M.Sc. degree in Food Science in 1997 from Mysore University She earned the Master of Science degree in Food Science and Technology from Texas A&M University in December 2003.

Her permanent address is D-4/11, Krishna Nagar Delhi, 110051 India and her e-mail address is sapna_1076@hotmail.com