ANTISTALING PROPERTIES OF AMYLASES, WHEAT GLUTEN AND CMC ON CORN TORTILLA

A Dissertation

by

FRANCISCO JAVIER BUESO UCLES

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

DOCTOR OF PHILOSOPHY

May 2003

Major Subject: Food Science and Technology

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Approved as to style and content by:

Lloyd W. Rooney
(Co-Chair of Committee)

Ralph D. Waniska
(Co-Chair of Committee)

Ronald L. Richter
(Member)

Rosana Moreira
(Member)

Lloyd W. Rooney
(Chair of Food Science and Technology Faculty)

Mark Hussey
(Head of Department)

May 2003

Major Subject: Food Science and Technology

ABSTRACT

Antistaling Properties of Amylases, Wheat Gluten and CMC on Corn Tortilla.

(May 2003)

Francisco Javier Bueso Ucles, B.Sc., Escuela Agricola Panamericana (Zamorano);

M.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Lloyd W. Rooney
Dr. Ralph D. Waniska

Antistaling properties of enzymes (xylanase, bacterial maltogenic and conventional α-amylases), CMC and vital wheat gluten on corn tortillas were evaluated during storage for up to 21 days. Effect of storage time (0-21 days) and temperature (-40, -20, 3, 10 and 21 ^{o}C) on tortilla staling was evaluated with or without additives.

Addition of 275-1650 AU of ICS maltogenic amylase effectively reduced amylopectin retrogradation without reducing tortilla yields, but did not improve tortilla flexibility.

The combination of 825 AU of ICS amylase (to interfere with intra-granular amylopectin re-crystallization) and 0.25% CMC (to create a more flexible inter-granular matrix than retrograded amylose) produced less stiff, equally flexible and less chewy tortillas than 0.5% CMC.

Corn tortilla staling followed the basic laws that control aging in starch-based semi-crystalline systems such as starch gels, bread and other baked products. Amylopectin re-crystallization was the driving force behind the staling of corn tortillas. Increasing levels of re-crystallized amylopectin measured by DSC correlated significantly with increased tortilla stiffness and reduction in tortilla rollability, pliability and rupture distance during storage.

Re-crystallization of amylopectin in fresh tortillas was not detected. It increased rapidly during the first 24 hr reaching a plateau after 7 days storage. The level of amylopectin re-crystallization on tortillas showed a bell-shaped trend along the evaluated storage temperature range with a maximum around 7 ^{o}C.

However, a negative linear relationship of peak pasting viscosity with storage temperature of tortilla extracts without additives after 21 days suggests other compounds besides amylopectin affect tortilla staling. Thus, interfering with amylopectin re-crystallization is not the only way to retard staling.

Further research is required to optimize the addition of maltogenic amylases in continuous processing lines that use fresh masa instead of nixtamalized corn flour, to determine how these amylases interfere with amylopectin re-crystallization and to elucidate if amylose retrogradation continues during storage and plays a role in tortilla staling.

DEDICATION

To my parents Pedro and Alicia, to my sister Claudia and to my family in Tegucigalpa (Francis, Dagoberto, Marvin, Waleska and Los Abuelos).

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CHAPTER I

INTRODUCTION

Corn tortillas have found their place in the North American mainstream diet thanks in part to the widespread popularity of Mexican and Southwestern cuisines. Tortilla versatility for serving and re-warming is another powerful reason for its popularity (TIA 2002). Consumers demand tortillas, which are soft with optimum flexibility and rollability, while rigid, firm and less rollable tortillas are undesirable (Guo 1998). Consumers also prefer tortillas that remain soft and rollable for a long period of time.

Corn tortillas made without additives stale fast, especially within the first 12 hr of storage, becoming rigid and dry. Commercial tortillas have a shelf life of approximately 25-60 d under refrigeration, thanks to the addition of gums (such as carboxy-methyl cellulose, CMC) that preserve flexibility and mold inhibitors that delay spoiling. This prolonged shelf life comes with a price: a significant change in tortilla flavor (tasteless or bitter), aroma (off-odors) and texture (rubbery).

So far, studies on corn tortilla staling agree that there is not a single additive able to produce tortillas with a shelf stability of at least 30 d without a significant decrease in quality. Most of these studies suggest testing additives that interfere with amylopectin retrogradation in combination with others that are able to maintain masa machinability and tortilla textural and sensory quality.

Starch retrogradation is considered a time/temperature-dependent polymer re-crystallization process. Many constitutive models have been developed to describe the viscoelastic behavior of synthetic polymers based on their molecular structure, and to explain how crystallization of molecules leads to polymer aging. This study aims to apply these molecular models to elucidate if some anti-staling ingredients work by interfering with the retrogradation of amylopectin in corn tortillas during storage or by some other mechanism.

This thesis follows the style and format of Cereal Chemistry.

Goal Objective

Improve the texture and shelf-stability of commercial corn tortillas by understanding the mechanisms behind the anti-staling properties of CMC, amylases and vital wheat gluten with the help of polymer aging theory.

Specific Objectives

1. Evaluate the anti-staling properties of bacterial maltogenic and non-maltogenic amylases on corn tortillas.
2. Determine the combination of CMC, amylase and vital wheat gluten that provides the softest, more flexible and less rubbery tortilla texture, and the longest shelf-stability.
3. Determine the effect of storage temperature on the staling rate of tortillas.

CHAPTER II

LITERATURE REVIEW

Corn Tortillas

Tortillas are flat, unleavened breads made from either corn or wheat. Corn tortillas, or "tlaxcallim," were the principal food of the meso-american civilizations. Today, corn tortillas are made from either corn cooked in a lime-based solution or by re-hydrating nixtamalized corn flour to produce masa, sheeting, forming and baking. (TIA 2002).

The Corn Tortilla Market

According to TIA (2002), tortilla is the fastest growing segment of the baking industry worldwide. North American and European tortilla markets continued to grow in 2002, with US sales estimated at more than $6 billion, up from $4.4 billion in 2001.

Approximately 44% ($2.64 billion) of US sales correspond to corn tortillas and 56% ($3.36 billion) to wheat tortillas. However, a survey conducted by Aspex Research in the U. S. on 2000 confirmed that corn tortilla sales have been consistently growing faster than wheat tortilla sales during the past five years, indicating that eventually corn tortillas will outsell wheat tortillas.

Corn table tortillas production has grown 57% within the last four years, with California being responsible for 39% of the U.S. total production (TIA 2002), followed by Texas, Colorado, Illinois, New Mexico and Georgia (Mabin 1999). The Tortilla Industry Association estimates that every U. S citizen consumes one tortilla per day.

The growing popularity of tortillas is attributed to the "bread-like" acceptance of tortillas by non-Hispanic cultures, and low costs, versatility, and healthy ingredients (Mabin 1999). Recent surveys indicate that corn tortilla consumers (specially Hispanics) want a “fresher” and “less rubbery” product, with fewer preservatives and a “just cooked flavor and aroma”. However, they also demand a long shelf life under refrigeration. Several processors (Mission Foods, etc) have endeavored in commercializing “fresher” tortillas (low preservatives) with lower shelf life (3-10 instead of 25-60 days) with not-so profitable results (TIA 2002). Logistic obstacles, such as having a good chain of retailers close to the biggest markets have hampered this approach.

Starch and Starch Granule Organization

Starch consists of two main polysaccharides, amylose and amylopectin. Both polysaccharides are based on chains of 1-4 linked α-D-glucose but whereas amylose is essentially linear, amylopectin is highly branched containing on average one branch point, which is 1-6 linked for every 20-25 straight chain residues. Most starches, corn included, contain between 20 and 25% amylose although some waxy starches contain very little, if any, amylose (<1%) (Parker and Ring 2001).

Typical molecular weights of extracted amylose are 10^5 to 10^6. In aqueous solution the amylose molecule behaves as a flexible coil with a hydrodynamic radius of 7-22 nm (Buleon et al.. 1998).

Amylopectin is one of the largest biopolymers known with typical molecular weights being in the region of 10^8 g/mol and a hydrodynamic radius of 21-75 nm. The branching of amylopectin is not random (Thompson 2000). There is a bimodal population of chains with two main populations with peak DP of 12-14 and 45 (Hizuruki 1985). The short chain fraction is the most abundant by weight and number.

The structure of different amylopectins is generally characteristic of a particular species. Current models (Buleon et al. 1998) of amylopectin structure depict short linear chains, 10 to 20 units long, arranged in clusters on longer chains, with the longer chains spanning more than one cluster. Typically, the cluster model is a two dimensional representation of a structure which must pack in the starch granule to account for a density of 1.5 g/cm^3.

The starch granule is a very complex macromolecular assembly whose exact structure has not been yet fully elucidated. Starch occurs naturally as water-insoluble granules whose form is characteristic of its botanical origin. When viewed under polarized light the granules are birefringent. As the radial refractive index is larger than the tangential refractive index a preferred radial distribution of chains is indicated (Buleon et al. 1998).

The starch granule is partially crystalline with crystallinities in the region of 30% being reported. A number of crystalline forms are known, the A form (Imberty et al. 1988) which is found in most cereal starches, including corn, consists of starch double helices packed in a monoclinic array. The B form (Wu and Sarko 1978), which is found in tubers, high amylose and retrograded cereal starches, is a more highly hydrated and open structure, consisting of double helices packed in an hexagonal array.

As waxy starches, containing only amylopectin, still have crystalline granules the participation of amylopectin chains within the crystalline domains is indicated. Examination of the products of acid etching of the starch granules has shown that the length of chain participating in the crystalline domains is comparable to the short chain fraction of amylopectin. Hence the suggestion that it is the short chains (DP 12-20) of amylopectin which form the double helices that originate the crystalline areas of the starch granule (Guilbot and Mercier 1985).

X-ray and neutron scattering experiments on starch granules have revealed a model of repeating amorphous and crystalline layers (Jenkins et al. 1998, 1993). A recent interpretation of this proposed lamellar structure is that the amylopectin forms a side chain liquid crystal structure (Waigh et al. 2000). Some areas that remain a focus of discussion are how is the amylose arranged, what is the significance of the variability of amylopectin structure, and the lengths of the chains of amylopectin, to its organization in the granule (Parker and Ring 2001).

Production of Corn Tortillas with Nixtamalized Corn Flour

Traditionally, corn tortillas are made by nixtamalizing the grain (cooking and steeping in a calcium hydroxide solution), washing and grinding with volcanic stones to produce masa. The masa may be fine or coarse, depending on the product characteristics, and it is sheeted and then baked in a three-tier oven. Lately, the use of nixtamalized corn flour (NCF) for production of tortillas has increased dramatically, due to advantages such as product flexibility, uniformity in the product, reduction in equipment and labor costs, and reduced sewage costs (Serna-Saldivar 1996).

Nixtamalized corn flour, also known as dry masa flour (DMF), is the product of controlled grinding and particle size formulation of corn that has been alkaline-cooked, washed, ground and dried. Flour is sieved into various particle sizes and reformulated according to particular specifications.

The re-hydrated NCF is less cohesive and elastic than fresh masa because of additional drying and grinding performed on the NCF process, which produces more mechanically damaged, gelatinized and retrograded starch in the intermediate and smaller particle size fractions. Therefore, products made from NCF stale at a faster rate than products made with fresh ground masa (Gomez et al. 1991). Addition of hydrocolloids, such as sodium carboxy-methyl cellulose (CMC) at 0.25-0.5% levels to NCF has helped overcome these deficiencies (Serna-Saldivar 1996).

NCF is normally reconstituted with water to produce masa and then processed into tortillas following the traditional sheeting and baking procedure (Almeida-Domínguez 1996). Tortillas are then cooled using wire belts and packaged in low-density polyethylene bags in stacks of 30 or 50 units. In Texas and the West Coast, the most popular package sizes are 50, 36 and 100 tortillas per bag, in that order (TIA 2002).

Corn starch changes during tortilla processing with NCF

Starch granules in raw corn exhibit birefringence. After nixtamalization, the majority of the starch granules are swollen, adhered to other granules, and exhibit partial or total birefringence. At this point, only 2% of starch is fully gelatinized and 15-25% is damaged (Gomez et al. 1991).

Masa grinding disrupts the grain structure, releasing starch granules from the endosperm cells and dispersing cellular components and starch polymers. Masa is a network of solubilized starch polymers supporting dispersed, native and partially gelatinized starch granules, cell fragments, and lipids (Gomez et al. 1990). Further starch damage (32-36 %) and gelatinization (<5%) occur during grinding due to a combination of previously damaged and swollen starches, high water content (51%), physical shear and warm to high temperatures (50-60 ^{o}C) (Gomez et al. 1992).

Additional drying and grinding performed on the NCF process produces more mechanically damaged, gelatinized and retrograded starch in the intermediate and smaller particle size fractions (Gomez et al. 1991) than in fresh masa. The net effect is a reduction in the total amount of enzyme-susceptible starch (29%) in NCF compared to fresh masa (55%). This factor becomes important when determining optimum levels of addition of amylases in NCF versus fresh masa.

Tortilla baking results in grain components being set into a three dimensional structure. Starch granules and endosperm pieces are glued together by amylose, protein, lipids and cell wall components (continuous phase). Starch gelatinization occurs during the 45-60 sec of baking time, making tortillas reach an internal temperature close to 91-93 ^{o}C (Aida et al. 1996) and yielding tortillas with approximately 12.5% gelatinized starch (Gomez et al. 1992) and 60% enzyme-susceptible starch .

Staling of Corn Tortillas

The process and mechanism of staling differs between maize tortillas and bread. Textural changes in tortillas occur faster. However, these textural changes can be reversed when tortillas are reheated. This is the reason why tortillas can be stored for long periods of time. Bread, on the other hand, becomes stale more slowly and these changes are only partially reversible upon reheating. Starch retrogradation is the most important reason for loss of tortilla texture, while in bread moisture migration from gluten to starch is also responsible for staling. Higher starch concentration and lower fat content of tortillas are also responsible for the increased staling rate compared to bread (Campas-Baypoli et al. 2002).

The quality of a corn tortilla changes dramatically within the first 24 hr and then shows smaller changes for the reminder of its shelf life (Fernandez et al. 1999, Limanond et al. 2001). Tortilla staling is identified by a gradual decrease in rollability and pliability, a gradual increase in firmness, and a more friable and brittle structure (Friend et al. 1992). The increase in firmness has probably been used to the largest extent to quantify staling in corn tortillas (Suhendro 1997; Quintero-Fuentes 1999; Yeggy 2000; Limanond et al. 2001).

Starch plays a significant role in staling and retrogradation of corn tortillas (Fernandez et al. 1999). The re-crystallization of gelatinized starch, also called starch retrogradation, is believed responsible for the texture changes that take place during the storage of corn tortillas and other starch-based systems. Most researchers attribute the changes in firmness of tortillas mainly to the physicochemical reactions of the starch components, specially the amylopectin fractions (Schoch and French 1947, Kulp and Ponte 1981). Therefore, anti-staling agents are substances that interfere in one way or another with the re-association of amylose, amylopectin or both.

During baking, starch granules gelatinize, that is their native crystalline structure is disrupted, but still maintains their granular identity (Hugh-Iten et al. 1999). The two starch polymers, amylose and amylopectin, tend to separate due to their thermodynamic immiscibility (Kalichevski and Ring 1987). Phase separation of the two starch polymers leads to the accumulation of amylose within the starch granules, but also in the intergranular space in the form of double helices (Conde-Petit et al. 1998).

Right out of the oven, amylose retrogrades very quickly, stabilizing the initial structure forming a more rigid, insoluble network. This process is thermo-reversible at 153 ^{o}C (Ring et al. 1987). Therefore, amylose retrogradation cannot be reversed even after normal reheating.

Retrogradation of amylopectin involves a crystallization process of the outer branches (DP 12-20). On examination of the behavior of amylopectins from different botanical sources it was observed that the bigger the abundance of the short chain fraction of amylopectin the greater the tendency to retrograde and crystallize was (Kalichevski et al. 1990). Wheat amylopectin, which has a relatively short, short chain fraction, shows a reduced tendency to retrograde than corn amylopectin (Shi and Seib 1992). Schiraldi et al. (1996) found that enthalpies of the endothermic transition for corn tortillas stored for 2 h were similar to the enthalpies of bread stored for 24 h at a similar temperature, indicating a faster retrogradation in tortilla than in bread.

In maize, amylopectin retrogradation was found proportional to the amount of short chains having a DP of 16-30 and inversely proportional to the level of short chains with a DP of 6-11 (Shi and Seib 1995). Treatment of starch with alpha and beta amylases shortens the short chain fraction and reduces the rate of retrogradation.

In contrast to amylose, the crystallization of amylopectin is a slow process continuing over several days or weeks (Miles et al. 1985). While re-crystallized amylopectin melts in the temperature range 45-64 ^{o}C (Campas-Baypoli et al. 2002), amylose crystallites do so only at much higher temperatures (120-170 ^{o}C) (Eerlingen, Jacobs & Delcour, 1994).

Amylopectin binds water and associates slowly, developing more perfect crystals than amylose, as staling progresses (Gudmundsson 1994), but after 24 hr storage yields brittle, less flexible tortillas (Fernandez et al. 1999). Since most normal starches are 70-80% amylopectin, their gelatinization and retrogradation processes are dominated by the non-equilibrium melting and recrystallization behavior of amylopectin, although combinations due to amylose are observed (Levine and Slade 1991).

Retrogradation produces crystalline forms that are different in nature from those present in the native starch granules. This is confirmed by changes in X-ray diffraction pattern from A-pattern in native cereal starches to a B-pattern in retrograded starches (Collison 1968).

Amylopectin re-crystallization is associated with the development of stiffness of the product and typically, at high water contents such as in tortillas, takes days or weeks to reach a plateau value (Suhendro 1997, Fernandez et-al 1999, Limanond et al. 2001). At the end of this time the extent of crystallinity of the amylopectin is comparable to that found in the native starch granule (around 30%) (Parker and Ring 2001).

The rigid crystalline (retrograded amylopectin) and the amorphous mobile components exist simultaneously in the system even after a relatively long storage time, where retrogradation reaches equilibrium. These observations are in agreement with the "fringed micelle" model used widely to describe partially crystalline synthetic polymers (Wunderlich 1976) and later to describe starch gels (Levine and Slade 1988).

Time and Temperature Dependence of Starch Retrogradation

The magnitude of staling is a function of aging time and temperature. The retrogradation phenomenon has been described as a non-equilibrium polymer crystallization process in starch-water polymer melts (Levine and Slade, 1990). Theories of polymer crystallization may provide fundamental understanding and further modeling of the retrogradation process of corn tortillas.

The rate of retrogradation of gelatinized waxy maize starch (Farhat et al. 2000), white bread (Russell 1985), cakes (Guy et al. 1983) and corn tortilla (Limanond et al. 2001) show a “bell-shaped” dependence on storage temperature in a range between T_g and T_m of the product. This behavior is in agreement with the general theory of crystallization, where the effect of temperature on the rate of crystallization is the result of its net effect on the nucleation and propagation rates, as reviewed by Levine and Slade (1990).

Maximum rate of retrogradation for cakes was at 25 ^oC (Guy et al. 1983), for corn tortillas was at 13 ^oC (Limanond et al. 2001) and for bread occurred at 4 ^oC (Russell 1985). Compared to storage at room temperature, storage of starch gels containing 45-50% water (like corn tortillas) at low temperatures but still above the glass transition temperature (Tg $\simeq$ -5 ^oC), increase retrogradation, especially during the first days of storage (Gudmunsson 1994). Storage at freeze temperatures below Tg virtually inhibits re-crystallization (Gudmunsson 1994) Higher temperatures (above 32 – 40 ^oC) effectively reduced retrogradation of wheat starch gels (Colwell et al. 1969) and corn tortillas (Limanond et al. 2001).

When a tortilla is baked in the oven, it transforms into an amorphous, rubbery material. A fresh tortilla, right out of the oven is a partially crystalline system due to retrograded amylose (Campas-Baypoli et al. 2002). Apart from the amorphous phase (amylopectin) it contains crystal nuclei (retrograded amylose) in the rubbery matrix (continuous phase). Below its T_g (-23 ^oC, according to Limanond et al. 2001) the amorphous phase is glassy, the composite material, therefore, will show the same aging behavior as a purely amorphous polymer.

Unlike amorphous polymers, however, semi-crystalline polymers age (re-crystallize) at temperatures above the T_g. Since the polymer chains adhere to the filler particles (amylopectin crystal nuclei created during retrogradation), the segmental mobility near the particle's surface will be reduced. Only far away from the particles, the mobility and other properties of the rubbery matrix will be equal to those of the pure rubber (tortilla in the oven). This implies that the glass transition of semi-crystalline materials (tortilla) will be broadened. The amorphous regions will have the same T_g as the fresh tortilla, but that of areas including crystallized (retrograded) regions will be higher (Struik 1978).

The broadening of the glass transition temperature explains why above the T_g of the bulk amorphous polymer (non-retrograded amylopectin) the composite possesses considerable viscoelasticity accompanied with aging. In conclusion, aging occurs in a wide range of temperatures above T_g and below T_m. When the polymer ages, its stress relaxation curve shifts along the time scale and the stiffness of the material increases (Struik 1978, Limanond et al. 2001).

Limanond et al. (2001) studied the crystallization rate of corn tortillas in the 6-35 ^{o}C storage temperature range. The crystallization rate (k) from the modified Avrami-nucleation model increased from 6 to 20 ^{o}C and started decreasing from 20 to 35 ^{o}C. The maximum crystallization was observed at 12.3 ^{o}C based on stiffness data from stress relaxation tests. As is known, the temperature dependence of the rate of formation of nuclei and the rate of crystal growth of polymer crystallization are bell shaped. Since the sub-ambient storage temperatures were much closer to the T'_g (-23 ^{o}C) than to T_m (90 ^{o}C) it was concluded that the tortilla crystallization process is strongly nucleation-limited.

In other words, tortillas stale at a slower rate when stored at room temperature (25 ^{o}C) than when refrigerated (4 ^{o}C). Limanond et al. (2001) states that the glass transition temperature of tortillas due to amylopectin crystallization increased from 39 to 48 ^{o}C after 48 hours of storage at room temperature. Most likely, this is the melting temperature of amylopectin increasing during storage, although Roos (1995) has suggested that increasing crystallinity in partially crystalline polymers increases the glass transition temperature of amorphous regions.

Tg is affected by the history of the sample (rate of cooling, time under storage, etc.), and depends upon its material composition and water content. The presence of large molecules like gums, improve tortilla flexibility by lowering the Tg (Cauvain 1998). Torres et al. (1993), found that the addition of CMC to wheat-flour tortillas containing 20% sorghum flour significantly decreased staling.

Antistaling Agents

Previous studies on corn tortilla shelf stability agree there is no single additive that significantly delays staling without causing a detriment in tortilla texture or its sensory quality.

Carboxymethylcellulose (CMC)

Sodium carboxymethylcellulose (CMC) is currently the most popular antistaling agent used in commercial tortillas. CMC is a linear, long chain, cold or hot water-soluble, anionic, chemically modified, cellulose ether (Keller 1986).

Purified cellulose from wood or cotton (DP 1000-2000) is converted to alkali cellulose by adding NaOH, a catalyst of the subsequent etherifying reaction using chloroacetic acid. In a cellulose molecule there are three hydroxyl groups available for etherifying per anhydrous glucose unit (AGU). The average number of hydroxyls substituted per AGU in a CMC molecule is known as the degree of substitution (DS), a key aspect in characterizing cellulose ethers solubility. With three OH groups present, the maximum DS is three. CMCs with low DS (0.7) provide thixotropy to aqueous systems (Feller and Wilt 1990).

Most polymers have a product code that is related to their major properties. For example, CMC 7HF from Aqualon has a DS of about 0.7 (indicated by the 7), a high viscosity (H) at 1% concentration (1500-2500 mPa.s) and a fine particle size (F).

CMC has a lower DP than cellulose, ranging from 50 (low viscosity) to 1000 (high viscosity). CMC 7HF has a 910 DP so it is considered a high viscosity polymer. The crystallinity of cellulose tends to be destroyed by treatment with alkali, so CMC is amorphous and rarely exhibits crystalline morphology or the fiber structure of cotton (Feller and Wilt 1990).

Solutions of the polymer are stable in the range of pH 4 to 10 (pH of commercial tortilla is 4.7-5.5). CMC is highly soluble in water due to its anionic character and possesses excellent temperature stability, which makes it suitable for baking (Feller and Wilt 1990).

When added in aqueous solutions at concentrations of 0.25% or higher CMC chains overlap causing formation of an amorphous network structure. With higher polymer concentration the polymer-polymer interactions (entanglements) become the main factor influencing the rheology of the CMC solution (Florjancic et al. 2002).

When CMC is mixed with a gel-forming material with tendency to cross-linking such as Xanthan gum, the rheological properties of the resulting network will depend on the relative concentrations of both polymers.

When low levels of Xanthan are mixed with CMC, the presence of linear unbranched CMC chains inhibits formation of extended junction (cross-linked) zones and induces the formation of small clusters of xanthan as a dispersed phase, surrounded with entangled (amorphous) CMC chains.

At a high content of Xanthan, the dispersion of small gel clusters display solid-like behavior at low stresses, and its rheological properties cannot be easily distinguished from those of weak gels (Florjancic et al. 2002). This example might be useful in understanding the way CMC maintains flexibility of tortillas by mixing with amylose in the continuous phase. Both molecules are essentially linear and have similar molecular weights (4.35×10^5 g/mol for CMC 7HF). However, amylose tends to re-crystallize very fast and CMC does not.

The recommended level for corn tortillas varies from 0.25-0.5% (Serna Saldívar et al. 1990). CMC improves tortilla texture, eliminates stickiness of packaged tortillas, increases yield and improves freeze-thaw stability. Suhendro (1997), Quintero-Fuentes (1999) and Yeggy (2000) reported that CMC increases rollability and extensibility of tortillas during storage. However, tortillas with 0.5% CMC tend to have a rubbery texture regarded as undesirable by some consumers (Quintero-Fuentes 1999). It is believed that CMC does not retard starch retrogradation of tortillas during storage. Instead, it creates a more flexible structure in the tortilla.

Vital wheat gluten

Wheat gluten has been evaluated as an antistaling agent in corn tortillas. Yau et al. (1994), after testing a series of additives added to wet masa, reported that a mixture of 0.5% CMC, 2% gluten and 3% sorbitol extended storage stability of tortillas to 12 days; compared to a three-day shelf life of control and 7 days for tortillas with 0.5% added CMC. Apparently, gluten modified the structure of masa and baked corn tortillas and had a synergistic interaction with CMC and starch. Gluten incorporation over 2% increased the number and size of burned spots in corn tortillas.

Lipids and surfactants

Polar lipids, e.g. monoglycerides, and related compounds are known to have an anti-staling effect on bread and extend its shelf life (Krog and Jensen 1970). There is evidence that interactions of amylopectin and lipids are negligible (Kugimiya et al. 1982). Lipids/surfactants retard retrogradation in bread by complexing with leached amylose on the surface of starch granules and possibly by acting as a barrier against water transport (D'Appolonia and Morad 1981). Studies on the antistaling properties of lipids in corn tortillas are scarce (Arambula-Villa 2001, Bueso et al. 2001). Arambula-Villa (2001) found that tortillas with 0.5% non-polar corn masa lipid fraction were more rollable than control after 24 h of storage. However Bueso et al. (2001) did not find a significant anti-staling effect of neutral lipids in corn tortillas when added at levels up to 2%. Higher levels of lipids significantly reduced tortilla pliability.

Barley Flour and β-glucans

β-Glucans consist of linear unbranched polysaccharides of linked β-(1-3)- and β-(1-4)-D-glucopyranose units that form "worm-like" cylindrical molecules containing up to 250,000 glucose residues ($6 \times 10^4 – 6 \times 10^6$ MW). β-Glucans, especially low molecular weight ones ($6 \times 10^4 – 9 \times 10^4$ MW), tend to form thermo-reversible, pseudoplastic gels by entanglement and cross-linking; as shorter chains rearrange easier to maximize linkages. These arrangements make β-glucans more soluble in water than cellulose (Jezquel 1998). Dehulled barley contains 33 g/kg dm of β-Glucans , while corn and sorghum have 1g/kg dm.

The potential of β-glucans from barley as anti-staling agents is being studied. Corn tortillas with 20% barley flour showed higher rupture distance (extensibility) than control tortillas after 9 days of storage at 4 ^{o}C (Mitre-Dieste 2001). Anti-staling properties of purified β-glucans is currently being evaluated.

Defatted soy flour

Reheated tortillas with 5% native defatted soy flour and 0.5% CMC stored for three weeks under refrigeration (4 ^{o}C) have shown significantly higher flexibility (subjective bending test), extensibility (rupture distance) and required less force to rupture than the control. Soy extracts and isolates were less effective anti-staling agents than native soy flour. Tortillas with native soy flour developed more air tunnels during baking. During storage, soy flour appeared to interfere with tortilla retrogradation, maintaining the flexibility of the continuous phase matrix and limiting crumb contraction.

