THE EFFECT OF ENZYMES AND HYDROCOLLOIDS ON THE TEXTURE OF TORTILLAS FROM FRESH NIXTAMALIZED MASA AND NIXTAMALIZED CORN FLOUR

A Thesis

by

ARTURO CARLOS GUTIERREZ DE VELASCO ALVAREZ

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

May 2004

Major Subject: Food Science and Technology

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ABSTRACT

The Effect of Enzymes and Hydrocolloids on the Texture of Tortillas from Fresh Nixtamalized Masa and Nixtamalized Corn Flour. (May 2004)

Arturo Carlos Gutierrez de Velasco Alvarez, B.S., Instituto Tecnologico y de Estudios Superiores de Monterrey

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The texture of tortillas was improved by the addition of maltogenic amylase and carboxymethylcellulose (CMC) and guar gum to fresh masa from ground nixtamal (FNM) and nixtamalized corn flour (NCF) masa. Differences in the performance of additives in tortillas held under refrigeration or ambient storage were documented.

For NCF tortillas, significant improvements were obtained in objective and subjective texture measurements by two treatments. Tortilla texture was improved by a treatment with a high enzyme level (170 mg/kg of maltogenic α-amylase, 0.14% CMC, 0.85% guar) as measured by objective tests and by a treatment with low enzyme level (60 mg/kg of maltogenic α-amylase, 0.43% CMC, 0.57% guar) as measured by subjective tests. The addition of maltogenic α-amylase (70 mg/kg) and CMC (0.35%) to FNM tortillas at levels similar to the low enzyme NCF treatment but with lower guar level (0.12%) improved tortilla texture.

The maltogenic α-amylase softened tortillas by trimming the starch structure. This allowed the guar to interfere with amylopectin re-crystallization inside gelatinized starch granules. The CMC created a more flexible inter-granular matrix that helped maintain the disrupted tortilla structure. Guar was ineffective in refrigerated tortillas, whereas, maltodextrins effectively improved refrigerated tortillas. The sequence of partial starch hydrolysis, warm holding condition, and time for guar to associate with starch and CMC was necessary to

improve tortilla texture. Thus, different additives may be required for cold versus room temperature storage.

Sugars increased in enzyme-treated tortillas during storage. This suggests that maltogenic α-amylase was only partially inactivated during baking of corn tortillas. Tortillas with more enzyme had lower and later pasting viscosity as measured by a Rapid Viscoanalyzer. Tortillas prepared from FNM also had lower and later pasting viscosity compared to NCF tortillas. Pasting viscosity of tortillas revealed intrinsic starch polymer characteristics and interactions.

Results of this study provide commercially applicable information about desired levels for the extent of starch hydrolysis, the type and amount of gums and starches, and product microstructure to delay staling of corn tortillas.

DEDICATION

To my father Arturo Gutierrez de Velasco who expected no less of me, to my mother Luz Amada Alvarez for her encouragement, to my wife Emma Cristina Rosas who supported me, and to my kids Arturo, Andres, Adrian, and Alonso for all their love.

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But all of this would not have happened without God’s help. Ahi me lo apuntas …

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CHAPTER I

INTRODUCTION

Tortilla History

Tortillas are unleavened flat breads mainly produced from corn or wheat. The word tortilla comes from the Spanish word “torta” which means “round cake”. Tortillas or “tlaxcallim” were the principal food of the Aztecs. In the United States, tortillas are not considered ethnic bread anymore; Americans consume tortillas as a substitute for bread from hot dog buns, sandwiches, and pizzas to casseroles (TIA 2003).

U.S. Market

In the U.S. Baking industry, the fastest growing sector is wheat and corn tortillas; in 2002 tortilla sales reached $4.5 billion dollars, which is expected to reach $5 billion by 2004. This rapid increase in acceptance is related to an increased Hispanic population and greater consumption by non-hispanic's (TIA 2003). Tortillas are the second most popular bread type in America, with 32% of the bakery market, white bread is the most popular with 34% market share (Aspex Research 2003).

Tortilla Staling

Staling is defined as the loss of freshness in tortillas, or can be described in terms of almost any change, short of microbial spoiling, that occurs during storage which makes baked products less acceptable to consumers (Zobel and Kulp 1996). Consumers prefer soft, flexible tortillas. However, during tortilla storage there are changes that cause firming. Texture changes are mainly related to modifications in the starch properties (Miranda-Lopez 1999).

This thesis follows the style and format of Cereal Chemistry.

The rapid hardening of corn is caused by a phenomenon called retrogradation. Retrogradation of gelatinized starch involves the recrystallization of both amylopectin and amylose (Quintero-Fuentes 1999). As a result, during storage of baked products they become firmer, less elastic and break upon bending (Quintero-Fuentes 1999). Methods to retard staling include storage at temperatures below Tg, reduction of moisture content; modification of starch structure to reduce intermolecular associations and disruption of the continuity of the gel network.

Objectives

The objectives of this study were:

1. Evaluate texture changes in tortillas from fresh nixtamalized masa (FNM) supplemented with amylases in combination with different hydrocolloids.
2. Determine the optimum combination of amylase and gums (CMC and guar)
3. Document changes that occur in masa and tortillas containing effective levels of amylases in corn tortillas.
4. Compare the results from FNM to NCF (nixtamalized corn flour) tortillas with texture improved by the use of enzymes and hydrocolloids.

Impact of Research

Few studies, if any, have been published on the use of amylases to retard the staling of tortillas made from fresh masa. Guidelines for fresh masa were established on how to use amylases as a processing aid to reduce firmness of stored tortillas. Enzymes in combination with hydrocolloids were able to produce a soft tortilla that is flexible with less rubbery texture. These studies provide information on how amylase affects tortillas made with fresh masa compared to dry masa flour tortillas.

CHAPTER II

LITERATURE REVIEW

Masa and Tortillas

Nixtamal

Tortillas are defined as flat round unleavened bread made from ground nixtamalized corn (Serna-Saldivar et al. 1990). The process of nixtamalization (from nauatl, nixtli: ashes, tamale: masa) is widely practiced in Mexico and Central America. This process consists of cooking the corn in the presence of lime (CaO), steeping and grinding to form the masa. Masa is shaped into a disk form, baked and cooled. The process variables are modified to obtain specific products from table tortillas to chips. To obtain table tortillas, the process is adjusted to produce high moisture masa by decreasing the particle size distribution. Tortillas from this masa will retain more water and remain rollable and pliable during storage (McDonough et al. 2001)

Cooking is a critical step in the nixtamalization process. Many variations in the process and ingredients determine the extent of corn cooking including the quality characteristics of the corn, interactions between temperature, time, lime concentration and distribution, mass of the cooked corn and agitation. Good indicators of cooking include water uptake of the kernel, ease of pericarp removal and softening of the kernel (McDonough et al. 2001).

Changes occur in the corn kernel during cooking, such as solubilization of cell wall polymers by increased pH and structural alterations on the outside surface and in the cell walls of the corneous and floury endosperm. The structural and chemical changes of the grain during processing affect texture, color, flavor and shelf life of the final nixtamalized product (Gomez et al. 1989).

During cooking, several granular and molecular forms of starch are produced by incomplete gelatinization and retrogradation. Half of the starch granules loose birefringence, indicating a high extent of gelatinization. Some granules swell and retain the structure of the endosperm cells while others are completely disrupted (Gomez et al. 1989).

The appearance of the protein bodies changes during nixtamalization. Zein from nixtamalized corn has higher molecular weight proteins compared to zein from raw corn. The glutelin fractions are also changed (Paredes-Lopez and Saharopolus-Paredes 1983) probably because of disulfide cross-linking of aminoacids and heat denaturation of proteins.

The cooked corn is steeped to distribute the moisture and lime, and to allow additional changes in kernel structure. Amylose and amylopectin anneal during steeping, resulting in partial recovery of the starch crystallinity. This annealing process is characterized by the realignment of starch chains in the amorphous phase (Gomez et al. 1989). Annealing of maize starches facilitates compartmentalization of intermixed amylose and amylopectin chains into amylose–amylose and amylopectin–amylopectin double helices that cause higher melting points and increased pasting temperatures, which reduce starch solubility (Tester et al. 2000).

After steeping, the corn is washed to remove the pericarp and excess lime, which decreases the pH (McDonough et al. 2001). The kernel loses starch, protein and lipids; the quantities lost are affected by the corn quality, extent of cooking, steeping time and shear stress (Pflugfelder 1986).

During the grinding process, the particle size is reduced; the heat and shear cause significant gelatinization of the starch granules. The reduction in size of masa particles depends on several factors, including the degree of cooking, shear resulting from the mill design, texture of the grinding stones, the gap between them and the flow rate of the product (McDonough et al. 2001). The tortillas are formed either pressed by rolls and cut or extruded into a round disk of various sizes and thickness depending on market demand.

Nixtamalized Corn Flour (NCF)

The process of masa production is altered for NCF. Facilities that use NCF reduce their labor, capital output on equipment and treatment of wastewater. The ingredient acquisition is simplified. Alterations in the process of NCF production cause changes in quality as compared to fresh masa products. Corn is usually undercooked (decreased time and temperatures) which causes decreased water absorption and weakening of the endosperm structure, restricted swelling of the starch granules and limited amylose leaching during the initial heating step (Gomez et al.1991). The cooked corn is steeped for less time, which limits water distribution and reorganization of the molecular structure compared to what occurs when fresh masa is produced (Gomez et al. 1990) After washing to remove excess lime and loose pericarp fragments, the corn is stone or hammer milled. The particles are dried under controlled conditions, usually in large tunnels or drying towers with a countercurrent hot air flow. The dried material is ground, sifted, large particles are reground and the resulting fractions are blended for specific applications (Serna-Saldivar et al. 1990).

Masa prepared from NCF is a less cohesive material than fresh masa because the dehydration produces more gelatinized and retrograded starch in the intermediate and smaller particle size fractions (Gomez et al. 1991). NCF absorbs less water compared to fresh masa (Pflugfelder 1986). Rheology of the masa from NCF differs from fresh masa, as the former develops higher viscosities and it is only partially hydrated during mixing (McDonough et al. 2001). Drying the NCF accelerates starch retrogradation by forming crystalline areas that act as a nucleation point for further starch associations. The modifications to produce NCF and the increased retrogradation during drying increase the rate of staling (Gomez et al. 1991).

Several attempts have been made using extrusion to produce NCF without the nixtamalization step but their success has been limited (Duran de Bazua et al. 1979, Johnson and Horner 1990, Gomez-Aldapa et al. 1999). The quality (flavor and rheology) of the resulting product was different (Gomez-Aldapa et al. 1999). Other efforts to make nixtamalized corn flour with reduced wastewater involve the use of enzymes (Sahai and Jackson 2001). The corn is cooked with low levels of lime at low temperatures in the presence of an alkaline protease enzyme. The enzymes hydrolyze proteins that attach the pericarp to the kernel allowing its removal during washing (Sahai and Jackson 2001). This technology has not been used commercially at this time.

Baking of Tortillas

The masa disks are baked at high temperatures (>250^{o}C) in gas-fired ovens for a short time (60 s) (Serna-Saldivar 1996). The degree of alteration of starch and proteins during baking depends on their position on the disk; the center is cooked more than the edges as indicated by a greater loss of birefringence in the former. The edges are dehydrated and insufficient water is present for complete gelatinization (Gomez et al. 1990). Rapid water evaporation from the tortilla surface and extensive starch gelatinization occur during baking (Gomez et al. 1992). Changes are evident in the proteins bodies that swell, loose their shape and in some cases are physically disrupted and may become part of the binding material that set the structure of the tortilla (Gomez et al. 1989).

Staling

The process of starch associations occurs during baking of tortillas. It is a progressive, spontaneous aggregation of the starch and other components that affect corn tortilla texture. The gelatinized starch continues to reassociate during staling into an ordered structure. The increase in firmness is primarily used to quantify staling (Suhendro 1997; Quintero-Fuentes 1999; Yeggy 2000; Limanond et al. 2002). Researchers have also developed or adapted a variety of techniques to measure the degree of staleness that include rheological (uniaxial compression, pasting properties, etc), thermal analysis, infrared spectroscopy, nuclear magnetic resonance, X-ray crystallography, conductance, microscopy and sensory methods (Gray and Bemiller 2003)

Extensive research has been done in bread and bread systems. In wheat bread, many approaches have been taken to reduce staling. The use of surfactants, amylolytic and non-amylolytic enzymes and modified atmosphere packing are examples of these (Heflich 1996). For wheat flour tortillas the addition of polyols and/or vital wheat gluten has retarded staling (Suhendro 1992). Pascut et al. (2001) demonstrated that gliadin or specially processed wheat proteins improved shelf stability of tortillas. Lowering the levels of leavening improves the stability of flour tortillas (Garza-Casso 2003). Arora (2003) found that a specific α-amylase improved the texture and retarded the staling of wheat tortillas.

Several authors (Bedolla 1983, Twillman and White 1988, Yau et al. 1994, Iturbe-Chinas et al. 1996, Suhendro 1997, Fernandez et al. 1999, Miranda-Lopez 1999, Quintero-Fuentes 1999, Yeggy 2000, Bueso-Ucles et al. 2001, Mitre-Dieste 2001, Limmanond et al. 2002, Bueso-Ucles 2003, Garza-Casso 2003, Gutierrez de Velasco et al 2003, Leal-Diaz 2003) have investigated staling in corn tortillas and mechanisms to limit staling; their approaches to limit staling are described here.

Amylases

Amylases are generally known as the enzymes that hydrolyze starch and glycogen (Hiromi 1998). Theories of the mechanism by which amylases may retard staling in bread and model systems include: **a)** the shortening of amylopectin chain length by enzymes reduce retrogradation tendencies of the amylopectin fraction (Zobel and Senti 1959); **b)** the oligosaccharides (DP 2-7) produced by the enzymes are themselves antistaling agents that have less ability to retrograde and inhibit cross linking between starch and gluten (Martin and Hoseney 1991); **c)** amylases act by removing the protruding amylopectin branches making them unavailable to cross-link. These would be the ones most likely to cross-link with amylose, since they are the most accessible or external and most susceptible to enzymatic attack (Lineback 1984); **d)** Amylases produce higher concentration of maltooligosacharides (DP 3-8) that impact Tg (glass transition temperature) resulting in a smaller ΔT (Tg-Tm) above Tg that retards staling by slowing the starch crystallization process (Slade and Levine 1991); **e)** amylases have the ability to produce a partially degraded amylopectin that is less prone to crystallize, and partially degrade amylose to a partially crystalline polymer network that resists later rearrangements (Hugh-Iten et al. 2001)

There are currently almost 20 different amylase products on the market as antistaling agents. Several authors have worked with amylases in corn tortillas prepared with NCF (Iturbe-Chinas et al. 1996, Suhendro 1997, Quintero-Fuentes 1999, Bueso-Ucles 2003). Amylases in masa may be difficult to handle due to excessive stickiness and even if the product can be processed, it can develop an unacceptable texture (Bowles 1996). Continued action of the enzyme in the finished product on the shelf is also a concern.

The maltogenic α-amylase (glucan 1,4 α-maltohydrolase, E.C. 3.2.1.133) has been the most useful antistaling agent in bread (Hebeda et al. 1990). This enzyme was cloned from a strain of the Bacillus family. It degrades amylopectin and amylose to maltose and longer maltodextrins and does not require an unblocked non-reducing end by an endo-type mechanism (Christophersen et al. 1998). Maltogenic α-amylase in corn tortillas act as an antistaling agent, the mechanisms of action are still to be elucidated. Iturbe-Chinas et al. (1996) evaluated the effect of several types of amylases on corn tortillas and suggested that could be used as a valuable additive to delay staling.

When used in tortillas, it must be combined with other additives like CMC (Suhendro 1997, Miranda-Lopez 1999, Bueso-Ucles 2003, Silva 2003), powdered cellulose or monoglycerides (Miranda-Lopez 1999) or waxy barley flour (Silva 2003) to obtain a softening effect without making the tortilla excessively crumbly.

Proteins

Alteration of the protein profile by addition, proteolysis and use of different cultivars has been tried with different degrees of success. Vital wheat gluten (VWG) retained the flexibility of tortillas when used at low pH (5.0) by delaying the reassociation of amylopectin. However, over 1% of VWG in the formula imparted an undesirable “wheat” flavor. At higher pH no improvement was observed on the texture of tortillas during storage (Yau et al. 1994, Suhendro 1997, Miranda-Lopez 1999, Bueso-Ucles 2003).

Native soy flour together with CMC improved the texture of tortillas with increased nutritional value. Soy flour formed brown spots on the surface of the tortilla (Suhendro et al. 2001). When zein was added to NCF (Yeggy 2000) the masa resembled over mixed wheat dough and the tortilla structure became weak and the staling increased. The cross-linked zein in corn contains high levels of proline (18%) is a structurally rigid aminoacid (Hoseney 1994) that could affect the texture of tortillas. Leal -Diaz (2003) evaluated cultivars with different protein ratios than normal food grade corn and resulted that quality protein maize and high protein corn in tortillas had an extend shelf stability. Alkaline affected the starch-protein complex, weakening the tortilla structure (Miranda-Lopez 1999).

Starch

A three-dimensional network is created by the gelation of amylose right after baking (Suhendro 1997). Amylose associates to form an insoluble network that causes a flexible gel structure in the corn tortilla (Fernandez et al. 1999). By changing the proportions of amylopectin and amylose in tortillas, the normal staling rate was altered during storage. Added amylopectin appears to impart desirable softness and extensibility to tortillas, but they became more brittle than control and amylose enhanced tortillas after 24 hr of storage. Hence, the stability of corn tortillas could be increased by finding ways to delay retrogradation of amylopectin.

Hydrocolloids

The most common hydrocolloids used in corn tortillas are CMC (Serna-Saldivar et al. 1990) and guar. Hydrocolloids are used in baked goods primarily to enhance finished product moistness (Heflich 1996). Some hydrocolloids (guar, xanthan, agar, pectin, etc.) absorb up to 6 times their weight in water. However, the increase in water absorption is relatively small because the levels used range from 0.01% to 0.5% of the formula (Rosell et al. 2001). The additional formula water may be insignificant; but the viscous, slippery mouth feel that the gums impart may be perceived as a beneficial increase in product moistness. In corn tortillas, the competition for available water in the formula reduces gelatinization, helping to retard the staling process (Yau et al. 1994).

Hydrocolloids can make the tortilla rubbery and elastic. This may be perceived as softer or fresher at low levels, but as tough or chewy at elevated concentrations. Gums function successfully in some applications and have no effect in others. Explanation for this variability is lacking (Heflich 1996). Some authors found antagonistic effects when bread was supplemented with hydrocolloids (Rossel et al. 2001). Other antagonistic effects have been found when hydrocolloids are used in corn tortillas (Yau et al. 1994, Yeggy 2000).

Mitre-Dieste (2001) substituted barley flours for nixtamalized corn flour at 10-25% to evaluate tortilla quality improvement as dietary fiber content increases. Beta-glucan content of flours correlated positively with water absorption and with improved texture. Recent trials were made with waxy barley flour with 10% beta-glucan. These tortillas had improved texture during storage and suggest that the hydrocolloids in barley may cause the texture improvements in tortillas (Silva 2003). When the experiment was repeated and compared to a purified beta-glucan (70%) from barley, the improvements were not as great as expected (Gutierrez de Velasco et al. 2003).

Lipids

The addition of monoglycerides (MG) has provided limited improvement in corn tortillas (Bedolla 1983). Subjective texture of these tortillas was not improved but had a reduced rupture force (softer) when compared to the control. When tortillas containing monoglycerides were stored at 4 and -20^{o}C, Twillman and White (1988) observed a reduced rupture force but no significant difference in subjective measurements. Bueso-Ucles et al. (2001) added shortening of different sources to NCF and observed some improvement in the machinability of masa and sensory properties of tortillas but no improvement of shelf stability. Fat may delay the re-association of starch molecules (Hoseney 1994).

Storage Temperature

Inhibition of staling can be obtained by the manipulation of crystallization with control of storage temperatures. To inhibit the recrystalization of amylopectin, an ideal condition would be to keep the temperature below the Tg (Quintero-Fuentes 1999, Limmanond et al. 2002, Bueso-Ucles 2003). However, the control of temperature is not practical. The rate of retrogradation of gelatinized starch of corn tortilla (Limanond et al. 2002) exhibited a “bell-shaped” dependence on storage temperature in a range between T_g (glass transition temperature) and T_m (glass melting temperature) of the product. This is a result of the combined effect of the nucleation and propagation rates. Limanond et al. (2002) found 13^{o}C to be the maximum rate of retrogradation for corn tortillas.

Other Approaches to Prevent Staling

Fernandez de Castro (1998) found a lower staling rate in tortillas at higher pH. When tortillas were supplemented with CMC, rollability scores were higher at lower pH, but a rubbery texture was detected. Yau et al. (1994) and Suhendro (1997) showed similar results. Different leavening systems have been applied to corn tortillas without success. TSPP was used by Garza-Casso (2003) and sodium bicarbonate by Miranda Lopez (1999).

CHAPTER III

MATERIALS AND METHODS

Nixtamalized Corn Flour (NCF)

Nixtamalized Corn Flour, Tortilla # 4 (Minsa, Muleshoe, TX, USA), without additives. One kg of nixtamalized corn flour (NCF) was mixed with a paddle for 5 min at low speed in a 20 qt mixer (Model A-200, Hobart, Troy, OH, USA) with preservatives, amylases, CMC, Guar.

Distilled water (1.2 kg/kg NCF) was added and mixed with a hook for 30 s at low speed and 90 s at medium speed to form the masa. Additional five parts of water per part of guar were added.

Fresh Nixtamal Masa, FNM

Masa Prepared at the CQL

Masa was also made with white food corn in our tortilleria. White food corn was obtained from a tortilla factory (Espiga de Oro, Houston, TX, USA) as a standard corn for nixtamalization.

Corn (100g) in nylon cooking trial bags was cooked at different times to determine the optimum water absorption. Steam kettle containing 45 l of water, 13.8 kg of filling corn and 150 g of lime ($Ca(OH)_{2,}$ 1% of corn), temperature raised to a soft boil (98^{o}C) and three samples added every 15 min after the initial boil at 0, 15, 30 and 45 min. The filling corn was used to keep a constant water to corn ratio of approximately 3:1 that was used in masa production, as the weight of corn for the cooking trials was only 1.2 kg (3 bags of 100 g each at 4 addition times). Optimum moisture absorption (55%) determined by lost weight after drying the nixtamal in a forced air oven (model 16, Precision Scientific Co. PS, Chicago, IL) for 72 hr at 60^{o}C. The test was done in triplicates.

For masa production, corn (3 bags of 2 kg each) was cooked in a kettle with 45 l of water, 9 kg of filling corn and 150 g of lime ($Ca(OH)_{2,}$ 1% of corn), for 35 min at a soft boil (98^{o}C), and steeped for 12 hr.

Each bag of cooked corn was washed in 5 L containers by adding warm water and draining until pH was lower than 8.5, usually 3-4 times. Moisture absorption (optimum set at 54.5%) and dry matter losses were recorded (Figure 1). We developed the following formulas for cooking time and nixtamal weight from the cooking trials for the corn used under our cooking conditions:

Cooking time= (54.5% [Nixtamal moisture] – 48.205) / 0.174= **36.2 min**
Nixtamal weight increase (%)= 0.0172* (cooking time (min))2 -0.3002 * (cooking time (min)) + 68.848= **80.5% @ 36.2 min cooking time**

From the total increase of nixtamal weight after washing, we determined optimum water absorption. Some adjustments were made by water addition during grinding so final masa moisture was 58%, the same level as NCF.

