

SHELF LIFE EXTENSION OF CORN TORTILLAS

by

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Abstract

The tortilla segment of the Mexican food market in the United States is rapidly growing. Tortillas are being used in many different mainstream applications, including wraps, lasagna, pizza, and appetizers. In 2000, the tortilla market was a \$4 billion industry and with more than 85 billion tortillas consumed in the United States alone. As Mexican food becomes more common in the American diet, consumers start to branch out into a more authentic presentation of Mexican food. This causes a shift in consumption from flour to corn tortillas. As the consumer demand for corn tortillas increases, food manufacturing companies are challenged with producing a tortilla that will retain its softness, pliability, foldability, and flavor while remaining safe for consumption over several months. Since tortillas have two modes of deterioration, mold and staling, there are several factors that need to be considered. Hurdle technology is employed to prevent mold growth. By adjusting water activity, pH, storage temperature, and addition of preservatives mold growth can be prevented for a period of several months. Retaining tortilla texture over time is much more complicated. Tortillas stale through a complicated process of starch retrogradation. During cooking, the starch granules gelatinize and amylose and amylopectin leech out of the granules. After the tortillas are baked, the starch immediately begins to retrograde. The amylose and amylopectin complex together form a matrix that stiffens the tortilla. Based on current research, the shelf life of a corn tortilla can be extended through a combination of CMC (0.5%), maltogenic amylase (1650 Activity Units), sorbitol (3%), glycerol (4%).

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CHAPTER 1 - Background Information

Tortilla Market

Tortillas are a Mexican flat bread that are traditionally round, thin, and made from unleavened cornmeal. They are consumed at almost every Mexican meal and are often used as eating utensils. The first tortilla dates back to 10,000 BC and was made from native corn with a dried kernel. Mayan legend says that tortillas were invented by a peasant for his hungry king (TIA, 2008). The traditional process for making tortillas is to boil the corn with lime, which will soften the kernels and will loosen the hulls. The grains are then ground on a stone tool called a metate and a dough is made. The dough is formed into small pieces that are patted by hand into thin disks and then they are baked on an earthenware or iron griddle called a comal. Today tortillas are made with either corn that is cooked in a lime based solution or using corn flour (TIA, 2008).

In the United States, tortillas are more common than any other ethnic bread (TIA, 2008). In the year 2000, the Tortilla Industry Association estimated that 85 billion tortillas (not including chips) were consumed in the United States. Hard and soft shell shelf stable meal kits were growing at 5.5% per year and the refrigerated sales were growing at 3.9% per year and corn tortilla sales were growing faster than flour (Stockwell, 2000). In 2004 tortillas consisted of 30% of all bread sales in the United States and were second to white bread sales. In 2005 and 2006, white bread sales started to decline due to low carb dieting. Tortilla sales did not see this decrease (AIB, 2008). A large contribution to the growth of the tortilla market in the United States is the infiltration of tortillas in the mainstream American diet. Tortillas are used in wraps, sandwiches, pizza, appetizers, lasagna, and they can be served hot or cold (TIA, 2008). As

consumers are introduced to Mexican foods, they start with preparing meal kits at home. These are a little easier for the consumer to understand and to feel confident with when preparing meals for their families. As they become more comfortable with the food, they start to venture outside of the kit and explore the components of the Mexican meal. Newer users typically prefer flour tortillas to corn tortillas, but as the consumer gets more familiar with authentic Mexican food, they venture out to the corn tortilla (Stockwell, 2000).

Tortilla Production

Flour Tortillas

To make a flour tortilla at home only flour, water, shortening, salt, and baking powder are used. When producing on an industrial scale where a longer shelf life is needed, the formula will require other ingredients to maintain the integrity of the tortilla over time, such as an acidulant, dough conditioners, gums, and antimicrobials (Stockwell, 2000).