Soy protein molecules are believed to combine with the retrograded amylose matrix to make it more flexible (Suhendro et al. 2001). Tortillas with 5% soy flour had acceptable sensory properties but tended to have more brown spots than tortillas with 0.5% CMC or without additives.

Conventional vs. maltogenic amylases

According to the classification of amylases, the α-amylase family (glycoside hydrolase family 13) is one of five structural families of starch-degrading hydrolases and includes endo-type enzymes specifically catalyzing the cleavage of the internal α-D-1, four glycosidic bonds of starch, and various oligosaccharides. Pullulanase cleaves the internal α-D-1, 6 glycosidic bonds of the substrate pullulan and amylopectin. Glucoamylases and β-amylases are exo-type enzymes cleaving glucose and maltose units, respectively, from the non-reducing end of starch materials by hydrolyzing α-D-1, 4 glycosidic bonds (Kim et al. 1999).

Several groups of starch-hydrolyzing enzymes are known to harbor more than single enzyme activity. One group of these, maltogenic amylases, exhibit unique characteristics that are different from other α-amylases in that they exhibit (i) a dual activity of α-D-1, 4 and α-D-1, 6-glycosidic bond cleavages that yield maltose; (ii) an activity of α-D-1, 4- to α-D-1, 3-, α-D-1, 4-, or α-D-1, 6-transglycosylation that generates oligosaccharides of DP 3-6; and (iii) an activity of cleaving acarbose, a pseudo-tetrasaccharide competitive inhibitor of α-amylases (Kim et al. 1999).

Some of these properties of maltogenic amylases, if not all, are shared by two other amylolytic enzymes with different names, including neopullulanases and cyclomaltodextrinases, both of which are homologous to maltogenic amylases with sequence identity of 40-86%. These three groups of amylases have intra-cellular activity in bacteria (*Bacillus sp*. and *Thermus sp*) and fungi (A. oryzae and P. expansum), unlike typical commercial α-amylases and pullulanases from *Bacillus subtilis* (Fresh-N® from EDC) and *Aspergillus* (Enzeco® from EDC) which have extra-cellular activity (Park et al. 2000).

The three groups of versatile amylases are high molecular weight (62-90 kDa for the monomers) amylases because of a unique addition of 130 residues at the N terminus compared with the conventional α-amylases containing the single activity of hydrolyzing α-D-1, 4-glucosidic bonds. This addition is the binding site for cyclodextrins and branched oligosaccharides, and the host for transglycosylation (Kim et al. 1999).

Maltogenic amylases like Novamyl® (from *Bacillus stearothermophilus*, 67 kDa per monomer) are normally in dimeric form in aqueous solution (Abe et al. 1996) unlike conventional α-amylases.

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

Amylase activity is expressed in activity units (AU), defined as the amount of enzyme (g or mg) necessary to release 1 g or mg of glucose equivalents from the substrate per unit of time (hr or 30 min) (Doyle et al. 1999).

Maltogenic amylases as antistaling agents

Glycosyl hydrolases (amylases) can act as antistaling agents (Kulp and Ponte 1981). The addition of amylases retards the firming of bread (Martin and Hoseney 1991) and inhibits the retrogradation of amylopectin as measured by DSC (Defloor and Delcour 1999). Dragsdorf and Varriano-Marston (1980) found that reduced firmness of bread with amylases supplementation correlated with decreased levels of starch crystallinity.

Amylases hydrolyze α-1,4 linkages within the amorphous region of the starch matrix during baking (Zobel and Senti 1959). Conventional α-amylases derived from bacterial (*Bacillus subtilis*) or fungal (*Aspergillus oryzae*) sources are not well suited for this purpose due to excessive or insufficient thermo stability, respectively (Hebeda et al. 1990).

Two different theories may explain why enzymes extend shelf-stability in baked products: 1) The shortening of amylopectin chain length by enzymes reduce retrogradation tendencies of amylopectin (Boyle and Hebeda 1990) and 2) It is the oligosaccharides (DP 2-7) produced by the enzymes that are themselves antistaling agents (Martin and Hoseney 1991).

In a detailed examination of the retrogradation of maize amylopectins retrogradation was directly proportional to the amount of chains of DP 16-30 and inversely proportional to the level of chains of DP 6-11 (Shi and Seib 1995). Treatment of starch with β-amylase (an exo-acting enzyme) shortened amylopectin chains and reduced the rate of retrogradation (Wursh and Gumy 1994). However, Gerrard et al. (1997) contend that staling rate was not related to the presence of dextrins in a specific size class and that these dextrins are just symptomatic of a modification to the starch that retards staling.

Barley malt (Suhendro 1997), bacterial (Suhendro 1997, Quintero-Fuentes 1999), and fungal α-amylases (Aida et al. 1996, Suhendro 1997) have been evaluated as antistaling agents on corn tortillas. Aida et al. (1996) found that addition of a conventional fungal α-amylase blend (10 AU g-1) extended shelf life of corn tortillas according to 75 panelists. However, Suhendro (1997) reported that low levels (0.0005%) of either bacterial or fungal amylases had a detrimental effect on masa characteristics and machinability, as well as tortilla rollability. Therefore, additive (s) that can increase viscosity and create a new network of viscoleastic structure to compensate for the weakened structure affected by the enzymes were needed. Suhendro (1997) found that a combination of 0.25-1% CMC and 0.005-0.01% barley malt produced masa with improved machinability and tortillas with better rollability than control after 12 days of storage.

Intermediate temperature stability (ITS) maltogenic enzymes, which have an optimum temperature range of 65-80 ^{o}C, were effective as antistaling agents for wheat dough systems (Hebeda et al. 1991). Maltogenic amylases have been tailored by genetic engineering to exhibit its maximal activity at about 80 ^{o}C, but with a level of 60% activity at room temperature (Fitter et al. 2001). This type of enzyme would be adequate for the tortilla system, since the rest period of masa and baking time are very short (10 min and 1 min, respectively) compared to bread. Therefore, the enzyme should hydrolyze amylopectin during the rest period at a higher rate than regular enzymes and could be inactivated before the tortilla comes out of the oven.

Novamyl® 1500 MG removes oligosaccharides in the DP 2-7 range from amylopectin and amylose; it does not cause gumminess as other bacterial amylases. Miranda (1999) used Novamyl® 1500 MG at levels of 0.04 % (600 maltogenic amylase units, MAU, per kg of NCF) in corn tortillas. Novamyl® activity was optimum at pH 5 and tortillas stored under refrigeration were more rollable and pliable than control; but they required more extension force to break. Miranda (1999) suggested that combinations of amylase and other additives, such as CMC and wheat gluten should reduce tortilla staling to a larger extent than using amylase alone.

Suhendro (1997) suggested that interaction effects between potential additives (gums, amylase, shortening, emulsifiers and gluten) that can improve corn tortilla texture need evaluation.

Methods for Studying Starch Retrogradation

Methods to study starch retrogradation can be classified as: (1) macroscopic techniques, i. e. those methods which monitor alterations in certain physical properties as manifestations of retrogradation, for example, mechanical and textural changes (rheological techniques, sensory evaluation of texture, DSC and light scattering), and (2) molecular techniques, which study changes in starch polymer conformation or water mobility in starch gels at molecular levels (X-ray diffractometry, nuclear magnetic resonance spectroscopy) (Karim et al. 2000).

Subjective and objective methods are commonly used to monitor changes in corn tortilla texture during storage and to monitor the effects of additives (CMC, barley malt, gluten, α-amylases and waxy cereal flours) (Suhendro 1997, Quintero-Fuentes 1999, Yeggy 2000). Suhendro (1997) developed and evaluated five objective corn tortilla texture measurement techniques (objective rollability, bending, tensile strength, puncture and stress relaxation). The objective rollability, bending and tensile techniques were simple and fast to run and correlated well to subjective rollability and flexibility scores. These techniques were sensitive to differences in corn tortilla texture due to storage time and additives.

The tensile technique only takes 15 s to run. In contrast, the stress-relaxation technique required a longer time to run (180 s). However, stress relaxation provided fundamental information on the viscoelastic properties of corn tortillas, which was not provided by the other techniques. Yeggy (2000) found that energy dissipated, a viscoelastic behavior parameter obtained from the stress relaxation test, correlated significantly with the subjective bending and pliability parameters and objective texture techniques. Guo (1998) recommended the 7-element Maxwell model to fit experimental data when evaluating corn tortilla under tension. Stiffness (Pa), relaxation moduli and energy dissipated (J/m^3) were the best parameters to predict the texture properties of corn tortilla.

Limanond et al. (2001) measured changes in tortilla viscoelasticity of corn tortillas during storage with stress relaxation. Tortillas received a 3% strain (linear viscoelasticity region) and force (N) required to maintain the strain was recorded for 180 s. Final stiffness (Y), also known as Young's equilibrium modulus was calculated with the 7-element Maxwell model and fed into the Avrami-nucleation model to estimate the rate (k) and degree of crystallization (X) of corn tortillas over a 3-day storage period at different temperatures (6-35 ^oC). The stress relaxation technique successfully detected textural differences between corn tortillas at various storage times and temperatures. The Avrami-nucleation model with final stiffness data was adequate to describe the staling of tortillas at the practical temperature range (6-30 ^oC).

3^{k-p} Fractional Factorial Experiments

The main justification for using three-level fractional factorial designs is run size economy. Take, for example, the 3^3 designs in 27 runs. Unless the experiment is not costly, it is more efficient to use one-third of the 3^3 design (Wu and Hamada 2000).

Response Surface Analysis

Modeling curvature effects can be very important when the objective of an experiment is to identify the combination of levels of the quantitative factors that leads to an optimum response. Response surface experiments can be used for this purpose (Neter et al. 1996). Response surface designs are generally used in the latter stages of an investigation, when five or fewer factors (ingredients, conditions) are under investigation.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for design, development, and formulation of new products, as well as in the improvement of existing products. Many product design and improvement involve formulation problems, in which two or more ingredients are mixed together. In such cases, the response variables of interest (tortilla rollability, stiffness, etc) in the product are a function of the proportions of the different ingredients used in its formulation (enzymes, gums, gluten, etc). This is a special type of response surface problem called a mixture problem (Myers and Montgomery 2002).

In general, the experimenter is concerned with a product involving a response y that depends on the controllable input variables $\xi_1, \xi_2, \xi_3 \ldots \xi_k$.
The relationship is

$$Y = f(\xi_1, \xi_2, \xi_3 \ldots \xi_k) + \varepsilon \tag{1}$$

Where the form of the true response function f is unknown and perhaps very complicated, and ε is a term that represents other sources of variability not accounted for in the surface response model *f*. "ε" will be considered as statistical error, assuming it to have a normal distribution with mean zero and variance σ^2.

The variables $\xi_1, \xi_2, \xi_3 \ldots \xi_k$ in equation (1) are usually called the natural variables, because they are expressed in the natural units of measurement (grams, degrees Celsius, Activity Units, etc). In RSM work it is convenient to transform natural variables to coded variables $x_1, x_2, x_3, \ldots, x_k$, which are dimension-less with mean zero and the same spread or standard deviation (-1,1).

Successful use of RSM is critically dependent upon the experimenter's ability to develop a suitable approximation for *f*. Usually; a low-order polynomial in some relatively small region of the independent variable space is appropriate. In many cases, either a first-order (linear) or a second-order (quadratic) model is used.

The first-order model is more suitable when the experimenter is interested in approximating the true response surface over a relatively small region of the independent variable range in a location where there is little curvature in *f*.

Often the curvature in the true response surface is strong enough that the linear model (even with the interaction term included) is inadequate. The second order (quadratic) model is widely used in RSM because it is very flexible, so it will often work well as an approximation to the true surface response. Also, there is considerable practical experience indicating that they work well in solving real response surface problems (Myers and Montgomery 2002).

Central Composite Designs

The central composite designs (CCD) are without a doubt the most popular class of second-order designs used in RSM. It was introduced by Box and Wilson (1951).

A CCD consists of the following three parts:

a) n_f cube points (or corner points) with x_i = -1,1 for i = 1,…., k. They form the factorial portion of the design.
b) n_c center points with x_i = 0 for i = 1,…., k.
c) 2k star points (or axial points) of the form (0,…, x_i,…, 0) with x_i = α, -α for i = 1,…,k.

How small can a fractional factorial design of the form 2^{k-p} for the factorial portion be so that the resulting CCD is a second-order design? The total number of parameters in a second order model is (k+1)(k+2)/2. Therefore the total number of distinct design points in a CCD, N = n_f+2k +1, must be at least (k+1)(k+2)/2.

For k = 3 (amylase, CMC and gluten), either the 2^{3-1} or the 2^3 design can be chosen for the factorial portion. If the 2^{3-1} design is chosen, then n_f = 4 and N = 4 +2(3) + 1 = 11 experimental units in the CCD. Since (k+1)(k+2)/2 = 10 for k = 3, this CCD covers the minimum number of experimental units required to calculate the parameters of a second-order model (Wu and Hamada 2000).

CHAPTER III

MATERIALS AND METHODS

Raw Materials

The raw materials used in this study are listed in table I.

Table I

Sources of Raw Materials and Range of Levels Used in Formulas

Ingredient	g/kg NCF (dry basis)
Nixtamalized corn flour (NCF)	1000 g
(Tortilla #4 with no additives. Minsa, Red Oak, IA)	
Fumaric acid powder (Balchem Co. Slate Hill, NY)	4
Potassium sorbate (ADM Arkady, Olathe, KS)	5
Vital wheat gluten (Midwest Grain Products Inc.,	0-20
Atchinson, KS)	
Novamyl 1500 MG, bacterial maltogenic amylase	0.08-0.4
(Novozymes North America, Franklinton, NC)	
Bacterial maltogenic amylase (Innovative Cereal Systems,	0.08-0.4
Wilsonville, OR)	
Fresh-N (Enzyme Development Co, NY, NY)	0.15
Xylanase (Enzyme Development Co, NY, NY)	0.15

Enzymes

Two maltogenic amylases (Novamyl® from Novozymes and an ICS amylase), one conventional bacterial amylase (Fresh-N) and one Xylanase (Enzeco Xylanase S200) were evaluated as anti-staling agents in this study.

A detailed description of the maltogenic amylases can be seen in table II.

Table II

Characteristics of Maltogenic Amylases

Enzyme	Origin	Mol Mass	Activity	Optimum		[a]Substrate Preference
		(kDa)	AU/g	(°C)	pH	
Novamyl	*Bacillus stearothermo*philus	69	1500	55	6	CD=MD>SS
ICS	*Bacillus subtilis*	69	11,000	40	6	CD=MD>SS

[a]CD, cyclodextrin, referring to the ∀-, ∃-, and (-CD; MD, maltodextrin; and SS soluble starch.

Fresh-N® is a conventional heat resistant ∀-amylase from *Bacillus subtilis* with an activity of 1000 AU/g. Recommended dosages are 0.1-0.4 g/kg NCF

Enzeco Xylanase BSX® is a xylanase preparation derived from *B. subitlis*. It is a powder standardized to 12,000 BXU/g. Its primary use is in baking and milling. Recommended levels are 0.1-0.9 g/kg NCF. Total arabinoxylan content of corn (43 g/kg dm) is lower than wheat (61-66 total and 11.8 g/kg dm soluble) and barley (76 total and 4.8 g/kg dm soluble).

CMC

Sodium carboxymethylcellulose (CMC) used in this study, with the trade name Blanose® 7HF cellulose gum, is a commercial product of Aqualon. The molecular mass determined by the producer is 4.35x 10^5 g/mol, with a degree of substitution in the range of 0.65-0.90, pH of 6.5-8.5, sodium fraction of 7-8.9% and an average viscosity of 2500 mPa.s at a 1% concentration (Florjancic et al. 2002).

Tortilla Preparation

Tortillas were prepared in the Cereal Quality Laboratory Pilot Plant at Texas A&M University. One kg of nixtamalized corn flour (NCF) was mixed with 5 g potassium sorbate, 4 g fumaric acid, CMC, amylases and vital wheat gluten for 5 minutes at low speed in a 20 qt mixer (Model A-200, Hobart, Troy, OH). Distilled water (1.2 kg/kg

NCF) was added and masa was formed with a hook for 30 s at low speed and 90 s at medium speed.

Masa was equilibrated in a polyethylene bag for 10 min before sheeting into 15 cm diameter, 30 g disks (Model CH4-STM, Superior Food Machinery, Inc., Pico Rivera, CA).

Tortillas were baked in a gas-fired three-tier (320 ^{o}C top, 270 ^{o}C middle and 220 ^{o}C bottom ^{o}C) oven (Model C-0440, Superior Food Machinery, Pico Rivera, CA) for 60 s, cooled and stored in polyethylene bags at temperatures ranging from –40 to 21 ^{o}C, depending on the type of study.

Starch Stabilization of Tortillas

Tortillas were stabilized with methanol for Differential Scanning Calorimetry (DSC) testing. A sample of 100 g of tortilla was mixed with 250 ml of methanol in a blender and ground for 2 min at maximum speed. The ground sample was filtered with vacuum using filter paper (Whatman #2) 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. Stabilized samples were stored at –40 ^{o}C until DSC testing.

Moisture and pH of Corn Tortillas

The moisture content of tortillas was determined by grinding the tortilla in a coffee grinder (Model KS M2, Braun Inc., Lynnfield, MA) for 45 sec, and drying 4 g of ground sample to constant weight in a forced-air oven at 105 ^{o}C for 48 hr (a variation of AACC method #44-15A 1995). The pH of tortillas was determined by using method #02-52 (AACC 1995).

Subjective Texture Evaluation of Tortillas

Subjective rollability and pliability of tortillas was evaluated 20 min, 1, 7, 14 and up to 21 d after baking.

Rollability was performed by rolling a tortilla around a 1 cm diameter dowel, and estimating the extent of cracking and breaking with a five-point subjective scale, defined as 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.

Squeezing a tortilla inside the palm of one hand, holding it for 2 s and then releasing it evaluated pliability. 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).

Objective Texture Evaluation of Tortillas

Stress relaxation (Limanond et al. et al. 2001) and 1-D extensibility (Suhendro et al. 1999) were performed on tortillas using a Texture Analyzer (model TA-XT2i, Texture Tech. Corp., Scarsdale, NY).

1-D extensibility

A tortilla strip (70*35 mm) was held between two tensile grips, with one end attached to the analyzer platform and the other end attached to the analyzer arm (Suhendro et al. 1999). The distance between the tensile grips was calibrated at 21.8 mm. During the test, the tortilla was pulled until it broke apart. The extensibility method was run using Texture Expert software in tension mode with the return to start option. The maximum force (N) and distance (mm) required to break apart the tortilla was calculated.

Stress relaxation

The stress relaxation method developed by Guo (1998) and modified by Limanond et al. et al. (2001) was used to determine the changes on final stiffness (Pa) and energy dissipated ($\mu J/m^3$) of tortillas during storage as a function of time and temperature.

A uniform tortilla strip (70 x 35 mm taken from the center of a baked tortilla) was clamped between two grips, with one end attached to the Texture Analyzer platform and the other end attached to the Texture Analyzer arm. The distance between the two arms was set to 21.8 mm. The Texture Analyzer system was set in the tension mode and the samples were tested at 3% strain levels (linear viscoleasticity region) for 180 sec. Pre and post-test speed was reduced to 0.5 mm/sec (compared to the 2 mm/sec speed used by Limanond et al. 2001) to avoid tortilla cracking in samples stored more than 3 d after baking. Test speed was 0.1 mm/sec.

The stress relaxation data (force as a function of time) were transformed into relaxation modulus, E, and then fitted to a generalized Maxwell model with seven parameters using a modification of the Matlab program developed by Spadaro (1996) and Guo et al.. (1999). Data were the transformed into compliance, stiffness and energy dissipated using Matlab software version 6.1 (Matlab 2001).

Further transformation into stiffness, Y, was carried out using Matlab Software (Matlab 2001):

$$Y(t) = \frac{\sigma_{ij}}{\varepsilon_{ij}} = \frac{(1/V) \int_V \sigma_{22} dV}{(1/V) \int_V \varepsilon_{22} dV},$$

Where σ_{ij} is the homogenized stress; ε_{ij} is the homogenized strain; V is the volume of the tortilla sample; σ_{22} and ε_{22} are the normal stress and strain acting in the plane perpendicular to x_2 in the direction of x_2, respectively (Insert figure). Stiffness is the ratio of homogeneous stress to the homogeneous strain, which may be referred to as the "modulus of elasticity" or "Young's modulus". This parameter indicates the hardness of materials. The higher value corresponds to a harder (firmer) material (more solid-like).

Differential Scanning Calorimetry (DSC)

Thermal analysis of methanol-stabilized samples of corn tortillas was performed in a Differential Scanning Calorimeter (Perkin Elmer, Norwalk, CN, Model DSC-1). Starch-stabilized tortilla extract samples (4 mg) were re-hydrated 20 min before heating with 8 mg of water and hermetically sealed in aluminum pans. Then the samples were heated at a rate of 10 ^{o}C/min from –40- to 100 ^{o}C.

The parameters evaluated were: ΔH (enthalpy of water and amylopectin crystal fusion in J/g), peak water and amylopectin melting temperature (T_p in ^{o}C), tortilla midpoint glass transition temperature (T_g).

Empirical Viscosity (RVA analysis)

Slurries (15% solids) of residues of 20 min and 21-day old tortillas extracted with methanol and dried at 50 ^{o}C for 3 hr were evaluated for pasting properties using a Rapid Viscoanalyzer (Model 3C, Newport Scientific, Narabenn, Australia). Samples were evaluated in duplicates. Slurries were held at 50 ^{o}C for 2 min, then heated to 95 ^{o}C at a rate of 7.5 ^{o}C/min, held at 95 ^{o}C for 4 min and cooled down back to 50 ^{o}C at a 7.5 ^{o}C/min rate. Total testing time was 22 min.

Experimental Designs

This study consisted of three separate but sequentially connected experiments. The fist one aimed to determine the optimum type and concentration of amylase that provides the best antistaling performance without compromising sensory quality and tortilla yields. The second study evaluated antistaling properties of combinations of additives (bacterial maltogenic amylase with CMC or wheat gluten). The third study determined the storage temperature at which corn tortillas stale the fastest.

Experiment 1: Optimizing addition of amylases and CMC

This experiment consisted of three phases:

Phase 1: maltogenic amylases evaluation

Two intermediate-temperature bacterial amylases (Novamyl 1500 MG and ICS maltogenic amylase) were evaluated at 4 concentrations (0, 0.4, 0.3, and 0.15 g/kg of NCF) in combination with three levels of CMC (0, 0.25 and 0.5% based on NCF weight). The provider's recommended concentration of Novamyl was 0.3-0.4 g/kg of NCF (450-600 AU/kg of NCF), whereas for the ICS amylase was 0.075-0.15 g/kg of NCF (825-1650 AU/kg of NCF). The 2 (amylase type) x 4 (amylase conc.) x 3 (CMC level) factorial was arranged in a randomized complete block design (RCBD) with three replications (processing days).

Tortillas were produced following the procedure described previously and stored at room temperature (21 ^{o}C) for 7 days. Tortillas were evaluated for subjective (rollability and pliability in triplicates) and objective texture (1-D extensibility) in quintuplicates 20 min, 5 h, 1 and 7 days after baking. Stress relaxation was also performed on tortillas in triplicates 20 min, 5 and 24 hr after baking.

Phase 2: comparison of maltogenic and non maltogenic amylases.

Two bacterial maltogenic amylases (Novamyl and ICS) were compared with a fungal conventional amylase (Fresh-n) and a xylanase for antistaling properties at two concentrations (0 and 0.15 g/kg of NCF) in combination with 0.25% CMC. Tortillas with no additives, or with 0.25% and 0.5% CMC only were used as controls. The experimental design was a RCBD with two replications (processing days).

Tortillas were produced following the procedure described previously and stored at room temperature (21 ^{o}C) for 7 days. Tortillas were evaluated for subjective (rollability and pliability in triplicates) and objective texture (1-D extensibility) in quintuplicates 20 min, 5 h, 1 and 7 days after baking. Stress relaxation was also performed on tortillas in triplicates 20 min, 5 and 24 hr after baking.

Phase 3: comparison of maltogenic amylases at equal number of AU

Novamyl and ICS bacterial maltogenic amylases were evaluated for antistaling properties at four levels (0, 75, 150 and 225 activity units/kg NCF) in combination with 0.25% CMC. Tortillas with no additives, or with 0.5% CMC only were used as controls.

Tortillas were produced following the procedure described previously and stored at room temperature (21 ^{o}C) for 7 days. Tortillas were evaluated for subjective (rollability and pliability in triplicates) and objective texture (1-D extensibility) in quintuplicates 20 min, 1, 14 and 21 days after baking.

Experiment 2: Antistaling properties of combinations of additives

Three concentrations of ICS maltogenic amylase (0, 825 and 1650 AU/kg of NCF), three levels of CMC (0, 0.25 and 0.5%) and three levels of vital wheat gluten (0, 1 and 2%) were evaluated for antistaling properties in an incomplete factorial design with three replications (processing days).

Eleven treatment combinations (Table III) were selected following a Central Composite Design (CCD). The full control treatment (no additives) was added to the CCD in order to have a starting point for comparisons.

Table III

Treatment Combinations Used for Evaluating Antistaling Properties of Maltogenic Amylase with CMC and/or Vital Wheat Gluten in a Central Composite Design

Treatment	CMC (%)	ICS Amylase (AU)	Gluten (%)
1	0.25	0	1
2	0.25	1650	1
3	0.25	825	0
4	0.25	825	2
5	0	825	1
6	0.5	825	1
7	0.5	0	0
8	0	0	2
9	0	1650	0
10	0.5	1650	2
11	0.25	825	1
12	0	0	0

Tortillas were produced following the procedure described previously and stored at room temperature (21 ^{o}C) for 14 days. Tortillas were evaluated for subjective (rollability and pliability in triplicates) and objective texture (1-D extensibility in quintuplicates and stress relaxation in triplicates) 20 min, 5 hr, 1, 7 and 14 days after baking.

Tortilla samples obtained from all treatments 20 min, 5 hr, 1, 7 and 14 days after baking were stabilized with methanol as previously described for DSC analysis.

Data was analyzed using response surface methodology (RSM) to generate second order regression models using SAS version 8.

Experiment 3: Temperature dependence of tortilla staling rate

Tortillas made with a combination of 1650 AU of ICS maltogenic amylase and 0.25% CMC were evaluated in comparison with control tortillas (no additives) and tortillas with 0.5% CMC only under five storage temperatures (-40, -20, 3, 10 and 21 ^{o}C). A split plot design with two replications was used to conduct the experiment. The main plots were the storage temperatures and the treatments were designed as the sub-plots.

Tortillas were produced following the procedure described previously and stored at the respective temperature for 21 days. Tortillas were stored individually in polyethylene bags at –20 ^{o}C in a Hotpoint refrigerator freezer (GE, model CTX21EAXFRWH,) with a 22 ft/min air flow, while a So-Low freezer (model PR120-12, Environmental Equipment Co., Cincinnati, Ohio) with an air flow of 8 ft/min was used for storage at –40 ^{o}C.

Tortillas were evaluated for subjective (rollability and pliability in triplicates) and objective texture (stress relaxation in triplicates) 20 min1, 7 and 21 days after baking. Frozen tortilla samples were microwaved for 10 sec to remove ice from the surface and then allowed to equilibrate to room temperature (21 ^{o}C) for 1 hr before performing texture evaluations.

Tortilla samples obtained from all treatments 20 min, 5 hr, 1, 7 and 21 days after baking were starch-stabilized with methanol as previously described for DSC analysis. RVA analysis was performed on 20 min and 21-day old tortilla extracts.

Statistical Analysis

Statistical analyses were performed using SAS version 8. Analysis of variance for experiments 1 and 3 was performed using PROC GLM, while RSM for experiment 2 was performed with PROC RSREG.

Tukey's means separation test was performed with the MEANS statement and the Tukey option (α = 0.05%). Tukey's Honest Significant Difference (HSD) was used for treatment comparisons in graphs and tables.

CHAPTER IV

OPTIMIZING ADDITION OF ENZYMES AND CMC TO CORN TORTILLAS

Evaluation of Maltogenic Amylases

No significant differences in tortilla moisture content were observed among treatments. Average tortilla moisture content was 47.1% and the CV was 0.83%. Addition of amylases did not significantly change tortilla pH. Average tortilla pH was 5.42 with a CV of 0.67%.