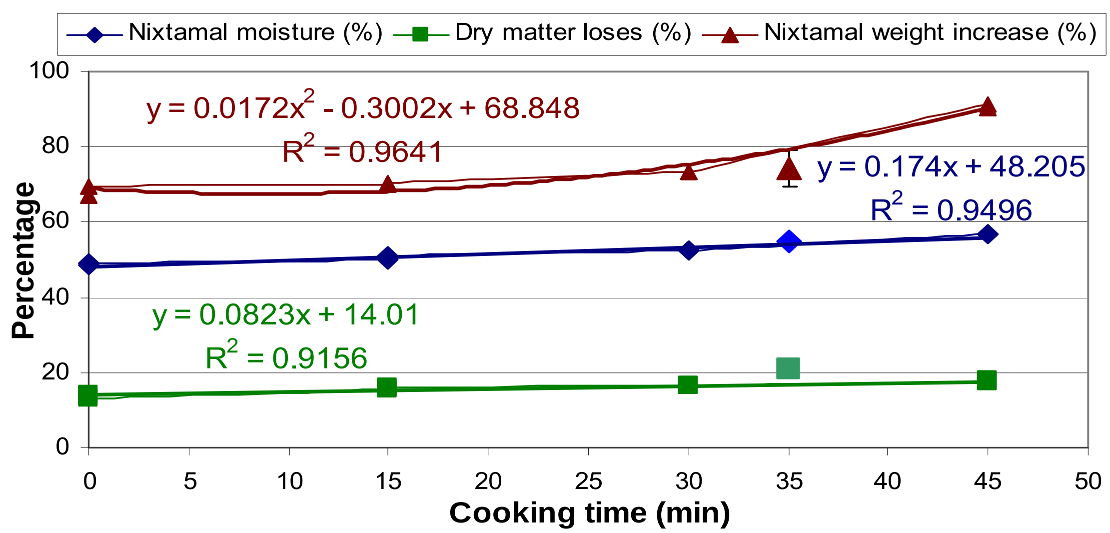

Figure 1 Cooking trials of standard corn and values for the selected cooking time for FNM (35 min). Note: trend lines and R^2 are included; values are averages of 3 replicates, 3 repetitions each.

Preservatives (0.5% numeric acid and 0.4% potassium sorbate, dm) were added and mixed before grinding. Nixtamal was ground using a lava stone grinder (model CG, Casa Herrera, Los Angeles, CA, USA). The predicted moisture was used to estimate the amount of water needed to add during grinding to adjust the masa to 58% moisture. Masa was stored at 40°C in an insulated chest (8.5 gal, model 1935, Rubbermaid, Wooster, OH, USA) with 3 polyethylene bags with 2 liters of hot (50 ± 10°C) water prior to sheeting. Additives and enzymes were added slowly while the FNM masa was mixed in a 20 qt mixer (Model A-200, Hobart, Troy, OH) for 30 s at low speed and 90 s at medium speed with a hook.

Masa from Other Sources

Masas from 3 different tortillerias was collected from the production site, kept warm in an insulated chest and processed within 5 hr after grinding. Masa from optimal cooked corn had 55 ± 1% moisture and did not require the addition of NCF for sheeting. Masa made with undercooked corn (< 40% moisture absorption) and overcooked (>55% moisture absorption) were also evaluated. Masa rheology and handling characteristics were measured subjectively for the masa after mixing.

Standard Baking Procedures

Masa was equilibrated in a polyethylene bag for 10 min, sheeted and die cut (15 cm diameter) into 30 g disks (Model CH4-STM, Superior Food Machinery, Inc., Pico Rivera, CA, USA). Tortillas were baked for 60 s in a gas-fired three-tier oven (320°C top, 270°C middle and 220°C bottom, (Model C-0440, Superior Food Machinery, Pico Rivera, CA, USA), cooled and stored in polyethylene bags under ambient (22°C) and refrigeration (4°C) temperatures. Objective and subjective texture were measured at 2 hr, 1, 4 and 7 d after baking. Moisture was measured in tortillas at 2 h and 7 days after baking.

Additives

Amylases

Maltogenic α-amylase (Bacterial)

Alpha amylase from *Bacilus subtilis* was used (Innovative Cereal Systems, Wilsonville, OR, USA). This enzyme is classified as a glucan 1,4-α -maltohydrolase, E.C. 3.2.1.133 with an activity of 10,000 units / g. One enzyme unit (maltogenic α-amylase unit, MAU) was defined as the amount of enzyme, which under standard assay conditions cleaved 1 μmol of maltotriose per min. This enzyme was cloned from a strain of the Bacillus family and prepared by submerged fermentation of Bacillus subtilis, strain DN 252, through recombinant DNA techniques. The gene was imported from *Bacillus stearothermophilus* (NCIB 11837). It degrades amylopectin and amylose to maltose and longer maltodextrins and does not require an unblocked non-reducing end by an endo-type mechanism (Christophersen et al. 1998).

Bacterial *α*-amylase

Alpha amylase from *Bacilus amyloliquefaciens*, (Novozymes, Novo Nordisk A/S, Bagsvaerd, Denmark, with 250 bacterial activity units (BAUN) / g. One BAUN is defined as the amount of enzyme, which under standard assay conditions dextrinizes 5.26 g of dry Merck's lintner starch (special for diastatic power determination) per hr.

Barley α- and β-amylase

Amylase type VIIIA from malted barley (*Hordeum vulgare* L.). (E.C. No. 232-565-6, E.C. 3.2.1.1). The enzyme has an activity of 2.0 units of α-amylase and 3.0 units of β-amylase per mg of solid at pH 4.8. One unit of enzyme liberates 1.0 mg of maltose from Merck's lintner starch (special for diastatic power determination) in 3 min at pH 6.9 and 20^{o}C.

Hydrocolloids

Sodium carboxymethylcellulose (CMC)

Ticalose CMC 2500 (TIC Gums, Belcamp, MD, USA) with degree of substitution in the range of 0.65-0.90, pH of 6.5-8.5, sodium fraction of 7-8.9% and a medium viscosity (typically 2,500 cps at a 1% concentration) was used.

Guar gum

Guar gum BLN-200-HV (TIC Gums, Belcamp, MD, USA). Guar is a natural galactomannan polymer with randomly oriented chains without a galactose free region. Forms a weak gel in the presence of water and has a medium viscosity (typically 3,500 cps at a 1% concentration)

Guar plus alginates

Fold n' flex (TIC Gums, Belcamp, MD, USA) is a mixture of guar and sodium alginate. Sodium alginate is the salt of the long-chain carbohydrate alginic acid, extracted from various species of brown algae (seaweed) and purified to a white powder. The alginates have different characteristics of viscosity and reactivity based on the specific algal source and the ions in solution. Alginic acid itself is insoluble, but the salts are hydrocolloids.

CMC, Maltodextrin, carrageenan mix

COLL 1023-TDx (TIC Gums, Belcamp, MD, USA) is a mixture of CMC, maltodextrins and carrageenan. Maltodextrins are non-sweet, nutritive saccharide polymers that consists of D- glucose units linked primarily by α-1,4 bonds and that had DEs (dextrose equivalents) of less than 20 [US FDA 21 CFR paragraph 184.1444]. Carrageenans are a family of linear sulfated food grade polysaccharides obtained from red seaweeds. They have the unique ability to form an almost infinite variety of gels at room temperature, rigid or compliant, tough or tender with high or low melting point. The gelation requires no refrigeration and the gels can be made stable through repeated freeze-thaw cycles. Carrageenan solutions will thicken, suspend and stabilize particulates as well as collodial dispersions and water/oil emulsions.

Preservatives

Preservatives were used to delay microbial spoilage. A combination of potassium sorbate (Fleishman's yeast, Greenville, TX, USA) at 0.25% (w/w) of masa and fumaric acid food grade fine granular (Bartek Ingredients, Ontario, Canada) at 0.20% (w/w) of masa was used.

Physical Characterization

Moisture

The one-stage moisture oven AACC method 44-15A (AACC 2000) with some modifications was used and consists of drying a sample of a tortilla taken with a 1" diameter cutter to attain a constant weight (1.6 $\pm$ 0.3 g). The sample is dried in a forced air oven (model 16, Precision Scientific, Chicago, IL) for 24 hr at 130^{o}C. Moisture was calculated by loss of weight using triplicates. Tortilla moisture was evaluated the day of processing and after 4 days.

pH

Masa was measured directly by inserting the electrode 4 times in different parts of the masa dough, 10 min before processing. Ten grams of a tortilla ground with a coffee grinder and mixed in 90 ml of distilled water were measured with a pH meter (model Φ 10, Beckman Instruments, Fullerton, CA). The electrode probe (Corning "3 in 1". Corning, Inc., New York, NY) was dipped in the water-tortilla solution and the pH measurement was recorded after 25±5 seconds. Tortilla pH was measured 1 hr after processing.

Water Absorption Index, Water Solubility Index (WAI/WSI)

Water absorption index and water solubility index were performed by the method of Anderson et al. (1969) with some modifications. A masa sample (1 g) in 20 ml of water, was agitated in a shaker (Eberbach Corp., Ann Arbor, MI, USA) for 10 min at 200 cycles per minute. Tubes were centrifuged (International Centrifuge, Boston, MS, USA) for 10 min at 600 rpm. The tube was placed on a 90-degree angle for 5 min and excess water was removed with a paper towel. The tube was weighed and dried in a forced air oven (model 16, Precision

Scientific Co. PS, Chicago, IL). Water absorption index was calculated as the weight of the gel formed per g of dry sample. Water solubility index was the difference between the dry matter used and the final weight of the dried gel. Analysis was done on 4 replicates.

Particle Size Distribution

A masa sample (20 g) was suspended in 50 ml of water for 12 hr. Masa was sieved in running water through 40, 60, 80, 100, 140 and 200 mesh (425, 250, 180, 150, 106, 75 μm average size respectively). Fractions were dried in a forced air oven (model 16, Precision Scientific Co. PS, Chicago, IL) for 24 hr at 130^{o}C. The smallest fraction (less than 75 μm) was estimated by subtracting from original dry the sample weight the dry weight of all the fractions, the moisture from the sample and the water solubles estimated from WSI. This corresponds to the 11 μm fraction reported in other studies (Gomez 1988).

Texture Analysis

Masa

Cohesiveness and stickiness was rated using a scale of 1 to 5. Cohesiveness refers to pressing the masa in the palm of the hand, while determining how it stays in one piece without crumbling apart. Stickiness refers to the difficulty of forming a ball with the same hand after the evaluation of cohesiveness.

Tortillas

Subjective pliability of tortillas, which measures the cracking and breaking of a tortilla at ambient temperature was evaluated by squeezing a tortilla inside the palm of one hand, holding it for 2 s and then releasing it. The five-point scale was defined as 1 = complete crumbling, 2 = almost total crumbling, 3 = a lot of cracking, no crumbling, 4 = isolated cracks and 5 = completely pliable (no cracks). This test was done for 5 replicates.

Subjective rollability of tortillas, which measures the cracking and breakage of a tortilla at ambient temperature, subjectively evaluates stability.

Rolling half of a tortilla around a 1.0 cm dowel, gives a subjective rollability score (RS) on the scale of 1 to 5, where 1 = unrollable, 2 = breaks on one side and cracks on the other, 3 = breaks on one side, 4 = cracks on one side only, 5 = rolls without cracking or breaking. This test was done for 5 replicates.

One-dimensional extensibility: test was conducted using the texture analyzer (model TA.XT2i Texture Analyzer, Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK), using the method of Suhendro et al. (1999). A tortilla strip (70x35 mm) held between two clamps, pulling apart until rupture. The extensibility test uses the return to start option, with tension mode and trigger force of 0.05 N. Pre and post-test speed of 10.0 mm/s, and test speed of 1.0 mm/s. Modulus of deformation (N/mm), rupture force (N) and rupture distance (mm) were recorded.

Rapid Viscoanalyzer (RVA)

Stabilized Starch Residue (SSR)

Starch was stabilized with methanol using the method of Fernandez et al. (1999) with the modifications described by Seetharaman et al. (2002). Stabilization of selected treatments improved with amylases was performed for tortillas and masa. Dried stabilized starch residue (SSR) samples were ground on an UDY Cyclone Sample Mill (Model 3010-030, Tecator / Udy, Fort Collins, CO) with a 1 mm mesh and stored at -30^{o}C until testing.

A 72 g sample was mixed with 180 mL of 0.05% (w/w) solution of mercuric chloride (Fisher Hannover Park, IL, USA) in methanol in a blender and ground for 3 min at maximum speed. The mercuric chloride was used to inactivate the enzyme as suggested by De Haas et al. (1978). The ground sample was filtered under vacuum using filter paper (Whatman #1) to remove the excess methanol. Another rinse with 250 ml of methanol for 2 min, followed by filtering was performed before drying the stabilized sample at 50^{o}C for 3 h in a forced-air oven. An aliquot of the liquid after extraction was saved for evaluation of dry matter weight losses during stabilization that was determined by recording the initial weight of the tortillas and the weight of the residue after drying.

RVA Profile and Settings

The SSR sample was brought to 28.0 g. of solution containing 15% solids in a 0.05% mercuric chloride solution. Special polymer coated canisters (Sipca Buff, Newport scientific, Warriewood, Australia) were used to prevent corrosion from the solution of mercury chloride.

The standard 1 profile was used in the Rapid Viscoanalyzer (Model RVA-4, Newport scientific, Warriewood, Australia). In this profile, the temperature is first maintained at 50°C for 1 min, raised to 95 °C at a rate of 12 °C/min, maintained for 2.5 min and reduced to 50°C at a rate of 12 °C/min, and a final hold of 2 min. Total test duration was 13 min.

Peak viscosity, viscosity at trough and final viscosity, peak time and pasting temperature were recorded in Rapid Viscoamylograph Units (RVU). These values from the RVA profile (standard 1) are defined as follows: **a)** pasting temperature in degrees Celsius defined as a rapid increase in the viscosity, higher than 15 RVU in 0.2 min., **b)** peak viscosity defined as the highest initial viscosity after the temperature increases from 50 to 95^{o}C and before a viscosity drop of at least 50 RVU; **c)** viscosity at trough, defined as the lower viscosity reading after the peak viscosity; **d)** final viscosity defined as the value in RVU after cooling to 50^{o}C (end of test: 13 min).

HPLC

Tortillas and masa from selected treatments improved with amylases and hydrocolloids were extracted and analyzed by cation exchange HPLC to calculate the amount of polymers, oligosaccharides and simple sugars as outlined by Jackson (1991) with some modifications. Tortilla starch fractions were solubilized in aqueous 0.05% (w/w) mercuric chloride in water in a water bath at 100^{o}C for 30 min. The samples were cooled to 60^{o}C in a water bath for 10 min and then sonicated in a Vortex Genie (model G560, Scientific Industries, Bohemia, NY, USA) at speed 8 for 30 sec. The solution was centrifuged (International Centrifuge, Boston, MS, USA) for 10 min at 600 rpm and subsequently filtered (1.2 µm paper filter) and injected (20 µL) into a HPSEC system consisting of 4 KS-series Shodex Ionpack columns (Showa Denko, Tokyo, Japan) connected in series to a refractive index detector (Waters model 410, Millipore Co., Milford, MA, U.S.A.). The mobile phase used consists of degassed, deionized and distilled water at a flow rate of 1.0 ml/min. Standard solutions of lyophilized maltose (Sigma Chemical Co, Saint Louis, MO, USA) and maltodextrins with dextrose equivalents 7, 11, 13 and 18 (Dry MD 7.0, 11, 13, 18, Cargill, Minneapolis, MN, USA). Results were analyzed using Milenium 32 software (Millipore Co., Milford, MA, U.S.A.). The peaks were defined for polymers from 10.0-16.0 min, 16.0-25.0 min for oligosaccharides and 25.0-30.0 min for low molecular weight (LMW) sugars. An ion peak corresponding to mercuric chloride present in the solution used for extraction was detected and its area was deducted from all chromatograms. Masa was compared to tortillas to observe changes in the starch structure and verify inactivation of enzymes. Tortillas stored for different times were analyzed to observe changes in the starch structure and verify inactivation of enzymes.

Response Surface Methodology (RSM)

This methodology is a collection of statistical and mathematical techniques useful for design and development, in which several ingredients are evaluated for their response (tortilla rollability, pliability, rupture force, etc) in the formula (Box et al. 1978). Response surface designs were used in the latter stages of this investigation, to optimize level addition.

Statistical Analysis

One-way analysis of variance (ANOVA) in a randomized experimental design was used to evaluate tortilla properties. Protected Fisher's LSD procedure with a confidence level of 95%. The statistical software SAS (version 8.0, SAS Institute, Cary NC) was used. When Response Surface Methodology was used, data was analyzed creating a second order regression models using SAS version 8.

CHAPTER IV

ADDITIVES THAT IMPROVE NCF AND FNM TORTILLA TEXTURE

The most appropriate enzymes and hydrocolloids were selected and evaluated for improvement of the objective and subjective texture of tortillas from NCF and FNM. Improvements were measured in tortillas after 7 days of storage under refrigeration. The function of additives in corn tortillas was evaluated.

Selection of Enzymes

FNM tortillas were treated with different amylolytic enzymes, selected according to baking temperature stability (Bacterial > Maltogenic > Barley). To develop a criterion for enzyme selection, NCF control tortillas were formulated with maltogenic α-amylase (0.15 g/kg flour or 68.2 mg/kg masa) based on the levels of Bueso-Ucles (2003) without CMC. Tortillas formulated with enzymes were compared to a control after storage for 7 d at 4^{o}C. This caused significant reduction of pliability and modulus of deformation. The enzyme type and level was selected from those that conform to the pliability and modulus of deformation reductions in the treated control tortillas. The moduli of deformation and pliability scores were recorded after storing treated tortillas for 7 d at 4^{o}C (Table I).

Table I Changes in subjective and objective measurements of FNM tortillas treated with different amylases after 7 d storage at 4°C[a].

	mg enz/ kg masa dmb	Machin-ability	Modulus of deformation (N/mm)	Pliability score	# of tortillas measured
CONTROL **NCF+ 1500 MAU amylase**	170.0	4.50	1.02	1.00	10
FNM + Bacterial amylase	6.2	4.50	0.96	1.00	20
FNM + Bacterial amylase	12.5	3.00	1.24	1.19	20
FNM + Maltogenic amylase	24.9	4.50	0.31	0.33	10
FNM + Maltogenic amylase	47.4	4.50	0.97	1.00	20
FNM + Maltogenic amylase	74.8	4.00	0.30	0.67	20
FNM + Maltogenic amylase	187.0	3.00	0.90	0.00	10
FNM + Barley amylase	24.9	5.00	0.02	-0.33	10
FNM + Barley amylase	37.4	5.00	0.70	0.67	10
FNM + Barley amylase	62.3	5.00	0.68	0.67	10
FNM + Barley amylase	74.8	5.00	-0.24	-0.33	5
FNM + Barley amylase	124.6	4.00	0.43	0.00	5

[a]Modulus of deformation and pliability scores are expressed as the difference between the mean values for the enzyme treated and the control tortillas from the same trial; values are means of the number of tortillas indicated in the last column; for measurement values, see 0, Table X.

Tortillas containing bacterial α-amylase (2.5 mg/kg masa) and maltogenic α-amylase (15 mg/kg masa) had the closest values to our ideal parameters (Table I). Tortillas with bacterial α-amylase complied with the criteria parameters, but caused stickiness in the masa, which decreased its machinability. Lowering its addition may improve the results, but thermal inactivation during baking is difficult to control. Tortillas treated with maltogenic α-amylase met our criteria. This enzyme was previously used in corn tortillas in accordance with previous research (Miranda-Lopez 1999, Quintero-Fuentes 1999, Bueso-Ucles 2003, Silva 2003).

Selection of Hydrocolloids

Hydrocolloids were tested along with enzymes in masa. Addition of hydrocolloids was expected to significantly improve the texture reflected by an increase in pliability and reduced modulus of deformation at 7 days of storage under refrigeration.

Three common additives (Table II) from a supplier of tortilla manufacturers were tested at different concentrations to meet our selection criteria of increased pliability scores, moisture retention and reduced modulus of deformation. The additives were used with the formula developed by Bueso-Ucles (2003) that included maltogenic α-amylase (1500 MAU/ kg flour) and CMC (0.25%) for tortillas prepared using NCF.

Table II Hydrocolloid treatments to improve texture and moisture retention of tortillas prepared using NCF.

	Additive (g / kg flour)				
Treatment code	**Maltogenic amylase**	**CMC**	**Tested additives**	**Hydrocolloids**	**Brand name**
Control	None	None	None	Standard control	
Ctrl improved	0.15	2.5		Improved control with CMC	
GG 0.4%	0.15	2.5	4.0	Guar gum	Guar BLN
GG 0.8%	0.15	2.5	8.0	Guar gum	Guar BLN
GG + Alg 0.4%	0.15	2.5	4.0	Guar gum and Na Alginate	Fold 'n Flex
GG + Alg 0.8%	0.15	2.5	8.0	Guar gum and Na Alginate	Fold 'n Flex
CMC + MD + Carr 0.4%	0.15	None	4.0	CMC, Maltodextrin, Carrageenan	Coll 1023-TX
CMC + MD + Carr 0.8%	0.15	None	8.0	CMC, Maltodextrin, Carrageenan	Coll 1023-TX

Tortillas from all treatments and control received the highest score for pliability (5.0) and the lowest modulus of deformation (>0.8N/mm) 20 min after baking (0, Table XI).

After 1 d of storage, significant differences in tortilla objective and subjective measurements (0, Table XII) were observed among treatments and storage temperatures. Changes in rupture force, rupture distance, modulus of deformation, rollability and pliability were further increased after 7 d of storage at 22^{o}C (0, Table XIII) and 4^{o}C 0, (Table XIV). Aging of tortillas decreased the rollability and pliability scores, the rupture distance and increased the rupture force and the modulus of deformation. Staling rate was initially higher; then decreased during storage (Miranda-Lopez 1999). These results were expected and are in agreement with former research (Iturbe –Chinas 1996, Suhendro 1997, Fernandez et al. 1999, Quintero-Fuentes 1999; Yeggy 2000, Limanond et al. 2002, Bueso-Ucles 2003, Silva 2003).

The treatments that contained 0.4% guar, 0.4% guar and sodium alginate and 0.4% CMC, maltodextrin and carrageenan had significantly higher pliability scores (Figure 2) than the improved control after 7d storage under ambient temperatures. For the tortillas stored under refrigeration, the treatment that contained 0.8% of CMC, maltodextrin and carrageenan had significantly higher pliability scores than the improved control. Increasing the percentage of guar or guar and sodium alginate from 0.4 to 0.8% did not result in significantly higher pliability scores of tortillas.

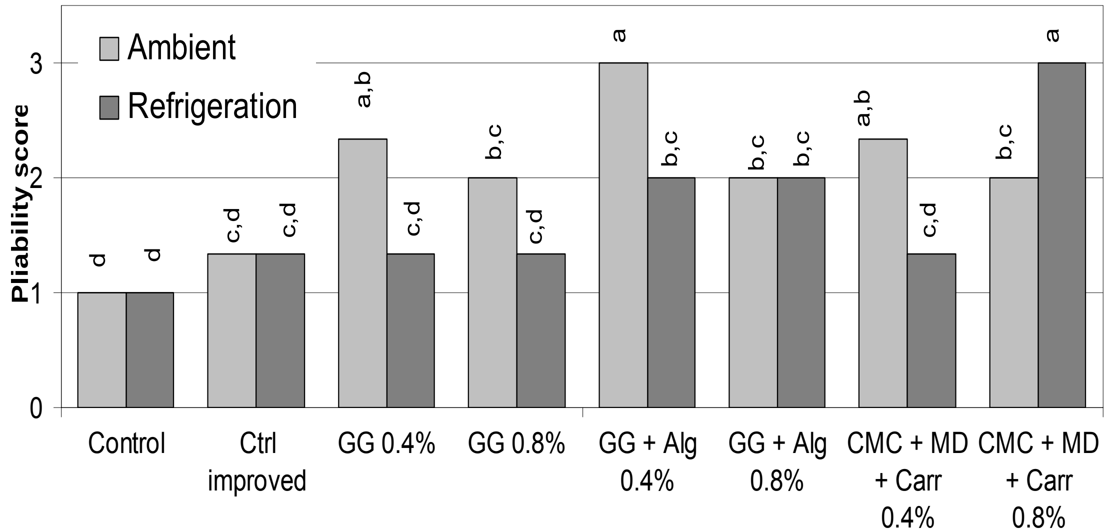

Figure 2 Pliability scores 7 days under ambient and refrigeration temperatures of corn tortillas containing different hydrocolloids and maltogenic α-amylase (1500 MAU / kg flour). Note: values are means of 2 replicates, 3 tortillas each; $LSD_{0.05}$ 0.9; columns with the same letter are not significantly different; see treatment codes in Table II.