The three ways that flour tortillas can be produced are using the hot press, hand stretch, and the dye cut method. Most flour tortillas today are produced using the hot press method. The dough is first mixed in a large mixer and then transported to a divider/rounder where the dough is divided into small dough balls of the desired weight. The small balls of dough pass through a proofer and are dropped onto the tortilla press where they are pressed between heated plates to form the tortillas. The tortillas then pass through an oven where they are cooked. This is where the leavening will occur, causing the tortillas to puff. Following cooking, the tortillas will pass into a cooling chamber where they will be cooled before packaging (Pacyniak, 1996).

The hand stretch method will form tortillas that have an irregular shape and different textures. In this method, the tortilla dough is mixed and then divided into dough balls before

they are sheeted to a general size and shape. The dough is then passed over a hot plate where it is stretched. These tortillas will also have stripes in them with a more homemade appearance. This method is often used to make tortillas that will be used for burritos (Ehli, 1986).

The third way that tortillas can be made is using the die cut method. This method is made by a machine rolling the dough into a long thin sheet and then the tortillas are cut out of the sheet. This method is often used when making soft corn tortillas or fried tortilla chips or shells. The texture of this type of flour tortilla is often unacceptable for restaurant use due to a less durable texture. This is the least expensive way to process tortillas due to lower priced equipment and less people operating the line (Ehli, 1986).

Corn Tortillas

Traditionally, corn tortillas were made using nixtamalized corn. In a process that dates back to 3500 BC, wood ash or slacked calcium hydroxide is added to corn and boiled. This softens the outer skin of the corn making it easier to grind and allowing the corn to absorb more water. This process also makes the vitamins and minerals in the corn more bioavailable and the starch matrix has greater gelatinization potential. Today when making nixtamalized corn, calcium oxide is added to boiling water. Calcium oxide converts to calcium hydroxide having the same effect on the corn. Corn is then washed to remove the excess calcium hydroxide and then allowed to cool before being milled. Once the corn is washed, it is left to steep to allow time for the equilibration and then the corn is milled. After the corn is milled, it can either be used to make corn tortillas or it can continue to go through drying and then ground and screened into different granulations in order to make corn masa flour (see Figure 1.1). Today it is much more common for the corn masa flour to be made and then shipped to another location to make

the corn tortillas. Using corn masa flour provides some control of the variability in the process of making fresh masa and the seasonal availability of raw corn (Trevino and Norton, 2006).

Corn tortillas are typically produced using the die-cut method. The masa or corn flour is first hydrated in a mixer to produce dough with a 58-59% moisture level. The dough then travels to a sheeter head where the dough is extruded and then die cut. The tortillas travel to a three pass oven. The oven will first sear the bottom of the tortilla, the second will sear the top, and then the tortillas will pass through a third time for a more controlled bake. Corn tortillas are cooled by passing them through a cooling area at ambient temperature before they are packaged (Pacyniak, 1996).

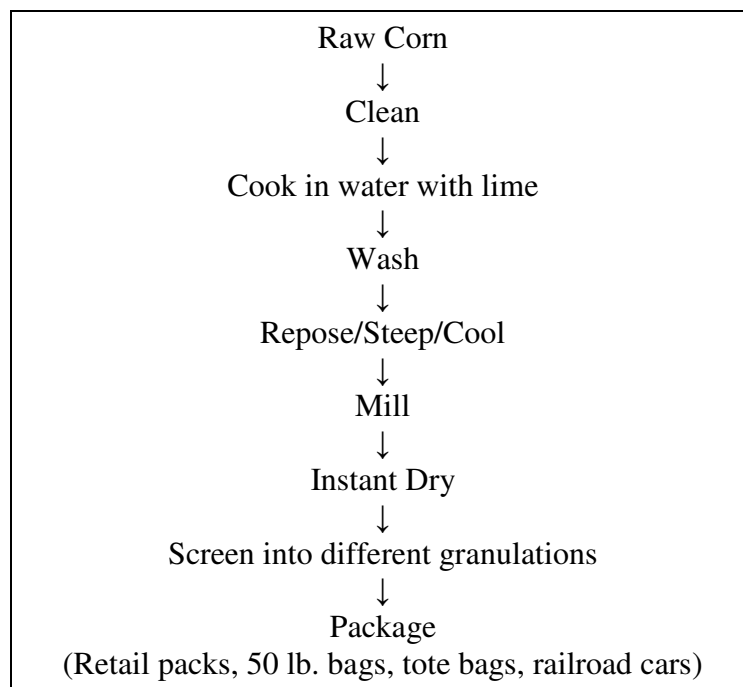


Figure 1.1 Process for Making Masa Flour (Trevino and Norton, 2006).