Tortilla yield (CV = 8.4%) was significantly affected by amylases and CMC (Fig. 1). In general, tortilla yields (control mean = 1 kg/kg NCF) were lower than the industry standard due to problems with the oven belts that caused tortilla folding and cracking.

Tortilla yield was significantly increased by adding 0.25% CMC compared to the control. Increasing CMC level to 0.5% did not significantly increase tortilla yield compared to 0.25% CMC.

Novamyl maltogenic amylase significantly reduced tortilla yield when 350 MAU or more were added. However, addition of 0.25% CMC or more allowed using up to 600 MAU of Novamyl without significant reductions in tortilla yield.

ICS maltogenic amylase reduced tortilla yield significantly when 1650 MAU or more were added compared to the control. Addition of 0.25% CMC did not prevent significant reductions in tortilla yield, but 0.5% did. Up to 3500 MAU of ICS amylase could be added to tortillas without significant reductions in tortilla yield when 0.5% CMC was added.

Neither 75 nor 150 AU of maltogenic amylases (Novamyl and ICS) combined with 0.25% CMC were enough to significantly decrease tortilla rupture force after 21 days of storage compared to the control (Appendix A.1, A.2 and A.4). However, significant improvements in tortilla rollability, pliability (Appendix A.3) and rupture distance were observed compared to the control. The combination of ICS amylase and 0.25% CMC produced significantly more rollable and pliable tortillas than 0.5% CMC.

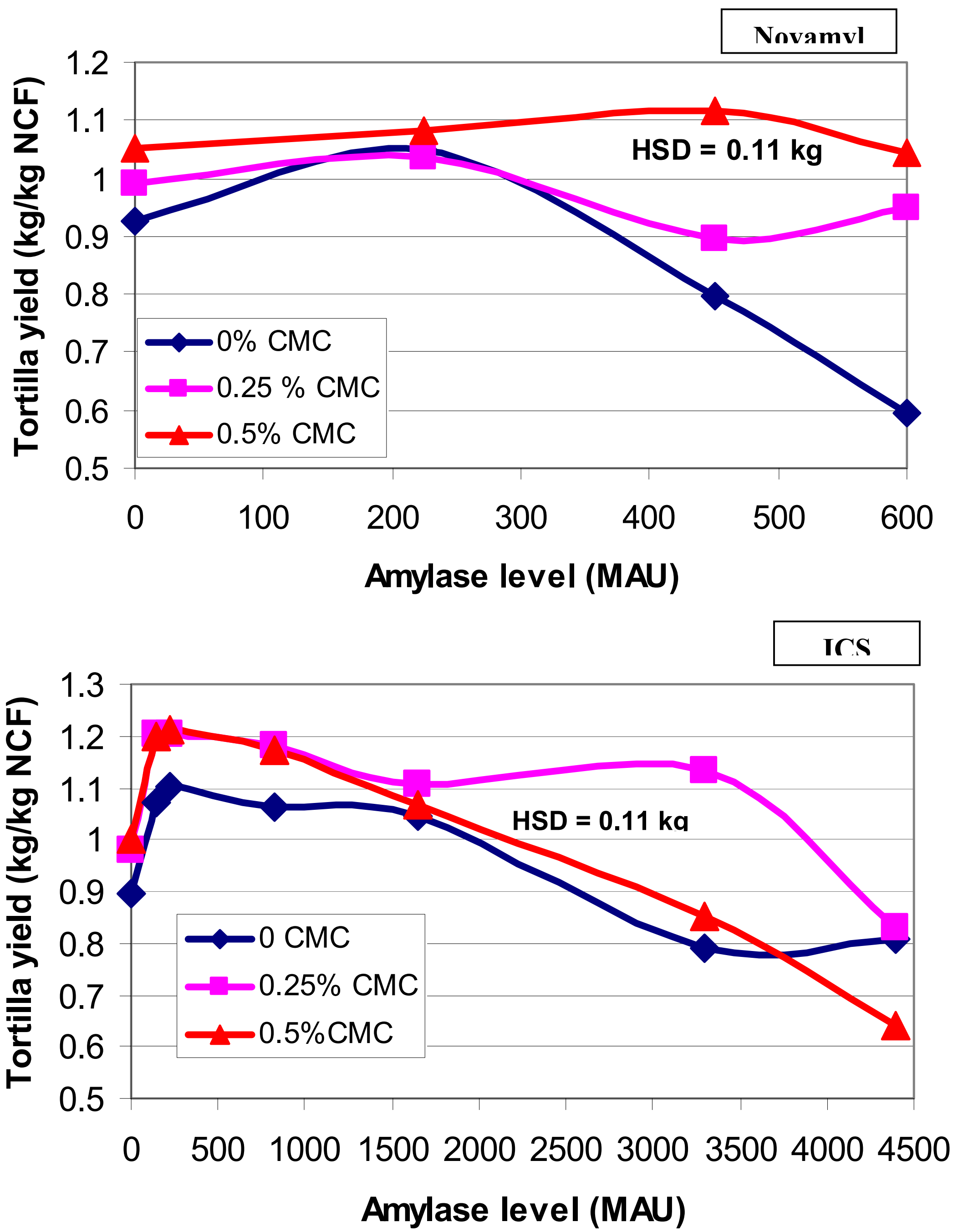


Fig. 1. Effect of Novamyl and ICS maltogenic amylases on corn tortilla yield.

These results suggest the improvements in tortilla texture were due mostly to the addition of 0.25% CMC and that amylases were not effective in softening the tortilla (reducing rupture force) when added at levels up to 150 AU. However 75 AU of ICS amylase improved tortilla flexibility (rollability, pliability and rupture distance) when combined with 0.25% CMC compared to 0.5% CMC and control.

A combination of 0.25% CMC and 225 AU of ICS maltogenic amylase was more effective in maintaining rollability of tortillas stored for 21 days than 0.5% CMC (Fig. 2). The combination of 0.25% CMC and 225 AU of Novamyl maintained tortilla rollability better than the control but not as well as 0.5% CMC or the combination of 0.25% CMC and 225 AU of ICS amylase.

After 21 days of storage at room temperature, none of the treatments prevented the drop of tortilla pliability below the acceptable level (score = 4). However, combinations of 0.25% CMC and 225 AU of amylase (Novamyl and ICS) preserved tortilla pliability as well as 0.5% CMC and better than the control after 21 days of storage (Fig. 3). Tortillas with 225 AU of ICS amylase were significantly more pliable than tortillas with 225 of Novamyl when 0.25% CMC was present.

Tortillas stored for 21 days required significantly more force to rupture (mean = 13.3 N) than fresh tortillas (mean = 3.5 N). Only the combination of 0.25% CMC and 225 AU of ICS amylase produced tortillas with significantly lower rupture force than the control (Fig. 4). This combination also produced tortillas with lower rupture force than the combination of 0.25% CMC and 225 AU of Novamyl.

Tortilla rupture distance also decreased dramatically after 21 days of storage (mean fresh tortilla = 10.4 mm vs. mean after 21 days = 2 mm). However, 0.5% CMC and a combination of 0.25% CMC and 225 AU of ICS amylase were equally effective in producing tortillas with significantly higher rupture distance than the control (Fig. 5).

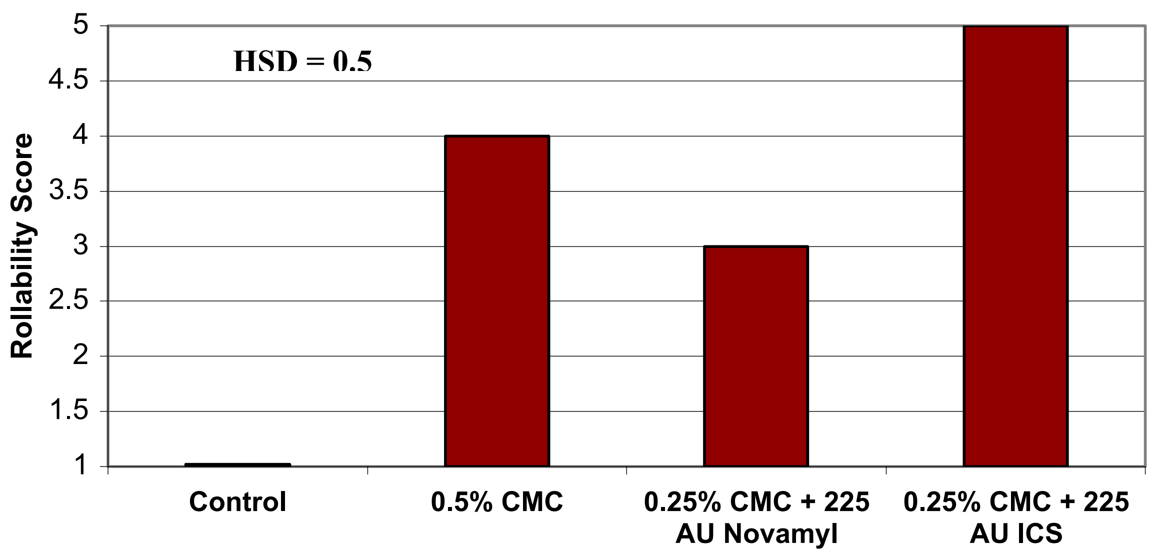


Fig. 2. Effect of 225 AU maltogenic amylase (Novamyl or ICS) and 0.25% CMC on the rollability of tortillas stored 21 days at room temperature.

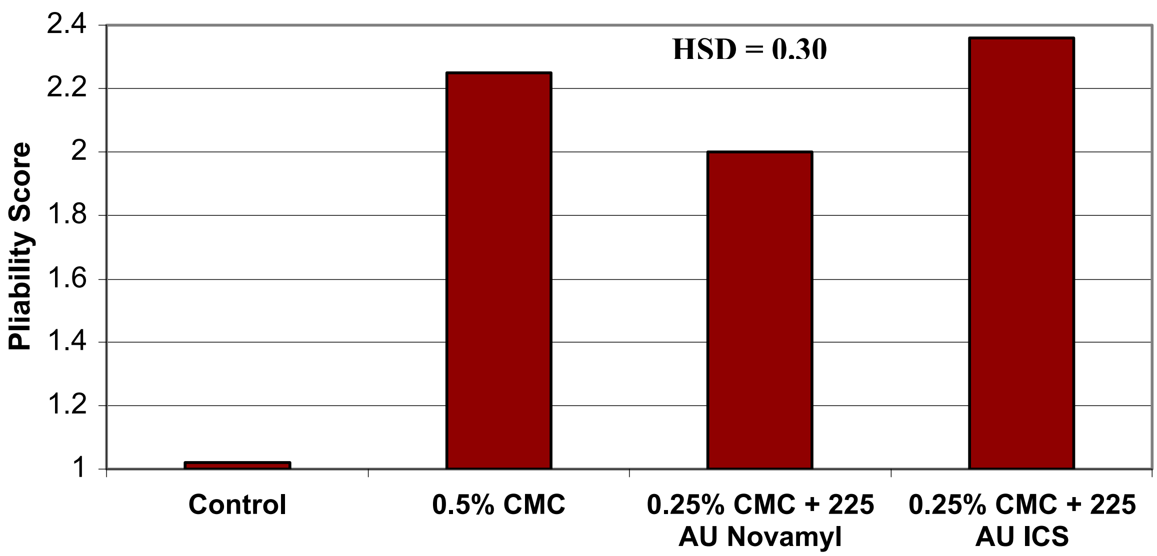


Fig. 3. Effect of 225 AU maltogenic amylase (Novamyl or ICS) and 0.25% CMC on the pliability of tortillas stored 21 days at room temperature.

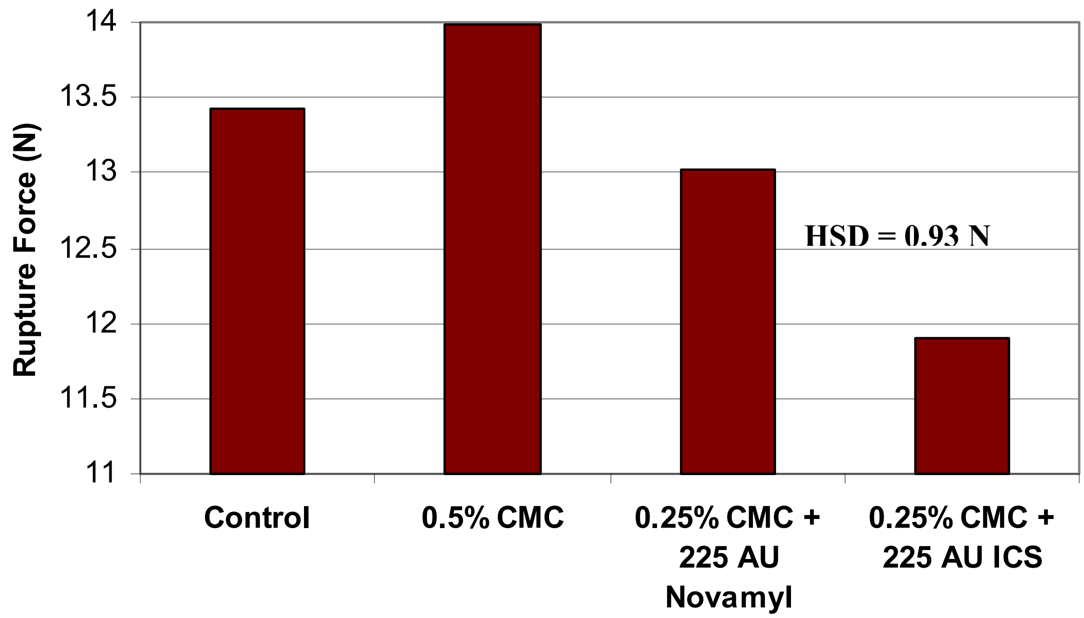


Fig. 4. Effect of 225 AU maltogenic amylase (Novamyl or ICS) and 0.25% CMC on the rupture force of tortillas stored 21 days at room temperature.

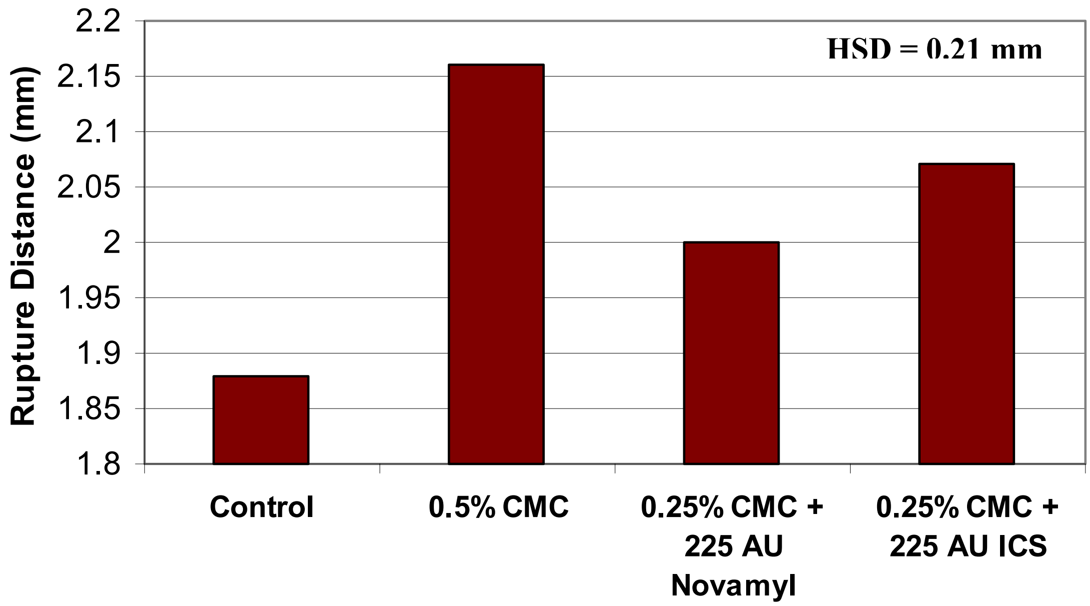


Fig. 5. Effect of 225 AU maltogenic amylase (Novamyl or ICS) and 0.25% CMC on the rupture distance of tortillas stored 21 days at room temperature.

Maltogenic vs. Non Maltogenic Enzymes

No significant differences in tortilla moisture content, pH or yield were observed when adding enzymes (0.15g/kg of NCF) and/or CMC (Table IV). The model explained differences among treatments poorly (Moisture R^2 = 0.66; pH R^2 = 0.42 and Yield R^2 = 0.59). Coefficients of variation (CV) were low for tortilla moisture content (1.67%), pH (2.2%) and yield (9.15%).

TABLE IV

Effect of CMC and Enzyme Combinations on Tortilla Yield, Moisture and pH

Treatment Combination		**Moisture**	**pH**	**Yield**
CMC (%)	**Enzyme (AU/kg NCF)**	**(%)**		**(kg/kg NCF)**
0	0	47.0	4.84	1.14
0.25	0	47.7	4.73	1.01
0.5	0	47.6	4.66	1.09
0	Novamyl (225)	47.7	4.79	1.04
0	ICS (225)	46.9	4.73	1.10
0	Fresh-N (225)	46.4	4.64	1.18
0	Xylanase (1800)	47.0	4.72	1.05
0.25	Novamyl (225)	46.4	4.81	1.06
0.25	ICS (225)	47.2	4.75	1.14
0.25	Fresh-N (225)	47.9	4.74	1.24
0.25	Xylanase (1800)	47.9	4.61	1.29
HSD (α=0.05)*		**1.7**	**0.25**	**0.29**

* Tukey's Honest Significant Difference for means separation.

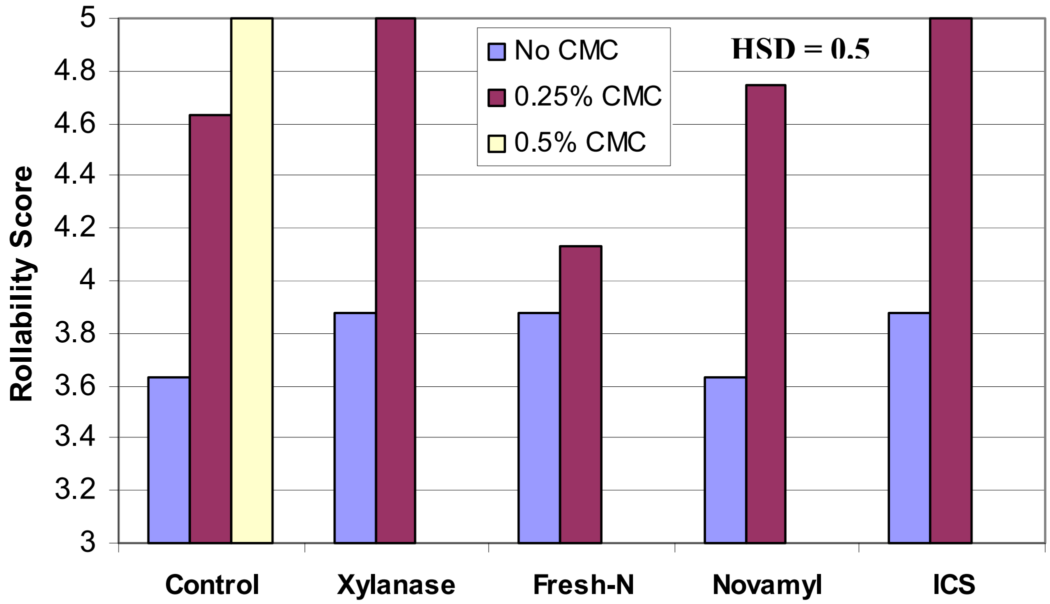


Fig. 6. Effect of enzymes and CMC on the rollability of tortillas stored 7 days at room temperature.

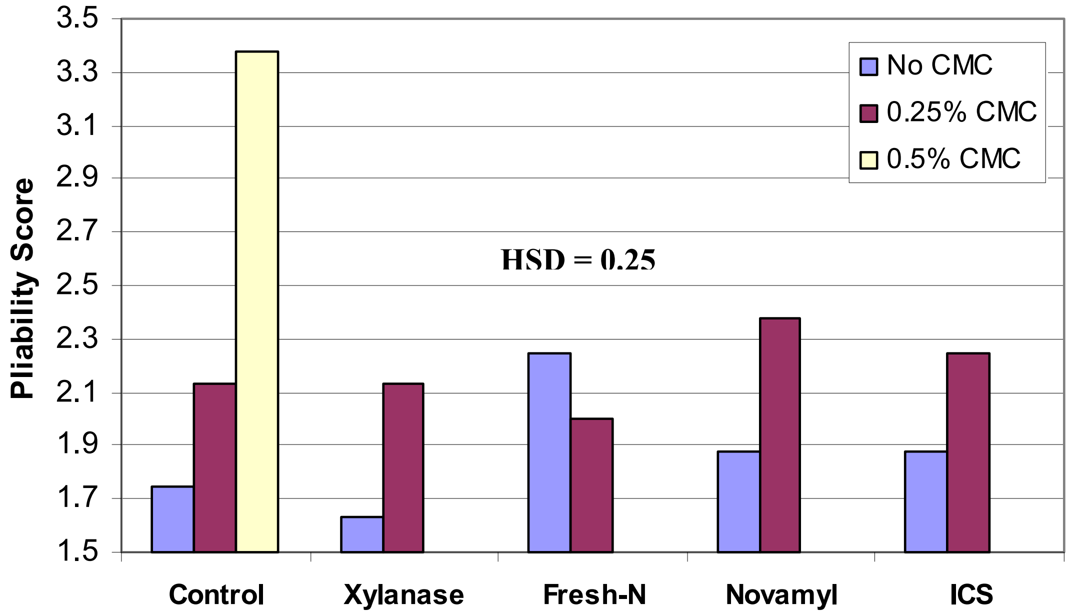


Fig. 7. Effect of enzymes and CMC on the pliability of tortillas stored 7 days at room temperature.

Rollability of control tortillas stored for 7 days was below the optimum (score = 4). Addition of 0.25-0.5% CMC maintained tortilla rollability above the optimum (Fig. 6). Enzymes did not improve rollability of tortillas stored 7 days compared to the control. Furthermore, addition of 225 AU of Fresh-N significantly reduced tortilla rollability when combined with 0.25% CMC compared to tortillas made with only 0.25% CMC.

Similarly, 0.25% CMC made tortillas significantly more pliable than control after 7 days of storage (Fig. 7). Tortillas with 0.5% CMC were the most pliable after seven days of storage. Enzymes, except for Fresh-N, did not significantly improve pliability of 7-day old tortillas compared to the control. Only a combination of 225 AU of Novamyl and 0.25% CMC produced tortillas with higher pliability than tortillas with 0.25%.

Tortillas with 0.25-0.5% CMC did not significantly reduce rupture force of tortillas stored 7 days compared to the control (Fig. 8). Maltogenic amylases (225 AU of Novamyl and ICS) were the only enzymes that significantly reduced tortilla rupture force after 7 days of storage compared to the control or to tortillas with 0.25-5% CMC. Tortillas with 225 AU of ICS amylase required significantly less force to rupture than tortillas with 225 AU of Novamyl after 7 days of storage. No positive interaction was observed between maltogenic amylases and CMC in reducing tortilla rupture force.

Rupture distance of tortillas stored for 7 days was significantly increased only by 0.5% CMC compared to the control (Fig. 9). Enzymes did not increase tortilla rupture distance. Furthermore, 225 AU of ICS maltogenic amylase significantly decreased tortilla rupture distance compared to the control. Combinations of individual enzymes with 0.25% CMC did not significantly increase tortilla rupture force compared to tortillas with 0.25% CMC only.

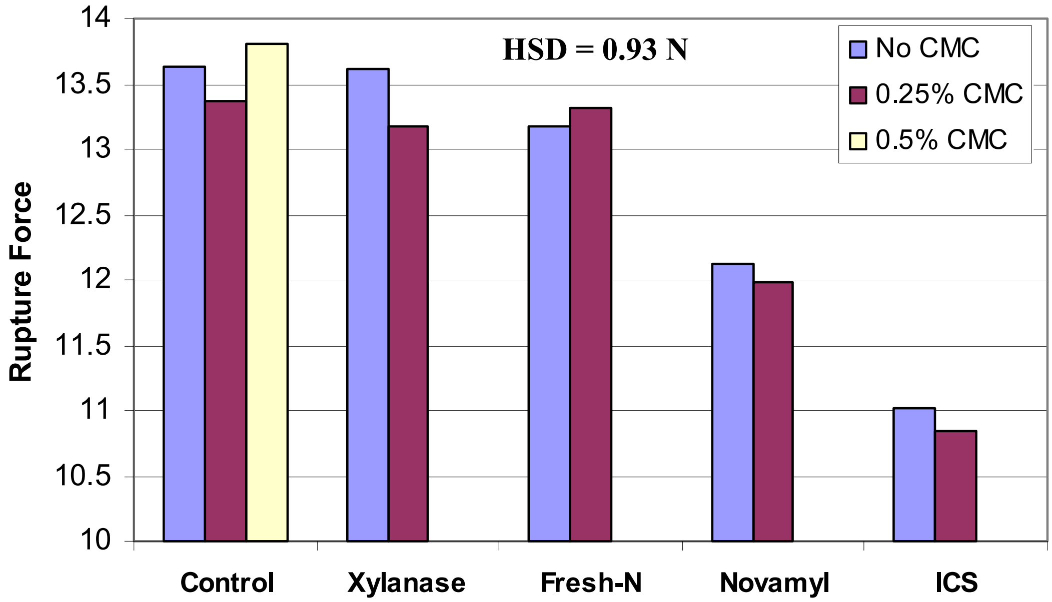


Fig. 8. Effect of enzymes and CMC on rupture force of tortillas stored 7 days at room temperature.

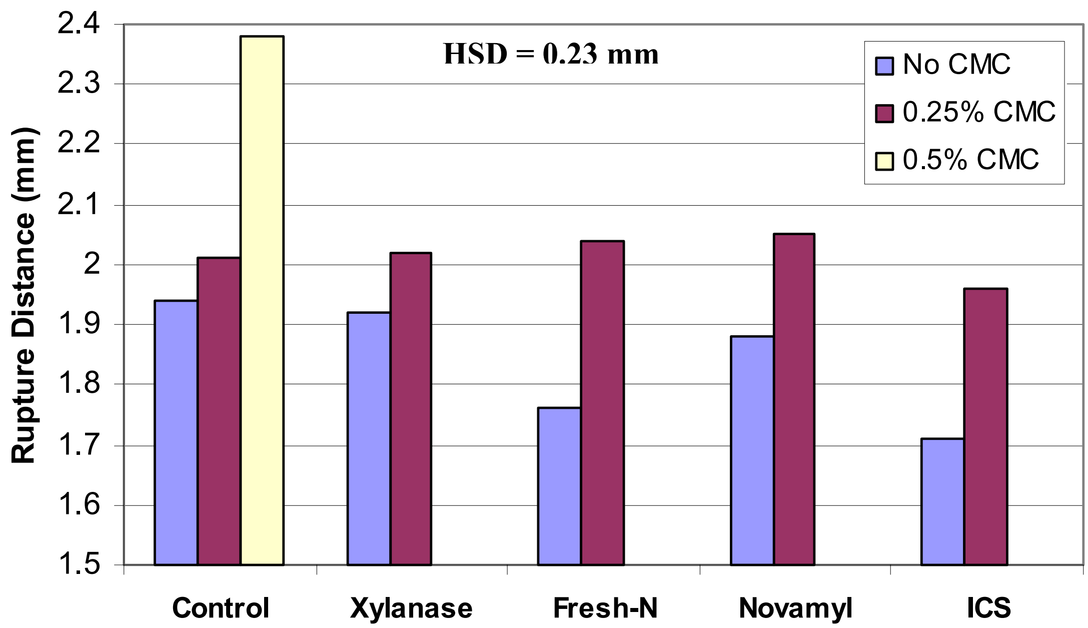


Fig. 9. Effect of enzymes and CMC on rupture distance of tortillas stored 7 days at room temperature.

Discussion

Commercial tortillas with 0.5% CMC, unlike tortillas made without additives can be stored under refrigeration (4 ^{o}C) for at least a month. Addition of 0.5% CMC improves masa cohesiveness and machinability (Gomez et al. 1991) and maintains tortilla flexibility during storage (Suhendro 1997; Quintero-Fuentes 1999; Yeggy 2000). Tortillas with 0.5% CMC became more rubbery and have a chewier texture than tortillas without additives (Quintero-Fuentes 1999).

Figure 1 shows that 0.25% CMC was needed to increase tortilla yields compared to the control and 0.5% did not contribute to further yield increases. Masa with 0.5% CMC was stickier than control and stuck more frequently to the oven belts than masas with 0.25% or no CMC. Therefore, gains in masa yield due to increased water holding capacity provided by 0.5% CMC were shadowed by greater tortilla loses by folding in the oven and subsequent non-uniform baking.