The treatments that contained 0.4% or 0.8% guar or 0.8% guar and sodium alginate had significantly lower modulus of deformation (Figure 3) than the improved control after 7d storage at 22^{o}C. For the tortillas stored at 4^{o}C, the treatments that contained 0.8% guar, 0.8% guar and sodium alginate or 0.8% CMC, maltodextrin and carrageenan had significantly lower modulus of deformation than the improved control.

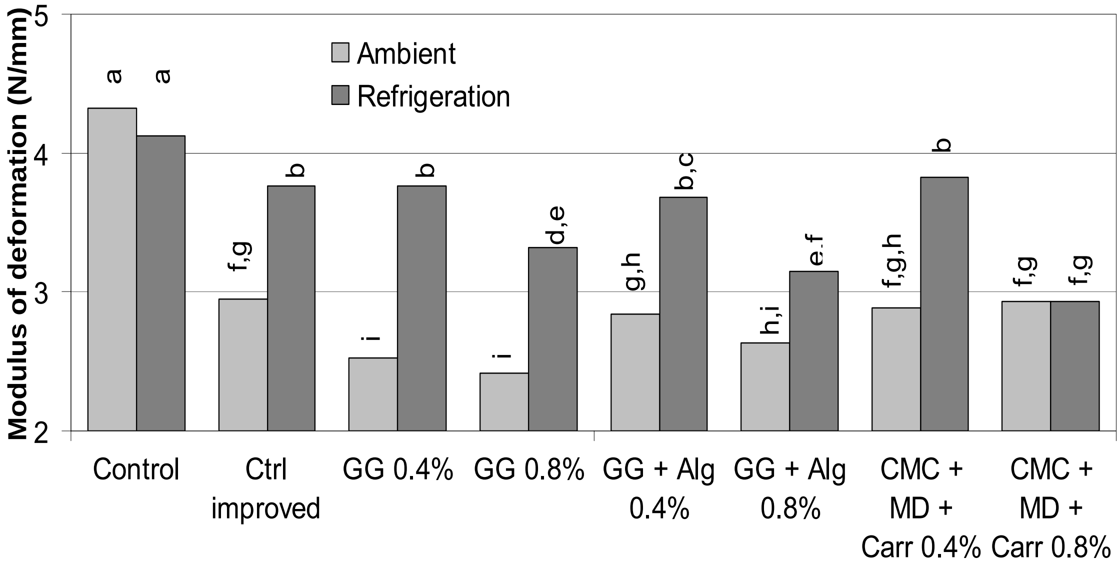

Figure 3 Modulus of deformation (N/mm) after 7 days under ambient and refrigeration temperatures of corn tortillas with different hydrocolloids and maltogenic α-amylase (1500 MAU / kg flour). Note: $LSD_{0.05}$ =0.3; columns with the same letter are not significantly different; values are means of 2 replicates, 5 tortillas each; see treatment codes in Table II.

Hydrocolloids delayed tortilla staling when used in conjunction with maltogenic α-amylase and CMC. Higher levels of additives (0.8%) in tortillas reduced the modulus of deformation but did not give higher pliability scores indicating that they could be disrupting the tortilla structure. The structure disruption may be at the threshold of detection for the subjective test, but was detected by the objective texture analysis. The only mixture that consistently improved the texture at low temperatures contained CMC, maltodextrin and carregeenan. Maltodextrins could be delaying tortilla staling by reducing available water, which decreases gelatinization of starch granules. This mechanism is different than changes in viscosities of gums

The function of guar as an antistaling agent is closely related to the interaction with starch, particularly with linear amylose, but also with amylopectin and the formation of these complexes inhibits staling by preventing amylose or amylopectin retrogradation (Kamel and Ponte 1993). Guar gum may also be adsorbed into the starch surface (Pisesookbunterng & D'appolonia 1983). Guar gum and other galactomannans develop a weak gel at concentrations as low as 0.5%. This gel breaks down on heating (>50°C) losing its structural integrity, which is restored if kept at ambient temperature for around 3 days. When freezing and thawing of galactomannans occurs in the presence of other polysaccharides, they separate, self-associate and form "gel-islands" (Dea et al. 1977). Guar must be adsorbed onto the starch surface to develop a film that delays starch retrogradation. The gelation of guar takes longer (>3 days) than the retrogradation of starch under refrigeration (1-2 days) loosing its effect as an antistaling agent under these conditions and segregating into "gel-islands".

Tortillas containing guar at 0.4% or 0.8%, 0.4% guar and sodium alginate and 0.4% CMC, maltodextrin and carrageenan had variable improvements compared to control tortillas (Table III).

Table III Summary of improvements to tortillas prepared using NCF, CMC and maltogenic α-amylase with different hydrocolloids[a].

	Pliability score*		Modulus of defrormation (N/mm)*	
Treatment	22°C	4°C	22°C	4°C
Control	1.0 [d]	1.0 [d]	4.3 [a]	4.1 [a]
Ctrl improved	1.3 [c,d]	1.3 [c,d]	3.0 [f,g]	3.8 [b]
GG 0.4%	2.3 [a,b]	1.3 [c,d]	2.5 [i]	3.8 [b]
GG 0.8%	2.0 [b,c]	1.3 [c,d]	2.4 [i]	3.3 [d,e]
GG + Alg 0.4%	3.0 [a]	2.0 [b,c]	2.8 [g,h]	3.7 [b,c]
GG + Alg 0.8%	2.0 [b,c]	2.0 [b,c]	2.6 [h, i]	3.1 [e,f]
CMC + MD + Carr 0.4%	2.3 [a,b]	1.3 [c,d]	2.9 [f,g,h]	3.8 [b]
CMC + MD + Carr 0.8%	2.0 [b,c]	3.0 [a]	2.9 [f,g]	2.9 [f,g]
$LSD_{0.05}$	**0.9**	**0.9**	**0.3**	**0.3**

[a]Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; treatment codes in Table II.

Tortillas containing guar at 0.4% had a better structure, indicated by a higher pliability score with a similar decrease in the modulus of deformation. An indication of disruption of the tortilla structure is when the modulus of deformation and the pliability scores decrease. Thus guar at 0.4% was selected for the RSM trials.

Levels of selected enzymes and hydrocolloids were adjusted for FNM and NCF tortillas. In the following chapter these levels will be optimized by response surface methodology. The center points for RSM were located for the different products. For:

FNM: Maltogenic α-amylase at 15 mg/kg masa (470 MAU/kg masa dm) from the results of these trials. CMC at 1% of masa (0.23% of dm) was estimated at similar levels to NCF at 0.28% dm. Central point of Guar (0.23% of dm) was set as equal to the level of CMC.

NCF: Guar gum at 0.5% of flour (0.57% dm) from the results of these trials. Maltogenic α-amylase at 1000 MAU/ kg flour (1100 MAU/kg dm) and 0.25% CMC (0.283% dm) from the formula developed by Bueso-Ucles (2003).

Summary

Significant new findings were found from these trials. Maltogenic and bacterial α-amylase were able to disrupt the starch structure in tortillas from FNM masa. Guar improved the texture of NCF tortillas with enzymes and CMC. Guar seems to have a complimentary functionality with amylases and CMC in NCF tortillas. NCF tortillas with combination of additives that included maltodextrins retained their freshness longer at lower temperatures of storage.

CHAPTER V

OPTIMIZING COMBINATIONS OF SELECTED ADDITIVES TO IMPROVE SHELF TEXTURE OF TORTILLAS FROM FNM AND NCF

Central composite design experiments using the best enzymes and hydrocolloids selected from previous trials were used to predict treatments for masa of different sources (NCF and FNM) that produce tortillas with improved texture. The central points of the central composite design were the optimum levels for maltogenic α-amylase, CMC and guar (Table IV). Pliability, rollability, rupture force and modulus of deformation of tortillas after 7 days of storage are reported in this section.

Table IV Levels used for central composite design to be used in NCF and FNM formulation of tortillas.

	NCF (RSM levels)			FNM (RSM levels)		
Additives on a dmb	**low (-1)**	**med (0)**	**high (+1)**	**low (-1)**	**med (0)**	**high (+1)**
Amylase (MAU/kg)	0	1,150	2,300	0	465	930
CMC (g/kg)	0.0	2.8	5.7	0.0	2.3	4.7
Guar (g/kg)	0.0	5.7	11.3	0.0	2.3	4.7

Several graphs are presented in each section to facilitate a better understanding of the impacts of the independent variables and their interactions on texture measurements of tortillas.

Optimization of Additives to NCF

Two different tendencies were observed in tortillas prepared using NCF. Objective and subjective measurements predicted different ingredient optimums. The subjective values of tortillas stored 7 d at 4^{o}C supported a treatment low in enzymes. Rollability scores (Figure 4) and pliability scores (Figure 5) of tortillas with the medium level of guar were retained longer with lower levels of maltogenic α-amylase and higher levels of CMC. Pliability scores (Figure 6) of tortillas without maltogenic α-amylase were retained longer with higher levels of CMC, with only a slight effect of guar.

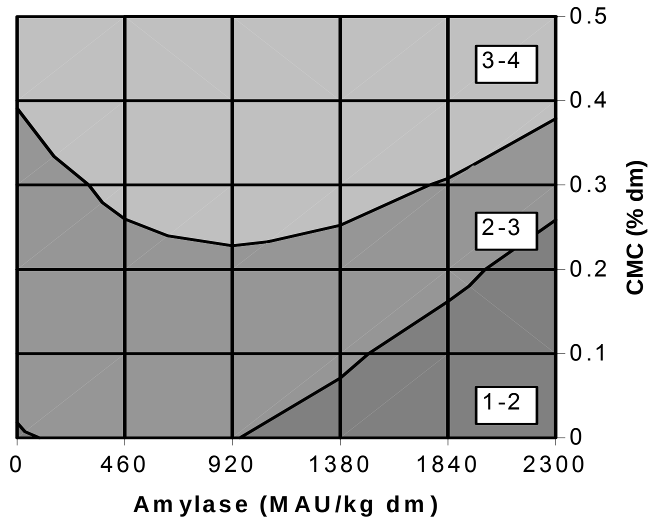

Figure 4 Rollability scores of NCF tortillas stored 7 days at 4°C, as affected by variable levels of maltogenic α–amylase and CMC and the medium level of guar (0.57% dm). Note: least significant difference ($LSD_{0.05}$) = 0.93.

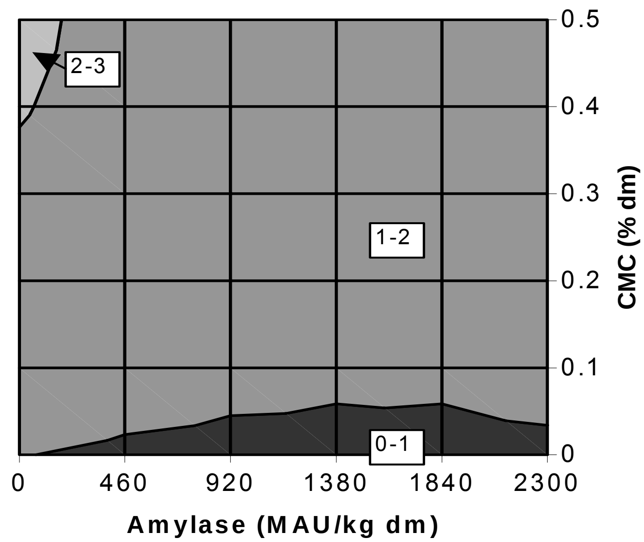

Figure 5 Pliability scores of NCF tortillas stored 7 days at 4°C, as affected by variable levels of maltogenic α–amylase and CMC and the medium level of guar (0.57% dm). Note: least significant difference ($LSD_{0.05}$) = 0.63.

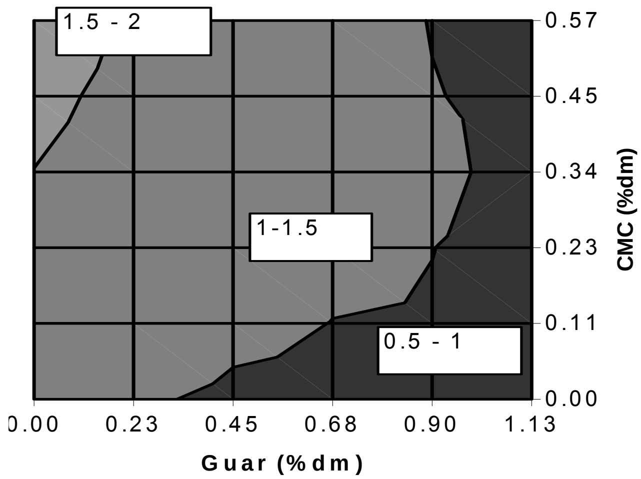

Figure 6 Pliability scores of NCF tortillas stored 7 days at 4°C, as affected by variable levels of CMC and guar and no α-amylase (0 MAU/kg dm). Note: least significant difference ($LSD_{0.05}$) = 0.63.

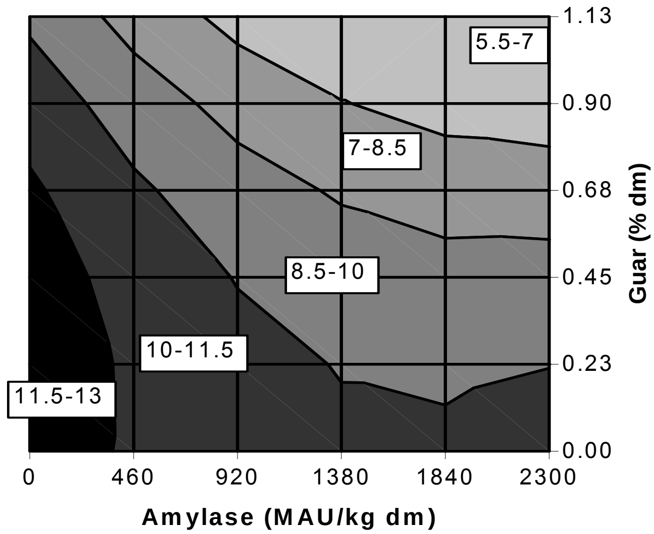

Figure 7 Rupture force (N) of NCF tortillas stored 7 days at 4°C, as affected by variable levels of maltogenic α–amylase and guar and no CMC. Note: least significant difference ($LSD_{0.05}$) = 1.25.

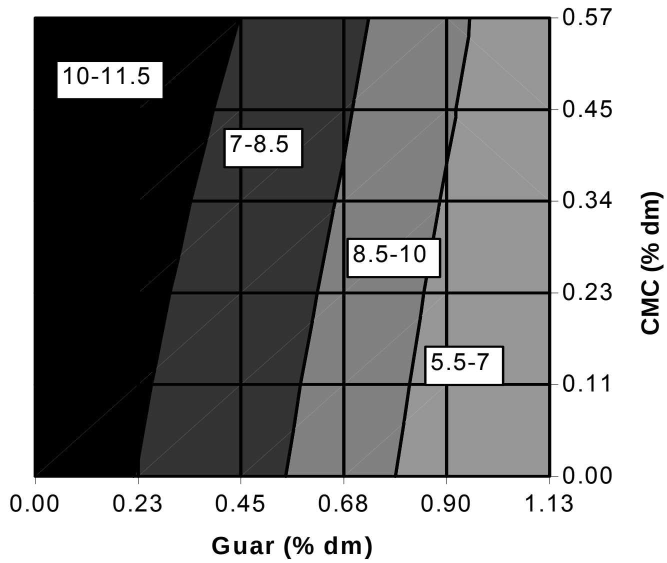

Figure 8 Rupture force (N) of NCF tortillas stored 7 days at 4°C, as affected by variable levels of CMC and guar and the high level maltogenic α-amylase (2300 MAU/kg dm). Note: least significant difference ($LSD_{0.05}$) = 1.25.

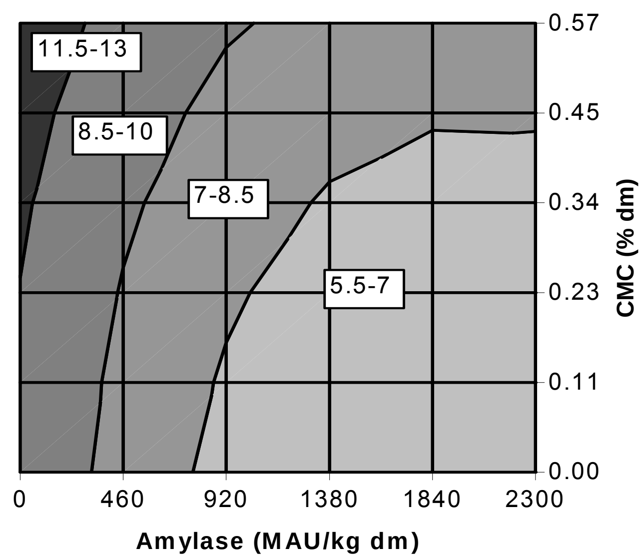

Figure 9 Rupture force (N) of NCF tortillas stored 7 days at 4°C, as affected by variable levels of maltogenic α–amylase and CMC and the high level of Guar (1.13% dm). Note: least significant difference ($LSD_{0.05}$) = 1.25.

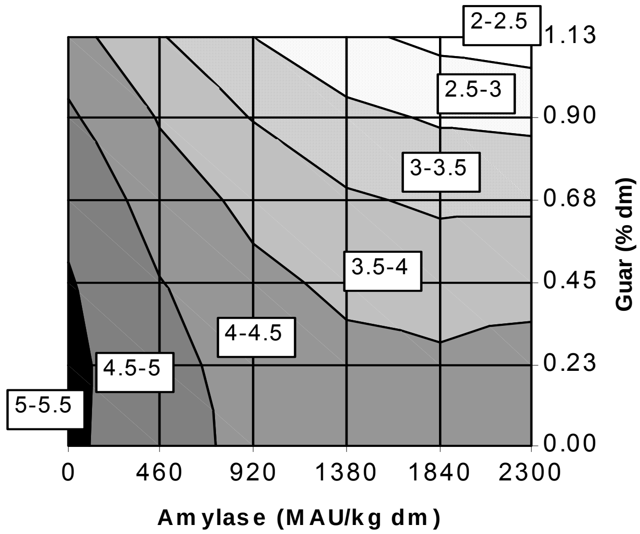

Figure 10 Modulus of deformation (N/mm) of NCF tortillas stored 7 days at 4°C, as affected by variable levels of α–amylase and guar and no CMC. Note: least significant difference (LSD $_{0.05}$) = 0.35.

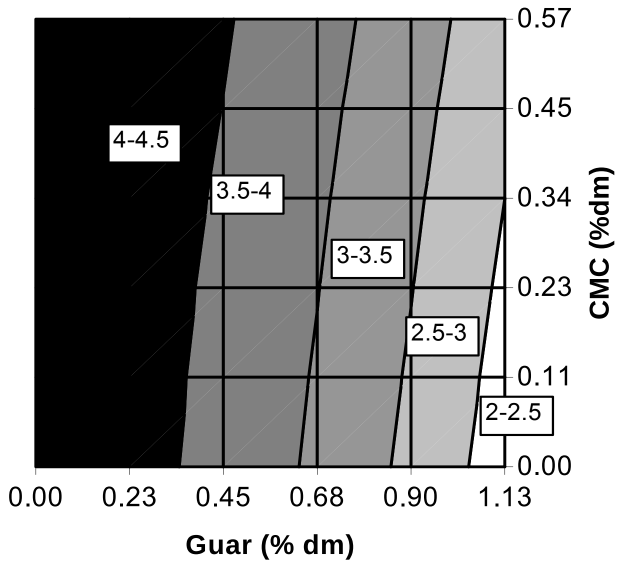

Figure 11 Modulus of deformation (N/mm) of NCF tortillas stored 7 days at 4°C, as affected by variable levels CMC and guar and the high level of maltogenic α-amylase (2300 MAU/kg dm). Note: least significant difference (LSD $_{0.05}$) = 0.35.

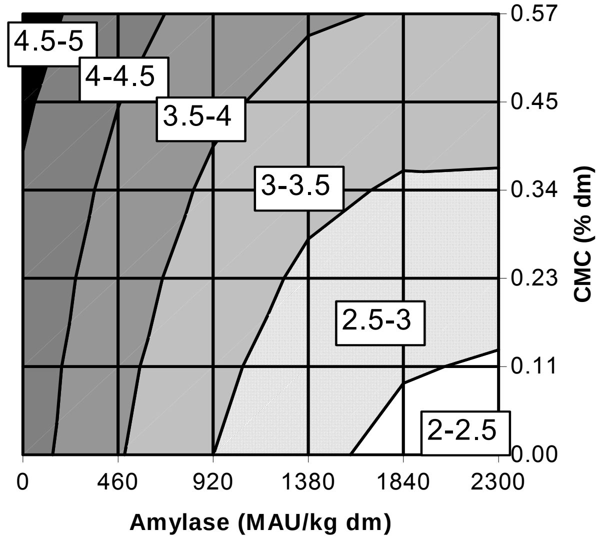

Figure 12 Modulus of deformation (N/mm) of NCF tortillas stored 7 days at 4^{o}C, as affected by variable levels of maltogenic α-amylase and CMC and the high level of guar (1.13% dm. Note: least significant difference ($LSD_{0.05}$) = 0.35.

These results support an optimum treatment with low maltogenic α-amylase, high CMC and medium level of guar. This confirms previous data on selection of hydrocolloids for NCF tortilla texture improvement, where medium levels (0.4%) of guar were optimum for delay of staling as measured by subjective pliability scores.

The objective values supported a treatment higher in enzymes. Rupture force (Figure 7) of tortillas without CMC decreased with higher levels of maltogenic α-amylase and guar. Rupture force (Figure 8) of tortillas with high levels of maltogenic α-amylase decreased with higher levels of guar and lower levels of CMC. Rupture force (Figure 9) of tortillas containing high levels of guar decreased with higher levels of maltogenic α-amylase and lower levels of CMC. Modulus of deformation (Figure 10) of tortillas with no CMC decreased with higher levels of maltogenic α-amylase and guar. Modulus of deformation (Figure 11) of tortillas with high levels of maltogenic α-amylase decreased with higher levels of guar and lower levels of CMC. Modulus of deformation (Figure 12) of tortillas with high levels of guar decreased with higher levels of maltogenic α-amylase and lower levels of CMC.

These results support a treatment high in maltogenic α-amylases, low in CMC and high in guar. High levels of maltogenic α-amylase disrupt the tortilla structure and low levels of CMC that do not form a secondary structure makes the tortilla softer (Miranda-Lopez 1999, Suhendro 1997, Bueso-Ucles 2003, Silva 2003). If the levels of maltogenic α-amylase are high and the CMC low, the tortilla crumbles. Optimized levels showed that even when the tortilla is softer it is not crumbly.

Optimization of Additives to FNM

Objective and subjective values were improved for FNM tortillas held 7 d at 4^{o}C. Rupture force (Figure 13) and modulus of deformation (Figure 14) of tortillas containing high levels of CMC decreased with higher levels of maltogenic α-amylase and lower levels of guar. Rollability (Figure 15) of tortillas with high levels of CMC was retained longer with higher levels of maltogenic α-amylase and lower levels of guar. Pliability (Figure 16) of tortillas without of guar was retained longer with higher levels of maltogenic α-amylase and lower levels of CMC. Modulus of deformation (Figure 17) decreased and rollability scores (Figure 18) of tortillas with high levels of maltogenic α-amylase were retained longer with higher levels of CMC and lower levels of guar. Modulus of deformation values (Figure 19) decreased and rollability scores (Figure 20) were retained longer in tortillas without guar gum and increasing levels of CMC and maltogenic α-amylases.