CHAPTER 2 - Starch

Starch Structure

Starch is a naturally water insoluble molecule of botanical origin. It is birefringent, meaning that when viewed under polarized light, a dark cross is seen (Buelon et al, 1998). Depending on the shape of the starch and its orientation to the light, the intensity of the cross will vary. The molecular structure of starch is made up of two compounds, amylose and amylopectin. In most types of starch, 72-82% is amylopectin and 18-33% is amylose. There are certain mutant genotypes of barley, rice, and maize that can have up to 70% amylose. Waxy types of starch are almost 100% amylopectin, having less than 1% amylose (Buelon et al, 1998).

Amylose is a linear molecule having (1->4) linked alpha-D-glucopyranosyl units. Some amylose molecules are slightly branched with a few (1->6) branched linkages (Buelon et al, 1998), but amylose behaves as a completely linear molecule. Amylose is a very large molecule with a molecular mass range of 3×10^5 to 9×10^6 daltons. A branched amylose molecule can have a molecular mass that is one to three times greater (Buelon et al, 1998).

Amylose can be described as either A or B type. Both types are arranged in a six fold left handed double helix (Buelon et al, 1998). Type A amylose is found in corn and most other cereal starches. The average chain length is 45 units and it is arranged in double helices in a monoclinic array (Imberty et al 1988). Type B amylose has a more open structure that is more hydrated. The double helices are arranged in a hexagonal array. This type of amylose is found in tubers and high amylose and retrograded starches (Wu and Sarko, 1978). The actual organization of the starch granule varies depending on the source (Buelon et al, 1998).

Amylose is broken down by β -amylase hydrolyzing the (1->4) linkages. β -amylase attacks the non-reducing end of the amylose molecule, making β -maltosyl units. β -amylase

cannot cleave the (1->6) branched bonds. A completely linear molecule will be converted entirely to maltose by β -amylase, while a branched molecule will also give one β -limit dextrin (Buelon et al, 1998).

Amylopectin is the other molecule that makes up starch. Unlike amylose, it is highly branched. Amylopectin is still made up of short chains of alpha-D-glucopyranosyl units, linked mostly through (1->4) linkages (Miles et al, 1985). However, in amylopectin 5-6% of its (1->6) bonds are at branch points (Buelon et al, 1998), which are amorphous regions of the starch (Imberty et al, 1988). These branch points are separated by 20-23 units and are in clusters throughout the molecule (Buelon et al, 1998). The linear amylose molecules are incorporated into the amorphous branched regions of the amylopectin (Imberty et al, 1988). Because of the large number of branch points, amylopectin is a very large molecule with a molecular mass that is greater than 1×10^8 Daltons (Buelon et al, 1998).

Amylopectin is made up of three different types of chains, referred to as A, B, and C. The A-type is the outer chain that is linked to the inner chains through a potential reducing end. The B-type is an inner chain that has other chains branching off of it. The C-type is linked to other branches, but it has the only reducing end. There are more A chains in the amylopectin molecule than there are B chains. Because of this, B type chains must have more than one A chain linked to them (Buelon et al, 1998).