Addition of maltogenic amylases was limited to 1650 AU for ICS and 350 AU for Novamyl due to significant reductions in tortilla yields (Fig. 1). Hydrolytic activity of these enzymes at the above-mentioned levels during masa formation and the rest period (17 min) was enough to reduce the cohesiveness of masa, increase the number of cracked tortillas during baking and consequently reduce tortilla yields.

Addition of 0.25%-0.50% CMC prevented reductions in tortilla yield when using more than 350 AU of Novamyl or above 1650 AU of ICS amylase. However, undesirable tortilla mushiness and sweetness was detected on tortillas at or above these levels.

Subsequent tests proved that there was no need to use such high levels of maltogenic amylases to obtain significantly softer tortillas after a week or more of storage at room temperature.

Addition of 225 AU of maltogenic amylases (Novamyl or ICS) significantly reduced tortilla rupture force compared to control and tortillas with 0.25-0.5% CMC after one week of storage (Fig. 4 and 8). Both amylases (at 225 AU/ kg of NCF) required only 18 min to effectively hydrolyze starch and produce tortillas with a reduced tendency to harden during storage. The higher hydrolytic activity of ICS maltogenic amylase than Novamyl at room temperature (Table II) might explain why tortillas with ICS amylase required significantly less force to rupture than tortillas with Novamyl after one week of storage.

The fact that 225 AU of Fresh-N, a conventional intermediate–temperature bacterial α-amylase, were unable to reduce tortilla rupture force in tortillas like 225 AU of maltogenic amylases suggests the basis of the anti-staling properties of maltogenic amylases is their particular ability to remove oligosaccharides (DP 2-7) from amylopectin (Boyle and Hebeda 1990). It could also mean that maltogenic amylases had a higher hydrolytic activity than Fresh-N at room temperature.

Lower rupture force does not exactly translate into higher tortilla flexibility, rollability or pliability. Tortillas with 0.5% CMC stored for one week or more were perfectly rollable (Fig 2, 6), significantly more pliable (Fig. 3,7) and extensible (higher rupture distance) than control tortillas (Fig. 5, 9), despite requiring a similar amount of force to rupture (Fig 4, 8). On the other hand, tortillas with maltogenic amylases required less force to rupture (were "softer") but were not significantly more extensible (more rollable, pliable and requiring more distance to rupture) than control.

Therefore, the anti-staling properties of CMC may be related to its ability to create a flexible amorphous matrix in the continuous phase of tortillas and not to interfering with amylopectin retrogradation during storage. The increased flexibility and cohesiveness of tortillas with CMC explains why they require more force to rupture without being harder, more brittle or less flexible than the control. Since tortillas with 0.5% CMC are regarded as "rubbery and chewy", addition might be limited to 0.25%.

This study supports the idea of combining 0.25% CMC with 225-1650 AU of ICS maltogenic amylase as a way of producing softer, less chewy and more flexible tortillas than 0.5% CMC after a week of storage at room temperature.

CHAPTER V

ANTISTALING PROPERTIES OF CMC, MALTOGENIC AMYLASE AND VITAL WHEAT GLUTEN

Masa Quality

All treatment combinations produced masas with optimum cohesiveness and without excessive stickiness. Therefore, masas were sheetable.

Tortilla Moisture, pH and Yield

Tortilla moisture content varied significantly ($P < 0.05$) among treatments (Table V, Appendix Table B.1). The second order model fit to observed data was low for moisture content ($R^2 = 0.40$). The coefficient of variation for tortilla moisture content was very low (1.42%), indicating precise measurements. Addition of gluten and the interaction of CMC and maltogenic amylase significantly reduced tortilla moisture content compared to the control (Table I). Tortillas with no additives (control) had significantly higher moisture content than other treatments, except for the combination of 0.25% CMC, 825 AU of amylase and 1% gluten.

Appendix B.1 and Table I show that tortilla pH was similar for all treatments ($P = 0.51$), with an overall mean value of 4.83 and a CV of 7.2%. The second order model fit was very low ($R^2 = 0.18$).

Tortilla yield varied significantly among treatments ($P < 0.001$) and the second order model explained observed data well ($R^2 = 0.77$), considering that an incomplete factorial design was used. Coefficient of variation was adequate (9.4 %). As expected, only the addition of CMC significantly increased tortilla yield (Appendix B.1 and Table V) compared to control. This increase in yield was caused by improved masa machinability that produced more acceptable tortillas, since moisture content of tortillas with CMC was actually lower than control tortillas. Addition of 0.5% CMC normally increases masa water absorption and produces tortillas with increased moisture content compared to control (Serna-Saldivar 1990, Quintero-Fuentes 1999), so these results are contradictory with past observations. Tortilla yields in this study were also lower than the industry standard due to tortillas folding and cracking in the oven belts. Addition of 1650 AU of maltogenic amylase did not significantly reduce tortilla yield (Table V) compared to control.

TABLE V

Effect of CMC, Maltogenic Amylase and Wheat Gluten Combinations on Tortilla Yield, Moisture and pH

Treatment Combination			Moisture	pH	Yield
CMC (%)	Amylase (MAU)	Gluten (%)	(%)		(kg/kg NCF)
0	0	0	48.5	4.84	0.93
0	0	2	47.1	4.88	0.99
0	825	1	47.3	4.84	1.03
0	1650	0	47.6	4.85	0.94
0.25	0	1	47.7	4.85	1.02
0.25	825	0	47.7	4.91	1.15
0.25	825	1	48.1	4.87	1.04
0.25	825	2	46.7	4.84	1.09
0.25	1650	1	47.7	4.83	1.09
0.5	0	0	47.3	4.89	1.05
0.5	825	1	47.4	4.91	1.10
0.5	1650	2	47.3	4.94	1.04
HSD (α=0.05)*			**0.6**	**0.16**	**0.10**

* Tukey's Honest Significant Difference for means separation.

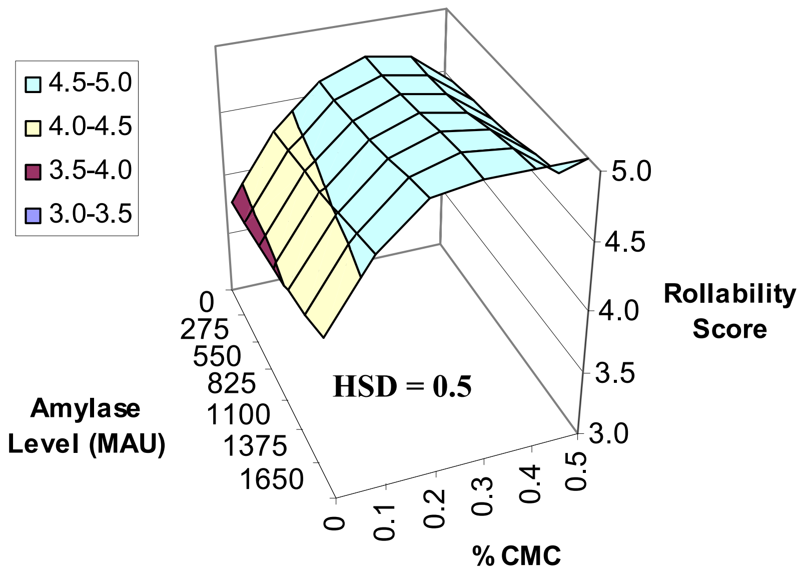
In general, all treatment combinations evaluated produced equal or higher tortilla yields than the control, despite having significantly lower moisture contents.

Changes in Tortilla Texture During Storage

Subjective texture evaluations

Tortillas from all treatments received the highest score (5) for pliability and rollability 20 min after baking (Appendix Tables B.3 and B.4). However, after 14 days of storage, highly significant differences in tortilla rollability (Fig. 10, Appendix Tables B.2 and B.3) and pliability (Fig. 10, Appendix Tables B.2 and B.4) were observed among treatments. The second order regression model explained changes due to addition of anti-staling agents in tortilla pliability (R^2 = 0.73) better than in tortilla rollability (R^2 = 0.60). This suggests that the subjective tortilla pliability test is more sensitive to textural differences than rollability. Coefficient of variation for tortilla rollability was 12.1% and for pliability was 19.2%, which indicates that the evaluator was more precise testing rollability than pliability.

Only the addition of CMC produced tortillas with significantly higher rollability than control after 14 days of storage at room temperature (Appendix Tables B.2). Maltogenic amylase did not reduce tortilla rollability when 1650 AU or less were added (Appendix B.3 and Fig. 10), indicating that starch breakdown was limited to the extent of not affecting tortilla flexibility and cohesiveness significantly. On the other hand, addition of at least 1% vital wheat gluten produced tortillas with significantly lower rollability than control after 14 days of storage (Fig. 10). When added in combination with CMC and amylase, wheat gluten did not significantly increase tortilla rollability during storage compared to treatments with combinations of CMC and amylase only. Therefore, vital wheat gluten at 1% level was ineffective in preserving of tortilla rollability by itself, and did not show a positive interaction with CMC and maltogenic amylase.



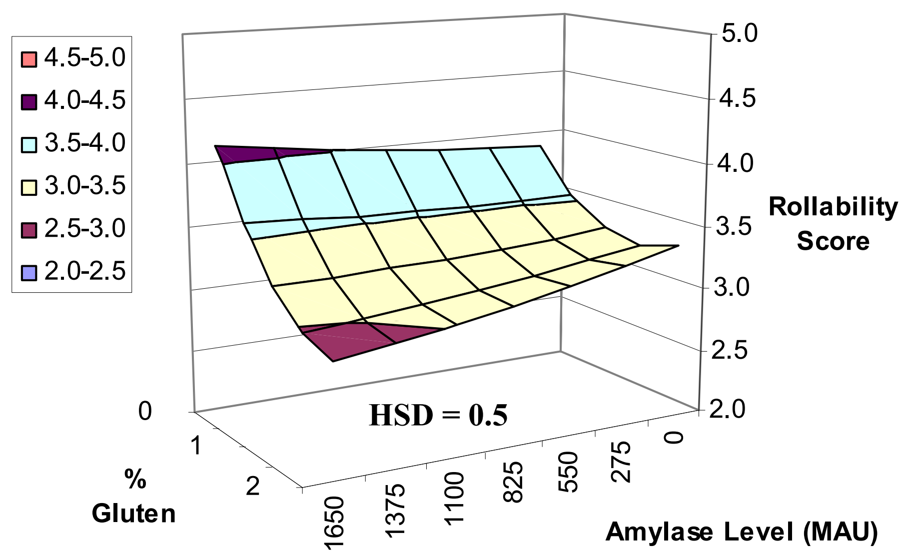


Fig. 10. Rollability of tortillas stored 14 days containing maltogenic amylase, CMC and vital wheat gluten.

Optimum tortilla rollability (> 4.75) was obtained when combining at least 0.25 % CMC and 825 AU of amylase or more (Fig.10).

When added alone CMC and maltogenic amylase were effective in preserving tortilla pliability after 14 days of storage compared to the control (Fig.11, Appendix Table B.4). Tortillas with up to 1% wheat gluten showed significantly higher pliability than control after 14 days of storage. Higher levels of gluten did not significantly increase pliability.

CMC added alone produced tortillas with higher pliability than control after 14 days of storage only when levels above 0.2% were used. To produce tortillas with a pliability score of 2 or higher, 0.5% CMC was needed if added alone (Fig. 11, Appendix Table B.4).

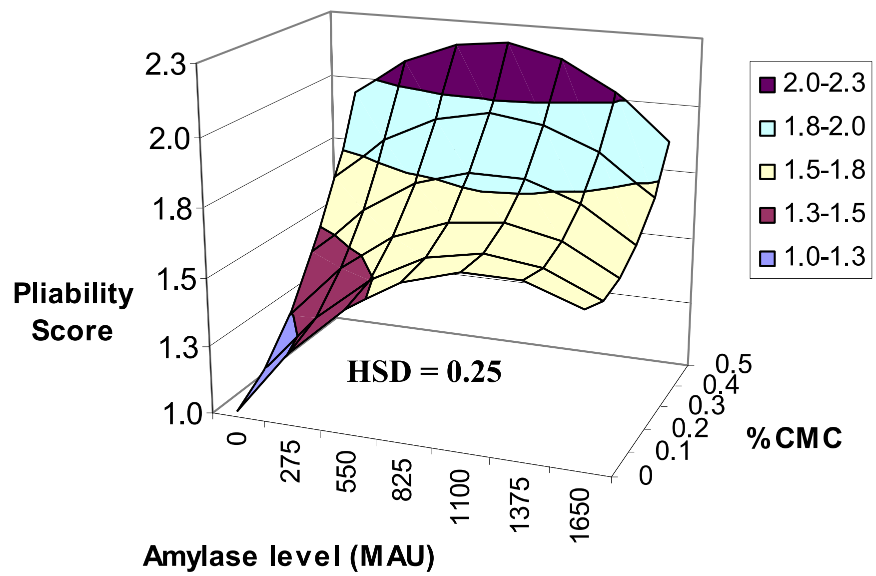
Addition of up to 825 AU of maltogenic amylase alone significantly increased pliability compared to control after 14 days of storage (Fig. 11), but not to similar levels than 0.5% CMC. Higher levels of amylase added alone did not significantly increase pliability, and Fig. 11 actually suggests a detrimental effect if more than 825 AU are added without CMC.

Combinations of 550-1100 AU of amylase and 1% gluten produced tortillas with pliability statistically similar to tortillas with 0.5% CMC, suggesting a synergy between the softening effect of the maltogenic amylase and the flexible matrix-building effect of gluten. However, only combinations of 0.5% CMC plus 550-1100 AU of amylase produced tortillas with a pliability score above 2 and significantly higher than 0.5% CMC alone (Fig. 11). This means that CMC was a better flexible-matrix builder than vital wheat gluten when interacting with the softening effect of amylase on retrograded starch, therefore producing more pliable tortillas after two weeks of storage.

Objective texture evaluations

Tortilla hardness: rupture force vs. stiffness

Appendix B.5 shows that significant differences in the amount of force required to rupture tortillas 20 min after baking were found among treatments ($P<0.001$). The model R^2 was 0.56 and the overall coefficient of variation was 8%, indicating that the repeatability of the rupture force measurement is good.



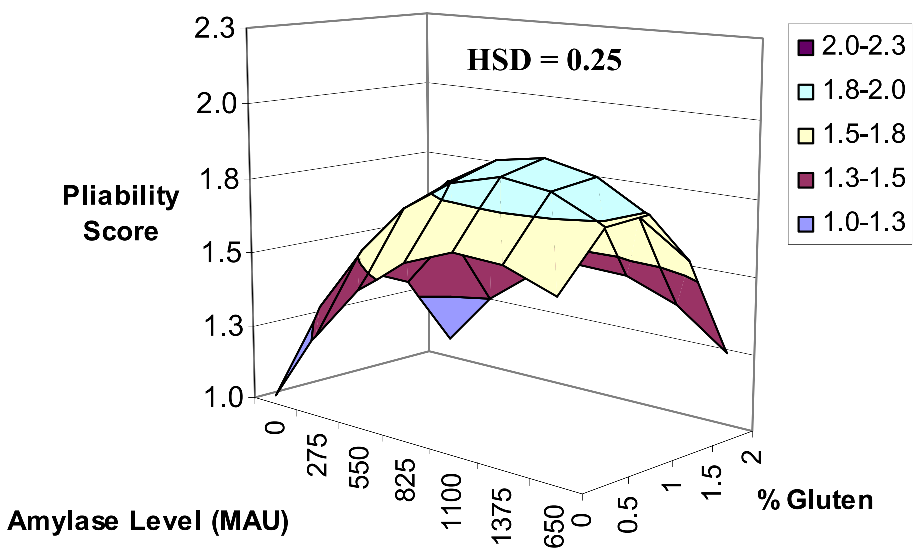


Fig. 11. Pliability of tortillas stored 14 days containing maltogenic amylase, CMC and vital wheat gluten.

Only 0.5% CMC added alone increased rupture force significantly in tortillas coming out of the oven compared to the control (Table VI, Appendix B.5). Neither maltogenic amylase nor wheat gluten working alone significantly changed tortilla rupture force 20 min after baking. However a significant interaction between CMC and amylase indicates that amylase had a softening effect on freshly baked tortillas only when CMC was present (Table VI, Appendix B.5). These small changes in texture had no significant effect on fresh tortilla rollability and pliability (20 min after baking).

TABLE VI

Effect of CMC, Maltogenic Amylase and Wheat Gluten Combinations on Stiffness and Rupture Force of Tortilla 20 Min After Baking

Treatment Combination			Rupture Force	Stiffness
CMC (%)	Amylase (MAU)	Gluten (%)	(N)	(x 10^6 Pa)
0	0	0	2.84	0.21
0	0	2	3.01	0.22
0	825	1	2.91	0.19
0	1650	0	3.05	0.23
0.25	0	1	3.04	0.19
0.25	825	0	2.91	0.19
0.25	825	1	2.99	0.24
0.25	825	2	3.32	0.22
0.25	1650	1	3.04	0.24
0.5	0	0	3.70	0.21
0.5	825	1	3.58	0.23
0.5	1650	2	3.29	0.25
HSD (α=0.05)*			**0.40**	**0.07**

* Tukey's Honest Significant Difference for means separation.

The stress relaxation test agreed with subjective texture measurements. It did not detect significant differences (P = 0.47) in tortilla stiffness among treatments 20 min after baking (Table VI, Appendix B.9). This also suggests that the 1-D extensibility indicator of hardness (rupture force) is more sensitive to smaller differences in tortilla texture than stiffness generated by the stress relaxation test. Model R^2 for stiffness was consequently very low (0.17) and the coefficient of variation very high (28 %) compared to tortilla rupture force measurements.

As expected, highly significant differences in tortilla texture among treatments were observed after 14 days of storage when measuring rupture force and stiffness (P<0.001) (Fig. 12 and 13; Appendix Tables B.6 and B.10). Here, stiffness explained differences in tortilla texture among treatments better than rupture force (R^2 stiffness = 0.85 vs. R^2 rupture force = 0.63) and was more consistent (CV stiffness = 9.7% vs. CV rupture force =12.4%).

CMC, maltogenic amylase and gluten changed rupture force and stiffness of 14-day old tortillas significantly (Appendix Tables B.7 and B.11) compared to the control. Maltogenic amylase accounted for most of these differences.

Tortillas with 0.5% CMC had similar rupture force than control regardless of the amylase level (Fig. 12). Reductions in tortilla rupture force when CMC was added at 0.25% were not significant.

The response surface model suggests that addition of at least 550 AU of maltogenic amylase significantly reduced tortilla rupture force (Fig. 12) 14 days after baking compared to the control. No significant interaction in rupture force between CMC and amylase was observed. This means that rupture force of tortillas was reduced by the amylase to the same extent regardless of the level of CMC added.

Significant reductions in tortilla rupture force were obtained by adding at least 1% wheat gluten. Reductions in tortilla rupture force were comparable for 1650 AU of amylase and 2% wheat gluten (Fig 12). This suggests that amylase and gluten were equally effective at producing softer tortillas after 14 days storage.

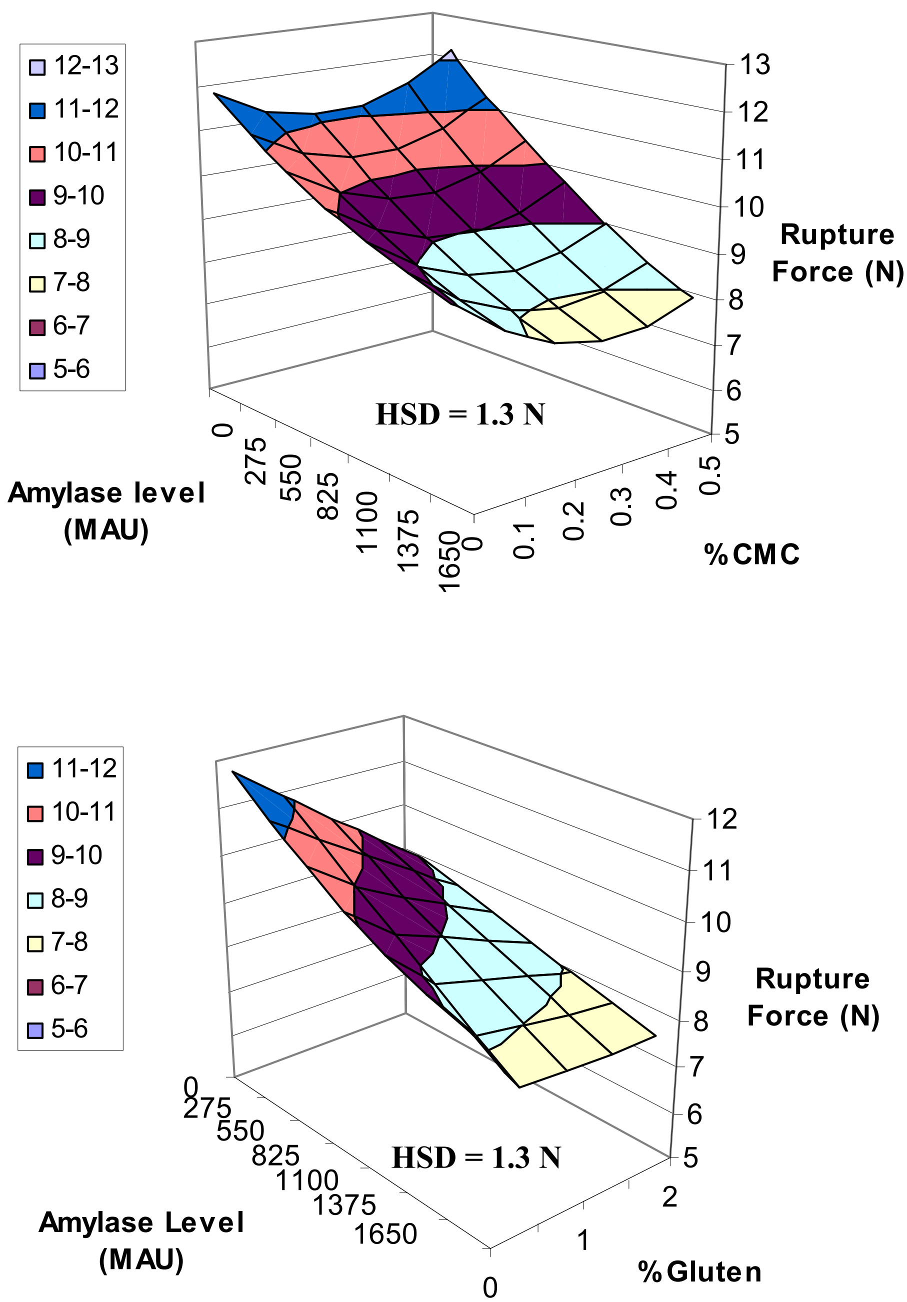
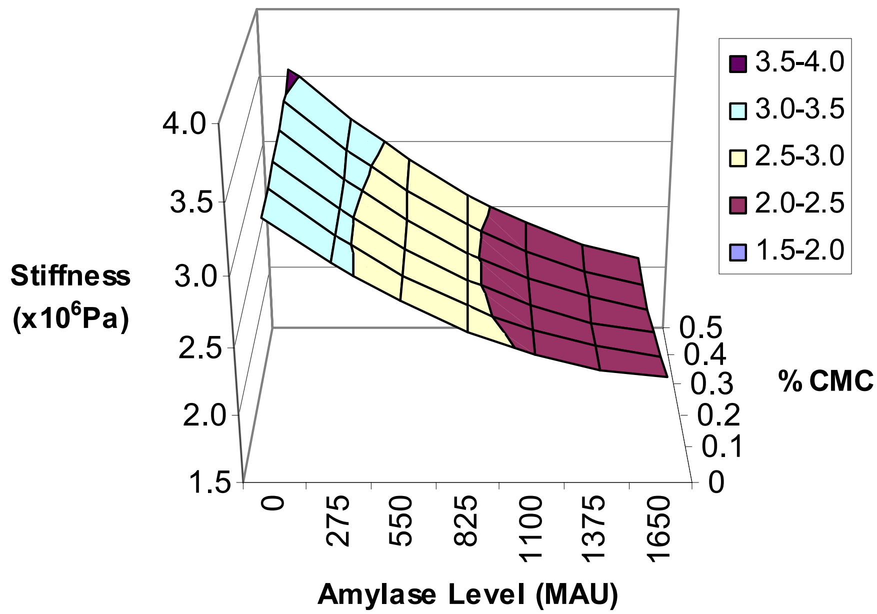


Fig. 12. Rupture force of tortillas stored 14 days containing maltogenic amylase, CMC and vital wheat gluten.



HSD = 0.21 E+6 Pa

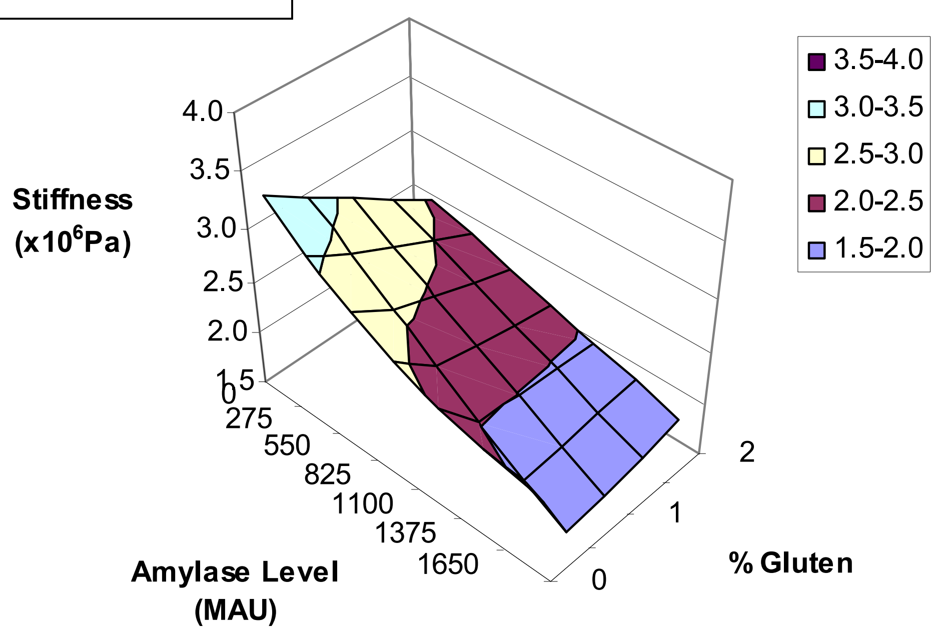


Fig. 13. Final stiffness of tortillas stored 14 days containing maltogenic amylase, CMC and vital wheat gluten.

When amylase and wheat gluten were combined, further reductions in tortilla rupture force were observed compared to the control and to treatments with only one of either additive, specially when gluten was added up to 1% mixed with up to 825 AU of amylase. Higher levels of gluten (> 1%) appeared to counteract the softening effect of amylase on tortillas when more than 825 AU were added.

Stiffness values confirmed the trends found with rupture distance. CMC, maltogenic amylase and wheat gluten significantly changed tortilla stiffness compared to control after 14 days of storage (Fig. 13, Appendix Tables B.10 and B11). However, the individual and combined effects of additives on tortilla firmness were more clearly seen with stiffness data than with rupture distance. Like rupture force, most of the variation in tortilla stiffness among treatments was generated by amylase level.

CMC significantly increased tortilla stiffness when added alone at levels over 0.25% compared to the control (Fig. 13). According to the response surface model, 275 AU of maltogenic amylase was enough to significantly decrease stiffness of 14 day-old tortillas. No significant interaction between amylase and CMC was observed for tortilla stiffness (Fig. 13). This means that tortilla stiffness always increased when CMC was added at higher levels, regardless of the level of amylase used.

Vital wheat gluten (1% or more) significantly decreased tortilla stiffness after 14 days of storage compared to the control (Fig. 13), confirming observations with rupture force data. However, unlike the rupture force data, the reduction in tortilla stiffness by amylase was significantly more dramatic than that of gluten. Stiffness is a better index of tortilla hardness than rupture force, since extensible materials (CMC, gluten) require a lot of force to break without being hard or brittle.

Addition of more than 1% gluten caused a subsequent reversal in the softening effect of amylase (increased stiffness from that point on) on tortillas when 825 AU or more was used, confirming the trend observed with rupture force.

Effect of additives on amylopectin retrogradation: DSC analysis

In fresh tortillas (20 min after baking), no native endothermic peak was detected by DSC in the 45 –65 ^{o}C temperature range (Appendix Fig. B.1) for any treatment. Melting of re-crystallized amylopectin usually occurs within this temperature range in starch-based products (Campas-Baypoli et al. 2002).