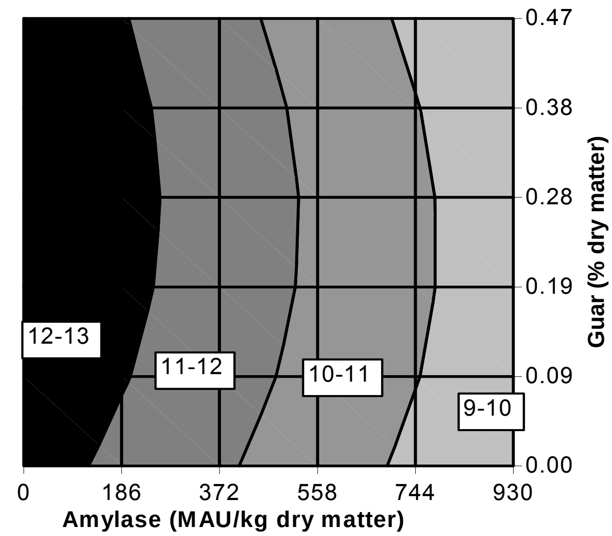

Figure 13 Rupture force (N) of FNM tortillas stored 7 days at 4°C, as affected by variable levels of guar and maltogenic α–amylase and the high level of CMC (0.47% dm). Note: least significant difference (LSD $_{0.05}$) = 1.12.

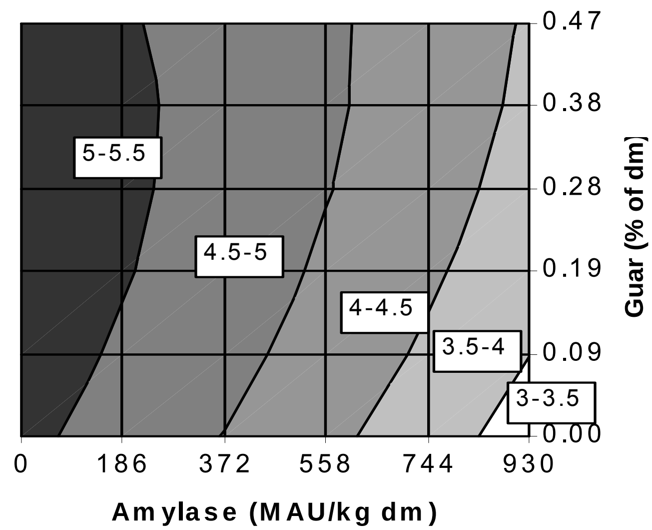

Figure 14 Modulus of deformation (N/mm) of FNM tortillas stored 7 days at 4°C, as affected by variable levels of guar and maltogenic α–amylase and the high level of CMC (0.47% dm). Note: least significant difference (LSD $_{0.05}$) = 0.36.

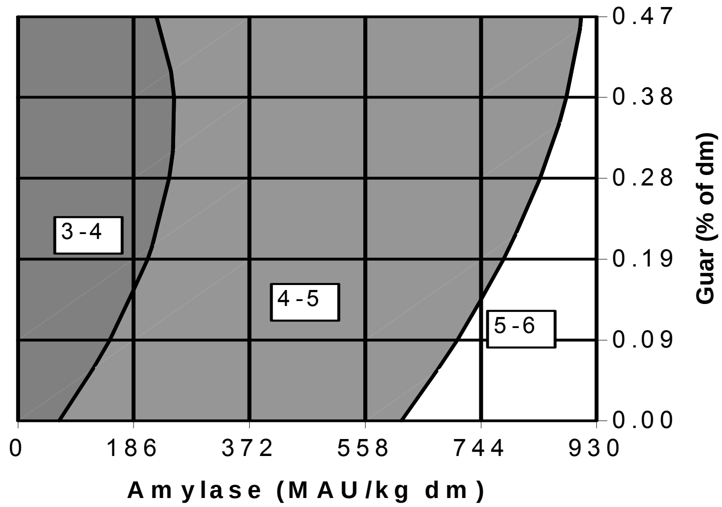

Figure 15 Rollability scores of FNM tortillas stored 7 days at 4°C, as affected by variable levels of guar and maltogenic α–amylase and the high level of CMC (0.47% dm). Note: least significant difference (LSD $_{0.05}$) = 0.84.

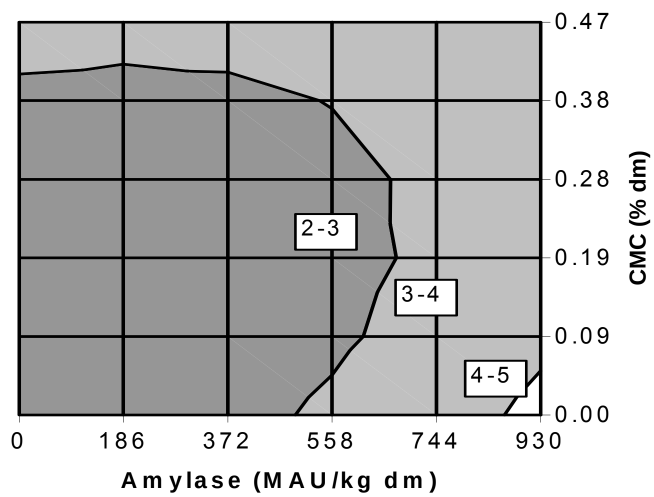

Figure 16 Pliability scores of FNM tortillas stored 7 days at 4°C, as affected by variable levels of CMC and maltogenic α–amylase and no guar. Note: least significant difference (LSD $_{0.05}$) = 0.98.

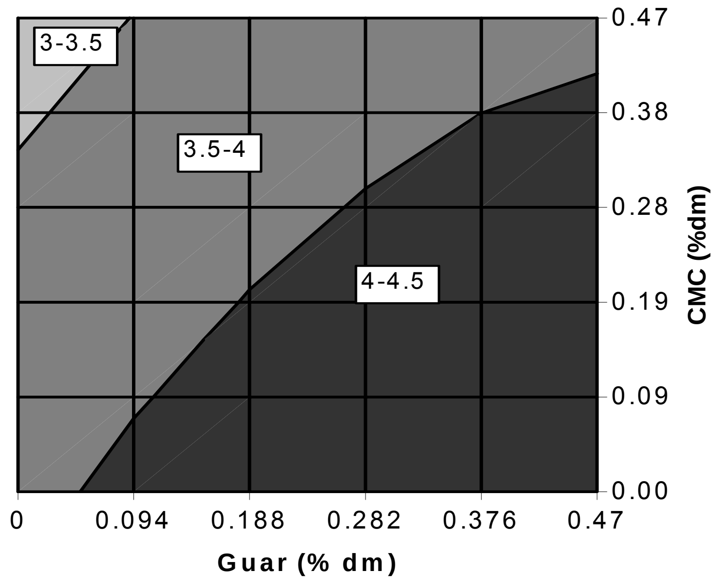

Figure 17 Modulus of deformation (N/mm) of FNM tortillas stored 7 days at 4°C, as affected by variable levels of guar and CMC and the high level of maltogenic α–amylase (930 MAU/kg dm). Note: least significant difference (LSD $_{0.05}$) = 0.36.

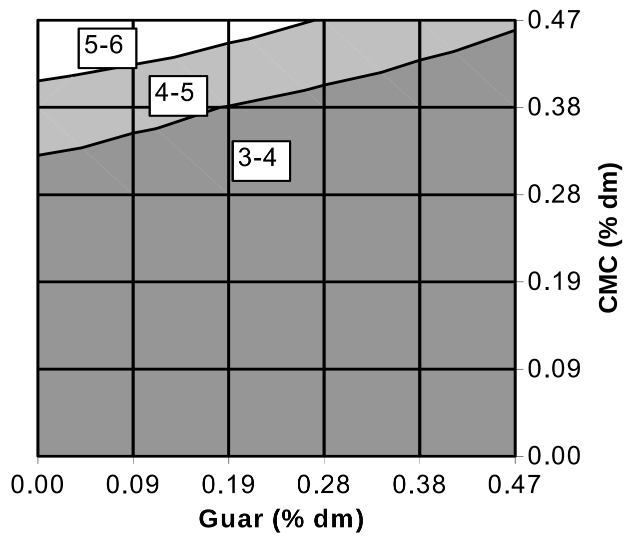

Figure 18 Rollability scores of FNM tortillas stored 7 days at 4°C, as affected by variable levels of guar and CMC and the high level of maltogenic α–amylase (930 MAU/kg dm). Note: least significant difference (LSD $_{0.05}$) = 0.84.

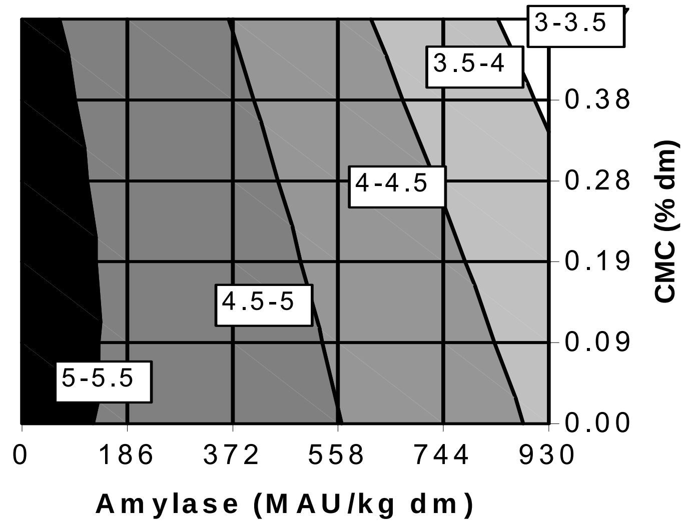

Figure 19 Modulus of deformation (N/mm) of FNM tortillas stored 7 days at 4°C, as affected by variable levels of maltogenic α–amylase and CMC and no guar. Note: least significant difference ($LSD_{0.05}$) = 0.37.

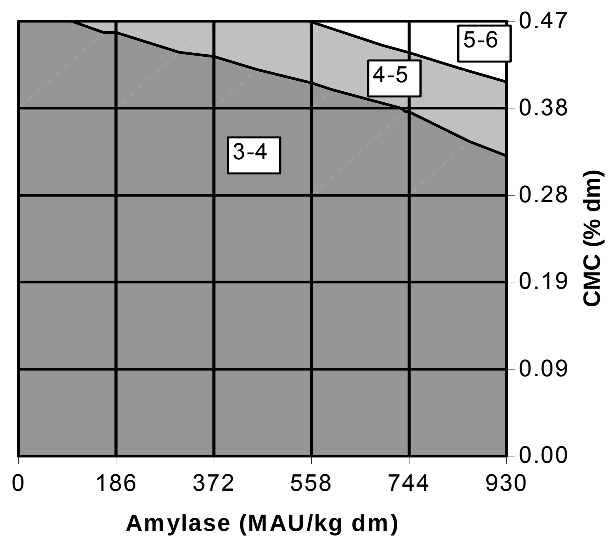

Figure 20 Rollability scores of FNM tortillas stored 7 days at 4°C, as affected by variable levels of CMC and maltogenic α–amylase and no guar. Note: least significant difference ($LSD_{0.05}$) =0.84.

Improvements of tortillas were observed by objective and subjective measurements with treatments with high levels of maltogenic α-amylase, high levels of CMC and low guar levels.

Level of Additives Selected for NCF and FNM Tortillas

The results from RSM trials are summarized in Table V. The levels of additives for NCF and FNM were selected, using the RSM results. To use a number different than zero, if results indicate low levels of additive, the middle between the medium and low points was used. In this point the RSM model was more robust. For the same reasons, if the results indicate high levels, a central value between the medium and high points was used.

Table V Texture measurements results by RSM at the optimum levels selected for NCF and FNM treatments[a].

Treatment	Supported by	Fixed additive
NCF + low amylase, high CMC, medium guar	Rollability score	High CMC
	Pliability score	High CMC
	Pliability	Low amylase
NCF + high amylase, low CMC, high guar	Rupture Force	Low CMC
	Rupture Force	High amylase
	Rupture Force	High guar
	Modulus of deformation	Low CMC
	Modulus of deformation	High amylase
	Modulus of deformation	High guar
FNM + high amylase, high CMC, low guar	Rupture Force	High CMC
	Modulus of deformation	High CMC
	Modulus of deformation	High amylase
	Modulus of deformation	Low guar
	Rollability score	High CMC
	Rollability score	High amylase
	Rollability score	Low guar
	Pliability score	Low CMC

[a]Tortillas were measured after storage for 7 days at 4^{o}C.

The levels of additives selected for further analysis were coded and formulated as:

- NCF ctrl: nixtamalized corn flour plus water and preservatives.
- NCF lo enz: same as NCF ctrl plus 60 mg/kg of maltogenic α-amylase, 0.43% CMC and 0.57% guar.
- NCF hi enz: same as NCF ctrl plus 170 mg/kg of maltogenic α-amylase, 0.14% CMC and 0.85% guar.
- FNM ctrl: fresh nixtamalized masa plus preservatives
- FNM + add: same as FNM ctrl plus 70 mg/kg of maltogenic α-amylase, 0.35% CMC and 0.12% guar.

Note: all values of additives are on a dry matter basis

Comparison of NCF and FNM

A summary of the treatments, including the formula proposed by Bueso-Ucles (2003) is presented in (Table VII). The levels of maltogenic α-amylase were the same for NCF hi enz treatment and Bueso 2003. The levels of maltogenic α-amylases for NCF lo enz and FNM + add treatments were similar. FNM + add was the treatment that needed lower levels of guar.

Table VI Optimum levels selected for improvement in texture of tortillas prepared from NCF and FNM for further analysis[a].

Additive per kg gm	FNM + add	NCF lo enz	NCF hi enz	Bueso 2003
Amylase (MAU/kg)	700	600	1,700	1,700
CMC (g/kg)	3.5	4.2	1.4	2.8
Guar (g/kg)	1.2	5.7	8.5	0.0

[a]Values from Bueso-Ucles (2003) are included as reference.

Similar levels of maltogenic α-amylase for FNM and NCF masas (FNM + add and NCF lo enz) coupled with different levels of CMC (3.5 and 4.2 g/kg, respectively) and guar (1.2 and 5.7 g/kg, respectively) indicate that the enzyme is able to hydrolyze the starch in the two products, but the action of the additives is different. The NCF hi enz treatment made tortillas soft as indicated by a decrease in the rupture force and modulus of deformation, but the rollability and pliability scores decreased to lower acceptable levels. The treatment was selected since the levels confirm those used by Bueso-Ucles (2003) for NCF tortillas.

The lowest modulus of deformation values obtained by RSM of for the optimum combination of additives was in the range of 3-3.5 N/mm for FNM tortillas and 2- 2.5 N/mm for NCF tortillas. This indicates faster starch retrogradation in the FNM tortillas. As discussed in Chapter IV, guar must form a weak gel before the starch retrogrades to delay staling of corn tortillas, and the time for gel formation is not available in FNM tortillas. The effect of guar resulted less important and could be due to a faster initial starch retrogradation of FNM tortillas under refrigeration than for FNM.

When comparing control tortillas (no additives) from FNM and NCF (Figure 21) after 1 and 4 d stored under refrigeration, the modulus of deformation, pliability and rollability scores were not significantly different. Tortillas from FNM had significantly lower rupture distance than NCF tortillas NCF after 1 and 4 d storage under.

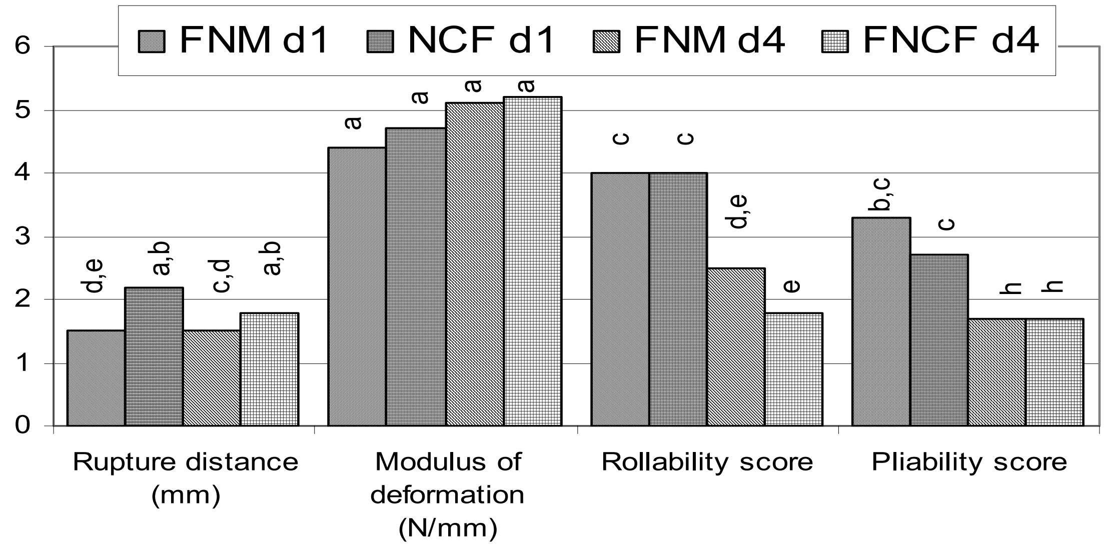

Figure 21 Texture values for FNM and NCF tortillas stored 1 and 4 d under refrigeration. See values in Appendix D, Table XXI and Table XXII.

A lower rupture distance in FNM leads while all other texture measurements result in similar values indicate faster starch retrogradation of FNM tortillas. Bueso-Ucles (2003) found that rupture distance and pliability scores were recommended as the best indicators for fast, empirical tortilla staling studies. Fernandez et al. (1999) suggested that “slow” intrapolymer associations of amylopectin double helices caused by a progressive spontaneous aggregation contribute to development of adverse textural properties in corn tortillas during storage. This process was initially faster for FNM tortillas.

CHAPTER VI

THE EFFECTS OF ADDITIVES IN THE PHYSICAL AND CHEMICAL PROPERTIES OF MASA AND TORTILLAS FROM FNM AND NCF

To understand the action of additives and their performances in various products (NCF and FNM), masas with optimized treatments were baked into tortillas and evaluated to determine differences in the physical and chemical properties. Measurements of texture, RVA properties and HPLC were conducted. Observations of changes due to additives and storage conditions of tortillas subject to different treatments were analyzed.

Physical Characterization

The NCF and FNM masas were similar in physical characteristics. The moisture of masa was successfully adjusted for the different products (Table VII). Particle size distributions of the FNM and NCF (Table XVII, 0) indicated that the smaller fractions were similar. FNM masa had more large size particles (425-850 :). The soluble fraction was larger for NCF (Figure 22).

Table VII Physical characteristics of nixtamalized corn flour and fresh nixtamal control masas[a].

	Moisture(%)		WAI (%)		WSI (%)	
Type of masa	**Avg**	**Std dev**	**Avg**	**Std dev**	**Avg**	**Std dev**
Nixtamalized corn flour	59.0	0.05	33.9	1.2	19.4	1.8
Fresh nixtamal	59.2	0.03	39.9	4.4	8.2	4.4

[a] WAI – water absorption index; WSI – water solubility index; WAI and WSI analyses were conducted on two replicates, 4 observations each. Moisture was conducted on 3 replicates, 2 repetitions each. Values in 0, Table XVII.

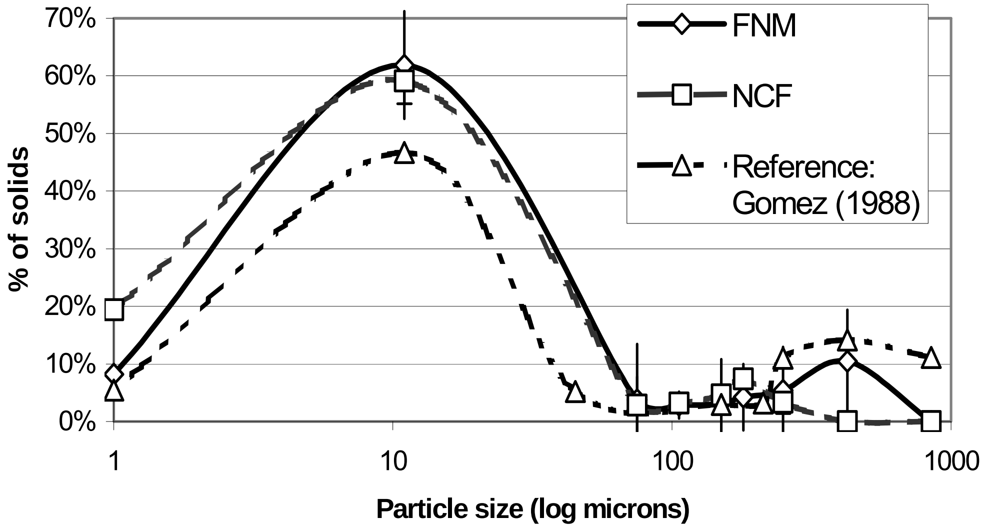

Figure 22 Particle size distribution for FNM and NCF masas. Note: values of minimum particle size + standard deviation from 2 replicates, 2 repetitions each; values and statistics in Table XVII, Appendix C.

Masa moisture content was similar for both products, indicating that the model developed was accurate in its prediction based on the nixtamal weight for the specific cooking conditions (see model in Chapter III). Equal moisture levels along with low proportions of large particles indicate that the masa was well ground and that the product was ideal for production of table tortillas.

Masas from NCF had a lower water absorption index (WAI) and a higher water solubility index (WSI) than FNM (Table VII). A higher WSI denotes higher levels of soluble material in the NCF masa and indicates that less soluble matter was removed during the alkaline cooking, steeping and washing of nixtamal. Corn in the NCF process is cooked less, steeped a shorter time and washed less. Gomez (1988) found that masas cooked longer had increased dissolved solids and modified starch. The pH of masa and tortillas out of the oven and the weight of 10 tortillas were similar for the treated and controls (0, Table XVII), which confirms the similarity of the products.

Texture during Storage

During aging of tortillas, their rollability, pliability scores and the rupture distance decreased and their rupture force and the modulus of deformation increased. The texture measurements had larger changes from the initial values (Table XX) to 1 d than from 1 d (Table XXI) to 4 d storage (Table XXII). All treatments had higher pliability scores than their controls at the corresponding storage time and temperature, except for the initial measurement where all scores were highest (Figure 23). At 4 d of storage, pliability scores of the treated tortillas were significantly higher than the control under equal storage conditions. Under ambient storage, the difference between treated and control tortillas were larger than under refrigerated storage. For NCF tortillas stored 4 d at 22°C, the treatment with lower enzyme level gave significantly higher pliability scores than the treatment with higher enzyme level. The pliability scores of tortillas stored 4 d under ambient and refrigeration temperatures were not significantly different for NCF tortillas with high enzyme levels and FNM tortillas with additives. Under ambient storage for 4 d, pliability and rollability were significantly higher for NCF tortillas with low enzyme treatment.