Starch granules are about 30% crystalline (Buelon et al, 1998). Since waxy starches are almost 100% amylopectin and they still have crystalline granules, it can be surmised that amylopectin contributes, at least in part, to the crystalline structure. The crystalline areas of the starch granule have chain lengths that are similar to the short chain areas of the amylopectin

molecule. It is thought that the short chains of amylopectin originate in the crystalline areas. (Ucles, 2008)

Starch Retrogradation

Starch retrogradation is the complex process where gelatinized starch moves from an amorphous state that is not in thermodynamic equilibrium to a more ordered crystalline state (Gudmundsson, 1994). Retrogradation can be thought of as two distinct processes. The first of these processes involves the amylose that is solubilized during gelatinization. The second process involves the amylopectin that remains within the starch granule (Miles et al, 1984). The result of retrogradation is that there is an increase of firmness that can be seen in the rheological properties. The starch loses its water holding capacity and moves back toward a more crystalline state. Starch retrogradation is the main factor in the staling of bread and other bakery products. It has a significant effect on the texture of the product, decreasing the consumer acceptance of the product the more the starch retrogrades. Both the rate of retrogradation and the extent are affected by the starch components, the water content of the product, the storage temperature of the product, and other substances added to the product, such as lipids and surfactants (Gudmundsson, 1994).

Before starch retrogradation can begin, the starch must first be gelatinized. In order for this to happen, the starch granule is heated to 60-70 °C and it will swell irreversibly to many times its original size. As the starch is gelatinized, the amylose and amylopectin leach out of the granule and amylose is preferentially solubilized. During gelatinization, the starch granule loses its birefringence and crystallinity. The granule can maintain its integrity as long as there is no shear and the temperature remains below 100°C. Once the starch is gelatinized and the matrix begins to cool, retrogradation begins immediately (Miles et al, 1984).

Both amylose and amylopectin are a part of the starch retrogradation. Amylose starts to retrograde rather quickly making it responsible for the short term changes such as immediate firming of the product or gel. Amylopectin changes are responsible for most of the starch retrogradation, including the long term rheological and structural changes (Gudmundsson, 1994).

In an experiment to describe starch retrogradation and the portions that amylose and amylopectin are responsible for, Miles et al (1984) made 10 and 20% starch gels and measured the development of the shear modulus. The shear modulus of the gels was measured and after 100-150 minutes it had reached a constant value. Since a maximum of 2.1 and 4.2% of amylose could have been solubilized in the 10 and 20% gel respectively, amylose solutions of this concentration were made. These amylose gels behaved very similar to the starch gels when developing opacity. However, after 48 hours, the amylose gels reached their crystallization limits, while the starch gels continued to crystallize in line with an increase in the shear modulus and the development of the Differential scanning calorimetry (DSC) exothermic transition at 63 °C. Once the gels were matured they were heated to 95 °C and then cooled to 26 °C. While the gels had behaved in a very similar fashion when developing their initial crystallinity, they had a very different reaction to this heat-cool cycle. The shear modulus of the starch gels decreased to what it had been 24 hours after the gel had been prepared. The amylose only gels showed no change upon being heated and then cooled. This suggests that the retrogradation that occurs within the amylose portion of the starch is not reversible, while the amylopectin portion of the retrogradation is reversible. It has been hypothesized that amylose, being a linear molecule, has more opportunity to form inter-chain associations with other amylose molecules, providing more stability of the amylose gel. When the gels were heated to 90 °C, 70% of the crystallinity was removed in the starch gel while only 25% was removed in amylose gel (Miles et al, 1984).

Miles et al (1984) also measured the x-ray diffraction patterns of the amylose and starch gels after storing them at 26 °C for 14 days. The x-ray diffraction patterns were B-type.

Another experiment was done where the starch granules were washed at 95 °C to remove the soluble amylose. The sample was cooled to 26 °C and the X-ray diffraction patterns were read. Initially, there was no diffraction pattern, but after storing for 14 days at 26 °C, the B-type X-ray diffraction appeared. After heating the sample to 70 °C, the crystalline structure disappears, again, demonstrating that the amylopectin retrogradation is reversible (Miles et al, 1984).