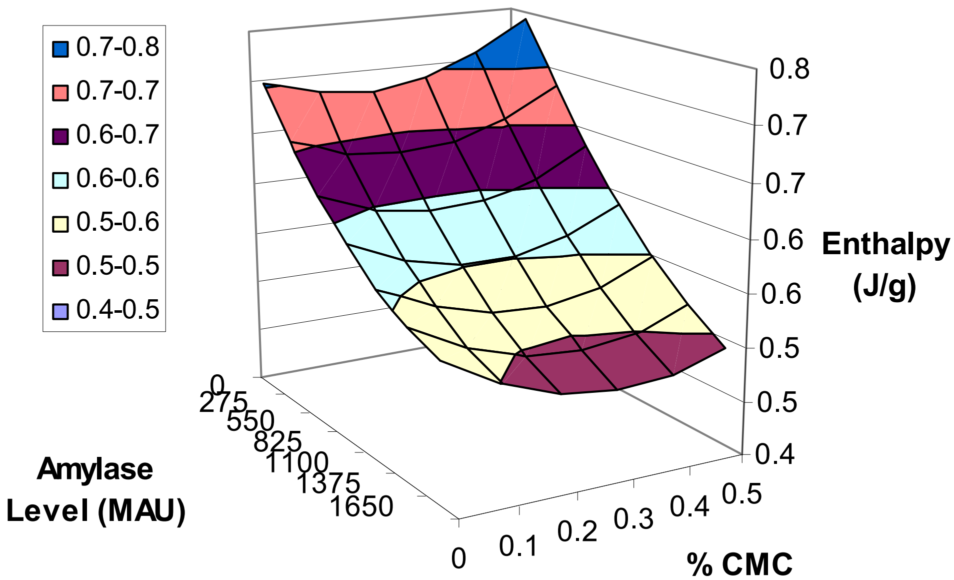
An endothermic melting peak was detected by DSC in methanol-stabilized tortilla extracts after 14 days storage (Appendix Fig. B.1). Onset of amylopectin melting occurred at 50.3 ^{o}C, peaked at 57.4 ^{o}C and ended at 65.9 ^{o}C. CMC, amylase and gluten did not alter these temperatures significantly for 14 day-old tortillas (Appendix B.13).

The endothermic amylopectin melting peak (peak value in mW and enthalpy in J/g) for tortillas stored 14 days was significantly reduced only by maltogenic amylase (Fig. 14 and Appendix B.14). The response surface model suggests 0.4% or more CMC significantly increases enthalpy of amylopectin melting compared to the control (Fig. 14). However, observed enthalpy values for tortillas with 0.5% were not significantly higher than control (Appendix Table B.15).

Gluten did not significantly change the enthalpy of amylopectin melting (Fig. 14, Appendix Tables B.14 and B.15). Maltogenic amylase was the only anti-staling agent that effectively interfered with amylopectin re-crystallization in tortillas during storage. The presence of CMC and or gluten (Fig. 14 and Appendix B.14) did not affect amylase activity and the enthalpy value.

Tortilla extensibility and flexibility: rupture distance vs. energy dissipated

Energy dissipated (J/m^3), an indicator of the tortilla viscous component obtained by stress relaxation, did not explain the variability in tortilla extensibility among treatments as well as the rupture distance indicator (obtained by the 1-D extensibility test) at any time evaluated (Table VII and Appendix Tables B.6, B.8, B.10 and B.12).



HSD = 0.15 J/g

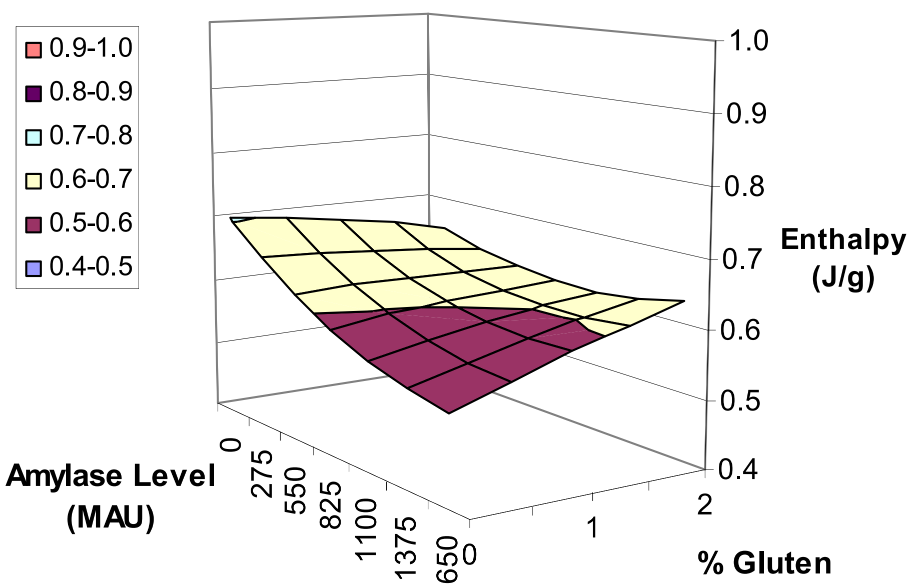


Fig. 14. Effect of ingredients on enthalpy of amylopectin melting of starch residues from tortillas stored 14 days.

Table VII

Comparison of Energy Dissipated and Rupture Distance as Indicators of Changes in Tortilla Flexibility Due to CMC, Maltogenic Amylase and Wheat Gluten During Storage

Indicator	Storage Time	Model Pr > f	R^2	% CV
Rupture Distance	20 Min	<0.001**	0.55	13.9
	14 Days	<0.001**	0.37	18.0
Energy Dissipated	20 Min	0.21	0.22	49.9
	14 Days	0.33	0.19	68.3

** Highly significant

Therefore, tortilla rupture distance was the best indicator of the effect of CMC, amylase and gluten on extensibility of fresh and stored tortillas.

In fresh tortillas (20 min after baking), only CMC (0.25% or more) significantly increased rupture distance, making tortillas more extensible than control (Appendix Tables B.5 and B.8). Gluten (2%), an additive which was supposed to make tortillas more extensible, did not significantly increase rupture distance compared to the control, while 1650 AU of amylase (a supposedly tortilla matrix weakener) did not significantly change tortilla rupture distance compared to control. However, a significant interaction between CMC and amylase was observed, indicating that an increase in the level of amylase while maintaining the level of CMC resulted in an increase in tortilla rupture force (extensibility) compared to using CMC alone. Amylase caused a more flexible and extensible CMC matrix in the tortilla continuous phase.

Significant reductions in rupture distance (extensibility) were observed in tortillas stored 14 days (mean = 1.72 mm) compared to fresh ones (mean = 8.8 mm) due to staling. Again, CMC (0.25% or more) was the only additive that made tortillas significantly more extensible than control after 14 days of storage (Fig.15).

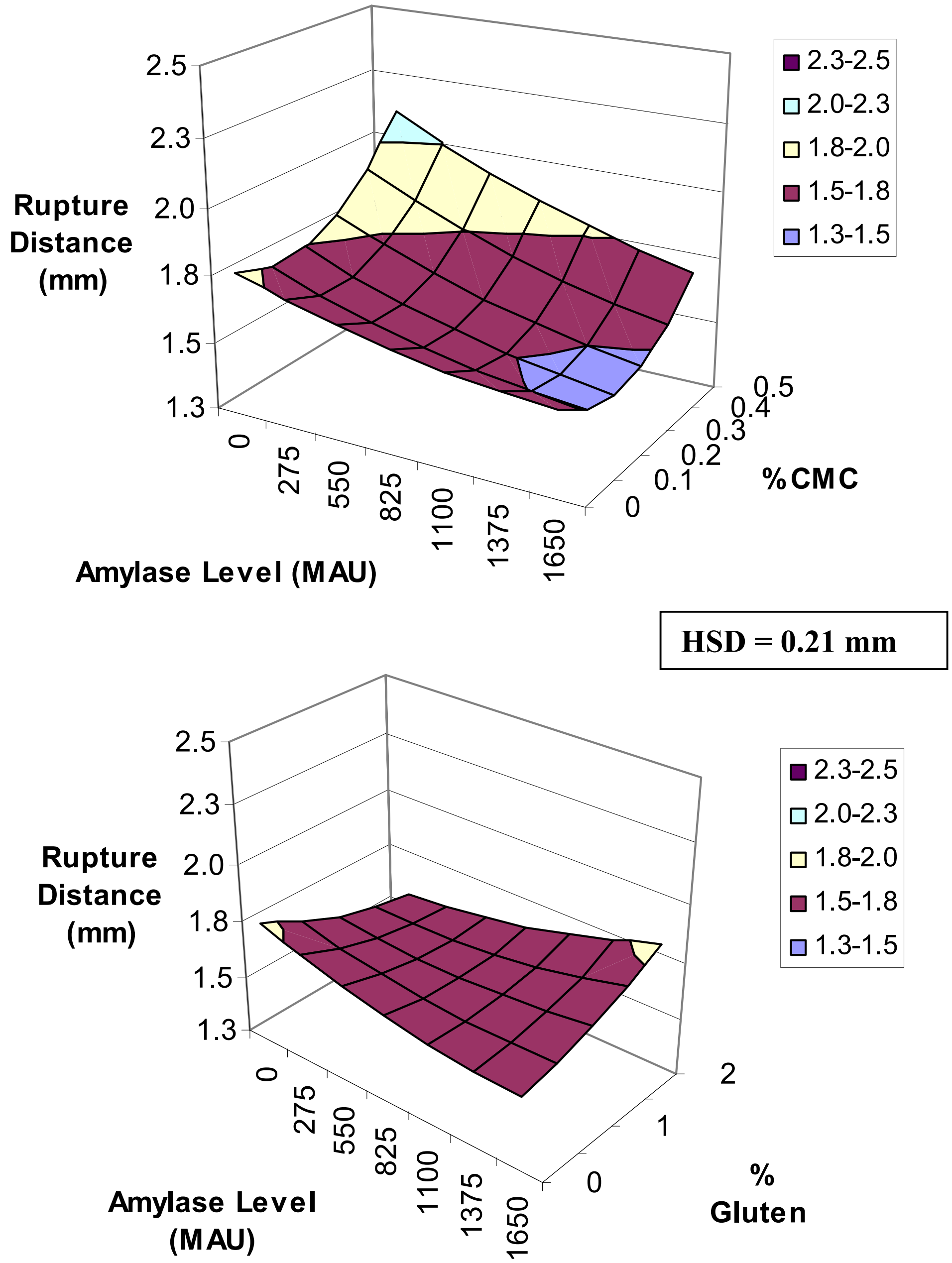


Fig. 15. Rupture distance of tortillas stored 14 days containing maltogenic amylase, CMC and vital wheat gluten.

Both amylase (550 AU or more) and gluten (1% or more) produced tortillas with significantly lower extensibility than the control when added alone. Amylase and gluten made tortillas softer but also less extensible. Combinations of amylase with CMC or gluten did not make tortillas significantly more extensible than the control after 14 days of storage.

Discussion

Effect of additives on masa mixing and sheeting

When masa for tortillas is produced using NCF, additives (CMC, wheat gluten and amylases) should normally be added as granular powders to facilitate mixing and storage. Nixtamalized corn flour for tortillas has a particle size distribution with a much lower proportion of particles that pass the 120 mesh (5.9%) than fresh masa (47%) (Gomez et al. 1992; Almeida-Dominguez 1996). This means that NCF is mostly comprised of pieces of endosperm and a small number of starch granules free of the protein matrix.

Dry mixing of NCF with the additives dispersed particles of CMC, amylase and gluten. The effectiveness of the maltogenic amylase as an hydrolytic anti-staling agent depended not only on how much of it was added, but on how much enzyme-susceptible starch was present in the NCF, how close to damaged starch particles the amylase was after mixing and how much time the amylase was given to work from activation (dry mixing) to inactivation (when tortilla reaches approx. 85 ^{o}C during baking). For all treatments in this study, 18 min passed from the start of dry mixing until tortilla baking was completed.

Hydration is the starting critical moment from the additive activation and tortilla structure formation points of view. ICS Amylase (MW = 69 kDa), being a much smaller molecule than CMC, wheat gluten and NCF particles (Florajancic et al. 2002; Gomez 1986), will certainly be dispersed in the continuous matrix formed by both hydrated NCF particles, CMC and wheat gluten.

Given that CMC has a higher affinity for water than the rest of the masa components, it is reasonable to believe that its linear molecules will tend to form an entangled amorphous matrix around hydrated NCF particles, therefore increasing the cohesiveness and flexibility of masa compared to the control and improving handling properties. Subjective masa texture observations and tortilla yield data (Table V) support this theory. At least 0.25% CMC was necessary to significantly improve masa machinability and consequently increase tortilla yields. Lower CMC levels might not provide enough molecules to entangle and create a sufficiently extended flexible network around hydrated NCF particles, free water, free starch and the other additives.

Hydrated vital wheat gluten will also tend to form a cross-linked and entangled amorphous matrix in between NCF particles as it does in bread dough. However, 2% wheat gluten apparently was not enough to generate a matrix as cohesive and flexible as CMC in the masa. Lower hydration capacity of vital gluten compared to CMC might also have confined matrix development to certain clusters in masa. This might be the reason why gluten was unable to improve masa machinability similar to CMC (Table V).

Even when CMC was added at the lowest level (0.25%) in combination with the highest gluten concentration (2%) it is likely that the CMC matrix predominated over the gluten matrix in masa. Combinations of gluten and CMC did not significantly improve tortilla yields compared to treatments with only CMC (Table V). Gluten matrix-forming activity might have been limited to areas engulfed by the predominant CMC matrix with scarce contribution to overall masa cohesiveness and flexibility (machinability).

0.25-0.5% CMC is added commercially to improve reconstituted dry masa cohesiveness and to increase machinability and tortilla yields (Serna-Saldivar 1996). Less than 2% gluten was not a total or partial substitute for CMC.

The maltogenic amylase dispersed in the aqueous phase acted on NCF particles. maltogenic amylase (1650 AU or less) did not affect handling properties of masa. The relatively low proportion of enzyme-susceptible starch (30% in NCF vs. 55% in fresh masa) available (Gomez et al. 1991), and the short time allowed for enzyme activity (17 min) before baking limits amylase activity in the masa. Therefore, dextrinization of starch was limited, there was no excessive water absorption and masas with amylase were equivalent to the control (Table V). Lower levels of amylases might be required when using fresh masa instead of NCF due to its higher level of enzyme-susceptible starch.

Effect of additives on fresh tortilla

Baking of tortillas took approx. 60 s. During this period, the tri-dimensional structure of the tortilla was set. Gelatinization of starch occurred, amylopectin crystallinity disappeared and double helices of amylose leached from the granule to form a flexible, amorphous, insoluble network upon retrogradation in the inter-granular aqueous continuous phase (Fernandez et al. 1999).

Addition of more than 0.25% CMC made fresh tortillas more elastic and cohesive than control, therefore requiring more extension force and distance to rupture (Table VI, Appendix B.5). Increase in fresh tortilla extensibility by adding CMC was not dramatic, since it was not detected by stress relaxation or by subjective rollability or pliability (Table VI, Appendix Tables B.3, B.4 and B.11).

A significantly higher distance was required to rupture fresh tortillas when 0.25% CMC was added. Increasing levels of amylase facilitated the formation and expansion of a flexible CMC matrix by limited hydrolysis of the retrograded amylose matrix during masa formation and tortilla baking.

Limited hydrolysis of amylose and amylopectin by 1650 AU of maltogenic amylase did not significantly weaken fresh tortilla structure to the point of reducing extensibility (lower rupture force and distance) compared to the control.

Fresh tortillas with amylase were not significantly softer than control (Table II). Gomez et al. (1991) and Fernandez et al. (1999) proposed retrograded (cross-linked) amylose gel as the “glue” that binds and holds the fresh tortilla together.

If that model is true, then maltogenic amylase activity did not significantly hydrolyze the amylose matrix during masa reconstitution and baking, and its anti-staling properties are not related to its activity on the amylose matrix.

Wheat gluten did not change fresh tortilla texture significantly, suggesting a lower ability to form a flexible matrix than CMC (Table VI, Appendix Tables B.5 and B.7). Furthermore, addition of vital wheat gluten should be limited to no more than 1%, since higher levels introduced a noticeable “wheat” flavor to the tortilla and produced a higher number of brown spots on the tortilla surface. This confirms the findings of Yau et al. (1994) and Miranda (1999).

Effect of additives on tortilla staling

A tortilla with acceptable texture should have a rollability and pliability score of at least 4, require no more than 4 N or 7 mm of extension to break. Stiffness values should be lower than $0.5x10^6$ Pa and energy dissipated at least $1x10^{-3}$ J/m^3. These values correspond to tortillas without additives stored for four hr at room temperature.

DSC analyses of fresh tortillas (20 min after baking) were unable to detect an endothermic melting peak corresponding to native or retrograded amylopectin within the 45-70 ^oC temperature range in any sample (Appendix Fig. B.1). DSC results suggest that amylopectin in tortillas lost its crystallinity during baking and was in an amorphous state 20 min after baking. No significant amylopectin re-crystallization (retrogradation) had occurred 20 min after baking and tortillas were perfectly rollable and pliable. This supports the theory that tortilla staling, just like bread staling, is a process dominated by the non-equilibrium re-crystallization of amylopectin (Levine and Slade 1991).

The appearance of a detectable endothermic melting peak at 57 ^oC in all tortilla samples stored for 14 days (Appendix Fig. B.1) further confirms the theory that correlates amylopectin re-crystallization with tortilla staling. Control tortillas stored for 14 days were significantly less rollable and less pliable than fresh tortillas to the point of being unacceptable (scores below 4).

Only maltogenic amylase significantly reduced amylopectin enthalpy of melting compared to the control. Since increasing levels of amylase also reduced tortilla stiffness and rupture force (Fig. 12 and 13), it can be concluded that the anti-staling properties of ICS maltogenic amylase rely on preventing the intra-granular re-crystallization of amylopectin in tortillas during storage. Boyle and Hebeda (1990) proposed that the reduction in length of amylopectin outer branches by removal of malto-oligosaccharides (DP 2-7) during mixing and baking was the mode of action of anti-staling maltogenic amylases. A higher proportion of short outer branches of amylopectin (DP 6-11) have been associated with reduced retrogradion in maize starches (Shi and Seib 1995).

Furthermore, no significant differences were found in the onset, peak and end temperatures of amylopectin melting due to amylase addition compared to the control and other treatments. This suggests that anti-staling properties of maltogenic amylase rely on reducing the degree of re-crystallization of amylopectin, and not in forcing the formation of a less perfect crystal structure with a lower melting point or a wider melting range.

Martin and Hoseney (1991) proposed malto-oligosaccharides have anti-staling properties for bread. However, Gerrard et al. (1997) contends that malto-oligosaccharides of DP 2-7 have no role as antistaling agents and are just by-products of maltogenic amylase activity on amylopectin. The role of malto-oligosaccharides generated by amylase activity in preventing amylopectin re-crystallization needs to be elucidated.

As little as 275 AU of ICS amylase were enough to produce a significant reduction in the enthalpy of amylopectin melting and, consequently produced softer tortillas (lower stiffness) than the control after 14 days of storage.

CMC and gluten did not reduce amylopectin enthalpy compared to the control in tortillas stored 14 days, confirming that their anti-staling properties do not rely on preventing amylopectin re-crystallization inside or outside the starch granule. As suggested in previous studies (Gomez et al. 1991; Suhendro 1997; Miranda 1999 and Quintero-Fuentes 1999), CMC and gluten most likely delay staling by creating a more flexible matrix than amylose alone in the tortilla inter-granular space.

Tortilla extensibility (Fig. 15) and subjective texture tests (Fig. 10 and 11) support the need for a two-way approach in delaying tortilla staling by using combinations of amylase (to reduce amylopectin re-crystallization) and an amorphous, matrix-forming additive (CMC) to counteract the collateral damage caused by the hydrolytic activity of amylase on the inter-granular amylose matrix and provide a more flexible “tortilla skeleton”. A combination of 0.25% CMC and 275-825 AU of maltogenic ICS amylase was found to produce tortillas with better texture. Tortillas were softer than control; and less chewy and equally flexible than tortillas with 0.5% CMC.

Based on the "fringed micelle" model developed for partially crystalline polymers (Wunderlich 1976) and adopted by food scientists such as Slade and Levine (1988) for starch gels and other starch-based products, a fresh tortilla could be viewed as a partially crystalline system with an amorphous phase (comprised of gelatinized starch granules full of amorphous amylopectin and remaining retrograded amylose) and a surrounding semi-crystalline but flexible matrix formed by retrograded amylose clusters and amorphous entangled amylose molecules. Amylopectin re-crystalliztation inside the starch granule during storage reduces the amorphous areas in the tortilla structure, increases rigidity and shrinks the overall structure. This may force the flexible amylose matrix to fail when the tortilla is rolled or squeezed.

When only amylases are used the intra-granular phase (amylopectin) tends to remain amorphous or become less crystalline over storage producing softer tortillas, but also reduces the flexibility of the amylose matrix by breaking down its molecules. By adding a network-forming additive immune to amylase activity (CMC, gluten, soy flour and beta-glucans), the loss of flexibility of the inter-granular amylose phase caused by amylase may be restored or improved. Mitre-Dieste (2002) reported that addition of 20% barley flour made corn tortillas more extensible than control after 9 days of storage under refrigeration.

Florjancic et al. (2002) reports that 0.25% CMC, when added in aqueous solutions, is enough to cause the formation of an amorphous matrix network structure. At higher CMC levels its polymer-polymer interactions (entanglements) become the main factor influencing the rheology of the system. Tortilla rupture force and distance data (Fig. 3 and 6) suggest the findings of Florjancic (2002) might be valid for explaining the anti-staling properties of CMC on corn tortillas at the inter-granular level.

A CMC and/or gluten network structure was generated during mixing and masa formation. The predominance of these amorphous networks over the partially crystalline matrix formed by a cross-linked polymer (amylose) during baking will depend on the relative concentrations of the polymers (Florjancic 2002), their chain length, tendency to cross-link, ionic charge and their temperature stability.

It is possible that a lower hydration capacity of vital wheat gluten compared to CMC and amylose molecules, coupled with lower heat stability explain the inability of gluten to maintain flexibility of tortillas as well as CMC at the levels evaluated in this study. Reductions in tortilla stiffness and extensibility by addition of 1% or more gluten might be caused by limited interference in the formation of the amylose network during baking at the inter-granular spaces. Similar effects have been seen by addition of 5% native soy flour to corn tortillas (Suhendro et al. 2001). When matrix-forming molecules are added in insufficient amounts to become the predominant continuous phase, they will be dispersed into clusters that will interfere with the amylose matrix at selected points. Tortillas with these additives (gluten or soy four) will be softer (shorter structure) but loss of extensibility during storage will not be reduced.

Wheat gluten might be useful as a softening agent at levels up to 1% in commercial tortillas as long as the cost is lower than adding 275 AU of a maltogenic amylase per kg of NCF. Addition of at least 0.25% CMC would be necessary to preserve tortilla flexibility.

CHAPTER VI

TEMPERATURE DEPENDENCE OF TORTILLA STALING RATE

Masa Quality

All three treatments produced masas with optimum cohesiveness and low stickiness. Therefore, masas were machinable and tortilla yields were not significantly different among treatments.

Tortilla pH and Moisture Content

Neither tortilla moisture content nor pH was significantly different among treatments (Table VIII). Moisture content CV was 1.22% and pH CV was 1.68%.

TABLE VIII

Effect of Maltogenic Amylase and CMC on Moisture Content and pH of Fresh Tortillas

Treatment	Moisture Content (%)	pH
Control	47.5	4.80
0.5% CMC	47.5	4.89
0.25% CMC + 1650 AU	47.4	4.90
HSD (0.05 %)	**0.68**	**0.10**

*Tukey's minimum significant difference

Effect of Storage Temperature on Tortilla Staling Rate

Tortillas individually packaged in polyethylene bags froze (-3 ^{o}C) after 6 min of storage in a –40 ^{o}C freezer while tortillas stored at –20 ^{o}C were frozen after 15 min of storage (Appendix Fig. C.1). It took two hr in both freezers for tortillas to reach the desired storage temperature (-20 and –40 ^{o}C respectively).

Subjective texture evaluations

Significant changes in tortilla rollability and pliability were detected during storage (Appendix Table C.1, C.2, C.3 and C.4). As observed in previous studies, tortilla pliability was the most sensitive indicator of changes in tortilla texture due to additives, storage time and/or temperature. The R^2 for tortilla rollability was 0.74 for the overall study and 0.81 at 21 days of storage, while the R^2 for pliability was 0.91 overall and 0.87 at 21 days. The evaluator however, appeared to be more precise in measuring tortilla rollability (overall CV = 12.3%, 21 days CV = 19.3%) than measuring pliability (overall CV = 16.7% , 21 days CV = 24.5%). Therefore, significant changes in tortilla texture due to treatments were detected with pliability after one day of storage at room temperature (Appendix C.4) while rollability differences were observed after 7 days (Appendix C.3).

Rollability and pliability of fresh tortillas (measured 20 min after baking) received a perfect score (5) regardless of the treatment, indicating that treatments did not exert noticeable changes to the texture of fresh tortillas at least when determined by subjective evaluations. (Appendix Tables C.3 and C.4).

No significant interaction was observed among additives and storage temperature (Appendix Tables C.1 and C.2) on tortilla rollability or pliability. This means the treatment that produced tortillas with the highest rollability and pliability did so regardless of the storage temperature.

After one day of storage, only control tortillas showed significant reductions in rollability compared with fresh tortillas (Fig. 16, Appendix Table C.3) especially for storage at 10 ^oC. Tortillas with 0.5% CMC and tortillas with 0.25% CMC plus 1650 AU of maltogenic amylase remained perfectly rollable (no significant reductions were observed) regardless of the storage temperature after one day.

Control tortillas stored for 7 days were significantly less rollable than fresh tortillas at every storage temperature evaluated (Fig. 16). Reductions in rollability were bigger when control tortillas were stored under refrigeration (3-10 ^oC) than at room (21 ^oC) or freezing temperatures (-20 ^oC or lower). Rollability of frozen control tortillas and of tortillas stored at room temperature was similar after seven days of storage. Tortillas without additives staled faster at refrigeration temperatures; staling rate at freezing was similar to staling rate at room temperature.

Tortillas with 0.5% CMC or with a combination of 0.25% CMC and 1650 AU of amylase showed significant reductions in rollability compared to fresh tortillas only when stored under refrigeration (3-10 ^{o}C). However, rollability of seven-day old tortillas with additives was significantly better than control tortillas stored under refrigeration (Fig. 16, Appendix Table C.3). Rollability of tortillas with 0.25% CMC and 1650 AU of amylase was similar to tortillas with only 0.5% CMC regardless of storage temperature, suggesting that both treatments are equally good in preserving rollability for a period of 7 days.

After 21 days of storage (Fig. 16 and Appendix Table C.3), control tortillas maintained an acceptable rollability (>4) only when stored frozen ($\leq$ -20 ^{o}C). Storage under refrigeration (3-10 ^{o}C) accentuated the loss of rollability in control tortillas compared to room temperature. Control tortillas stored at 3 ^{o}C had the lowest rollability.

Unlike control tortillas, tortillas with additives remained perfectly rollable after 21 days when stored either frozen or at room temperature (Fig. 16, Appendix Table C.3). At refrigeration temperatures, rollability of tortillas with additives fell under the acceptable level (<4). However, tortillas with additives were significantly more rollable than control tortillas when stored either at room temperature or under refrigeration.

Tortillas with 0.5% CMC stored for 21 days showed the lowest rollability when stored at 10 ^{o}C (score = 2.75), unlike control tortillas which were least rollable when stored at 3 ^{o}C (score = 2.00). At 10 ^{o}C, only tortillas with 0.25% CMC and 1650 AU amylase showed acceptable rollability after 21 days of storage. As storage temperature was reduced below 10 ^{o}C, rollability of tortillas with a combination of CMC and amylase decreased significantly and reached its lowest point at 3 ^{o}C (score = 3.25).