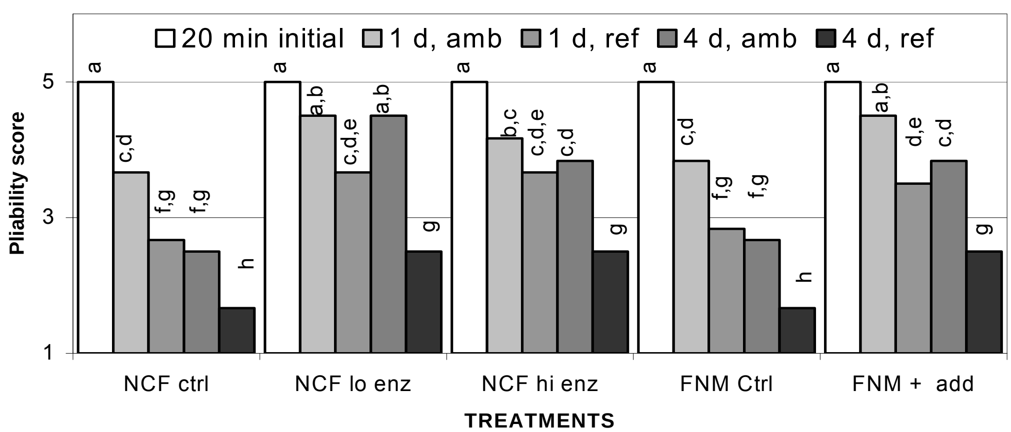

Figure 23 Pliability scores of corn tortillas stored for different times under ambient and refrigeration temperatures. Values are means of 2 replicates, 3 repetitions each. Note: LSD0.05=0.6; treatments with the same letter are not significantly different.

The modulus of deformation (Figure 24) had a large increase from the initial measurement at 20 min to the measurement at 1 d of storage at ambient temperatures. Refrigerated storage made tortillas firmer with a higher modulus of deformation than at ambient temperature storage. After 1 d, each treated tortilla had a lower modulus of deformation than their respective controls. NCF tortillas with higher enzyme levels had significantly lower modulus of deformation than the tortillas with lower enzyme levels. The values of modulus of deformation for tortillas stored 4 d under ambient and refrigeration temperatures were not significantly different for NCF tortillas with high enzyme levels and FNM tortillas with additives. These values were significantly higher for NCF tortillas with low enzyme treatment.

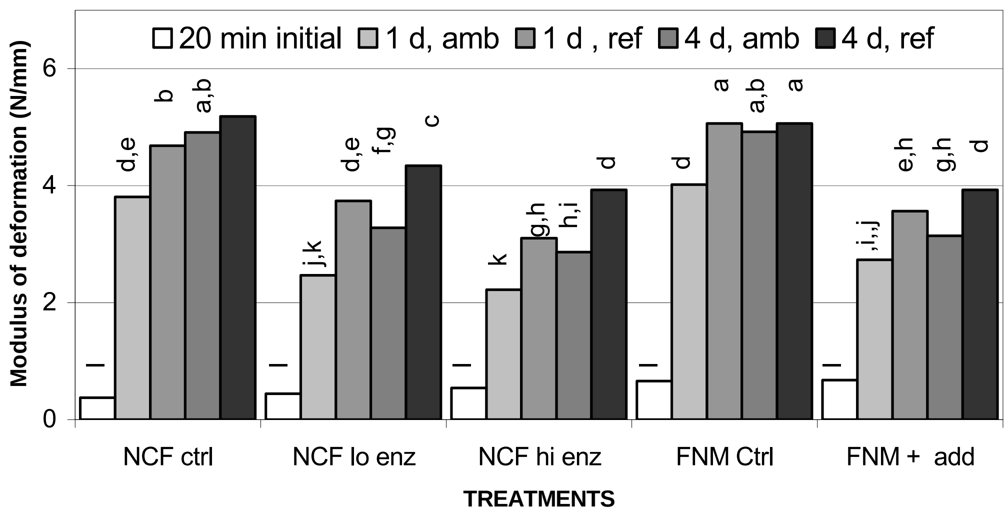

Figure 24 Modulus of deformation of corn tortillas stored for different times. Values are means of 2 replicates, 3 repetitions each. Note: LSD0.05=0.3; treatments with the same letter are not significantly different.

The delay in the reduction of the pliability scores and lower increase of modulus of deformation indicate that the treatments improved the texture of FNM and NCF tortillas. Under refrigeration, the additives were less effective in delaying staling. The modulus of deformation measures the force required to deform the sample (Steffe 1996). That is how much the material yields for each unit of force (N) applied and is related to the strength of the material. Larger values of modulus of deformation are usually associated with strong, brittle materials. Tortillas with lower modulus of deformation have a disrupted or less rigid structure that results in softer texture.

The highest values for pliability after 4 d of storage at 22°C were observed for the NCF lo enz treatment, while the lowest value of modulus of deformation was observed for the NCF hi enz treatment. This confirms that tortillas from NCF cannot be significantly improved in objective and subjective texture measurements with a single treatment. This must be considered when developing formulas for tortillas that include the ingredients used in this research.

FNM tortillas with additives showed significant improvements under ambient and refrigeration storage. The improvements in texture were greater for ambient storage than refrigeration. The level of staling in corn tortillas is a function of time and storage temperature. Bueso-Ucles (2003) indicated that maximum loss of flexibility of tortillas occurred when stored under refrigeration (3-10 °C). The loss of enzyme activity and decreased mobility of guar and CMC during refrigeration storage are influencing factors.

This analysis implies that the treatment of FNM tortillas resulted in texture similar to the NCF hi enz treatment. Further improvements could result if the cooking time and grinding conditions were optimized for the additives selected.

Loss of Solids during the Preparation of Stabilized Starch Residue

The weight of solids lost due to solubilization during the preparation of stabilized starch residue for RVA analysis was examined. The treated tortillas had significantly higher solids loss compared to their respective controls. Solids losses were significantly higher for the NCF control masa and tortillas than those for FNM control in masa and tortillas. Significantly fewer solids were extracted as the tortillas aged (Figure 25).

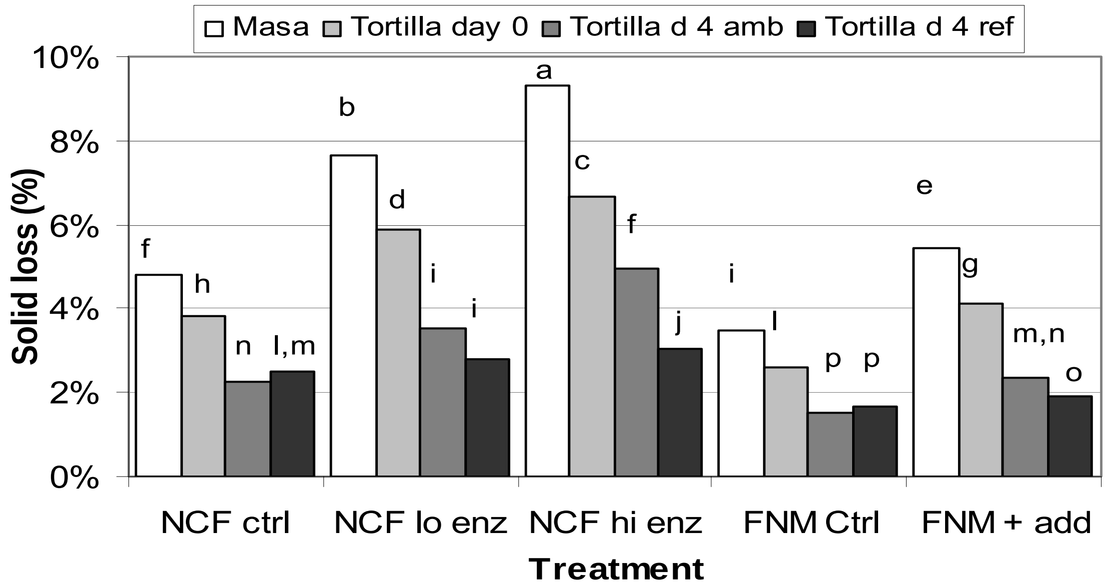

Figure 25 Loss of solids during preparation of stabilized starch residue (SSR) from tortillas with different treatments and storage conditions. Treatments with the same letter are not significantly different; values are means of 2 replicates with 4 repetitions each. Note: LSD0.05= 0.15%. Values in Table XXIV.

The differential loss of solids (ΔLS) was defined as the difference between solid loss of treated tortillas and their respective control. The ΔLS (Figure 26) of treated NCF tortillas was higher for NCF hi enz than for NCF lo enz masa and tortillas under both storage conditions. The ΔLS was significantly lower after 4 d for tortillas at refrigerated storage than at ambient storage. The ΔLS decreased with processing, that is from treated masa to initial tortillas. The ΔLS decreased with increasing storage time and at lower temperatures of storage for tortillas from FNM + add and NCF lo enz treatments.

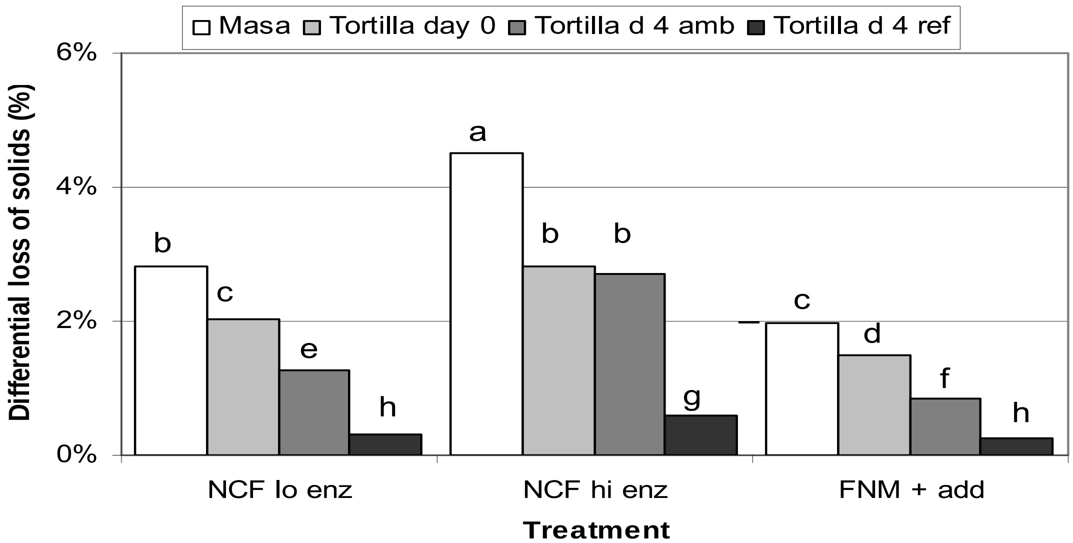

Figure 26 Differential loss of solids (ΔLS) during the preparation of stabilized starch residue (SSR) of tortillas with different treatments and storage conditions. Values are the difference between solid loss of treated tortillas and their respective control (Values in Table XXV). Note: LSD0.05= 0.2%; treatments with the same letter are not significantly different.

Moderate amounts of solids (< 10% dm) were soluble in methanol but not retained on the filter paper containing the SSR. The differences observed in the loss of solids for the different products (NCF higher than FNM) can be explained by the amount of soluble solids removed during corn processing into masas. Lower amounts of solubles are removed during NCF production. Corn is usually undercooked (decreased time and temperatures) which causes decreased water absorption and weakening of the endosperm structure, restricted swelling of the starch granules and limited amylose leaching during the initial heating step (Gomez et al.1991). The cooked corn is steeped for less time, which limits water distribution and reorganization of the molecular structure compared to what occurs when fresh masa is produced (Gomez et al. 1990). The nixtamal is washed to a lesser extent since it is cooked under lower lime concentration; and it is easier to decrease the calcium hydroxide levels and lower the pH.

The ΔLS was closely related to the retrogradation of the tortilla and reduced activity of enzymes. Retrogradation of starch limits its solubility. On aging, the percent of soluble fraction decreased due to retrogradation of amylose and amylopectin (Fernandez et al. 1999) and amylose-lipid complex formation (Miranda-Lopez 1999). Higher solubility of solids is an indication of starch solubility and thus a rapid test of starch retrogradation, and might be used to indicate the functionality of enzymes and of certain antistaling agents.

The lower ΔLS after 4 d storage for tortillas held under refrigeration compared to ambient conditions, demonstrates that the enzyme is still solubilizing carbohydrates during storage and the lower temperature reduces the enzyme activity. The ΔLS of tortillas before storage and after 4 days at ambient temperatures were not significantly different, indicating that the enzyme hydrolyzes starch, which helps delay the staling process.

Rapid Viscoanalyzer (RVA)

Pasting properties of masa and tortillas were evaluated using RVA analysis to provide information about the action of amylases and hydrocolloids at different concentrations on the insoluble fraction of tortillas. Differences were detected in the viscosity measurement and pasting behavior (Figure 27 and 0, Table XXIII) of SSR prepared from tortillas. Significantly lower peak viscosities and higher pasting temperatures were observed in SSR from NCF tortillas than their controls 20 min after production and after storage for 4 d at 22^{o}C. For the SSR of NCF tortillas stored at 4^{o}C, the viscosities reached levels similar to those of SSR prepared from the initial control tortillas. Higher enzyme levels significantly increased the pasting temperature of SSR of tortillas from NCF prepared 20 min after baking and tortillas stored 4d at 22^{o}C. When comparing tortillas from NCF stored 4 d at 22 and 4^{o}C, the increase in the pasting temperature was significantly higher for SSR of tortillas with NCF hi enz treatment than for the control or NCF lo enz.

Differences were detected in peak viscosities of SSR from FNM tortillas. SSR of FNM treated tortillas had significantly lower peak viscosities than their respective controls at 4 d storage at 22 and 4°C. Peak viscosities of SSR of initial and stored tortillas were not significantly different.

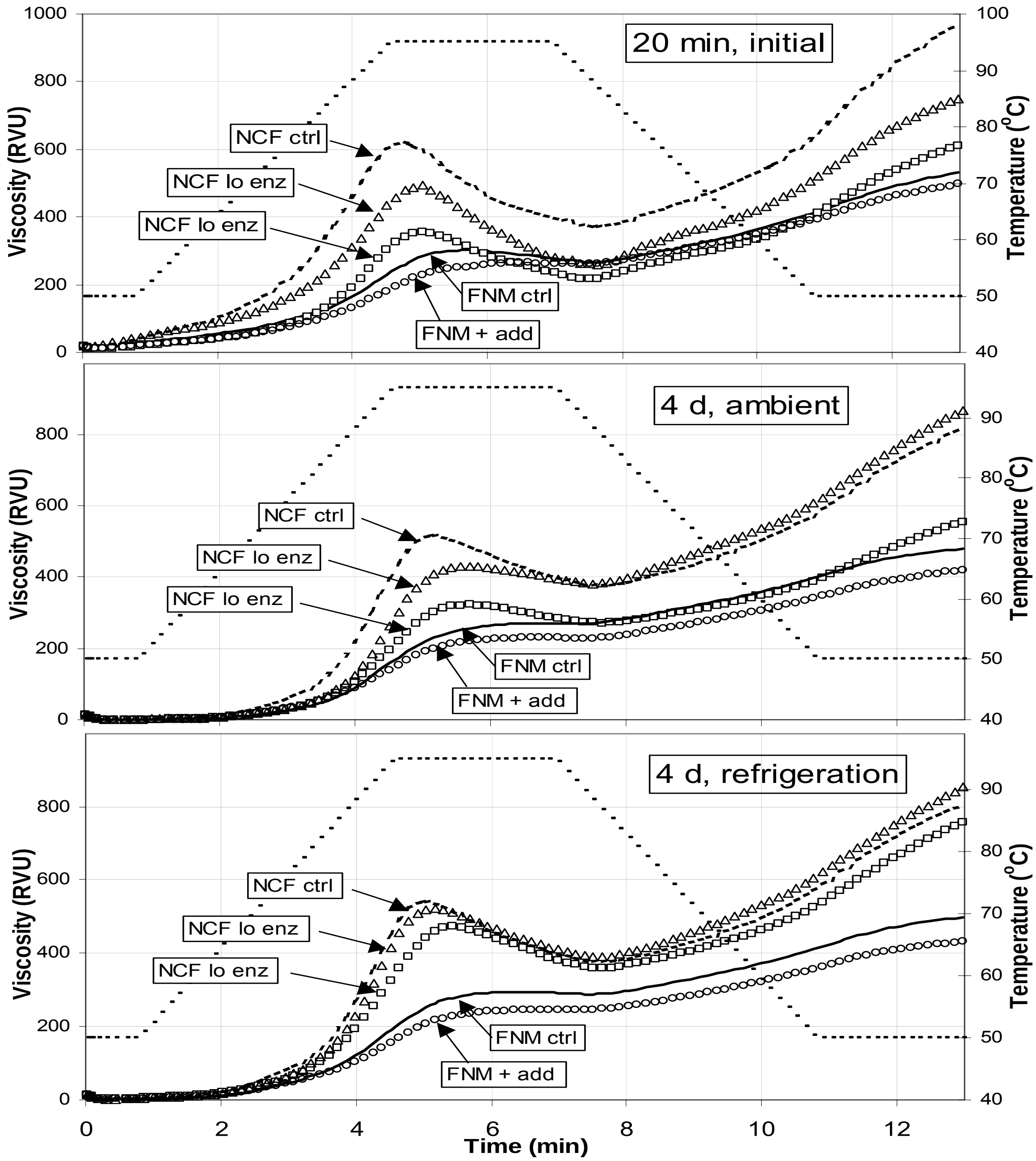

Figure 27 Pasting viscosities of stabilized starch residues (SSR)(15% solids) using a Rapid Viscoanalyzer prepared from NCF (nixtamalized corn flour) and FNM (fresh nixtamal masa) tortillas stored for different times and temperatures. Note: $LSD_{0.05}$ peak viscosity =42 RVU; $LSD_{0.05}$ pasting temperature= 3.3°C; temperatures in secondary y-axis.

Lower viscosities in treated NCF tortillas indicate a modification in the starch that affects its interaction with other polymers, like CMC and guar. Under refrigeration, accelerated staling and a decreased rate of enzyme hydrolysis occurred. This is indicated by reduced differences in viscosities. The viscosities of SSR prepared from NCF tortillas increased to the original values of the SSR from tortillas prepared 20 min after baking. Guar and CMC in tortillas stored under refrigeration were only partially bound to the starch chains, compared to starch binding in tortillas stored at ambient temperatures. Guar and CMC were solubilized by the increased temperature and applied shear in the RVA. Guar needs time (>3 days) and temperatures from 20 to 40^{o}C after it has been exposed to heat during baking to be adsorbed on the starch surface (Dea et al. 1977). If these conditions are not met, guar separates into "gel-islands" that are not soluble and causes increased viscosity. CMC has a similar reaction, but its binding to the tortilla structure occurs immediately after baking and develops lower viscosities (2500 cps in 1% solution) than guar (3500 cps in 1% solution). CMC properties and the fact that it is present at lower levels in the NCF hi enz treated tortilla, indicate that guar is responsible for most viscosity changes in the RVA viscosities of SSR of NCF tortillas after refrigerated storage.

The significant differences in peak viscosities are indications of differences in the insoluble fraction of tortillas caused by variations in processing of FNM and NCF. Differences in cooking time, steeping and drying of nixtamal significantly affects the pasting viscosities in a RVA (Almeida -Dominguez et al. 1996, Sahai et al. 1999, Sahai et al. 2001). Longer steeping time after cooking increases annealing of the starch granules, which reduces their swelling capacity (Tester et al. 2000) as observed in samples from FNM. Changes in masa pasting viscosity reflect differences in organization of starch molecules and degree of granule swelling. Physicochemical interactions of starch, non-starch polymers, and lime during alkaline cooking also affect the pasting properties (Robles et al. 1988).

The significant differences observed in viscosities from NCF and FNM are related to degree of granule swelling. Bueso-Ucles (2003) found that maltogenic α-amylase relied on preventing intra-granular reassociation to prevent corn tortilla staling and suggested that this action took place on the outer branches. Our results suggest that the enzyme hydrolysis, when the dispersion of the enzyme is allowed, also takes place in the inside of the granule, as indicated by differences in the swelling capacity. For NCF more internal granule hydrolysis took place as the flour was hydrated with the enzyme added, and the solution could flow trough the granules pores. For FNM the process was delayed due to the limited enzyme distribution, as the enzymes did not disperse into the hydrated annealed starch granules.

The results of RVA analysis of SSR at 15% solids concentration detected differences due to the effect of storage in SSR from NCF tortillas but not in FNM tortillas. The solids concentration of SSR slurry was increased from 15 to 20% (Figure 28) to enlarge differences in viscosities. The SSR from NCF lo enz treatment and control tortillas with 4 days ambient storage developed excessive viscosity that was above the detection limits of the RVA. However, the SSR from NCF hi enz with 4 d ambient storage developed high viscosities in a typical RVA curve. The RVA was able to generate a typical viscosity curve only for the SSR prepared from FNM + add treated tortillas, but not for the control. The peak viscosities for NCF hi enz and FNM + add were similar at 20% solids.

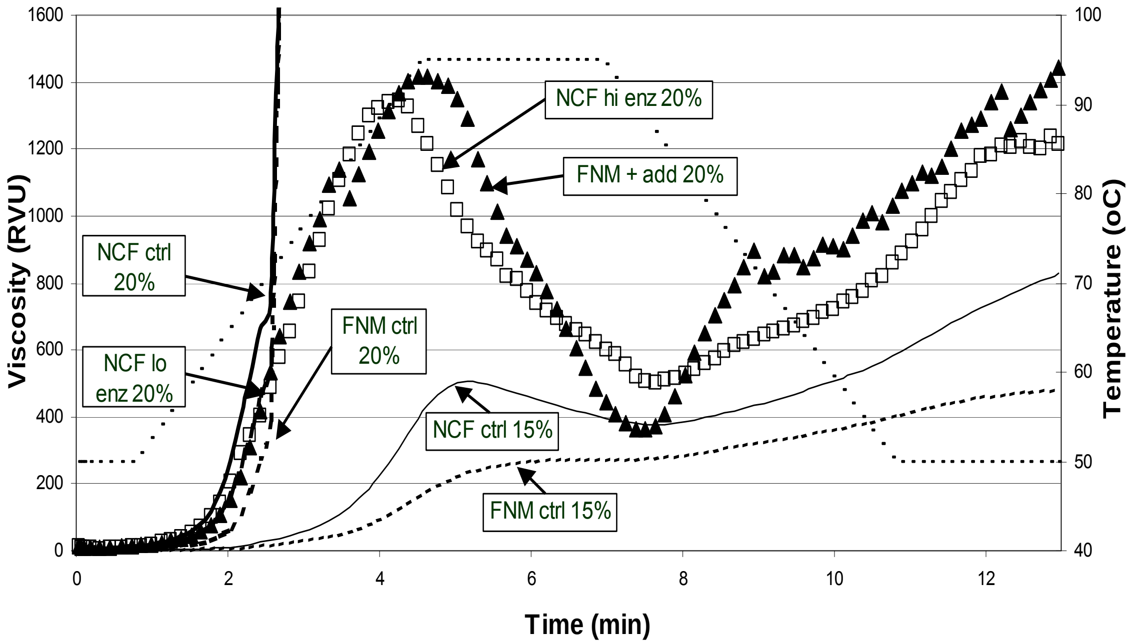

Figure 28 Pasting viscosities solutions of 20% solids of stabilized starch residues (SSR) prepared from NCF (nixtamalized corn flour) and FNM (fresh nixtamal masa) tortillas stored 4 d at ambient temperature using a Rapid Viscoanalyzer. Note: temperatures in secondary axis.

Increasing the solids gave significant differences in the RVA curves of SSR of control and treated tortillas from FNM and NCF. This indicates that the method of masa preparation and enzyme treatment significantly changed the viscosity properties of the SSR of the tortillas. The SSR from FNM tortillas treated with 60 mg /kg dm (FNM + add) and from NCF tortillas treated with 170 mg /kg dm (NCF hi enz) had reduced viscosity observed as a distinctive RVA curve of stabilized starch. This was not observed in the SSR from NCF tortillas treated with 70 mg /kg dm (NCF hi enz), indicating that lower levels of enzymes were needed for FNM to show differences.