Other research has been done on waxy maize starches that are greater than 98% amylopectin. In a study by Ring et al (1987) it was found that it takes several weeks for the amylopectin gel to approach a limiting value for the shear modulus. This is much longer than the 100 minutes that it took the amylose gels to reach a limiting value (Miles et al, 1984). During gelatinization, it took 4-5 days for amylopectin gel to reach a limiting value on the turbidity while the shear modulus increases only slightly in this time. If the amylopectin gel is melted and brought up to a temperature of 40-60 °C, the turbidity is lost, showing that the changes that happen to the amylopectin during retrogradation are thermo-reversible (Ring et al 1987). About 30% of an amylopectin gel is crystalline, with a wide range in size and the perfection of the crystals. The outer chains in the amylopectin, the β -limit dextrans do not gelatinize, they crystallize (Ring et al 1987).

Starch retrogradation is highly dependent on several variables, including the storage temperature, concentration of the starch, water content of the product, botanical source of the starch and other ingredients added to the product (Gudmundsson, 1994). Various studies have been done (Gudmundsson, 1994; Ring et al, 1987) showing that the storage temperature has a strong effect on the retrogradation of the starch gel. When compared to a gel that is stored at

room temperature and is 45-50% water, retrogradation speed increases when stored at a low temperature that is above glass temperature, especially for the first few days (Gudmundsson, 1994). When studying the amylopectin behavior in starch retrogradation, Ring et al (1987) worked at a temperature of 1 °C in order to increase the speed of retrogradation. They also found that increasing the starch concentration of the gel would increase the speed of retrogradation. Gudmundsson (1994) found that retrogradation could be reduced by storing at a temperature above 32-40 °C or even stopped when stored at a freezing temperature.

The water content of the starch gel is very important for the rate of recrystallization. The rate of retrogradation depends on the water content of the gel during aging. The maximum amount of recrystallization will happen in a gel that has 50-55% starch. The recrystallization rate of the gel depends on the glass transition temperature (T_g), or the temperature which an amorphous solid becomes brittle. Water works as a plasticizing agent, controlling the T_g of the gel. When the water content is very low, the T_g is above room temperature and the gel is viscous, hindering the mobility of the molecules. As the water content of the gel increases to 50%, the recrystallization of the gel will increase due to an increase in molecular mobility. As the water content moves up from 50-90% the solution is diluted and the retrogradation of the starch decreases (Gudmundsson 1994).

Sugars, surfactants and lipids all have an affect on the retrogradation of starch within a gel. Sugar increases the T_g by limiting the mobility of the chains within the amorphous gel. Lipids and surfactants will not only hinder mobility of molecules, but they will actually form complexes with amylose molecules, but not amylopectin. The mechanism for how this happens is not yet known, but there are several ideas as to why lipids and surfactants inhibit retrogradation. It is possible that the complex that is formed with the amylose interferes either

directly with amylopectin retrogradation in an unknown way or indirectly by changing the distribution of water in the system. It is thought that some retrogradation is a result of crystallization between amylose and amylopectin molecules and lipids could prevent amylose from being a part of this process. It is also possible that there is a small amount of direct interaction with the amylopectin, forming an amylopectin complex (Gundmundsson 1994).

The botanical source of the starch is also important when looking at the rate of retrogradation. As reported by Gundmundsson (1994), there are differences in starch retrogradation despite similar amylose to amylopectin ratios. This says that the difference in the structure of the amylopectin has an effect on the rate and extent of retrogradation. An increase in the number of short chains (greater than 15 glucose units) in the amylopectin complex increase the rate and extent of retrogradation in the starch. Chains that are very short (6-9 glucose units) retard or even inhibit the retrogradation of the starch (Gundmundsson, 1994).

Measuring Starch Retrogradation

Starch retrogradation can be measured in several different ways. Some of the most common methods are X-ray diffraction, thermal methods such as Differential Scanning Calorimetry (DSC), and rheological studies. There are two different techniques that these methods are employing; macroscopic and molecular techniques. Macroscopic techniques look at larger changes in the gel or the product as a representation of the changes that are happening during retrogradation. Molecular techniques observe the changes that are taking place within the starch polymer or the water within the starch matrix (Karim et al, 2000).