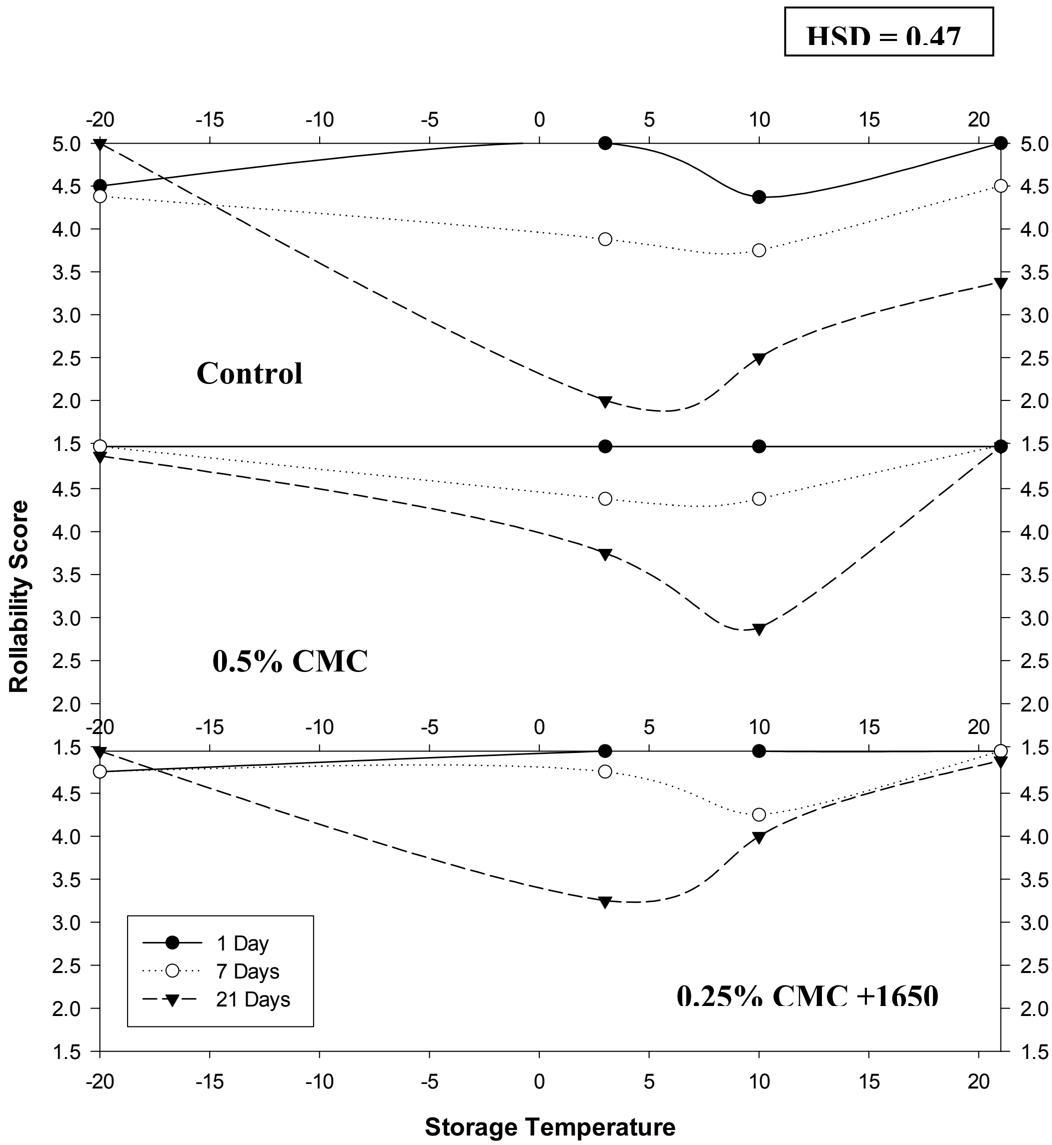


Fig. 16. Effect of storage temperature on rollability of tortillas with added CMC and maltogenic amylase after 1, 7 and 21 days.

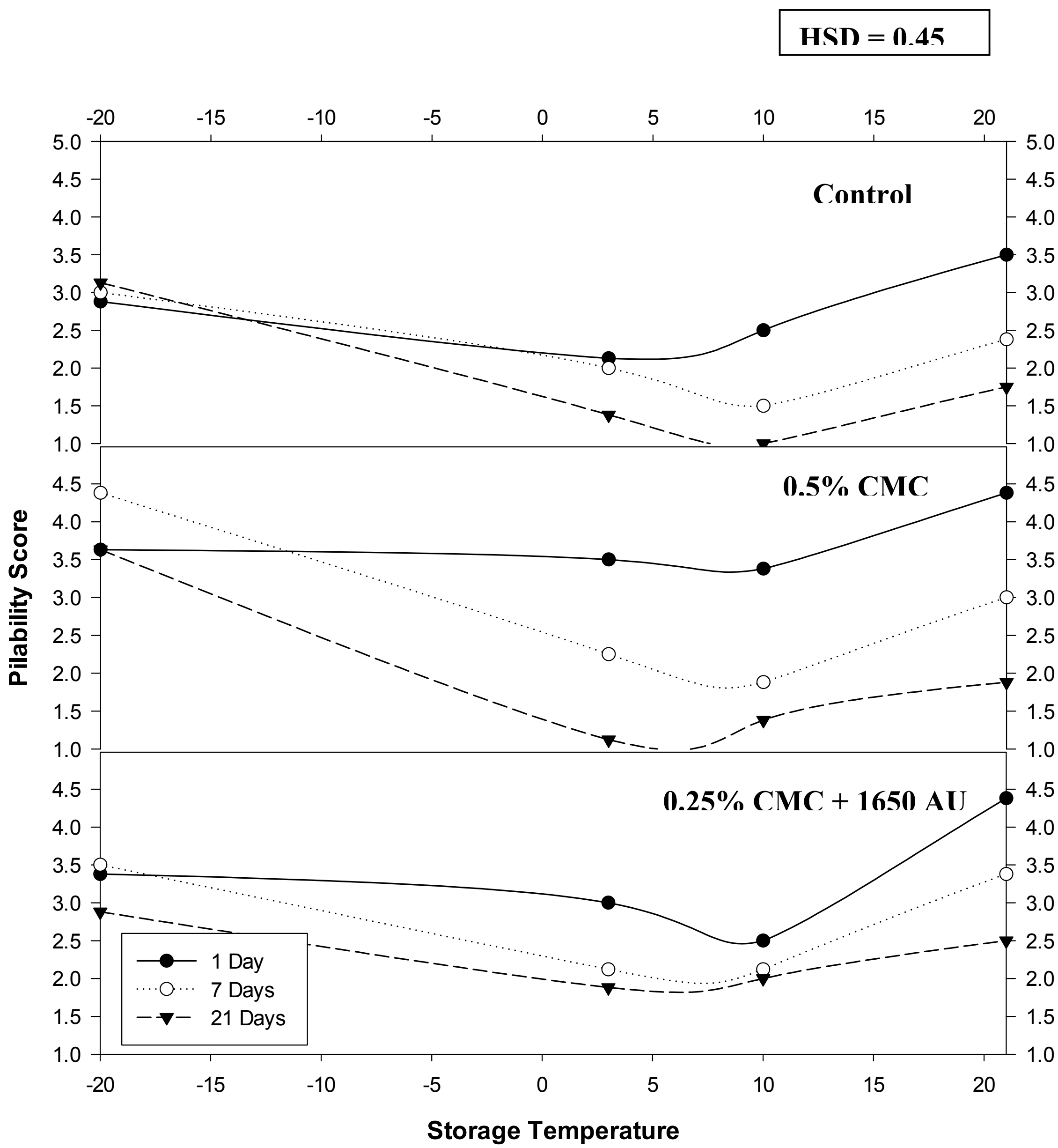


Fig. 17. Effect of storage temperature on pliability of tortillas with added CMC and maltogenic amylase after 1, 7 and 21 days.

Significant reductions in pliability were observed in tortillas after just one day of storage compared to fresh tortillas regardless of the treatment (Fig. 17 and Appendix Table C.4). The bell-shaped relationship between storage temperature and tortilla pliability was visible for all treatments after just one day, compared to the rollability curve that was visible only after a week of storage.

Storage temperatures below 21 ^{o}C significantly reduced pliability of tortillas with or without additives below the level of acceptability (score = 4) after one day of storage (Fig. 17, Appendix C.4). At room temperature, however, tortillas with additives stored for one day were significantly more pliable than the control, staying above the acceptable level. Tortillas without additives were least pliable when stored at 3^{o}C while tortillas with additives were least pliable at 10 ^{o}C. Loss of tortilla pliability under storage at freezing temperatures ($\leq$20 ^{o}C) was significantly higher than at room temperature but not as high as under refrigeration (3-10 ^{o}C) for treatments with less than 0.5% CMC. Tortillas with 0.5% CMC were more pliable than tortillas with the combination of 0.25% CMC and 1650 AU of amylase under refrigeration after one day of storage.

After seven days of storage, pliability of tortillas significantly decreased compared with tortillas evaluated after one day of storage at room and refrigeration temperatures (Fig. 17). Reductions in pliability for frozen tortillas were significant only for tortillas with less than 0.5% CMC. Again, storage at refrigeration temperatures produced tortillas with least pliability, treatment notwithstanding. After one week of storage, freezing preserved tortilla pliability better than room and refrigeration temperatures.

Similar tendencies were observed for 7 and 21 days of storage (Fig. 17). Freezing preserved tortilla pliability better than storage at room temperature; refrigeration (3-10 ^{o}C) caused the biggest losses in pliability. However, tortillas stored frozen at –40 ^{o}C were significantly more pliable than tortillas stored at any other temperature, especially when 0.5% CMC was added (Appendix Table C.4). CMC may have provided improved freeze-thaw stability to tortillas.

A combination of 0.25% CMC and 1650 AU of maltogenic amylase produced tortillas with higher pliability than control and tortillas with only 0.5% CMC stored at room temperature and under refrigeration (Fig. 17).

Objective texture evaluations

Significant changes in tortilla stiffness were observed during 21 days of storage (Fig. 18 and Appendix Tables C.5, C.6 and C.7). The model explained differences in stiffness among treatments well (R^2 = 0.94 overall and R^2 = 0.91 at 21 days). Precision of the stress relaxation method to estimate stiffness of tortillas was good (CV= 11.2% overall, CV= 16% at 21 days).

Storage temperature and additives significantly changed tortilla stiffness (Appendix C.5 and C.6). The temperature * additives interaction was also significant indicating that the effect of amylase and CMC was not the same on tortilla stiffness at different storage temperatures.

Most of the variation in tortilla stiffness was due to storage time (Appendix C.5). When tortilla stiffness was evaluated 21 days after storage most of the differences among treatments were caused by storage temperature and then by the additives (Appendix Table C.6).

Appendix Table C.7 shows stiffness significantly increased during storage at room temperature (21 ^{o}C), especially during the first week, and then reached a plateau.

Fresh tortillas (20 min after baking) had similar stiffness regardless of the treatment (Appendix Table C.7). Differences in tortilla stiffness due to additives were significant after one day of storage (similar to tortilla pliability data), but they were more dramatic seven days after baking (Fig. 18).

A combination of 0.25% CMC and 1650 AU of maltogenic amylase significantly reduced the stiffness of tortillas stored 21 days at room temperature compared to the control and to tortillas with 0.5% CMC. Control and 0.5% CMC tortillas had similar stiffness seven days of storage (Fig. 18).

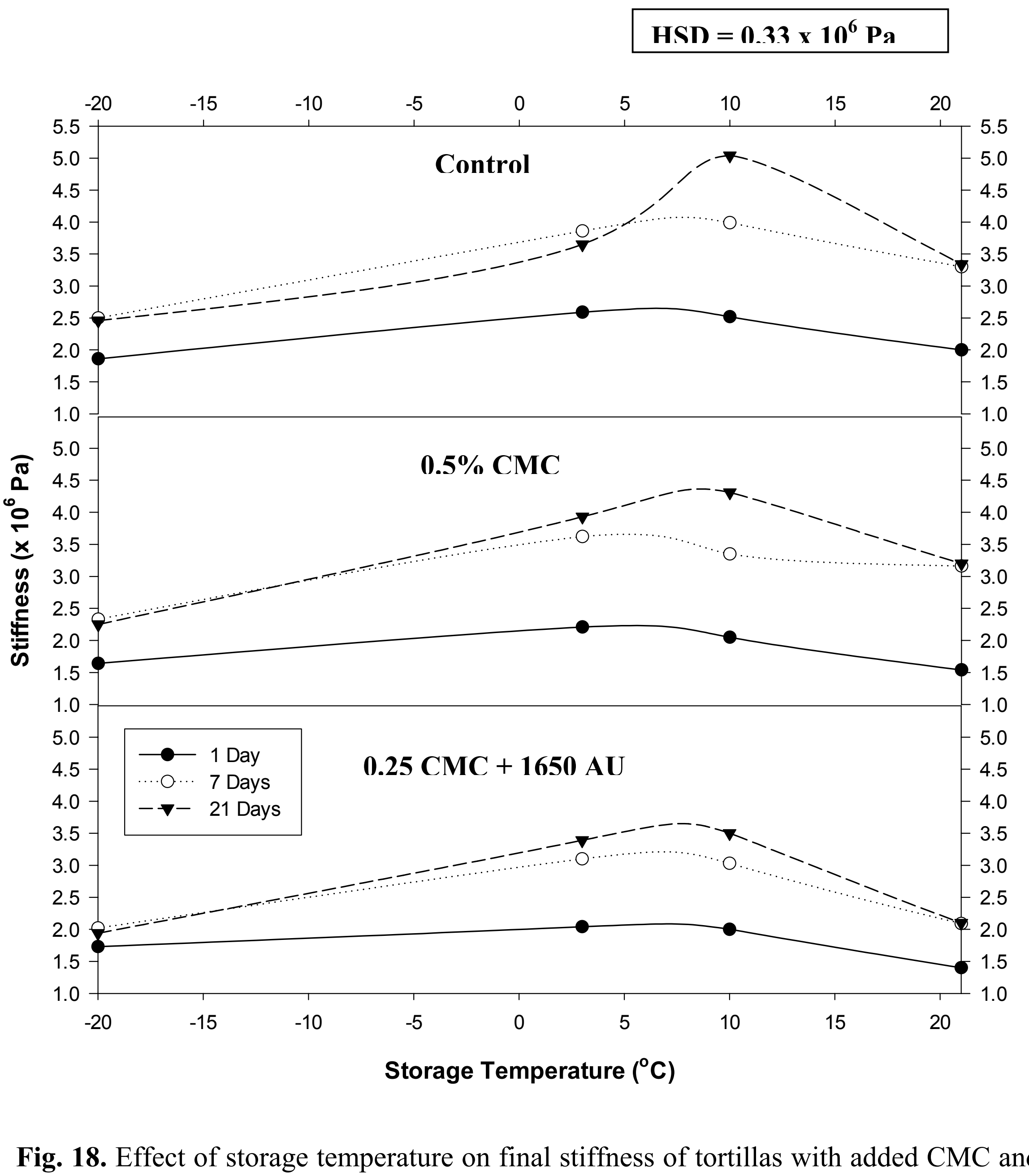


Fig. 18. Effect of storage temperature on final stiffness of tortillas with added CMC and maltogenic amylase after 1, 7 and 21 days.

A bell-shaped curve was observed for tortilla stiffness dependence on storage temperature one day after baking (Fig. 18). Stiffness of tortillas stored frozen ($t \leq -20\ ^oC$) showed similar stiffness than tortillas stored at room temperature (21 ^oC) after one day (Fig. 18 and Appendix Table C.7). Tortillas stored under refrigeration (3-10 ^oC) were significantly stiffer than tortillas stored frozen or at room temperature.

One-day old tortillas with a combination of 0.25% CMC and 1650 AU of maltogenic amylase were significantly less stiff than control tortillas at all storage temperatures, except for –20 ^oC (Fig. 18). Tortillas with 0.5% CMC were significantly less stiff than control tortillas only when storage was conducted under room and refrigeration temperatures.

Stiffness of tortillas with 0.25% and 1650 AU amylase stored for 7 days was similar when stored frozen or at room temperature, and was only significantly higher when stored under refrigeration (Fig. 18 and Appendix Table C.7).

Tortillas with no additives or with only 0.5% CMC were significantly less stiff when stored frozen than when stored at room temperature and stiffest when stored under refrigeration. Stiffness of tortillas without additives and of tortillas with 0.5% CMC was similar at all storage temperature evaluated seven days after baking. Tortillas with 0.25% CMC and 1650 AU amylase were significantly less stiff than control and tortillas with 0.5% without regard to storage temperature.

Tortillas became stiffer when stored at 10 ^oC for 21 days than at any other temperature evaluated (Fig. 18). Tortillas without additives and with 0.5% CMC became significantly stiffer after 7 days when stored at 10 ^oC, while tortillas with CMC and amylase did not. This indicates that amylase was more effective in producing less stiff tortillas than CMC even at the storage temperature with the highest stiffening rate (10^oC).

DSC analysis

DSC analysis of tortilla extracts methanol-stabilized 20 min after baking showed a re-crystallized amylopectin melting peak at 57 ^{o}C, unlike the previous study, where no peak was observed (Appendix Table C.11).

Storage temperature significantly changed melting enthalpy of re-crystallized amylopectin in tortillas during storage (Fig. 19, Appendix Tables C. 9, C.10, C11). Tortillas stored under refrigeration temperatures (3-10 ^{o}C) for more than a week showed higher enthalpy values than tortillas stored frozen or at room temperature (Fig. 19, Appendix Table C.11). Amylase reduced the enthalpy during amylopectin melting of tortillas stored at freezing and room temperatures for 21 days compared to control and 0.5% CMC tortillas. However, under refrigeration temperatures this reduction was not statistically significant.

RVA analysis

Pasting viscosity of tortilla extracts progressively decreased as storage time increased (Fig. 20), which indicated significant starch retrogradation.

RVA pasting viscosity of methanol-stabilized 21-day old tortilla extracts (Fig. 20 and Appendix Table C.12) showed a different relationship with storage temperature than other staling indicators used in this study. RVA pasting viscosity of 21-day old control tortilla extracts decreased as storage temperature increased. Pasting viscosity of 21-day old tortillas with CMC and/or amylase did not show a consistent temperature dependence trend (bell-shaped curve with a peak at 3-10 ^{o}C) as observed by subjective and objective texture measurement methods, and DSC analysis (Appendix Table C.12).

RVA analysis however, showed that tortilla extracts with amylase that were stabilized after 21 days of storage developed higher pasting viscosities than control and 0.5% CMC tortillas. This confirms the antistaling properties of maltogenic amylase.

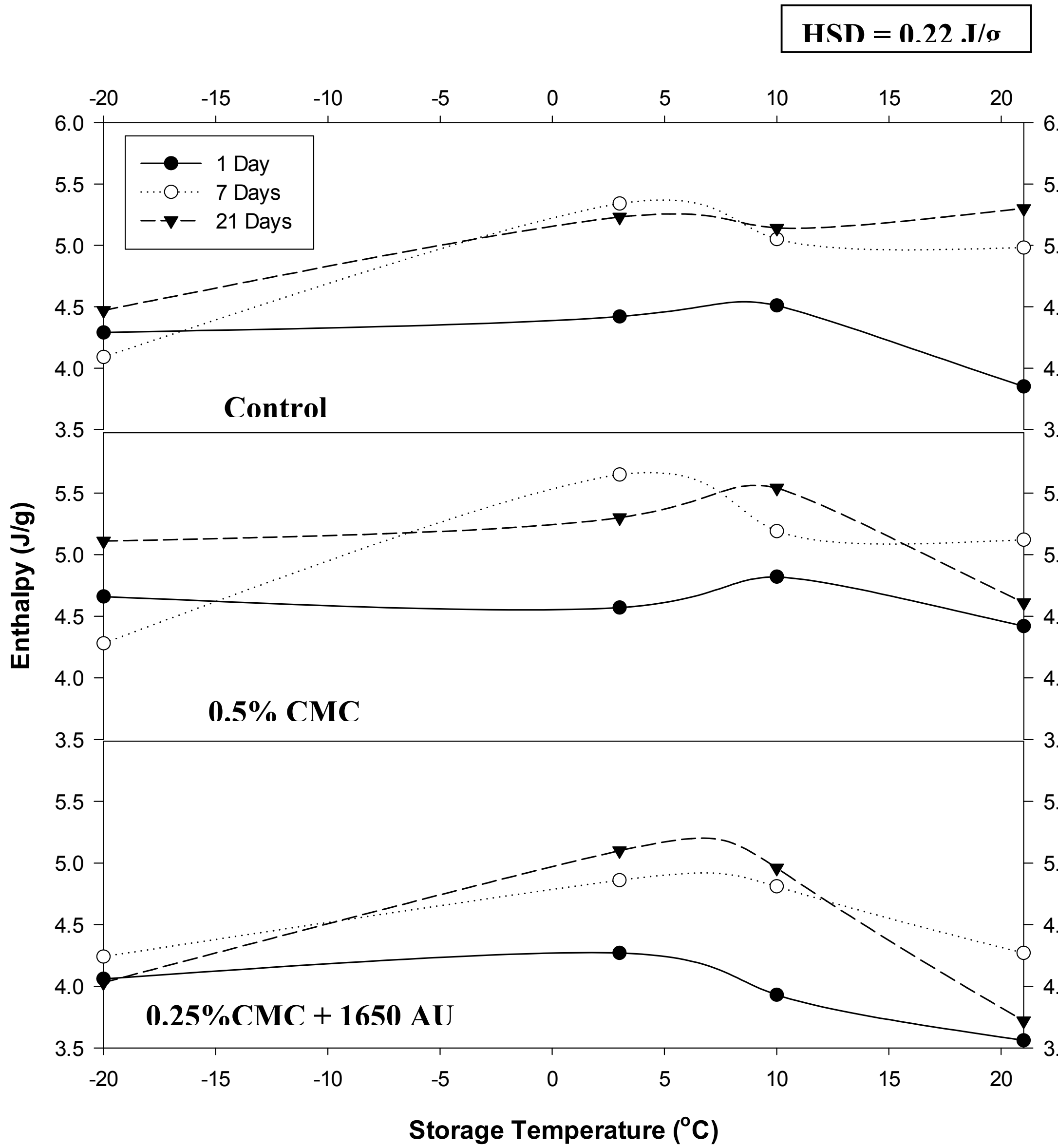


Fig. 19. Effect of storage temperature on amylopectin enthalpy of melting of tortillas with added CMC and maltogenic amylase after 1,7 and 21 days.

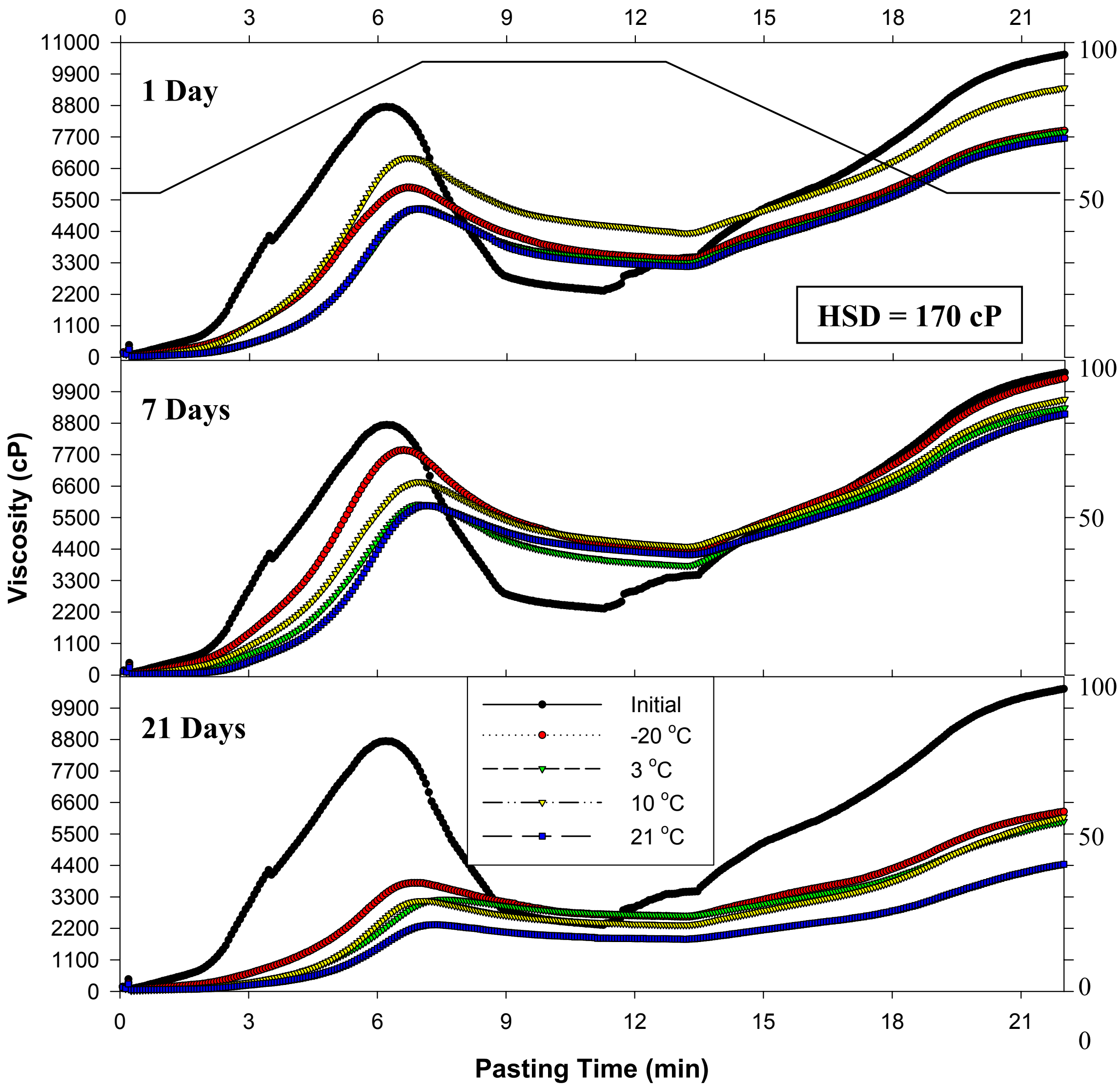


Fig. 20. Effect of storage temperature on pasting viscosity of 15% solids slurries of methanol-extracted residue of fresh, 1, 7, and 21-day old control tortillas measured with the RVA.

Changes in tortilla viscous component: energy dissipated

Appendix Table C.8 shows that energy dissipated (J/m^3) by tortillas decreased significantly during storage, and especially during the first 24 hr (1.22 $x10^{-4}$ J/m^3) compared to fresh tortillas (8.25 $x10^{-4}$ J/m^3). A staled tortilla shows more solid behavior and less viscous properties than a fresh tortilla, therefore dissipating less energy when deformed.

Significant differences in energy dissipated, an indicator of viscous (flow) behavior in materials evaluated by stress relaxation, were observed among tortilla treatments until 7 days of storage (Fig. 21, Appendix Table C.8). Energy dissipated was a less precise indicator of changes in tortilla texture (overall CV = 76%, at 21 days CV = 68%) than stiffness, pliability and rollability. The model R^2 for Energy dissipated was 0.73 for the complete set of data and 0.41 for measurements taken 21 days after baking.

Energy dissipated by tortillas decreased dramatically after just one day of storage and reached the lowest point after one week of storage at room temperature, with no significant changes occurring afterwards (Appendix Table C.8). Energy dissipated (ED) of fresh tortillas with 0.25% CMC and 1650 AU at room temperature was significantly higher than ED of tortillas with no additives and tortillas with 0.5% CMC.

Tortillas stored for one day dissipated less energy during the stress relaxation test when stored under refrigeration (3-10 ^oC) than those stored frozen (-20 ^oC) or at room temperature (Fig 21). A combination of 0.25% CMC and 1650 AU of amylase produced tortillas that dissipated significantly more energy than control and 0.5% CMC after one day of storage under either refrigeration or room temperatures.

Tortillas stored one and three weeks dissipated less energy when refrigerated than frozen (Fig. 21, Appendix table C.8). Seven and 21-day old tortillas with amylase and CMC dissipated more energy than other treatments only when stored frozen. When tortillas were stored for 7 to 21 days at room temperature or under refrigeration additives did not increase energy dissipation compared to the control.