Grinding the nixtamal in the preparation of fresh masa separates the starch granules by tearing the endosperm cell wall (Gomez et al. 1989). The protein matrix does not retain the granules as the nixtamalization process weakened it. For NCF, the nixtamal is coarsely ground and then dried. This results in granules that are clumped together. Further grinding with a hammer mill reduces the particle size but some granules remain attached to each other by the protein matrix. The free granules are easier to hydrolyze by the enzyme in the FNM masa and this is reflected in the lower levels of enzyme needed to improve the texture.

In NCF production drying to produce the flour accelerates starch retrogradation by forming crystalline areas that act as a nucleation point for further starch associations (Gomez et al. 1991) that limit enzyme hydrolysis. Large differences in the two products are also due to the structural conformation of starch, CMC and guar. The interaction between these 3 polymers affects viscosity development, while the enzyme treatment affected their interactions during storage at ambient temperatures.

HPLC

Carbohydrates were extracted by solubilization from masa and tortilla samples in 0.05% (w/w) $HgCl_2$ water solution heated in a water bath at 100^{o}C for 30 min, centrifuged and filtered as described in the Material and Methods chapter. The amounts of extracted carbohydrates from masas and tortillas were affected by type of product (NCF, FNM), stage of processing (masa, tortilla) and storage temperature (initial, 4 d ambient, 4 d refrigerated) (Figure 29). HPLC size exclusion chromatography provided information about the action of maltogenic α-amylase at different concentrations in corn tortillas. Values of extracted carbohydrates from masas and tortillas are presented 0, Table XXVI and Figure 30.

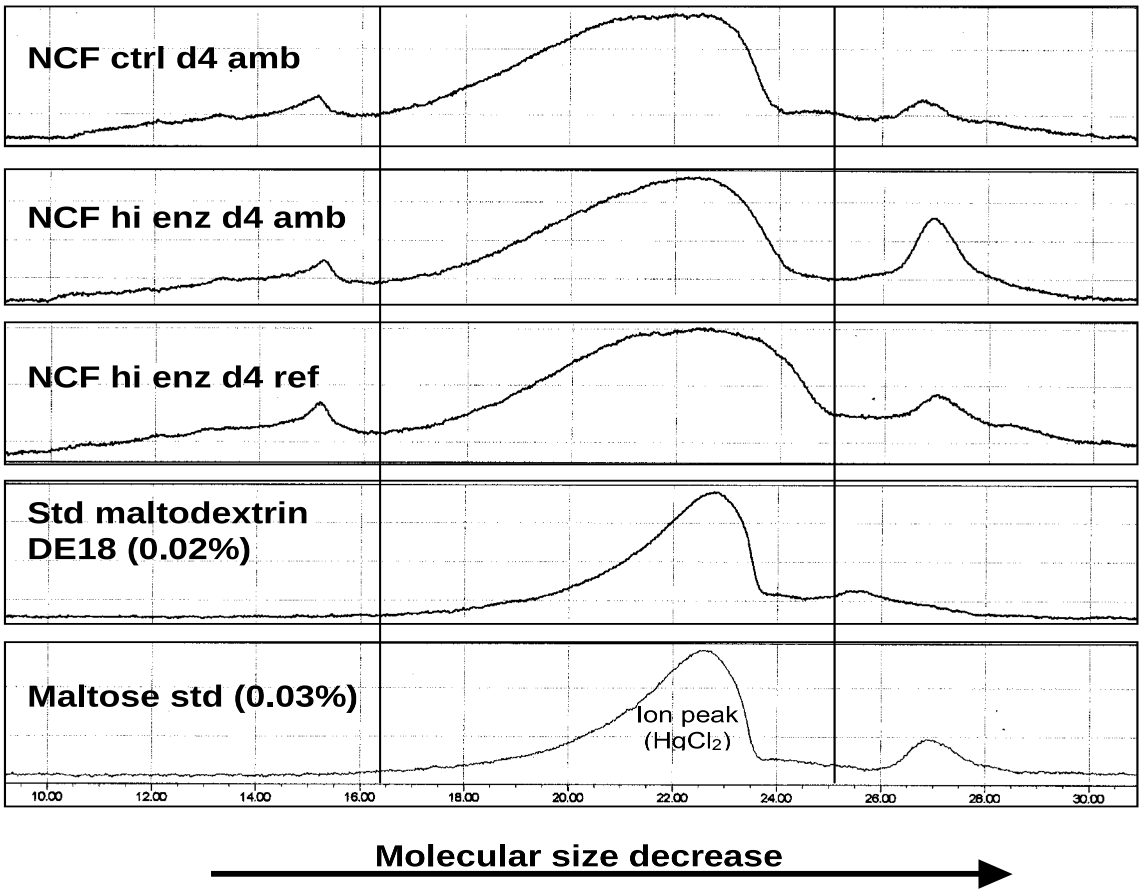

Figure 29 HPLC chromatograms of selected tortilla extractions and standard curves, showing the 3 regions defined as polymers (10-16 min), oligosaccharides (6-25 min) and low molecular weight sugars (25-30 min).

The low molecular weight (LMW) sugars had the largest changes after 4 days. Larger increases in LMW sugars occurred during storage at ambient temperature compared to refrigeration or control tortillas. The amount of polymers and oligosaccharides extracted did not show distinct patterns due to maltogenic α-amylase treatment or storage conditions. Changes in extraction are calculated from the values of extracted carbohydrates of treated masa or tortilla minus the values of their respective controls.

To analyze the storage effect of tortillas on the extraction of carbohydrates, differences in extraction (0, Table XXVII) from initial tortillas and those stored at 22 and 4^{o}C were compared. No significant increase was detected from initial treated tortillas from NCF and FNM to those stored 4 d at 4^{o}C. Larger increases in the extraction of carbohydrates from NCF than from FNM treated tortillas were detected between those stored at 22 and 4^{o}C

The increase of LMW sugars due to processing in the treated FNM and NCF tortillas indicates early hydrolysis of the starch polymers. Levels of sugars that increased when masas were treated with maltogenic α-amylase indicate that some starch polymers and granules were readily available for enzyme modification. Gomez (1988) found low levels of LMW sugars in the control masa from FNM but not from depolymerization to form dextrins during nixtamalization or grinding.

When maltogenic α-amylase is used in bread, the enzyme is inactivated during baking (Hebeda et al. 1990) thus it was defined as an intermediate temperature stability (ITS) enzyme. The results indicate that the enzyme is not inactivated during corn tortilla baking, suggesting that the enzyme inactivation process is time and temperature dependent. The short time, high temperature process of tortilla baking is less severe than the longer time, high temperature of bread baking. This agrees with Seetharaman et al. (2002) who compared the process of wheat tortillas with buns. The less severe processing conditions of flour tortillas caused less dispersion of amylose and amylopectin, reduced retrogradation of amylose, but greater retrogradation of amylopectin. The enzyme hydrolysis continued during storage and was affected by storage temperature indicated by greater extraction of carbohydrates at higher temperatures.

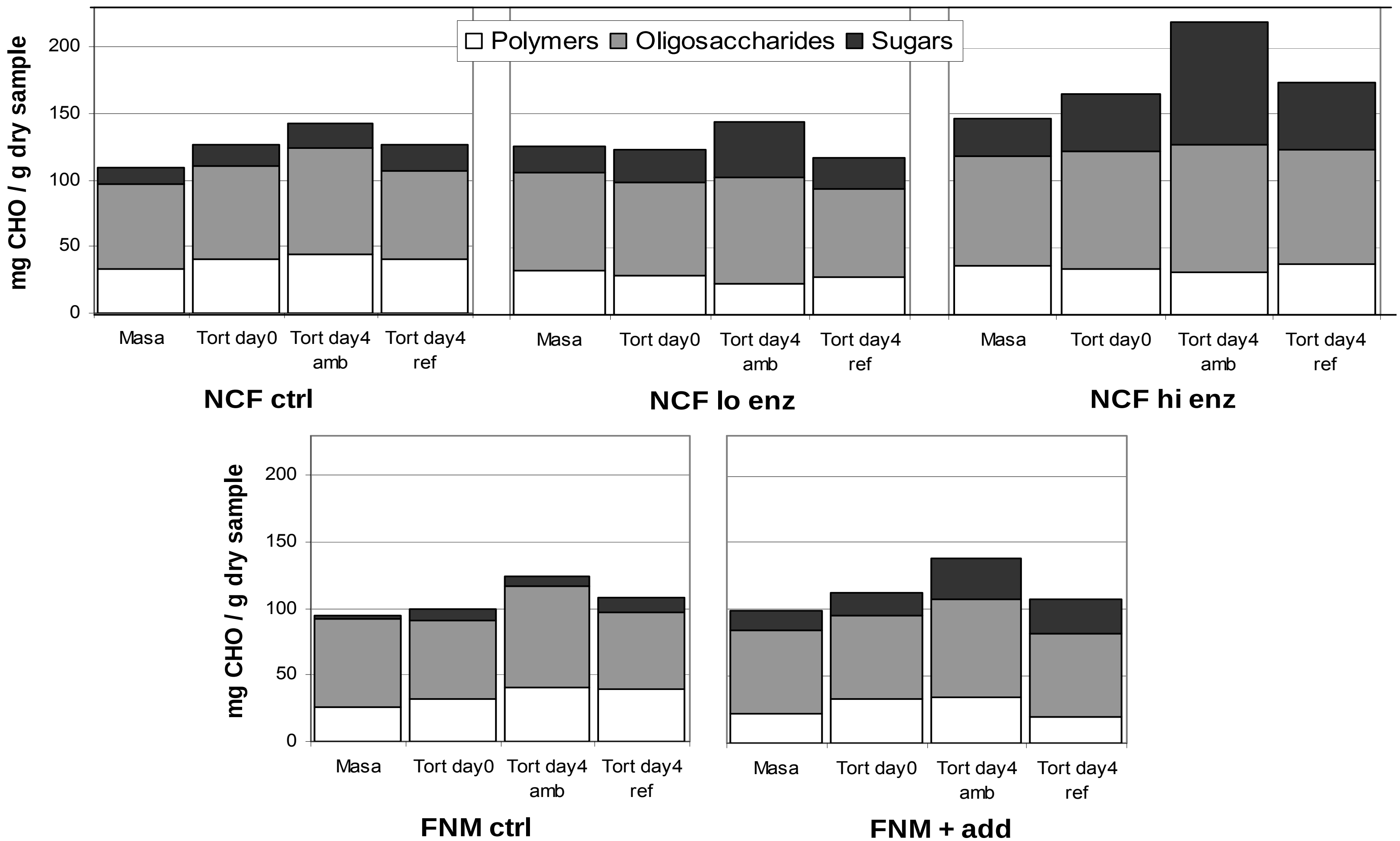

Figure 30 HPLC carbohydrate profiles of extracts from tortillas containing enzymes and hydrocolloids treatment at different stages of processing and storage. Note: masa and tortilla starch fractions were solubilized for extraction in 0.05% (w/w) $HgCl_2$ water solution heated in a water bath at 100^{o}C for 30 min.

Greater extraction of carbohydrates from NCF tortillas than from FNM tortillas indicates that the enzyme is easier to disperse inside the NCF starch granules. The enzyme was added to NCF while hydrating the flour and for FNM after the starch was hydrated. This restricts hydrolysis in FNM as the enzyme distribution depends more in dispersion in the liquid phase than on the flow of the solution into the starch granules during hydration.

Correlations

Selected measurements from the characterization of the tortillas treated with enzymes and hydrocolloids were correlated to each other (0, Table XXIX and Table XXX). Data were grouped according to specific storage conditions (4 d, 22^{o}C and 4d, 4^{o}C) and analyzed. At 4 d, ambient storage, significant correlations were found between increased maltogenic α-amylase level in tortillas and pliability scores (amylase: $r = 0.52$, CMC $r = 0.76$, guar $r = 0.59$; $N= 30$, $p<0.01$), but not under refrigerated storage. Moduli of deformation were not significantly correlated to pliability scores of tortillas under ambient storage.

Non-significant correlations were observed for pliability scores with other measurements stored under refrigeration. Lower pliability scores of tortillas at lower temperatures were caused by the reduced action of maltogenic α-amylase and guar under refrigeration. Rapid changes during refrigerated storage makes it difficult to distinguish the accelerated retrogradation by subjective texture measurements of tortillas due to staling, indicating that refrigeration cannot be considered an accelerated staling test. Other changes occur in the tortilla components, like moisture migration, protein and lipid structural changes and polymer aggregation. Pliability scores loose sensitivity when approaching lower values thus become inadequate for refrigeration and/or extended storage tests.

A separate analysis for the correlations of NCF tortillas at 4 d storage at 22^{o}C (Table VIII) and at 4^{o}C (Table IX) was performed and correlations were observed. Significant correlations were found between pasting temperature values from the RVA analysis of SSR from NCF tortillas stored 4 d and pliability scores (r = 0.75, N= 12, p<0.01) at 22^{o}C and modulus of deformation at 22^{o}C and 4^{o}C (r=-0.98 and r=-0.84 respectively, N=12, p<0.01). The amount of solids lost during the preparation of SSR from NCF tortillas was significantly correlated with values of modulus of deformation for tortillas stored for 4 d at ambient and refrigeration (r=-0.94 and r=-0.96 respectively, N=12, p<0.01).

RVA parameters were significantly correlated with most of the other parameters, especially with texture measurements. When all tortillas were included in the analysis, no significant correlation was found with RVA parameters indicating that the settings for this test are ideal to detect staling in tortillas from NCF but not from FNM.

Significant correlations between the amount of solids lost during the preparation of SSR from NCF tortillas and modulus of deformation values indicate that the two measurements are good indicators of corn tortilla staling and could be used to assess the action of antistaling additives. Moduli of deformation values are obtained faster, but a Texture Analyzer is needed. Solids lost during the preparation of SSR for RVA could be included as part of the analysis in additive assessment for corn tortillas. The selection of the most appropriate analysis will depend on the available testing equipment.

Our tests were not able to find consistent significant correlations between peak viscosities, pasting temperature and tortilla staling as those found by Sahai et al. (2001) and Bueso-Ucles (2003). The addition of small amount of gums can markedly increase the pasting viscosities, and pasting viscosities can be affected by interactions with sugars. Sahai et al. (2001) concluded that masa dough textural and RVA characteristics may be influenced by the status of starch polymer structures formed during nixtamalization. These results suggest that the variability caused by different additives has to be considered when selecting an appropriate RVA profile.

Table VIII Pearson correlation coefficients (r_{xy}) of pasting, tortilla texture and soluble sugars of NCF tortillas containing known levels of maltogenic α-amylase, CMC and guar after 4 d of storage at 22°C.

Tortillas from NCF 4 day ambient storage		Enzyme level	CMC level	Guar level	Peak Viscosity (RVU)	Pasting temp (oC)	Solid loss %	Mod. Def. (N/ mm)	Pliability score	Sugars extracted
Enzyme level (MAU/kg dmb)	r	**1**								
	N	45								
CMC level (g/kg dmb)	r	0.17	**1**							
	N	45	45							
Guar level (g/kg dmb)	r	0.94	0.50	**1**						
	N	45	45	45						
Peak Viscosity (RVU)	r	-0.98*	-0.152	-0.91*	**1**					
	N	12	12	12	12					
Pasting temp (oC)	r	0.86*	0.60	0.97*	-0.85	**1**				
	N	12	12	12	12	12				
Solid loss %	r	0.99*	0.30	0.98*	-0.97*	0.91*	**1**			
	N	18	18	18	12	12	18			
Mod. Def. (N/ mm)	r	0.82*	-0.57*	-0.92*	0.89*	-0.98*	-0.94*	**1**		
	N	45	45	45	12	12	18	45		
Pliability score	r	0.45	0.81*	0.69*	-0.481	0.75*	0.549	-0.69	**1**	
	N	18	18	18	12	12	18	18	18	
Sugars extracted	r	0.94*	0.09	0.86*	-0.91*	0.77*	0.93*	-0.82*	0.42	**1**
	N	12	12	12	12	12	12	12	12	12

* Correlation is significant at the 0.01 level (2-tailed).

Table IX Pearson correlation coefficients (r_{xy}) of pasting, tortilla texture and soluble sugars of NCF tortillas containing known levels of maltogenic α-amylase, CMC and guar after 4 d of storage at 4°C.

Tortillas from NCF 4 day refrigerated storage		Enzyme level	CMC level	Guar level	Peak Viscosity (RVU)	Pasting temp (oC)	Solid loss %	Mod. Def. (N/ mm)	Pliability score	Sugars extracted
Enzyme level (MAU/kg dmb)	r	**1**								
	N	45								
CMC level (g/kg dmb)	r	0.17	**1**							
	N	45	45							
Guar level (g/kg dmb)	r	0.94	0.50	**1**						
	N	45	45	45						
Peak Viscosity (RVU)	r	-0.92*	-0.059	-0.83*	**1**					
	N	12	12	12	12					
Pasting temp (oC)	r	0.79*	0.30	0.80*	-0.759	**1**				
	N	12	12	12	12	12				
Solid loss %	r	0.98*	0.33	0.98*	-0.87*	0.79*	**1**			
	N	18	18	18	12	12	18			
Mod. Def. (N/ mm)	r	-0.80*	-0.43*	-0.859	-0.81*	-0.84*	-0.96*	**1**		
	N	45	45	45	12	12	18	45		
Pliability score	r	0.35	0.35	0.43	-0.30	0.24	0.40	-0.25	**1**	
	N	18	18	18	12	12	18	18	18	
Sugars extracted	r	0.80*	-0.068	0.68	-0.81*	0.64	0.75*	-0.75*	-0.08	**1**
	N	12	12	12	12	12	12	12	12	12

* Correlation is significant at the 0.01 level (2-tailed).

Observations and Summary

Dry Masa

Treatments of maltogenic α-amylase, CMC and guar delayed staling of table tortillas from nixtamalized corn flour and fresh nixtamalized masa. NCF tortillas were improved by the treatment with higher enzyme level (NCF hi enz: 170 mg/kg of maltogenic α-amylase, 0.14% CMC and 0.85% guar) as measured objectively and by the treatment with lower enzyme level (NCF lo enz: 60 mg/kg of maltogenic α-amylase, 0.43% CMC and 0.57% guar) as measured subjectively. Subjective texture measurements are more reliable as they are analogous to the use of tortillas by the consumer, thus the lower enzyme treatment was better. Lower levels of enzymes than those suggested in previous research were needed when guar and CMC were included at optimized levels.

Fresh Masa

FNM tortillas from treated masa (FNM + 70 mg/kg of maltogenic α-amylase, 0.35% CMC and 0.12% guar) showed significant improvements under ambient conditions; the levels of enzymes were similar to those used for NCF lo enz treatment and lower levels of guar. The starch granules before pasting were less susceptible to enzyme hydrolysis than those from NCF masa due to restricted enzyme dispersion. Faster retrogradation in tortillas from FNM impedes guar from being adsorbed by starch polymers.

Mechanisms of Action

Separate studies helped clarify the differences between products and their interaction with additives. The insoluble fraction was analyzed with RVA techniques, and the soluble fraction was quantified by loss of solids and its composition revealed by HPLC. These studies confirmed the extended starch hydrolysis by maltogenic α-amylase during storage of tortillas that were not completely inactivated by heat treatment during baking.

From the trials in this chapter, we assume that the function of maltogenic α-amylase is to soften the tortilla structure by trimming the starch polymers, which allows guar to interfere with amylopectin re-crystallization inside gelatinized starch granules. CMC creates a more flexible inter-granular matrix than re-crystallized amylose and helps maintain the disrupted tortilla structure. The time frame is important in the use of these 3 additives: the hydrolysis caused by maltogenic α-amylase needs to occur early in the process to provide enough time for guar to be adsorbed on the starch surface, and the CMC network has to be developed in the oven to reinforce the structure lost by the enzyme hydrolysis. When a higher temperature of storage delayed starch retrogradation and starch hydrolysis was not interrupted, guar helped tortillas to retain a fresh texture longer by interfering with starch polymers association. At low storage temperature the texture improvement was limited.

Enzyme hydrolysis occurred on leached starch polymers and in pasted starch granules in masas. For NCF this happened when the flour was hydrated and the enzyme was added, as the polymers were readily available for hydrolysis and the enzyme was free to flow to the granules interior. For FNM the process was delayed due to the limited enzyme distribution. Apparently the soluble starch in FNM retained the enzymes (due to available substrate and increased viscosity) and the enzymes did not disperse into the hydrated annealed starch granules as during hydration of the NCF dry particles.

Although CMC imparts a chewy texture, it could not be completely eliminated, as under refrigeration, a reduced activity of maltogenic α-amylase and lack of interaction of guar with starch polymers suggest that CMC is the additive responsible for extension in pliability retention under these conditions. Hugh-Iten et al. (2003) found that maltogenic α-amylase combines a good balance between weakening and strengthening of the supramolecular starch structure in bread. Observations in that research indicated that the strengthening of the structure is accomplished by promotion of amylose ordering, but in corn tortillas we had to rely on CMC to obtain the desired strength to prevent tortillas from crumbling.

CHAPTER VII

SUMMARY

Effects of Enzymes and Hydrocolloids on Texture

Treatment of masas from nixtamalized corn flour and fresh nixtamalized masa with maltogenic α-amylase and hydrocolloids improved the texture of tortillas. Relevant information includes:

a) Lower levels of enzymes (60 mg/kg dm) than those suggested in previous research (170 mg/kg dm) were needed when guar and CMC were included at optimized levels. The cost of additional ingredients would have to be taken into consideration to verify possible savings.

b) Maltogenic α-amylase continues working during ambient storage of the baked corn tortilla and helps to extend the softening effect.

c) Guar was ineffective in delaying staling under refrigeration. The rapid starch retrogradation at lower temperatures impedes the functionality of guar to form chain-to-chain interactions with starch polymers. A less substituted galactomannan molecule that shows greater tendency to associate with starch polymers is recommended for tortillas stored at low temperatures as it may be adsorbed faster onto starch and may form a weak gel at low temperatures.

d) Mixtures of dextrins, CMC and carrageenan proved to be effective under refrigeration and may be an alternate additive to consider. It is important to design a system for tortillas stored under refrigeration.

e) Although CMC imparts a chewy texture, it could not be completely eliminated. Under refrigeration, the reduced activity of maltogenic α-amylase and lack of interaction of guar with starch polymers suggests that CMC is the additive responsible for the retention of pliability under refrigeration. The potential of combinations of dextrins and gums with enzymes might be important.

f) Some additives had little or no improvements in texture, under the settings used. Bacterial α-amylase seems to have a different pattern of hydrolysis that is not compatible with corn tortillas. Barley α and β-amylase had low temperature stability that makes them unfit for corn tortillas. Guar gum with sodium alginate had limited improvements

Differences and Similarities between FNM and NCF

Dry masa flour and fresh nixtamal have significantly different characteristics that affect how the enzymes and additives interact in each of the systems. The intrinsic starch polymer characteristics, polymer organization and the physicochemical interactions of starch and non-starch polymers probably contributed to differences in additive functionality. The most relevant differences and similarities observed were:

a) Enzyme hydrolysis started at different time in FNM and NCF. For NCF it started when the flour was hydrated and the enzyme was added, as the enzyme is more evenly distributed inside the starch granules. For FNM the process was delayed due to already hydrated annealed starch granules, which limited enzymes migration into the granules.

b) FNM and NCF masas were treated with similar levels of maltogenic α-amylase (60 – 70 mg/kg dm) to obtain similar improvements in texture. Theses levels of enzyme did not decrease the machinability of masa. The use of enzymes in fresh nixtamal masa is not a problem.

c) The effect of guar was less important for FNM tortillas and could be due to a faster initial starch retrogradation, especially under refrigerated storage.

d) Large differences were found by RVA analysis in slurries of 20% of stabilized starch of treated tortillas from FNM and NCF. The changes were due to enzyme hydrolysis of the interior of starch granules that altered their swelling capacity. The changes were much greater for dry masa flour residues from tortillas than for the fresh masa tortilla starch residue. This clearly indicates that the enzymes work more efficiently on dry masa tortillas.

General Observations

Valuable knowledge on measuring texture improvement of tortillas was gained during this study that may be valuable for further research and for industrial applications:

a) Tortilla surface appearance and smoothness: Observations of the tortillas immediately after baking showed definite textural changes in those that were enzyme treated. The tortillas treated with maltogenic α-amylase had a smooth appearance compared with the untreated tortillas. To observe these changes, extreme care must be taken in the baking procedures. The parts of equipment prior to the oven that are in contact with the masa were completely disassembled and washed between treatments to prevent enzyme cross-contamination and to remove any leftover masa that may be dried and cause a rough surface in the new masa disks. The cutting wires of the masa sheeter must be in optimum condition, clean and tense. These precautions gave a valuable subjective texture test based on the smoothness of the tortillas that were enzyme treated that proved helpful in this research.

b) Tortilla package softness: The additives used in this research produced tortillas that were softer during storage. The difference between untreated and treated tortillas, after they were reheated was minimal and only perceptible to a few trained frequent consumers. The advantage of a soft tortilla relates to the consumer's rapid test made at the point of sale where buying decisions are made. The package is touched and rated by how the tortillas stick to each other, how the stack bends without breaking and how much moisture has evaporated and condensed in the bag. The additives I used were able to address these issues, and have a definite impact on the consumer's perception of a "good tortilla". This situation is similar to a loaf of bread in that the consumer tests freshness by squeezing the loaf to see how soft it is.

c) Accelerated staling: Rapid changes occurred during refrigerated storage besides accelerated retrogradation that affects the softness perception. Additive functionality for softness improvement has to be selected considering that consumers usually use refrigerated storage. In this case, lack of functionality of an additive could be an expense without benefits. I found that guar was ineffective for refrigerated tortillas.

d) Texture measurements: Pliability measurements lose sensitivity when approaching lower scores (advanced staling) and become inadequate to evaluate texture of tortilla stored under refrigeration and/or extended storage tests. In this case, subjective rollability becomes the measurement of choice, but it also loses sensitivity at lower scores. Under these conditions, modulus of deformation becomes a more reliable measurement. For a typical staling evaluation under refrigeration it is recommended to rely on pliability from day 1 to 4, on rollability from day 5 to 7 and from 8 days and over on modulus of deformation.

e) RVA analysis: RVA was able to detect changes that were related to enzyme hydrolysis of the starch granule than to staling. At 20% solids, indications of significant changes in the swelling capacity of starch granules were observed and were related to enzyme modification. This analysis provides useful information but it is complicated to interpret the results when different variables are measured at the same time.

f) Masa from overcooked nixtamal: To use the additive levels proposed in this manuscript, masa from overcooked nixtamal (see 0) should contain at least 50% of masa from nixtamalized corn flour. Higher proportions of overcooked masa causes stickiness by excessive starch hydrolysis that would make it difficult to process into tortillas.

Further Research

Other additives may have to be included in the formula to overcome issues such as rapid staling of tortillas under refrigerated storage, as amylases and guar had limited action under these conditions. NCF tortillas with a combination of additives that included maltodextrins retained their freshness longer at lower temperatures of storage. This and other additives that might work under typical cold storage conditions need to be evaluated.

A closer look at the function of structure in tortillas (X-ray diffraction, dynamic rheometry, etc) made with different masas (FNM and NCF) to further elucidate their differences would be valuable.

Proteases and xylanases could be used to improve the texture and to further determine the differences between FNM and NCF masas. Other type of amylases with intermediate or high temperature stability should be tested.

There is a need to understand the hydrolysis of enzymes before, during and after baking to elucidate when and how long they need to work in corn tortillas. Solubility of solids during the preparation of SSR could be used as a quick test to understand the hydrolysis process.

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APPENDICES

APPENDIX A

ADDITIVES SELECTION

Table X Modulus of deformation and pliability scores of tortillas from FNM treated with different amylases and their controls after storage for 7 d at 4°C.

	mg enz/ kg masa dmb	Modulus of deformation (N/mm)		Pliability score		Machin-ability	# of tortillas measured
		Control	Treated	Control	Treated		
CONTROL **NCF+ 1500 MAU amylase**	170.0	4.40	3.38	2.0	1.00	4.50	10
FNM + Bacterial amylase	6.2	6.71	5.74	3.3	2.33	4.50	20
FNM + Bacterial amylase	12.5	5.51	4.27	3.0	1.82	3.00	20
FNM + Maltogenic amylase	24.9	4.95	4.64	3.0	2.67	4.50	10
FNM + Maltogenic amylase	47.4	6.62	5.65	3.3	2.34	4.50	20
FNM + Maltogenic amylase	74.8	4.95	4.65	3.0	2.33	4.00	20
FNM + Maltogenic amylase	187.0	5.90	5.00	3.0	3.00	3.00	10
FNM + Barley amylase	24.9	4.60	4.58	2.8	3.17	5.00	10
FNM + Barley amylase	37.4	6.06	5.36	3.0	2.33	5.00	10
FNM + Barley amylase	62.3	7.35	6.67	3.7	3.00	5.00	10
FNM + Barley amylase	74.8	4.95	5.19	3.0	3.33	5.00	5
FNM + Barley amylase	124.6	5.16	4.73	2.8	2.83	4.00	5

Table XI Objective and subjective texture values of NCF tortillas treated with hydrocolloids of variable types and levels out of the oven[a].

	Rupture force (N)		Rupture distance (mm)		Mod. of def. (N/mm)		Rollability Score		Pliability score	
	Average	std dev	Average	std dev	Average	std dev	Average	std dev	Average	std dev
Treatment	2.9[b]	0.1	4.7[d]	0.5	0.7[a]	0.0	5.0[a]	0.0	5.0[a]	0.0
Control	2.6[c]	0.3	10.8[a]	3.0	0.4[d]	0.0	5.0[a]	0.0	5.0[a]	0.0
Ctrl improved	2.7[b,c]	0.3	9.0[b]	1.7	0.4[c]	0.0	5.0[a]	0.0	5.0[a]	0.0
GG 0.4%	3.1[a]	0.3	5.0[d]	0.5	0.7[a]	0.1	5.0[a]	0.0	5.0[a]	0.0
GG 0.8%	2.9[a,b]	0.2	6.7[c]	0.7	0.5[b]	0.0	5.0[a]	0.0	5.0[a]	0.0
GG+Alg 0.4%	2.6[c]	0.4	10.8[a]	2.5	0.4[d]	0.0	5.0[a]	0.0	5.0[a]	0.0
GG+Alg 0.8%	2.7[b,c]	0.2	9.0[b]	0.5	0.4[c]	0.1	5.0[a]	0.0	5.0[a]	0.0
CMC+MD+Carr 0.4%	2.9[a,b]	0.2	6.7[c]	1.2	0.7[a]	0.0	5.0[a]	0.0	5.0[a]	0.0
CMC+MD+Carr 0.8%	2.9[a,b]	0.2	6.6[c]	0.7	0.5[b]	0.0	5.0[a]	0.0	5.0[a]	0.0
$LSD_{0.05}$	**0.2**		**1.6**		**0.03**		**0.8**		**0.8**	

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; treatment codes in Table II; means are from 2 replications, 5 tortillas each for rupture distance, rupture force and modulus of deformation, and 3 tortillas each for rollability and pliability scores.

Table XII Objective and subjective texture values of NCF tortillas treated with hydrocolloids of variable types and levels stored 1 d at 22 and 4^{o}C^{a}.

	1 d, 22^{o}C									
	Rupture force (N)		Rupture distance (mm)		Mod. of def. (N/mm)		Rollability Score		Pliability score	
Treatment	Average	std dev	Average	std dev	Average	std dev	Average	std dev	Average	std dev
Control	9.8^{a}	0.5	$2.4^{b,c,d,e}$	0.3	2.9^{a}	0.2	4.7^{a}	0.6	2.7^{c}	0.6
Ctrl improved	$5.9^{e,f,g}$	0.9	$2.3^{b,c,d,e}$	0.1	$1.1^{g,h}$	0.3	5.0^{a}	0.0	$3.7^{b,c}$	0.6
GG 0.4%	$5.8^{f,g,h}$	0.9	$2.0^{a,b}$	0.4	$1.5^{d,e,f}$	0.2	4.7^{a}	0.6	$4.0^{a,b}$	0.0
GG 0.8%	$5.0^{g,h}$	0.6	1.6^{a}	0.4	$1.5^{d,e}$	0.2	5.0^{a}	0.0	$4.3^{a,b}$	0.6
GG+Alg 0.4%	$6.5^{d,e,f}$	0.2	$2.8^{c,d,e}$	0.5	$1.5^{d,e}$	0.1	5.0^{a}	0.0	$4.0^{a,b}$	0.0
GG+Alg 0.8%	4.8^{h}	1.4	$2.2^{a,b}$	0.5	1.0^{h}	0.2	5.0^{a}	0.0	$4.3^{a,b}$	0.6
CMC+MD+Carr 0.4%	$6.8^{d,e}$	0.4	$2.3^{c,b}$	0.2	$1.2^{g,h}$	0.1	5.0^{a}	0.0	$3.7^{b,c}$	0.6
CMC+MD+Carr 0.8%	$7.1^{c,d}$	0.7	2.8^{e}	0.4	1.5^{d}	0.2	5.0^{a}	0.0	$4.3^{a,b}$	0.6
$LSD_{0.05}$	**1.0**		**0.5**		**0.2**		**0.5**		**0.8**	
	1 d, 4^{o}C									
	Rupture force (N)		Rupture distance (mm)		Mod. of def. (N/mm)		Rollability Score		Pliability score	
Treatment	Average	std dev	Average	std dev	Average	std dev	Average	std dev	Average	std dev
Control	11.3^{a}	0.7	$2.0^{c,d}$	0.3	$3.6^{b,c}$	0.4	4.7^{a}	0.6	2.3^{c}	0.6
Ctrl improved	$9.7^{b,c}$	0.5	$2.0^{c,d}$	0.4	$3.7^{a,b,c}$	0.1	5.0^{a}	0.0	$3.3^{a,b}$	0.6
GG 0.4%	$7.4^{e,f}$	0.4	$1.8^{b,c,d}$	0.3	$2.0^{g,h}$	0.3	4.7^{a}	0.6	$3.3^{a,b}$	0.6
GG 0.8%	6.1^{g}	1.8	1.2^{a}	0.5	2.7^{e}	0.5	4.7^{a}	0.6	$3.3^{a,b}$	0.6
GG+Alg 0.4%	$8.0^{d,e}$	0.7	$2.0^{b,c,d}$	0.4	3.2^{d}	0.2	5.0^{a}	0.0	4.0^{a}	0.0
GG+Alg 0.8%	$6.7^{f,g}$	0.3	$1.5^{a,b}$	0.4	1.7^{h}	0.2	5.0^{a}	0.0	$3.0^{b,c}$	0.0
CMC+MD+Carr 0.4%	$8.7^{c,d}$	1.0	$1.9^{b,c,d}$	0.4	$2.6^{e,f}$	0.3	4.7^{a}	0.6	$3.0^{b,c}$	0.0
CMC+MD+Carr 0.8%	$10.1^{a,b}$	0.9	2.3^{d}	0.4	$3.7^{a,b}$	0.3	5.0^{a}	0.0	$3.3^{a,b}$	0.6
$LSD_{0.05}$	**1.3**		**0.5**		**0.3**		**0.8**		**0.8**	

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; treatment codes in Table II; means are from 2 replications, 5 tortillas each for rupture distance, rupture force and modulus of deformation. 3 tortillas each for rollability and pliability scores.

Table XIII Objective and subjective texture values of NCF tortillas treated with hydrocolloids of variable types and levels stored 7 days at 22°C[a].

	Ambient, d7									
	Rupture force (N)		Rupture distance (mm)		Mod. of def. (N/mm)		Rollability Score		Pliability score	
Treatment	Average	std dev	Average	std dev	Average	std dev	Average	std dev	Average	std dev
Control	11.7 [a]	0.2	1.9 [d,e]	0.1	4.3 [a]	0.1	1.3 [e]	0.6	1.0 [d]	0.0
Ctrl improved	7.3 [e]	1.0	1.6 [b,c,d]	0.4	3.0 [c]	0.2	4.0 [b]	0.0	1.3 [c,d]	0.6
GG 0.4%	6.1 [f]	1.0	1.5 [a,b,c]	0.3	2.5 [f,g]	0.3	3.7 [b,c]	0.6	2.3 [a,b]	0.6
GG 0.8%	5.3 [f]	0.6	1.2 [a]	0.3	2.4 [g]	0.2	1.7 [e]	0.6	2.0 [b,c]	1.0
GG+Alg 0.4%	7.7 [d,e]	0.3	2.1 [e,f]	0.2	2.8 [c,d,e]	0.1	3.3 [b,c,d]	0.6	3.0 [a]	0.0
GG+Alg 0.8%	5.7 [f]	0.4	1.3 [a]	0.2	2.6 [e,f,g]	0.2	3.0 [c,d]	1.0	2.0 [b,c]	0.0
CMC+MD+Carr 0.4%	7.4 [e]	0.7	1.7 [c,d]	0.2	2.9 [c,d,e]	0.2	2.7 [d]	0.6	2.3 [a,b]	0.6
CMC+MD+Carr 0.8%	7.7 [d,e]	0.6	1.9 [d,e,f]	0.3	2.9 [c,d]	0.1	4.0 [b]	0.0	2.0 [b,c]	0.0
$LSD_{0.05}$	**1.0**		**0.3**		**0.3**		**0.9**		**0.7**	

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; treatment codes in Table II; means are from 2 replications, 5 tortillas each for rupture distance, rupture force and modulus of deformation. 3 tortillas each fro rollability and pliability scores.

Table XIV Objective and subjective texture values of NCF tortillas treated with hydrocolloids of variable types and levels stored 7 days at 4°C[a].

	Refrigerated, d7									
	Rupture force (N)		Rupture distance (mm)		Mod. of def. (N/mm)		Rollability Score		Pliability score	
Treatment	Average	std dev	Average	std dev	Average	std dev	Average	std dev	Average	std dev
Control	10.5 [a]	1.1	1.6 [a,b,c,d,e]	0.2	4.1 [a]	0.3	1.3 [f]	0.6	1.0 [b]	0.0
Ctrl improved	9.4 [a,b]	0.8	1.6 [a,b,c,d,e]	0.4	3.8 [b]	0.3	3.0 [b,c,d]	0.0	1.3 [b]	0.6
GG 0.4%	6.1 [e]	1.0	1.5 [a,b,c,d]	0.3	2.5 [h]	0.3	3.0 [b,c,d]	0.0	1.3 [b]	0.6
GG 0.8%	7.6 [d]	1.9	1.1 [a]	0.3	3.3 [e,f]	0.4	2.7 [c,d,e]	0.6	1.3 [b]	0.6
GG+Alg 0.4%	9.0 [b,c]	1.4	1.7 [b,c,d,e]	0.5	3.7 [b,c,d]	0.3	3.7 [b]	0.6	2.0 [a,b]	0.0
GG+Alg 0.8%	7.4 [d,e]	1.2	1.3 [a,b]	0.3	3.1 [f,g]	0.3	2.0 [e,f]	0.0	2.0 [a,b]	1.0
CMC+MD+Carr 0.4%	9.7 [a,b]	1.1	1.6 [b,c,d,e]	0.4	3.8 [a,b]	0.2	3.3 [b,c]	0.6	1.3 [b]	0.6
CMC+MD+Carr 0.8%	7.7 [c,d]	0.6	1.9 [d,e]	0.3	2.9 [g]	0.1	4.7 [a]	0.6	3.0 [a]	1.0
$LSD_{0.05}$	**1.3**		**0.5**		**0.3**		**0.8**		**0.9**	

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; treatment codes in Table II; means are from 2 replications, 5 tortillas each for rupture distance, rupture force and modulus of deformation. 3 tortillas each for rollability and pliability scores.

Table XV Moisture of NCF tortillas stored 7 days at 22°C and 4°C, with added hydrocolloids of variable types and levels[a].

	Average moisture (%)		
Treatment	**Initial**	**7 d, 22°C**	**7 d, 4°C**
Control	45.6 c	46.0 e	44.7 c
Ctrl improved	47.2 a,b,c	47.5 c,d	47.2 a,b
GG 0.4%	47.9 a,b	48.2 b,c	47.2 a,b
GG 0.8%	46.8 bc,	49.6 a	47.9 a,b
GG + Alg 0.4%	47.8 a,b	48.7 b	48.1 a,b
GG + Alg 0.8%	48.0 a,b	48.7 b	45.7 b,c
CMC + MD + Carr 0.4%	48.4 a,b	47.5 c,d	47.8 a,b
CMC + MD + Carr 0.8%	48.5 a,b	47.5 c,d	46.8 a,b,c
$LSD_{0.05}$	**2.0**	**0.9**	**2.5**

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; treatment codes in Table II; means are average of 2 replicates, 2 tortillas each.

APPENDIX B

OPTIMIZATION BY RSM

Table XVI Values of intercept, correlations and least significant differences from the central composite design optimization of FNM and NCF tortillas. Tortillas were stored 7 days under refrigeration (4^oC)[a].

FNM day 7	Rupture Force	Young's Modulus	Rollability	Pliability
intercept	11.27362	5.11061	3.36111	2.322222
amy	-0.005685	-0.001818	-0.000938	0.001375
cmc	2.88985	0.849675	-32.569444	-4.638889
guar	7.78535	1.271425	7.847222	1.194444
amy*amy	-0.000000574	-0.000002864	0.000005208	0.00000833
amy*cmc	-0.01155	-0.007859	0.023264	-0.015972
cmc*cmc	14.37	-4.44875	174.305556	48.61111
amy*guar	-0.005461	0.006845	-0.013194	-0.022222
cmc*guar	4.4435	10.07425	-60.4166667	9.16667
guar*guar	-34.4475	-13.4375	-2.777778	11.11111
$LSD_{0.05}$	**1.1224**	**0.3644**	**0.8426**	**0.983**

NCF day 7	Rupture Force	Young's Modulus	Rollability	Pliability
intercept	12.325988	5.097518	1.360544	1.125624
amy	-0.030118	-0.012446	0.016156	-0.002939
cmc	-2.345557	-1.524969	3.795918	3.491156
guar	1.398021	0.369516	0.564626	-0.587755
amy*amy	0.000107	0.00004485	-0.000084	1.17E-05
amy*cmc	0.026367	0.007376	0.025306	0.010385
cmc*cmc	2.976355	1.242445	2.503401	-3.464853
amy*guar	-0.018245	-0.006304	-0.017347	0.001859
cmc*guar	2.970939	1.918014	-4.272109	-3.256236
guar*guar	-4.003911	-1.252589	1.292517	0.46712
$LSD_{0.05}^{**}$	**1.2516**	**0.3524**	**0.9315**	**0.628**

[a] $LSD_{0.05}$ Least significant difference of the values obtained on the objective and subjective measurements of tortillas is from 10 tortillas of each treatment.

APPENDIX C

PHYSICAL PROPERTIES

Table XVII Selected physical characteristics of masa and tortillas from NCF and FNM treated with enzymes and hydrocolloids before processing and out of the oven (20 min) after baking[a].

	Masa moisture (%)		pH Masa		pH tortillas		Weight of 10 tortillas	
Treatment	**Average**	**std dev**	**Average**	**std dev**	**Average**	**std dev**	**Average**	**std dev**
FNM ctrl	59.2%	0.6%	5.8	0.2	5.4	0.3	237.9	1.4
FNM + add	58.7%	1.0%	5.9	0.1	5.4	0.1	238.1	0.9
NCF ctrl	59.0%	2.0%	5.7	0.1	5.5	0.1	234.9	1.2
NCF lo enz	59.0%	1.2%	5.7	0.1	5.4	0.3	235.3	1.2
NCF hi enz	58.2%	0.5%	5.8	0.2	5.5	0.2	237.5	1.7
$LSD_{0.05}$	**2.0%**							

[a] $LSD_{0.05}$ = Least significant difference for means separation; values are means of 2 replicates 2 samples of tortillas each. Treatment codes in Table VI of Chapter V.

Table XVIII Moisture content tortillas from NCF and FNM treated with enzymes and hydrocolloids, out of the oven (20 min) and after 4 d of storage at 22 and 4°C[a].

	Masa Moisture (%)			**Tortilla Moisture (%)**								
	Initial			**Initial**			**4 d, 22 °C**			**4 d, 4 °C**		
Treatment	**Average**		**std dev**	**Average**		**std dev**	**Average**		**std dev**	**Average**		**std dev**
FNM ctrl	59.2%	a	0.6%	46.6%	c,d	1.0%	46.0%	c,d	1.0%	46.0%	b,c,d	1.0%
FNM + add	58.7%	a	0.4%	46.1%	b,c	2.0%	46.3%	b,c	1.9%	46.6%	b	1.0%
NCF ctrl	59.0%	a	0.1%	45.8%	b,c,d	0.5%	45.7%	b,c,d	0.4%	46.6%	d	0.6%
NCF lo enz	59.0%	a	0.6%	47.3%	b,c,d	1.5%	47.1%	b,c,d	0.4%	47.9%	b,c,d	1.3%
NCF hi enz	58.2%	a	0.7%	46.1%	b,c,d	1.2%	46.6%	b,c,d	0.5%	44.9%	b,c,d	0.5%
$LSD_{0.05}$	**2.0%**											

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; values are means of 2 replicates 3 tortillas each; treatment codes in Table VI of Chapter V.

Table XIX Particle size distribution of masas from NCF, FNM and reference[a].

	FNM masa		NCF masa			Reference	
Particle size range (microns)	%	std dev	%	std dev	**Avg. size (microns)**	%	std dev
>850	0.0	0.0	0.0	0.0	>850	11.1	0.2
425-850	10.5	8.9	0.0	0.0	425-850	14.2	0.3
250-425	5.4	7.5	3.3	1.9	250-425	11.0	0.3
180-250	4.3	5.7	7.4	0.2	212-250	3.2	0.5
150-180	3.2	7.6	4.6	0.7	150-212	3.0	0.2
106-150	2.8	2.3	3.2	0.3	45-150	5.2	0.0
75-106	3.8	9.7	2.9	4.9	11-45	46.7	0.9
11-75	61.9	9.3	59.1	4.0	solubles	5.5	0.1
solubles	8.2	1.4	19.4	0.4			

[a] Reference is from Gomez (1988), optimum cooked, fine ground masa; values are averages of 2 replicates, 2 repetitions each.

APPENDIX D

TEXTURE ANALYSIS

Table XX Objective and subjective texture values of tortillas from NCF and FNM treated with enzymes and hydrocolloids stored 4 days at ambient (22°C) and refrigerated (4°C) temperatures[a].

Day 0		**Rupture Force (N)**		**Rupture distance (mm)**		**Mod. deformation (N/mm)**		**Rollability score**		**Pliability score**	
Treatment	**Storage temp.**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**
NCF ctrl	22°C	2.6^{c}	0.3	10.8^{a}	3.0	0.38^{d}	0.0	5.0^{a}	0.0	5.0^{a}	0.0
NCF lo enz	22°C	$2.7^{b,c}$	0.3	9.0^{b}	2.5	0.44^{c}	0.0	5.0^{a}	0.0	5.0^{a}	0.0
NCF hi enz	22°C	$2.9^{a,b}$	0.2	6.7^{c}	0.7	0.54^{b}	0.0	5.0^{a}	0.0	5.0^{a}	0.0
FNM Ctrl	22°C	2.9^{b}	0.1	4.7^{d}	0.5	0.66^{a}	0.0	5.0^{a}	0.0	5.0^{a}	0.0
FNM + add	22°C	3.1^{a}	0.3	5.0^{d}	0.5	0.68^{a}	0.1	5.0^{a}	0.0	5.0^{a}	0.0
$LSD_{0.05}$		**0.2**		**1.6**		**0.03**		**0.8**		**0.8**	

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; values are means of 2 tortillas 10 repetitions each for objective measurements and 5 tortillas each for subjective measurements; treatment codes in Table VI of Chapter V.