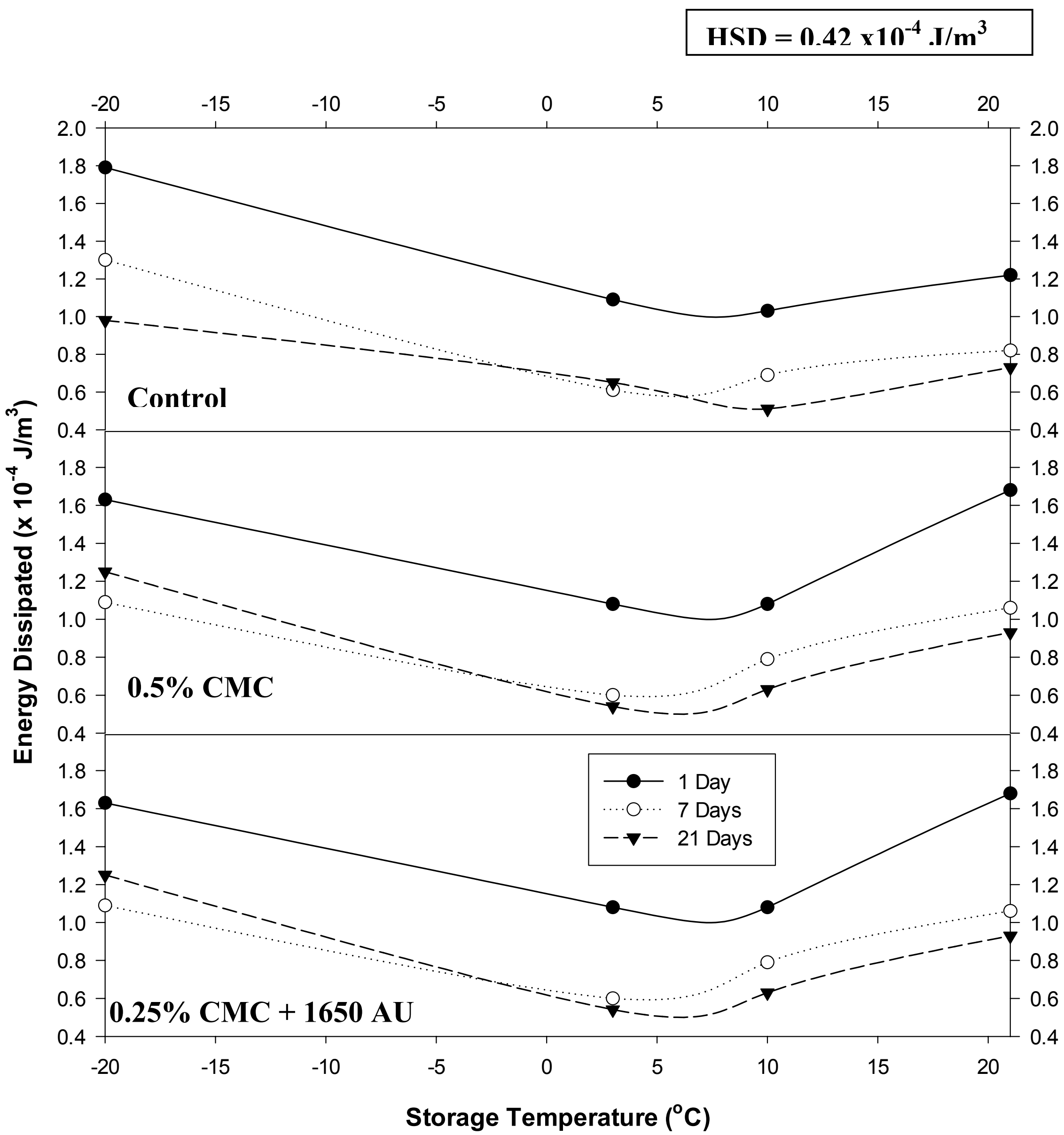


Fig. 21. Effect of storage temperature on energy dissipated of tortillas with added CMC and maltogenic amylase after 1, 7 and 21days.

Discussion

Results from this study support the findings of Limanond et al. (2001) stating the level of staling in corn tortillas is a function of not only time but also of storage temperature. Tortilla rollability and pliability, and stiffness and energy dissipated obtained by stress relaxation, and DSC showed a bell-shaped dependence on storage temperature from –40 to 21 ^{o}C. Only pasting viscosity measured by RVA showed a linear inverse relationship with storage temperature, suggesting other tortilla compounds (besides amylopectin) may re-associate during storage

Tortillas stored at –40 ^{o}C were not always consistent with this model, especially on objective texture measurements. Tortillas with and without additives reacted differently to freezing-thawing processes and confounded the effect of the –40 storage temperature. Also, temperature fluctuations in the –40 ^{o}C freezer occurred because of the air conditioning system of the building. Therefore, data from samples stored at –40 ^{o}C were not considered for this discussion.

Results are in accordance with theories of crystallization reviewed by Levine and Slade (1991) for starch gels, and applied by different scientists to study the retrogradation of gelatinization of waxy maize starch (Farhart et al. 2000) and corn tortillas (Limanond et al. 2001).

Corn masa (Fig. 22) and tortillas (Fig. 23 and 24), according to these theories, are semi-crystalline systems that, unlike amorphous polymers, age at temperatures above the T_g and below the T_m. Gomez et al. (1992) had already proposed graphic models of masa and tortilla structure (fresh and staled). Fig. 22, 23 and 24 use Gomez et al. (1992) framework and adds representations of retrograded amylose and CMC matrices in the intergranular space, and the hydrolytic activity of maltogenic amylases inside the starch granule.

Staling of tortillas during storage has been attributed to the non-equilibrium re-crystallization of amylopectin (Fernandez et al. 1999, Limanond et al. 2001). Also, amylopectin re-crystallization is the driving force behind tortilla staling. However, RVA data vindicates previous research that suggests other compounds such as amylose may be involved in the staling process (Seetharaman et al. 2002), depending on its degree of dispersion in the intergranular tortilla matrix.

Limanond et al. (2001) reported that tortilla Tg was –23 ^oC and amylopectin T_m was 90 ^oC. However, DSC results support the findings of Campas-Baypoli et al. (2002) indicating that re-crystallized amylopectin melts in the 45-64 ^oC range with a peak at 57 ^oC.

Therefore, if tortillas age like a typical semi-crystalline system, staling should occur in the –23 ^oC to 57 ^oC range showing a maximum rate somewhere around the middle point of this range (17 ^oC). Staling below Tg (-23 ^oC) would be minimal due to lack of molecular mobility (Struik 1978).

Limanond et al. (2001) estimated by linear regression that 13 ^oC was the temperature where maximum rate of corn tortilla retrogradation occurred. Her conclusion was that in tortilla staling, crystal nucleation predominated over crystal growth at least during the storage period she covered (12 days). This shifted the maximum staling rate closer to T_g than to T_m.

Subjective rollability and pliability (Fig. 16 and 17), Energy dissipated (Fig 21) and DSC data (Fig. 19) indicated that maximum loss of flexibility of tortillas occurred during refrigerated storage (3-10 ^oC). Tortilla pliability and energy dissipated were particularly sensitive indicators, showing a bell-shaped curve along the temperature range as early as one day after baking but more clearly after seven days of storage (Fig. 17, 21).

Tortilla stiffness (estimated by stress relaxation) was a more precise indicator of texture changes during storage than subjective tests and energy dissipated (higher model fit and lower % CV). Stiffness data of tortillas stored for one day confirmed a higher degree of staling in control tortillas stored under refrigeration. Stiffness data after one or three weeks of storage, however, clearly indicate that tortillas with or without additives stored at 10 ^oC became stiffer than at any other temperature evaluated.

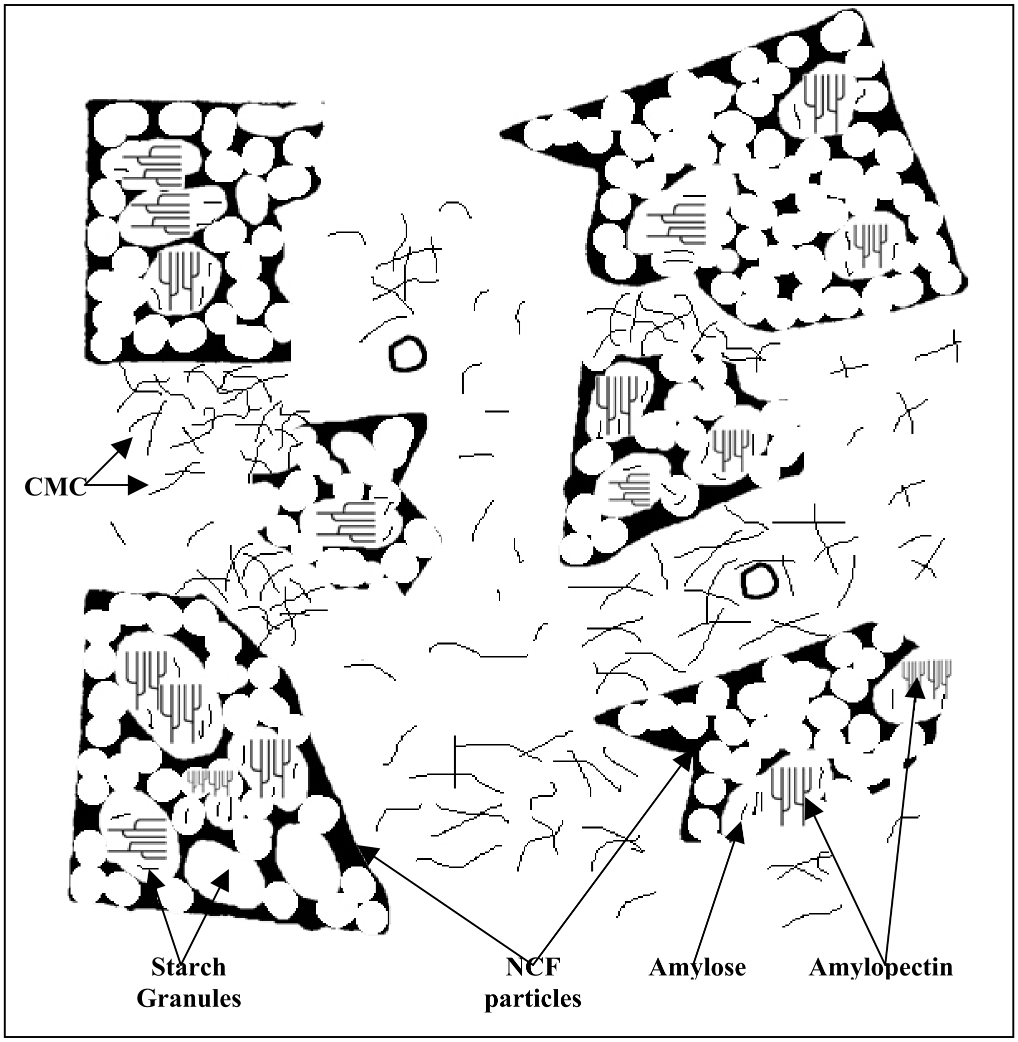


Fig. 22. Structure of masa made with nixtamalized corn flour.

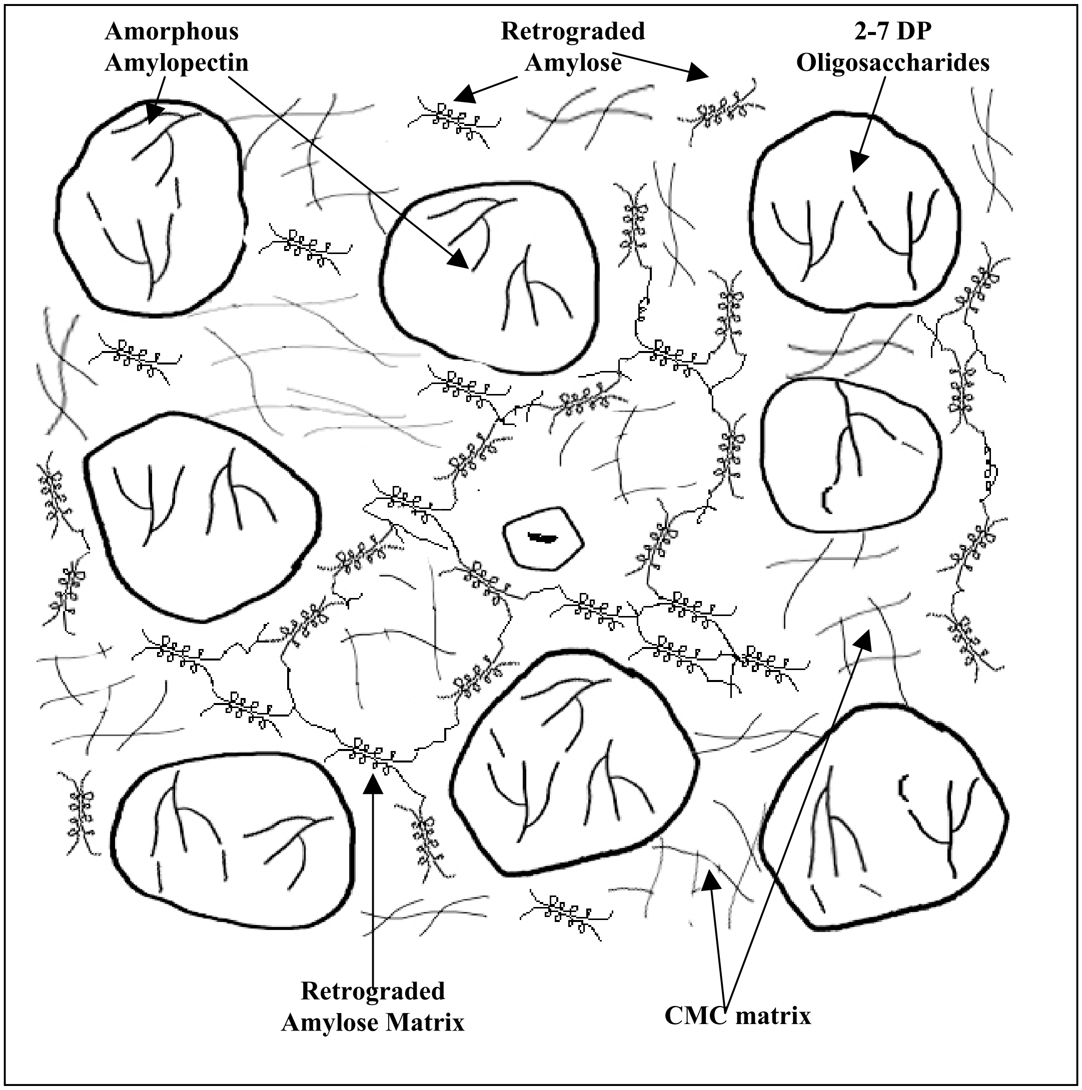


Fig. 23. Structure of a fresh tortilla.

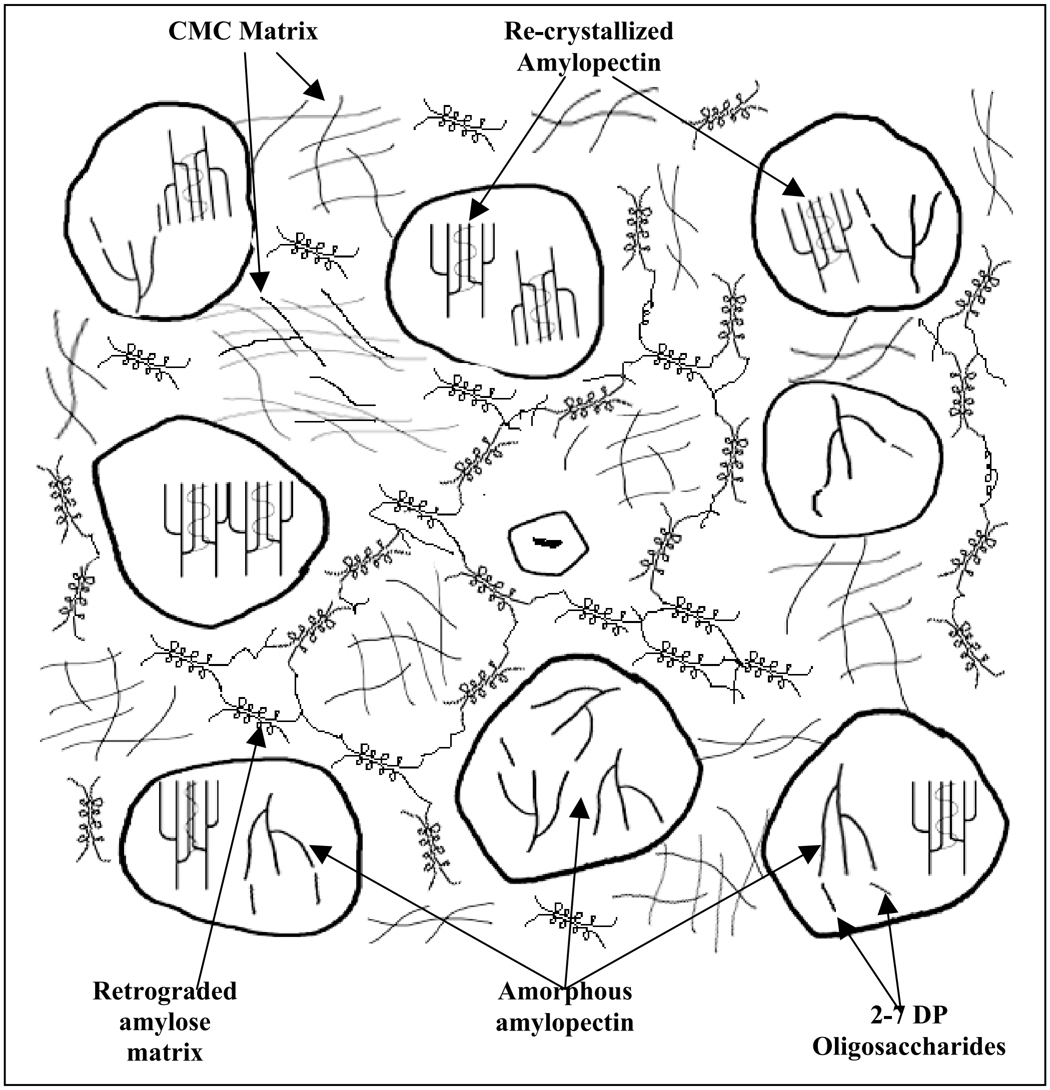


Fig. 24. Structure of a staled tortilla.

Given the high correlation found between stiffness data and enthalpy of amylopectin melting in the previous study, it could be inferred that amylopectin retrogradation was maximized when tortillas were stored at 10 ^{o}C. However, the DSC (Fig. 19) showed that control tortillas reached maximum amylopectin retrogradation when stored at 7 ^{o}C for at least a week.

Tortillas without additives stored at freezing temperatures ($\leq$-20 ^{o}C) for 21 days were more pliable (Fig. 17), less stiff (Fig. 19) and showed lower amylopectin melting enthalpy values than tortillas stored at room temperature. Freezing and thawing of tortilla samples might have damaged tortilla structure, therefore producing misleadingly lower stiffness values. However, the structural damage caused by freezing and thawing, if any, did not appear to be extensive enough to reduce pliability and rollability of control tortillas stored at –20 ^{o}C (Fig. 16-17). Therefore, results from this study suggest tortillas stored frozen ($\leq$-20 ^{o}C) staled less than tortillas stored under refrigeration or at room temperature. Further studies are required to confirm these findings. Estimation of staling in frozen tortillas should rely less on indirect textural techniques in favor of procedures that measure tortilla re-crystallization more directly (DSC, X-ray diffraction or ESEM microscopy).

Whatever the case, these results support the findings of Limanond et al. (2001) and the theory of crystallization of semi-crystalline materials (Struik 1978). Freezing temperatures close or below tortilla Tg limit the mobility of amylopectin and other amorphous molecules and therefore reduce their chance of getting close to each other to crosslink.

Tortillas staled (became stiffer, less rollable and pliable, and had bigger enthalpies of amylopectin melting) during storage (Fig. 16-21) even when frozen at –40 ^{o}C. DSC analysis of fresh and 21-day old tortillas did not detect any glass transition in the –40 to 45 ^{o}C range. This suggests that the Tg of corn tortillas might be well below the –23 ^{o}C proposed by Limanond et al. (2001) or that amylopectin is able to crosslink even below Tg and the crystallization theory of semi-crystalline polymers does not fully apply to corn tortillas. Further research is required in this area, taking special care of flash freezing fresh tortillas (with liquid nitrogen) immediately after cooling to avoid staling during the time it takes the tortillas to reach freezer temperature (-20 or –40 ^{o}C)

As for the effect of additives on tortilla staling at different storage temperatures, the combination of 0.25% and 1650 AU made 21 day-old tortillas more pliable, rollable and less stiff than the control when stored under refrigeration or at room temperature. Furthermore, this combination of additives reduced amylopectin re-crystallization (Fig. 19) made tortillas less stiff and more pliable than tortillas with 0.5% CMC regardless of the storage temperature 21 days after baking (Fig. 17, 18). This confirms the effectiveness of maltogenic amylase as an anti-staling agent.

Tortillas with 0.5% CMC were significantly more pliable than other treatments when stored frozen for 21 days because CMC gave them higher freeze-thaw stability than control and because amylase alone weakened the tortilla structure and made them more susceptible to crumble after one freeze-thaw cycle.

The most practical conclusion of this study is that adding 0.25% CMC and 1650 AU of amylase is the best option to maintain tortilla softness and flexibility both at room temperature and under refrigeration for at least three weeks. This means prolonging the shelf-life of tortillas both in the supermarket (room temperature) and at home (refrigerated).

As for the possibility of commercializing tortilla-based products without additives (anti-molding, anti-staling agents), freezing might be the only option to keep staling at a minimum. Even so, a modified starch or any other additive that provides freeze-thaw stability might be necessary.

Tortillas behaved like a semi-crystalline system under storage. The fringed micelle model proposed for starch gels by Levine and Slade (1991) appears to work for corn tortillas too.

CHAPTER VII

CONCLUSIONS

The results from this study give a clearer picture of the mechanisms that lead to corn tortilla staling and how tortilla components, additives and external factors such as storage temperature and time accelerate or delay this process.

Basic theories of staling of starch-based products seem to apply to the corn tortilla system, with some differences inherent to its particular nature. Results from this study support the theory that amylopectin re-crystallization is the driving force behind the staling of corn tortillas during storage.

Re-crystallization of amylopectin as measured by DSC was similar to increased stiffness and reductions in tortilla rollability, pliability and extensibility over time and temperature. Re-crystallization of amylopectin in fresh tortillas was low or non detectable, it increased quickly during the first 24 hr and reached a plateau after 7 days of storage. Amylopectin re-crystallization also showed a bell-shaped trend with storage temperature with a maximum around 7 ^{o}C.

Staling of corn tortilla follows the basic laws that control aging in semi-crystalline systems such as polymer melts, starch gels and other baked products. However, there are indications that these theories may not fully apply to corn tortillas. RVA data suggests other molecules (possibly amylose) tend to retrograde in a different fashion than amylopectin during storage. More research is required to determine the role of amylose in tortilla staling during storage and to establish the glass transition temperature of a corn tortilla as a starting point to confirm if tortillas stale below it.

Even though amylopectin re-crystallization is the main force behind tortilla staling, this does not mean that interfering with this process is the only way to retard staling. Addition of 275-1650 AU of ICS maltogenic amylase effectively reduced amylopectin retrogradation, but was not able to maintain tortilla flexibility.

Delaying tortilla staling requires a two-pronged approach: interfering with amylopectin re-crystallization inside gelatinized starch granules and creating a more flexible inter-granular matrix than re-crystallized amylose provides (with CMC, ß-glucans, pentosans or soybean proteins). The combination of 825 AU of ICS maltogenic amylase and 0.25% CMC appeared to do that. Tortillas with this combination of additives were softer, equally flexible and less chewy than tortillas with only 0.5% CMC. This combination of additives makes stored tortillas resemble more closely the original texture of a fresh tortilla without additives than using only 0.5% CMC.

No single objective texture measurement test fully described the changes detected in tortilla texture during storage by subjective means. Tortilla rollability remains the basic subjective indicator of tortilla texture. However, changes in tortilla texture were detected faster using subjective pliability than rollability, and pliability correlated better with tortilla stiffness and enthalpy of amylopectin retrogradation.

Tortilla rupture force was a misleading indicator of tortilla hardness. Tortillas with CMC required more force to rupture because they were more extensible and not because they were harder or more brittle. Tortilla final stiffness obtained by stress relaxation was a better indicator of tortilla hardness while rupture distance explained changes in tortilla extensibility better.

For fast, empirical tortilla staling studies subjective pliability and rupture distance are recommended as the best indicators. For fundamental studies evaluating effects of additives at the molecular level, DSC enthalpy of amylopectin retrogradation or final stiffness should be measured.

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APPENDIX A

OPTIMIZING ADDITION OF AMYLASES AND CMC

TABLE A.1
ANOVA Summary for Rupture Force and Distance Measured 20 min , 1, 14 and 21 Days After Baking on Tortillas with Maltogenic Amylase (0,75 and 150 AU of Novamyl or ICS) and/or CMC

Source	Rupture Force (N)		Rupture Distance (mm)	
	F Value	Pr > F	F Value	Pr > F
Model	81.15	<0.001**	89.5	<0.001**
Additives	3.56	0.005	0.67	0.65
Day	609.38	<0.001**	677.95	<0.001**
Additives*Day	1.37	0.18	1.42	0.16

* Statistically significant
** Highly significant

TABLE A.2
ANOVA Summary for Rupture Force and Distance Measured 21 Days After Baking on Tortillas with Maltogenic Amylase (0,75 and 150 AU of Novamyl or ICS) and/or CMC

Source	Rupture Force (N)		Rupture Distance (mm)	
	F Value	Pr > F	F Value	Pr > F
Additives	0.51	0.77	1.98	0.1187

* Statistically significant
** Highly significant

TABLE A.3
Subjective Texture Measurements Evaluated 21 Days After Baking on Tortillas with Maltogenic Amylase (Novamyl or ICS) and/or CMC

Treatment Combination			Rollability	Pliability
CMC (%)	**Amylase**	**(MAU)**	**(1-5 scale)**	**(1-5 scale)**
0	No	0	1	1
0.5	No	0	4	2
0.25	Novamyl	75	3.5	2
0.25	Novamyl	150	3	2
0.25	ICS	75	5	2.25
0.25	ICS	150	4.5	2.25
	HSD (α=0.05)*		**0.50**	**0.30**

* Tukey's Honest Significant Difference for means separation.

TABLE A.4
Objective Texture Measurements Evaluated 21 Days After Baking on Tortillas with Maltogenic Amylase (Novamyl or ICS) and/or CMC

Treatment Combination			Rupture Force	Rupture Distance
CMC (%)	**Amylase**	**(AU)**	**(N)**	**(mm)**
0	No	0	12.96	1.88
0.5	No	0	13.98	2.16
0.25	Novamyl	75	13.28	1.62
0.25	Novamyl	150	13.66	1.86
0.25	ICS	75	13.44	2.34
0.25	ICS	150	12.58	2.11
	HSD (α=0.05)*		**0.93**	**0.21**

* Tukey's Honest Significant Difference for means separation.

APPENDIX B

ANTISTALING PROPERTIES OF COMBINATIONS OF ADDITIVES

TABLE B.1
ANOVA Summary for Tortilla Moisture Content, pH and Yield

Source	Moisture (%)		pH		Yield (kg/kg NCF)	
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Model	3.00	0.002*	0.95	0.506	5.72	<0.001**
Block	3.39	0.041*	0.13	0.876	29.61	<0.001**
CMC	1.09	0.343	1.23	0.299	4.26	0.027
Amylase	0.65	0.526	0.30	0.739	1.34	0.282
Gluten	6.35	0.003*	0.44	0.647	0.06	0.946
CMC*Amylase	3.90	0.007*	2.02	0.104	0.71	0.596
CMC*Amylase*Gluten	0.50	0.482	0.12	0.727	1.05	0.317

* Statistically significant
** Highly significant

TABLE B.2
ANOVA Summary for Tortilla Rollability and Pliability Measured After 14 Days of Storage

Source	Rollability		Pliability	
	F Value	Pr > F	F Value	Pr > F
Model	4.26	<0.001**	8.08	<0.001**
Block	8.18	<0.001**	7.15	0.011*
CMC	20.05	<0.001**	24.24	<0.001**
Amylase	1.22	0.307	8.87	<0.001**
Gluten	2.15	0.132	4.95	0.012*
CMC*Amylase	0.93	0.459	3.43	0.018*
CMC*Amylase*Gluten	0.51	0.479	0.15	0.458

* Statistically significant
** Highly significant

TABLE B.3
Tortilla Rollability Measured During 14 Days of Storage

Treatment Combination			Days of Storage					
CMC (%)	**Amylase (MAU)**	**Gluten (%)**	**0**	**0.2**	**1**	**3**	**7**	**14**
0	0	0	5	5	4.92	4.17	3.67	3.88
0	0	2	5	5	5	433	4.83	3.38
0	825	1	5	5	5	4.92	4.92	3.25
0	1650	0	5	5	5	4.67	4.5	4.12
0.25	0	1	5	5	5	4.92	4.92	4.63
0.25	825	0	5	5	5	4.83	492	4.88
0.25	825	1	5	5	5	5	5	4.50
0.25	825	2	5	5	5	5	4.83	4.75
0.25	1650	1	5	5	5	5	4.67	4.50
0.5	0	0	5	5	5	5	4.67	4.50
0.5	825	1	5	5	5	5	5	4.63
0.5	1650	2	5	5	5	5	5	5.00
HSD (α=0.05)*			**0.25**					

* Tukey's Honest Significant Difference for means separation.