Table XXI Objective and subjective texture values of tortillas from NCF and FNM treated with enzymes and hydrocolloids stored 4 days at ambient (22°C) and refrigerated (4°C) temperatures[a].

Day 1		Rupture Force (N)		Rupture distance (mm)		Mod. deformation (N/mm)		Rollability score		Pliability score	
Treatment	**Storage temp.**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**
NCF ctrl	22°C	9.3 [c,d]	2.1	2.2 [a,b]	0.8	3.2 [c]	0.5	4.7 [a,b]	0.6	3.7 [a,b]	0.6
NCF lo enz	22°C	6.8 [f,g]	0.7	2.5 [a]	0.5	2.4 [d]	0.1	5.0 [a]	0.0	4.3 [a]	0.6
NCF hi enz	22°C	6.2 [g,h]	0.8	1.9 [b,c,d]	0.4	2.4 [d]	0.1	5.0 [a]	0.0	4.0 [a,b]	0.0
FNM Ctrl	22°C	8.1 [d,e]	0.3	1.8 [b,c,d,e]	0.2	3.2 [c]	0.2	4.3 [b,c]	0.6	3.3 [b,c]	0.6
FNM + add	22°C	5.4 [h]	0.2	1.8 [b,c,d,e]	0.2	2.2 [d]	0.0	4.7 [a,b]	0.6	4.0 [a,b]	0.0
NCF ctrl	4°C	13.2 [a]	0.9	2.2 [a,b]	0.5	4.7 [a]	0.3	4.0 [c]	0.0	2.7 [c]	0.6
NCF lo enz	4°C	9.9 [b,c]	0.9	2.1 [a,b,c]	0.3	3.7 [b]	0.2	5.0 [a]	0.0	4.0 [a,b]	0.0
NCF hi enz	4°C	8.1 [d,e]	0.9	1.4 [e]	0.2	3.4 [b,c]	0.3	5.0 [a]	0.0	3.7 [a,b]	0.6
FNM Ctrl	4°C	10.6 [b]	0.6	1.5 [d,e]	0.2	4.4 [a]	0.2	4.0 [c]	0.0	3.3 [b,c]	0.6
FNM + add	4°C	7.5 [e,f]	0.7	1.6 [c,d,e]	0.1	3.1 [c]	0.3	4.7 [a,b]	0.6	3.3 [b,c]	0.6
$LSD_{0.05}$		**1.2**		**0.5**		**0.3**		**0.6**		**0.8**	

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; values are means of 2 tortillas 10 repetitions each for objective measurements and 5 tortillas each for subjective measurements; treatment codes in Table VI of Chapter V.

Table XXII Objective and subjective texture values of tortillas from NCF and FNM treated with enzymes and hydrocolloids stored 4 days at ambient (22°C) and refrigerated (4°C) temperatures[a].

Day 4		**Rupture Force (N)**		**Rupture distance (mm)**		**Mod. deformation (N/mm)**		**Rollability score**		**Pliability score**	
Treatment	**Storage temp.**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**	**Avg**	**std dev**
NCF ctrl	22°C	13.1 [a,b]	2.0	1.9 [a]	0.5	4.9 [a,b]	0.5	3.3 [c]	0.8	2.5 [f,g]	0.5
NCF lo enz	22°C	8.4 [d,e]	1.0	1.9 [a]	0.3	3.3 [f,g]	0.2	5.0 [a,b]	0.0	4.5 [a,b]	0.5
NCF hi enz	22°C	6.8 [f]	0.8	1.5 [c,d]	0.2	2.9 [h,ii]	0.2	4.3 [a,b]	0.5	3.8 [c,d]	0.4
FNM Ctrl	22°C	11.9 [a,b]	1.9	1.6 [b,c]	0.2	4.9 [a,b]	0.8	3.2 [c,d]	1.0	2.7 [f,g]	0.8
FNM + add	22°C	7.3 [e,f]	1.8	1.6 [c,d]	0.2	3.1 [g,h]	0.8	4.3 [a,b]	0.5	3.8 [c,d]	0.4
NCF ctrl	4°C	13.4 [a,b]	1.6	1.8 [a,b]	0.4	5.2 [a]	0.4	1.8 [e]	1.0	1.7 [h]	0.8
NCF lo enz	4°C	11.4 [c]	0.7	2.0 [a]	0.3	4.3 [c]	0.2	4.2 [b]	0.4	2.5 [g]	0.4
NCF hi enz	4°C	9.2 [d]	1.1	1.2 [e]	0.2	3.9 [d]	0.3	3.3 [c]	0.5	2.5 [g]	1.0
FNM Ctrl	4°C	12.0 [b,c]	2.2	1.5 [c,d]	0.2	5.1 [a]	0.8	2.5 [d,e]	0.8	1.7 [h]	0.5
FNM + add	4°C	9.0 [d]	1.7	1.4 [d,e]	0.3	3.9 [d]	0.7	3.7 [b,c]	0.5	2.5 [g]	0.5
$LSD_{0.05}$		**1.1**		**0.2**		**0.3**		**0.8**		**0.6**	

[a] Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; values are means of 2 tortillas 10 repetitions each for objective measurements and 5 tortillas each for subjective measurements; treatment codes in Table VI of Chapter V.

APPENDIX E

RVA ANALYSIS

Table XXIII Values of specific points of interest from RVA viscosity development of stabilized starch residue (SSR) of tortillas from FNM and NCF, under variable time, storage and treatment conditions[a].

Treat-ment	Storage	Visc. @ peak (RVU)	Visc. @ Trough (RVU)	Final Visc. (RVU)	Peak time (min)	Pasting temp (°C)
FNM Ctrl	20 min	301 g,h	266 c,d	533 f,g	5.7 d,e	80.3 b,c,d
FNM + add	20 min	280 f,g	254 c,d	498 g,h	6.3 b,c	83.8 a
NCF ctrl	20 min	619 a	368 a	972 a	4.7 g	63.3 h
NCF lo enz	20 min	499 b	257 c,d	752 d	4.9 f,g	70.6 g
NCF hi enz	20 min	355 d,e	213 f	610 e,f	5.0 f,g	76.0 e,f
FNM Ctrl	4 d, 22 °C	283 f,g,h	265 d	483 g,h	6.6 a,b	81.8 a,b,c
FNM + add	4 d, 22 °C	237 i	224 e,f	419 h	6.6 a,b	83.2 a,b
NCF ctrl	4 d, 22 °C	496 b	367 a	828 b,c,d	5.1 f	73.8 f,g
NCF lo enz	4 d, 22 °C	437 c	384 a	880 b	5.7 d,e	78.6 c,d,e
NCF hi enz	4 d, 22 °C	323 e,f	270 c,d	557 f,g	5.6 e	79.9 c,d
FNM Ctrl	4 d, 4 °C	309 f,g	297 b,c	520 g	6.2 b,c	77.9 e,d
FNM + add	4 d, 4 °C	253 h,i	240 d,e,f	430 h	6.8 a	84.8 a
NCF ctrl	4 d, 4 °C	538 b	374 a	800 c,d	5.0 f,g	71.0 g
NCF lo enz	4 d, 4 °C	518 b	389 a	838 b,c	5.2 f	73.5 f,g
NCF hi enz	4 d, 4 °C	461 d	349 b,c	760 e	5.3 e,f	75.7 f
$LSD_{0.05}$		**42.3**	**31.3**	**79.5**	**0.40**	**3.32**

[a]Means in the same column followed by the same letter are not significantly different; $LSD_{0.05}$ = Least significant difference for means separation; values are average of 3 replicates, 2 repetitions each; treatment codes in Table VI of Chapter V.

Table XXIV Loss of solids (on dm) during the preparation of stabilized starch residue (SSR) of tortillas with different treatments and storage conditions[a].

	Masa		Tortilla day 0		Tortilla d 4, 22°C		Tortilla d 4, 4°C	
Treatment	% average loss	std dev	% average loss	std dev	% average loss	std dev	% average loss	std dev
NCF ctrl	4.8% f	0.03%	3.8% h	0.06%	2.3% n	0.02%	2.5% l,m	0.05%
NCF lo enz	7.6% b	0.21%	5.9% d	0.03%	3.5% i	0.02%	2.8% k	0.03%
NCF hi enz	9.3% a	0.18%	6.7% c	0.04%	5.0% f	0.02%	3.1% j	0.02%
FNM Ctrl	3.5% i	0.07%	2.6% l	0.01%	1.5% p	0.01%	1.7% p	0.03%
FNM + add	5.4% e	0.06%	4.1% g	0.04%	2.4% m,n	0.04%	1.9% o	0.07%

[a] $LSD_{0.05}$ (Least significant difference for means separation)= 0.15%; means in the same column followed by the same letter are not significantly different; values are means of 2 replicates with 4 repetitions each

Table XXV Increase of solids loss (on dm) during the preparation of stabilized starch residue (SSR) of tortillas with different treatments and storage conditions[a].

	Masa		Tortilla day 0		Tortilla d 4, 22°C		Tortilla d 4, 4°C	
Treatment	% average loss	std dev	% average loss	std dev	% average loss	std dev	% average loss	std dev
NCF lo enz	2.8% b	0.06%	2.0% c	0.03%	1.3% e	0.02%	0.3% h	0.03%
NCF hi enz	4.5% a	0.18%	2.8% b	0.04%	2.7% b	0.02%	0.6% g	0.02%
FNM + add	2.0% c	0.21%	1.5% d	0.04%	0.8% f	0.04%	0.2% h	0.07%

[a] $LSD_{0.05}$ (Least significant difference for means separation)= 0.2%; means in the same column followed by the same letter are not significantly different; values are means of 2 replicates with 4 repetitions each; values are difference between solid loss of the treatment and their respective control

APPENDIX F

HPLC

Table XXVI Carbohydrates extracted from masa and tortillas from FNM and NCF subject to different treatments of enzymes and hydrocolloids and stored for variable storage conditions of time and temperature[a].

Treatment	Masa day 0		Tortilla day 0		Tortilla 4 day 22°C		Tortilla 4 day 4°C	
	Average	std dev	Average	std dev	Average	std dev	Average	std dev
Polymers								
NCF ctrl	32.8	18	40.4	8	43.7	9	40.9	10
NCF lo enz	32.7	10	29.9	9	23.4	13	28.4	6
NCF hi enz	36.3	5	34.8	13	42.8	12	38.2	5
FNM Ctrl	25.5	4	32.0	3	40.7	8	39.0	2
FNM + add	22.4	7	32.9	7	34.2	4	19.4	4
Oligosaccharides								
NCF ctrl	64.3	14	84.8	14	80.8	6	66.4	13
NCF lo enz	80.4	16	69.8	18	79.6	21	54.6	10
NCF hi enz	74.0	8	87.2	12	88.3	13	84.9	16
FNM Ctrl	67.1	22	58.8	5	76.2	6	58.3	11
FNM + add	62.1	18	62.7	12	73.8	20	54.1	13
LMW sugars								
NCF ctrl	12.2	5	15.8	7	17.6	9	19.4	7
NCF lo enz	19.4	12	23.3	11	41.1	17	23.0	1
NCF hi enz	28.7	7	42.8	16	98.4	17	50.5	17
FNM Ctrl	1.8	5	8.8	5	7.0	8	10.7	3
FNM + add	14.9	2	16.4	4	30.1	6	24.8	6
Total extracted carbohydrates								
NCF ctrl	109.3		140.9		142.1		126.6	
NCF lo enz	132.5		123.0		144.1		106.0	
NCF hi enz	139.0		164.8		229.4		173.7	
FNM Ctrl	94.4		99.6		124.0		108.0	
FNM + add	99.4		112.0		138.2		98.3	

[a]Values are means of 2 replicates, 2 repetitions each.

Table XXVII Differences in carbohydrates extracted from masa and tortillas from FNM and NCF subject to different treatments of enzymes and hydrocolloids and stored for variable storage conditions of time and temperature[a].

	Treatment (mg CHO/g)				Tortilla storage (mg CHO/g)		
	Polymer						
Treatment***	Masa day 0	Tortilla day 0	Tortilla 4 day 22°C	Tortilla 4 day 4°C	0d-4d @ 22°C	0d-4d @ 4°C	4d @ 22oC-4d @ 4°C
NCF lo enz	-0.1	-10.5	-20.3	-12.5	-9.8	-2.0	-7.8
NCF hi enz	3.5	-5.5	-0.9	-2.7	4.6	2.9	1.7
FNM + add	-3.1	1.0	-6.5	-19.6	-7.5	-20.6	13.1
	Oligosaccharides						
NCF lo enz	16.0	-15.0	-1.3	-11.7	13.7	3.3	10.5
NCF hi enz	9.7	2.4	7.4	18.5	5.0	16.1	-11.1
FNM + add	-5.0	3.8	-2.4	-4.2	-6.2	-8.0	1.8
	Sugars						
NCF lo enz	7.2	7.5	23.6	3.6	16.0	-3.9	19.9
NCF hi enz	16.5	27.0	80.8	31.2	53.8	4.1	49.7
FNM + add	13.0	7.6	23.1	14.1	15.5	6.5	9.0
	Total extracted carbohydrates						
NCF lo enz	23.2	-17.9	2.0	-20.6	19.9	-2.7	22.6
NCF hi enz	29.7	23.9	87.3	47.0	63.4	23.2	40.2
FNM + add	5.0	12.4	14.2	-9.7	1.8	-22.1	23.9

[a]Values are the difference between the amount of carbohydrate extracted for the treatment and its respective control.

APPENDIX G

CORRELATIONS

Table XXVIII RVA, texture and HPLC measurements of tortillas subject to improvements by enzymes and hydrocolloids (controls included), with different storage times (initial, stored 4 d at 4°C and 22°C)[a].

Treatment	Storage	Enzyme level (MAU/kg dmb)	CMC (g/kg dmb)	Guar (g/kg dmb)	Peak Visc (RVU)	Pasting temp (°C)	Solid loss %	Mod. Def. (N/mm)	Pliability Score	LMW sugars (mg/g dmb)	Total CHO (mg/g dmb)
NCF ctrl	amb	0	0.00	0.00	495.9	73.8	0.0412	4.6	2.3	17.6	142.1
NCF lo enz	amb	600	4.25	5.66	436.5	78.6	0.0384	3.4	4.7	41.1	144.1
NCF hi enz	amb	1700	1.42	8.49	323.2	79.9	0.0665	3.0	3.7	98.4	229.4
FNM Ctrl	amb	0	0.00	0.00	282.6	81.8	0.0261	4.4	2.0	7.0	124.0
FNM + add	amb	700	3.49	1.16	237.1	83.2	0.0588	2.7	3.7	30.1	138.2
NCF ctrl	ref	0	0.00	0.00	537.6	71.0	0.0237	5.0	1.3	19.4	126.6
NCF lo enz	ref	600	4.25	5.66	517.7	73.5	0.0226	4.3	3.0	23.0	106.0
NCF hi enz	ref	1700	1.42	8.49	460.8	75.7	0.0496	4.0	3.3	50.5	173.7
FNM Ctrl	ref	0	0.00	0.00	309.1	77.9	0.0152	4.6	1.3	10.7	108.0
FNM + add	ref	700	3.49	1.16	253.0	84.8	0.0354	3.5	3.0	24.8	98.3

[a]Number of observations: peak viscosity and pasting temperature: 40, solid loss: 80, modulus of deformation: 50, pliability: 30, sugars and total carbohydrates extracted: 40.

Table XXIX Pearson correlation coefficients (r_{xy}) of pasting, tortilla texture and soluble sugars of NCF and FNM tortillas containing known levels of maltogenic α-amylase, CMC and guar after 4 d of storage at 22°C.

All tortillas 4 day ambient storage		Enzyme level	CMC level	Guar level	Peak Viscosity (RVU)	Pasting temp (oC)	Solid loss %	Mod. Def. (N/ mm)	Pliability score	Sugars extracted
Enzyme level (MAU/kg dmb)	r	**1**								
	N	75								
CMC level (g/kg dmb)	r	0.35	**1**							
	N	75	75							
Guar level (g/kg dmb)	r	0.89	0.40	**1**						
	N	75	75	75						
Peak Viscosity (RVU)	r	-0.29	-0.13	0.03	**1**					
	N	20	20	20	20					
Pasting temp (oC)	r	0.27	0.35	0.05	-0.94	**1**				
	N	20	20	20	20	20				
Solid loss %	r	0.91*	0.33	0.97*	0.10	-0.069	**1**			
	N	30	30	30	20	20	30			
Mod. Def. (N/ mm)	r	-0.74*	-0.65*	-0.67*	0.03	-0.10	-0.82*	**1**		
	N	75	75	75	20	20	30	75		
Pliability score	r	0.52*	0.76*	0.59*	-0.121	0.26	0.53	-0.593	**1**	
	N	30	30	30	20	20	30	30	30	
Sugars extracted	r	0.93*	0.22	0.89*	-0.075	0.05	0.93*	-0.84*	0.49*	**1**
	N	20	20	20	20	20	20	20	20	20

* Correlation is significant at the 0.01 level (2-tailed).

Table XXX Pearson correlation coefficients (r_{xy}) of pasting, tortilla texture and soluble sugars of NCF and FNM tortillas containing known levels of maltogenic α-amylase, CMC and guar after 4 d of storage at 4°C.

All tort 4 day refrigarated storage		Enzyme level	CMC level	Guar level	Peak Viscosity (RVU)	Pasting temp (oC)	Solid loss %	Mod. Def. (N/ mm)	Pliability score	Sugars extracted
Enzyme level (MAU/kg dmb)	r	**1**								
	N	75								
CMC level (g/kg dmb)	r	0.35	**1**							
	N	75	75							
Guar level (g/kg dmb)	r	0.89	0.40	**1**						
	N	75	75	75						
Peak Viscosity (RVU)	r	0.07	-0.07	0.39	**1**					
	N	20	20	20	20					
Pasting temp (oC)	r	0.15	0.31	-0.17	-0.87	**1**				
	N	20	20	20	20	20				
Solid loss %	r	0.66*	0.22	0.84*	0.78*	-0.519	**1**			
	N	30	30	30	20	20	30			
Mod. Def. (N/ mm)	r	-0.61*	-0.51*	-0.48*	-0.279	0.18	-0.47*	**1**		
	N	75	75	75	20	20	30	75		
Pliability score	r	0.41	0.45	0.38	-0.11	0.25	0.25	-0.09	**1**	
	N	30	30	30	20	20	30	30	30	
Sugars extracted	r	0.83*	0.13	0.73*	0.17	0.02	0.64*	-0.59*	0.08	**1**
	N	20	20	20	20	20	20	20	20	20

* Correlation is significant at the 0.01 level (2-tailed).

APPENDIX H

TEXTURE CHANGES IN TORTILLAS FROM TREATED FNM MASA MADE WITH UNDER, OVER AND OPTIMUM COOKED NIXTAMAL

A comparison of the response that maltogenic α-amylase has on tortillas from masa prepared from nixtamal with different degrees of cooking was performed. Masas were acquired from outside sources (0) or prepared at our facilities. Maltogenic α-amylase was added to the masa and baked into tortillas, pliability scores and modulus of deformation values were recorded after 7 days of storage.

Maltogenic α-amylase at 15 mg/kg masa behaved in a similar way in masas from optimum cooked nixtamal as in masa form overcooked nixtamal (Table XXXI) indicated by similar reduction in the modulus of deformation and pliability score of tortillas stored 7 d under refrigeration, compared to their respective controls under similar storage conditions. The undercooked control masa had low cohesiveness and had low changes when treated with enzymes.

Table XXXI Changes in subjective and objective measurements of tortillas treated with different levels of maltogenic α-amylases. Modulus of deformation and pliability scores are expressed as the differential between the average value for the enzyme and the control from the same trial.

	mg enz/ kg masa	Masa moisture (%)	MAU/ kg dm	Change of mod. of deformatio	Change of pliability score	Machin-ability values
Optimum cook	15	59.2	368	0.89	0.96	4.50
Optimum cook	30	59.2	735	0.47	-0.30	4.50
Optimum cook	50	59.2	1225	0.80	0.50	4.00
Overcooked	10	58.5	241	-0.31	-0.33	4.50
Overcooked	15	58.5	361	0.97	1.00	4.50
Overcooked	30	58.5	723	0.30	-0.67	4.00
Overcooked	75	58.5	1807	0.90	0.00	3.00
Undercooked	15	54.5	330	0.14	0.10	4.00
Undercooked	30	54.5	659	0.21	0.30	4.00

The enzyme at the levels used did not affect the undercooked masa. Not many repetitions done on undercooked nixtamal masa, as the quality of the tortillas was always poor as they were brittle and stiff. The enzymes at the levels used did not affect the properties of the tortillas.

These observations are in agreement with Gomez (1988) that analyzed masas with different degrees of cooking. Undercooked masa and native corn had similar high levels of soluble starch, while optimum and overcooked masa had lower levels. The reduction of soluble starch was the result of annealing during nixtamal steeping. Masas with similar levels of soluble starch have comparable results in the modification caused by enzymes.

Observations of the tortillas immediately after baking showed definite textural changes on those that were enzyme treated. The tortillas treated with maltogenic α-amylase had a smooth appearance compared with the untreated tortillas. To observe these changes, extreme care has to be taken in the baking procedures. The parts of equipment prior to the oven that are in touch with the masa were completely disassembled and washed between treatments to prevent enzyme cross-contamination and to remove any leftover masa that may be dried and cause a rough surface in the new masa disks. Attention has to be given to the cutting wires of the masa sheeter to assure that they are in optimum conditions and tense. These precautions gave place to a valuable subjective texture test based on the smoothness of the tortillas subject to enzyme treatment that proved to be helpful in this research.

APPENDIX I

MASA SUPPLIERS FOR THIS RESEARCH

El Milagro of Texas, Inc.
Raphael Lopez (Owner)
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Tel 512 477-6476

La Espiga de Oro
Alfredo Lira (Owner)
Hayde Villa (QC manager)
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La Poblana
Hector Villarreal (Owner)
7648 Canal Street
Houston, TX 77012
Tel 713-921-4760

APPENDIX J

NOMENCLATURE

CMC: Sodium Carymethylcellulose.
FNM: Fresh nixtamal masa.
FNM ctrl: Tortillas from fresh nixtamalized masa plus preservatives
FNM + add: Tortillas treated same as FNM ctrl plus 70 mg/kg of maltogenic α-amylase, 0.35% CMC and 0.12% guar. Formula was developed from RSM studies.
g: grams.
gal: Gallons.
HPLC: High performance liquid chromatography.
L: Liter.
LMW: Low molecular weight.
MAU: Maltogenic activity units.
min: Minutes.
N/mm: Newton per millimeter, units of modulus of deformation.
N: Newton.
NCF: Nixtamalized corn flour.
NCF ctrl: Tortillas from nixtamalized corn flour plus water and preservatives
NCF lo enz: Tortillas treated same as NCF ctrl plus 60 mg/kg of maltogenic α-amylase, 0.43% CMC and 0.57% guar. Formula was developed from RSM studies.
NCF hi enz: Tortillas treated same as NCF ctrl plus 170 mg/kg of maltogenic α-amylase, 0.14% CMC and 0.85% guar. Formula was developed from RSM studies.
PS: Pliability score.
qt: Quarts.
s: Seconds.
SSR: Stabilized starch residue.

VITA

Arturo Carlos Gutierrez de Velasco Alvarez was born on September 26, 1967 in Mexico City. He graduated from high school from Centro de Estudios Cristobal Colon in 1985. He graduated with honors as a B.A. in biochemistry engineering from Instituto Tecnologico y de Estudios Superiores de Monterrey in December 1989. He worked in the food industry from 1990 to 2002 in shrimp and catfish aquaculture, mushroom growing and processing, and in the first Mexican microbrewery.

He enrolled into Texas A&M University for a Master of Science degree in food science and technology and graduated in May 2004.

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