TABLE B.4
Tortilla Pliability Measured During 14 Days of Storage

Treatment Combination			Days of Storage					
CMC (%)	**Amylase (MAU)**	**Gluten (%)**	**0**	**0.2**	**1**	**3**	**7**	**14**
0	0	0	5	5	3.17	1.42	1.33	1
0	0	2	5	4.92	3	1.75	1.42	1.13
0	825	1	5	5	3.25	1.58	1.75	1.75
0	1650	0	5	5	3.16	1.67	1.83	1.63
0.25	0	1	5	5	3.92	2.25	1.67	1.75
0.25	825	0	5	5	4	3.16	2.41	1.63
0.25	825	1	5	5	4.42	2.17	2.67	2.63
0.25	825	2	5	5	4.25	2.17	2.08	1.75
0.25	1650	1	5	4.92	3.92	2.33	1.92	1.75
0.5	0	0	5	5	4.33	3.41	2.33	2.00
0.5	825	1	5	5	4.58	3.42	2.67	2.63
0.5	1650	2	5	5	4.42	2.75	3.67	2.25
HSD (α=0.05)*			**0.50**					

* Tukey's Honest Significant Difference for means separation.

TABLE B.5
ANOVA Summary for Tortilla Rupture Force and Distance Measured 20 min After Baking

Source	Rupture Force (N)		Rupture Distance (mm)	
	F Value	Pr > F	F Value	Pr > F
Model	8.77	<0.001**	8.55	<0.001**
Block	2.00	0.161	6.08	0.016*
CMC	38.28	<0.001**	39.17	<0.001**
Amylase	0.77	0.465	0.23	0.796
Gluten	0.36	0.695	1.34	0.266
CMC*Amylase	5.66	<0.001**	3.24	0.016*
CMC*Amylase*Gluten	1.72	0.193	2.03	0.158

* Statistically significant
** Highly significant

TABLE B.6
ANOVA Summary for Tortilla Rupture Force and Distance Measured After 14 Days of Storage

Source	Rupture Force (N)		Rupture Distance (mm)	
	F Value	Pr > F	F Value	Pr > F
Model	11.81	<0.001**	4.11	<0.001**
Block	1.09	0.298	3.7	0.057
CMC	16.56	<0.001**	17.11	<0.001**
Amylase	29.07	<0.001**	0.73	0.486
Gluten	16.62	<0.001**	0.26	0.769
CMC*Amylase	1.01	0.408	0.41	0.802
CMC*Amylase*Gluten	12.07	<0.001**	7.83	0.006*

* Statistically significant
** Highly significant

TABLE B.7
Tortilla Rupture Force (N) Measured During 14 Days of Storage

Treatment Combination			Days of Storage			
CMC (%)	**Amylase (MAU)**	**Gluten (%)**	**0**	**0.2**	**3**	**14**
0	0	0	2.84	5.42	10.94	12.29
0	0	2	3.01	4.83	7.91	9.01
0	825	1	2.91	4.73	7.83	8.83
0	1650	0	3.05	4.93	9.33	9.15
0.25	0	1	3.04	5.11	8.66	9.33
0.25	825	0	2.91	4.81	9.12	8.49
0.25	825	1	2.99	5.13	8.09	8.85
0.25	825	2	3.32	4.60	7.91	8.05
0.25	1650	1	3.04	4.72	7.74	7.87
0.5	0	0	3.70	6.92	11.64	12.24
0.5	825	1	3.58	5.48	8.20	9.51
0.5	1650	2	3.29	4.63	8.32	8.21
HSD (α=0.05)*			**1.3 N**			

* Tukey's Honest Significant Difference for means separation.

TABLE B.8
Tortilla Rupture Distance (mm) Measured During 14 Days of Storage

Treatment Combination			Days of Storage			
CMC (%)	**Amylase (MAU)**	**Gluten (%)**	**0**	**0.2**	**3**	**14**
0	0	0	6.93	3.08	1.88	1.87
0	0	2	8.32	3.54	1.79	1.54
0	825	1	7.48	3.49	1.62	1.51
0	1650	0	8.00	3.49	1.82	1.56
0.25	0	1	9.02	3.88	2.01	1.58
0.25	825	0	8.61	3.89	1.93	1.48
0.25	825	1	7.68	3.89	1.90	1.57
0.25	825	2	8.77	4.34	1.75	1.76
0.25	1650	1	8.83	4.06	1.90	1.67
0.5	0	0	9.87	4.23	2.34	2.09
0.5	825	1	11.50	4.19	2.06	2.00
0.5	1650	2	10.40	4.54	2.35	2.03
HSD (α=0.05)*			**0.80**		**0.21**	

* Tukey's Honest Significant Difference for means separation.

TABLE B.9
ANOVA Summary for Tortilla Final Stiffness and Energy Dissipated Measured 20 min After Baking

Source	Final Stiffness (Pa)		Energy Dissipated (J/m^3)	
	F Value	Pr > F	F Value	Pr > F
Model	0.99	0.472	1.36	0.209
Block	0.37	0.547	3.65	0.061
CMC	0.76	0.472	2.56	0.086
Amylase	1.97	0.148	0.34	0.711
Gluten	0.45	0.638	1.47	0.237
CMC*Amylase	1.26	0.294	0.48	0.747
CMC*Amylase*Gluten	0.06	0.804	2.00	0.612

* Statistically significant
** Highly significant

TABLE B.10
ANOVA Summary for Tortilla Final Stiffness and Energy Dissipated Measured After 14 Days of Storage

Source	Final Stiffness (Pa)		Energy Dissipated (J/m^3)	
	F Value	Pr > F	F Value	Pr > F
Model	27.06	<0.001**	1.17	0.328
Block	29.11	<0.001**	4.98	0.029*
CMC	10.18	<0.001**	0.40	0.674
Amylase	85.81	<0.001**	0.08	0.921
Gluten	40.29	<0.001**	0.12	0.886
CMC*Amylase	4.95	0.002*	1.01	0.407
CMC*Amylase*Gluten	3.29	0.074	3.75	0.058

* Statistically significant
** Highly significant

TABLE B.11
Tortilla Final Stiffness (x 10^6 Pa) Measured During 14 Days of Storage

Treatment Combination			Days of Storage	
CMC (%)	**Amylase (MAU)**	**Gluten (%)**	**0**	**14**
0	0	0	0.21	3.39
0	0	2	0.22	2.47
0	825	1	0.19	2.43
0	1650	0	0.23	2.30
0.25	0	1	0.16	2.79
0.25	825	0	0.19	2.51
0.25	825	1	0.24	2.42
0.25	825	2	0.22	1.95
0.25	1650	1	0.24	2.11
0.5	0	0	0.21	3.58
0.5	825	1	0.23	2.15
0.5	1650	2	0.25	2.00
	HSD (α=0.05)*		**0.21 x 10^6 Pa**	

* Tukey's Honest Significant Difference for means separation.

TABLE B.12
Tortilla Energy Dissipated (x 10^{-4} J/m^3) Measured During 14 Days of Storage

Treatment Combination			Days of Storage	
CMC (%)	**Amylase (MAU)**	**Gluten (%)**	**0**	**14**
0	0	0	23.7	2.0
0	0	2	13.7	1.1
0	825	1	16.9	1.1
0	1650	0	20.2	1.2
0.25	0	1	19.0	1.0
0.25	825	0	20.6	1.0
0.25	825	1	14.2	1.2
0.25	825	2	20.7	1.5
0.25	1650	1	18.3	1.4
0.5	0	0	13.9	0.7
0.5	825	1	10.7	1.3
0.5	1650	2	15.1	1.3
	HSD (α=0.05)*		**3.9 x 10^{-4} Pa**	

* Tukey's Honest Significant Difference for means separation.

TABLE B.13
ANOVA Summary for Amylopectin Onset, Peak and End Temperatures of Melting Measured by DSC on Tortillas Stored 14 Days

Source	Onset (°C)		Peak (°C)		End (°C))	
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Model	0.99	0.482	3.20	0.004	1.15	0.358
Block	4.04	0.050*	26.58	<0.001**	4.11	0.050*
CMC	0.26	0.772	2.17	0.129	0.43	0.655
Amylase	2.91	0.068	2.01	0.149	2.94	0.066
Gluten	0.12	0.887	0.32	0.729	0.09	0.914
CMC*Amylase	0.30	0.877	0.64	0.639	0.34	0.852
CMC*Amylase*Gluten	0.01	0.971	0.22	0.642	1.37	0.249

* Statistically significant
** Highly significant

TABLE B.14
ANOVA Summary for Amylopectin Peak value and Enthalpy of Melting Measured by DSC on Tortillas Stored 14 Days

Source	Peak (mW)		Enthalpy (J/g)	
	F Value	Pr > F	F Value	Pr > F
Model	2.79	0.009*	3.07	0.005*
Block	3.52	0.068	9.62	0.004*
CMC	2.45	0.101	2.81	0.074
Amylase	10.43	<0.001**	6.37	0.004*
Gluten	0.89	0.421	2.13	0.134
CMC*Amylase	0.39	0.814	0.96	0.441
CMC*Amylase*Gluten	0.86	0.356	0.72	0.401

* Statistically significant
** Highly significant

TABLE B.15
Tortilla Amylopectin Melting Peak DSC Data Measured After 14 Days of Storage

Treatment Combination			Onset	Peak	Enthalpy
CMC (%)	**Amylase (MAU)**	**Gluten (%)**	**(°C)**		**(J/g)**
0	0	0	51.0	57.3	0.68
0	0	2	50.5	57.4	0.64
0	825	1	48.7	57.2	0.62
0	1650	0	51.6	57.3	0.52
0.25	0	1	50.9	57.7	0.64
0.25	825	0	49.5	57.1	0.60
0.25	825	1	50.2	57.2	0.39
0.25	825	2	48.9	57.5	0.41
0.25	1650	1	50.7	57.3	0.44
0.5	0	0	51.2	57.9	0.73
0.5	825	1	48.9	57.3	0.44
0.5	1650	2	52.0	57.9	0.28
	HSD (α=0.05)*		**3.1**	**1.0**	**0.15**

* Tukey's Honest Significant Difference for means separation.

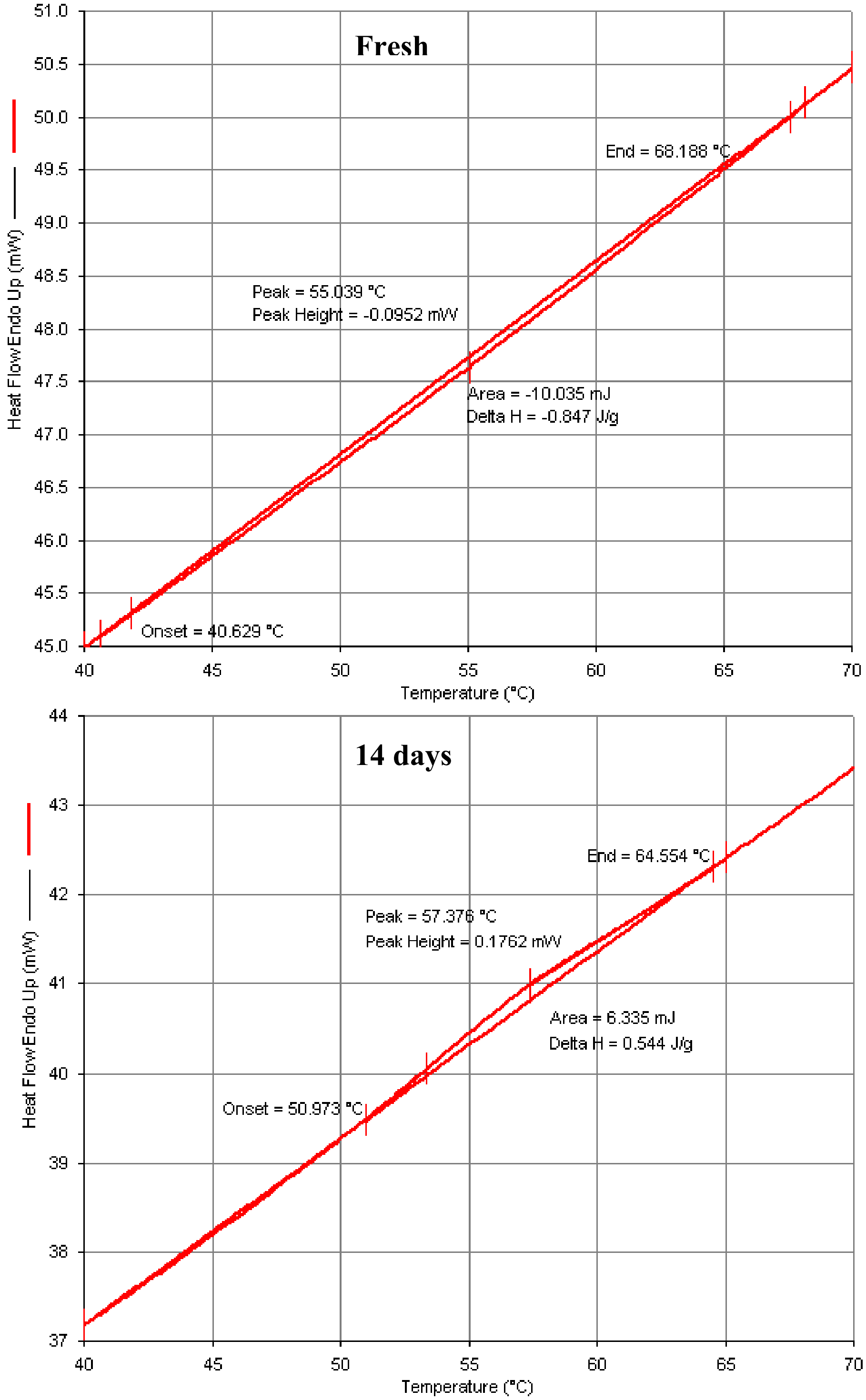

Figure B.1 Melting peak of amylopectin in fresh and 14 day-old tortillas with no additives as recorded by DSC

APPENDIX C

TEMPERATURE DEPENDENCE OF TORTILLA STALING RATE

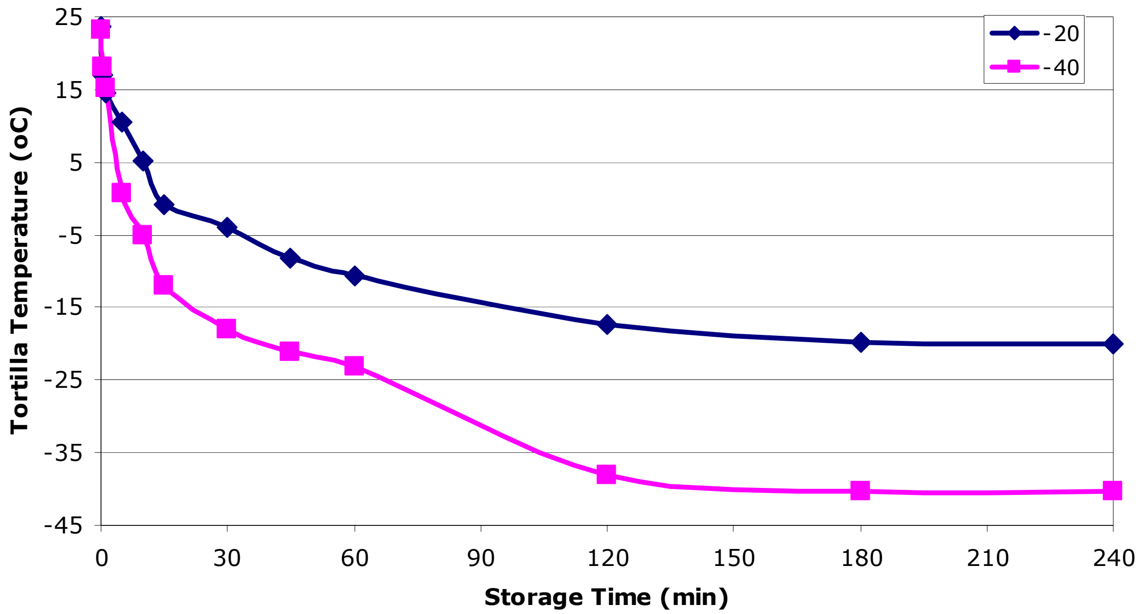

Fig. C.1. Tortilla internal temperature drop during storage in –20 and –40 °C freezers.

TABLE C.1
ANOVA Summary for Rollability and Pliability of Tortillas Stored at Temperatures between –40 and 21 ^{o}C

Source	Rollability		Pliability	
	F Value	**Pr > F**	**F Value**	**Pr > F**
Model	2.89	<0.001**	9.81	<0.001**
Block	12.42	<0.001**	1.49	0.22
Temperature	6.71	<0.001**	20.84	<0.001**
Additives	7.66	0.001*	8.98	<0.001**
Additives*Temp	0.29	0.97	1.16	0.34
Storage Days	16.59	<0.001**	126.10	<0.001**
Additives*Days	1.58	0.17	1.65	0.15
Temp*Days	3.76	<0.001**	5.99	<0.001**
Add*Temp*Days	0.51	0.96	0.68	0.23

* Statistically significant
** Highly significant

TABLE C.2

ANOVA Summary for Rollability and Pliability of 21-Day Old Tortillas Stored at Temperatures between –40 and 21 ^{o}C

Source	Rollability		Pliability	
	F Value	**Pr > F**	**F Value**	**Pr > F**
Model	3.84	0.008*	6.48	<0.001**
Block	8.81	0.01*	4.35	0.06
Temperature	8.45	0.001*	20.33	<0.001**
Additives	4.24	0.04*	1.19	0.33
Additives*Temp	0.82	0.60	1.14	0.39

* Statistically significant
** Highly significant

TABLE C.3

Changes in Tortilla Rollability During 21 Days of Storage at Temperatures in the –40 to 21 ^{o}C Range

Treatment	Days	-40 ^{o}C	-20 ^{o}C	3 ^{o}C	10 ^{o}C	21 ^{o}C
Control	0					5.00
	1	4.75	4.50	5.00	4.38	5.00
	7	4.25	4.37	3.87	3.75	4.50
	21	4.50	5.00	2.00	2.50	3.38
0.5% CMC	0					5.00
	1	5.00	5.00	5.00	5.00	5.00
	7	5.00	5.00	4.38	4.38	5.00
	21	4.88	4.88	3.75	2.88	5.00
0.25% CMC	0					5.00
+	1	5.00	4.75	5.00	5.00	5.00
1650 AU	7	5.00	4.75	4.75	4.25	.00
ICS Amylase	21	5.00	5.00	3.25	4.00	4.88
HSD (α=0.05)*				**0.47**		

* Tukey's Honest Significant Difference for means separation.

TABLE C.4

Changes in Tortilla Pliability During 21 Days of Storage at Temperatures in the –40 to 21 °C Range

Treatment	Days	-40 °C	-20 °C	3 °C	10 °C	21 °C
Control	0					5.00
	1	3.38	2.88	2.13	2.50	3.50
	7	2.50	3.00	2.00	1.50	2.38
	21	3.63	3.13	1.38	1.00	1.75
0.5% CMC	0					5.00
	1	3.75	3.63	3.50	3.38	4.38
	7	4.50	4.38	2.25	1.88	3.00
	21	4.50	3.63	1.13	1.38	1.88
0.25% CMC	0					5.00
+	1	3.25	3.38	3.00	2.50	4.38
1650 AU	7	2.88	3.5	2.13	2.13	3.38
ICS Amylase	21	3.50	2.88	1.88	2.00	2.50
HSD (α=0.05)*				**0.45**		

* Tukey's Honest Significant Difference for means separation.

TABLE C.5
ANOVA Summary for Final Stiffness and Energy Dissipated of Tortillas Stored at Temperatures between –40 and 21 ^{o}C

Source	Final Stiffness (Pa)		Energy Dissipated (J/m^3)	
	F Value	Pr > F	F Value	Pr > F
Model	82.75	<0.001**	13.84	<0.001**
Block	64.86	<0.001**	3.35	0.068
Temperature	108.47	<0.001**	4.27	0.002*
Additives	82.54	<0.001**	2.97	0.052
Additives*Temp	3.60	<0.001**	0.43	0.90
Storage Days	1294.83	<0.001**	253.08	<0.001**
Additives*Days	10.41	<0.001**	1.40	0.22
Temp*Days	23.60	<0.001**	1.89	0.04*
Add*Temp*Days	0.06	0.01*	0.43	0.99

* Statistically significant
** Highly significant

TABLE C.6
ANOVA Summary for Final Stiffness and Energy Dissipated Measured After 21 Days of Storage on Tortillas Maintained at Temperatures Between –40 and 21 ^{o}C

Source	Final Stiffness (Pa)		Energy Dissipated (J/m^3)	
	F Value	Pr > F	F Value	Pr > F
Model	20.51	<0.001**	1.48	0.099
Block	0.21	0.65	0.10	0.75
Temperature	102.32	<0.001**	5.35	<0.001**
Additives	51.30	<0.001**	0.16	0.85
Additives*Temp	4.59	<0.001**	1.17	0.33

* Statistically significant
** Highly significant

TABLE C.7

Changes in Tortilla Final Stiffness (x10^6 Pa) During 21 Days of Storage at Temperatures in the –40 to 21 °C Range

Treatment	Days	-40 °C	-20 °C	3 °C	10 °C	21 °C
Control	0					0.37
	1	2.05	1.87	2.59	2.53	2.00
	7	2.45	2.51	3.86	3.99	3.30
	21	3.12	2.46	3.65	5.04	3.34
0.5% CMC	0					0.35
	1	2.07	1.64	2.21	2.05	1.54
	7	2.72	2.33	3.62	3.35	3.16
	21	2.44	2.26	3.93	4.31	3.20
0.25% CMC	0					0.35
+	1	1.62	1.73	2.05	2.00	1.41
1650 AU	7	2.03	2.02	3.10	3.04	2.10
ICS Amylase	21	2.15	1.94	3.40	3.50	2.11
HSD (α=0.05)*				**0.33 x 10^6 Pa**		

* Tukey's Honest Significant Difference for means separation.

TABLE C.8

Changes in Tortilla Energy Dissipated (x10^{-4} J/m^3) During 21 Days of Storage at Temperatures in the –40 to 21 ^oC Range

Treatment	Days	-40 ^oC	-20 ^oC	3 ^oC	10 ^oC	21 ^oC
Control	0					8.25
	1	1.31	1.79	1.10	1.03	1.22
	7	1.7	1.30	0.61	0.69	0.82
	21	0.95	0.98	0.65	0.51	1.80
0.5% CMC	0					8.27
	1	1.26	1.63	1.08	1.09	1.68
	7	0.94	1.09	0.60	0.79	0.70
	21	1.41	1.25	0.54	0.63	0.93
0.25% CMC	0					10.20
+	1	1.80	1.58	1.37	1.38	1.92
1650 AU	7	1.32	1.31	0.81	0.81	1.22
ICS Amylase	21	1.56	1.42	0.55	0.64	1.08
HSD (α=0.05)*				**0.42 x 10^{-4} J/m^3**		

* Tukey's Honest Significant Difference for means separation.

TABLE C.9
ANOVA Summary for Amylopectin Enthalpy and Peak Temperature of Melting of Tortillas Stored at Temperatures Between –40 and 21 ^{o}C

Source	Peak Temperature (Pa)		Enthalpy (J/g)	
	F Value	Pr > F	F Value	Pr > F
Model	17.08	<0.001**	91.07	<0.001**
Temperature	147.51	<0.001**	25.91	<0.001**
Additives	0.84	0.43	16.40	<0.001**
Additives*Temp	2.08	0.05	9.23	<0.001**
Storage Days	28.27	<0.001**	1562.69	<0.001**
Additives*Days	0.66	0.69	12.37	<0.001**
Temp*Days	18.87	<0.001**	10.04	<0.001**
Add*Temp*Days	2.27	0.006*	4.46	<0.001**

* Statistically significant
** Highly significant

TABLE C.10
ANOVA Summary for Amylopectin Enthalpy and Peak Temperature of Melting Measured After 21 Days of Storage on Tortillas Maintained at Temperatures Between –40 and 21 ^{o}C

Source	Peak Temperature (Pa)		Enthalpy (J/g)	
	F Value	Pr > F	F Value	Pr > F
Model	29.99	<0.001**	15.92	<0.001**
Temperature	101.31	<0.001**	21.06	<0.001**
Additives	1.80	0.19	41.50	<0.001**
Additives*Temp	1.37	0.28	6.95	<0.001**

* Statistically significant
** Highly significant

TABLE C.11

Changes in Tortilla Amylopectin Enthalpy of Melting During 21 Days of storage at Temperatures in the –40 to 21 °C Range

Treatment	Days	-40 °C	-20 °C	3 °C	10 °C	21 °C
Control	0					1.48
	1	3.06	4.29	4.42	4.51	3.85
	7	5.32	4.09	5.34	5.05	4.98
	21	4.55	4.47	5.23	5.14	5.30
0.5% CMC	0					1.75
	1	4.44	4.66	4.57	4.82	4.42
	7	4.74	4.28	5.65	5.19	5.12
	21	5.11	5.11	5.30	5.54	4.61
0.25% CMC	0					1.83
+	1	4.11	4.06	4.27	3.93	3.56
1650 AU	7	4.72	4.24	4.86	4.81	4.27
ICS Amylase	21	4.09	4.03	5.10	4.96	3.72
HSD (α=0.05)*				**0.22 J/g**		

* Tukey's Honest Significant Difference for means separation.

TABLE C.12

Changes in Tortilla Peak Pasting Temperature and Viscosity During 21 Days of Storage at Temperatures in the –40 to 21 °C Range as Measured by the RVA

Treatment	**Storage Temperature (°C)**	**Paste Temperature (°C)**		**Paste Viscosity (cP)**	
		20 Min	**21 Days**	**20 Min**	**21 Days**
Control	-40		68.4		2120
	-20		61.4		3157
	3		69.9		2545
	10		70.4		2641
	21	57.9	75.2	5292	1896
0.5% CMC	-40		61.7		3870
	-20		61.5		3394
	3		59.5		4912
	10		61.0		4170
	21	59.4	68.4	5586	3363
0.25% CMC	-40		58.2		4740
+	-20		57.9		5845
1650 AU	3		62.0		4283
ICS Amylase	10		58.9		5504
	21	58.0	60.5	6844	5375
HSD (α=0.05)*		**1.21**		**170**	

* Tukey's Honest Significant Difference for means separation.

APPENDIX D

FURTHER RESEARCH

FURTHER RESEARCH

- Optimize addition of maltogenic amylase in corn tortillas produced with fresh masa as opposed to using NCF. Determine the best moment to mix (grinding step is suggested) and adequate concentration of amylase considering a continuous process.
- Evaluate other types of CMC with lower viscosities than 7HF in order to reduce tortilla chewiness without compromising flexibility.
- Evaluate combinations of maltogenic amylases (both fungal and bacterial) with other matrix forming additives that may be able to substitute CMC totally or partially (Beta-glucans, pentosans, etc) at a lower cost. Evaluate the effect of chain length, branching and tendency to cross-link.
- Determine the mode of action of maltogenic amylases in preventing amylopectin re-crystallization. Use HPLC to determine the types of oligosaccharides produced by the amylase hydrolytic activity on amylose and amylopectin of corn tortillas. Then, add these oligosaccharides to tortillas to determine if re-crystallization of amylopectin is reduced by hydrolysis of its outer branches or by interference.
- Establish the role of amylose in corn tortilla staling. Use DSC to evaluate changes in the retrograded amylose-lipid (150 ^{o}C) complex during storage. Evaluate the effect of amylases, CMC and other anti-staling agents on the re-crystallization of amylose. Sample and stabilize tortillas during baking and coming right out of the oven.
- Determine tortilla Tg. Store tortillas at –60 to 4 ^{o}C and estimate tortilla Tg by DSC. With enough points within this temperature range it might be possible to find the temperature at which tortillas do not stale or at the very least extrapolate it to the point were tortilla re-crystallization rate becomes zero.

VITA

Francisco Bueso was born in San Pedro Sula, Honduras on May 26th, 1972. He graduated from La Salle Institute High School, San Pedro Sula, Honduras in 1989 and received his Bachelor of Science degree in agronomy from the Escuela Agrícola Panamericana (Zamorano), Honduras in 1994. In 1995 he enrolled at Texas A&M University as a graduate student and obtained his Master of Science degree in food science and technology in 1997.

From January 1998 to December 1999 he worked as an Assistant Professor/ Grains Processing Plant Manager at Zamorano. In January 2000 he started his Ph. D. program at Texas A&M University. He received his Doctor of Philosophy degree in food science and technology in May 2003.

The author's permanent address is: Colonia Aurora, 16 ave, 7 y 8 calle # 782, San Pedro Sula, Honduras. His e-mail is: fjbueso@hotmail.com.