Starch granules are made up of crystalline micelles in layers. Since starch granules are partially crystalline, they give a distinct X-ray diffraction pattern. These patterns show the double helicies in the molecular structure, but it cannot detect irregular structures. There are

three different patterns that can be seen in X-ray diffraction; A-type, B-type, and C-type. The A-type is seen in most cereal starches including corn. B-type is found in tubers, high amylose starches. C-type patterns are found in retrograded starches (Karim et al, 2000). X-ray diffraction measures the recrystallization process through the diffraction patterns. Diffraction studies show the slowly developing B-type diffraction pattern (Gudmundsson, 1994). Miles et al (1985) used X-ray diffraction to show that the amylose separates into polymer rich and polymer deficient regions during gelatinization. They used X-ray diffraction to show that the amylose and starch gels initially develop crystallinity at a similar rate. X-ray diffraction has also been used to show that moisture content is very important in the retrogradation of starch gels. In samples with a moisture content greater than 43%, B-patterns developed while samples with a moisture content below 29% developed A-patterns. C-patterns were seen at moisture contents between 29 and 43% (Karim et al, 2000).

Heat is either absorbed or released whenever a substance goes through a change in physical state, reacts chemically, or has a change in crystalline structure. Thermal analysis, such as DSC can be used to measure this energy change. DSC measures the difference in energy that is put into a substance and a reference material. This energy is measured as a function of temperature. Both the test substance and the reference material undergo the same heating or cooling program (Karim et al, 2000). In DSC, a temperature balance is maintained. There is a thermal transition where the test substance absorbs energy. In order to maintain the balance, an increase in energy is put into the substance. DSC measures this energy and records it as a peak, showing either an endothermic or exothermic transition. DSC measures the temperature at which the recrystallized amylopectin melts (Karim et al, 2000).

DSC studies are effective in measuring long term changes than happen in the amylopectin. There is a melting endotherm that increases slowly over time that can be seen in bread, starch, and amylopectin gels. This endotherm is not present in amylose gels (Gudmundsson, 1994). There is an endothermic peak at 153 °C, showing the crystallinity in an amylose gel (Ring et al, 1987). When DSC studies were done on gels with a mixture of amylose and amylopectin, it was shown that the melting endotherm decrease as the amount of amylose increased. In starch gels and stale breads, the melting endotherm can be reversed completely. Immediately after heating a starch gel, no endotherm is reached (Gudmundsson, 1994).

Rheological techniques are macroscopic techniques. These techniques are better able to assess the properties of the entire three dimensional matrix. There is a marked change in how the starch gel or product behaves as starch retrogrades, and using rheological or mechanical testing is very effective in measuring this. Often, rheological techniques will destroy the material being tested by either using shear or larger forces. This impairs the ability to also measure the viscoelastic properties of the starch system (Karim, et al, 2000).

Rheological studies largely confirm information that is given through DSC and X-ray diffraction. They have been used to show that an amylose gel quickly retrogrades to a constant value, while a starch gel (with both amylose and amylopectin) retrogrades more slowly over time (Gudmundsson, 1994). Rheological tests are useful because they correlate very well with actual sensory attributes of the product or gel. Rheological testing can be either dynamic or uniaxial compression. The uniaxial compression tests work best with very firm gels, while dynamic testing works well when studying a soft gel. In order to ensure that the results are accurate, it is necessary to do several repetitions of the test due to variations both within a given sample and between samples (Karim et al, 2000).

Uniaxial compression testing can be done using the Instrumental Texture Profile Analysis (TPA) method. This method actually simulates two bites by compressing the sample, releasing the compression, and then compressing again. The resulting data gives a graph such as the one seen in Figure 2.1 (Karim et al, 2000). This test measures the force that is required to compress the sample. The maximum force at the end of the first compression represents the hardness of the sample. Fracturability is represented by the first maximum on the graph. Other information about the sample, such as cohesiveness, springiness, stickiness, gumminess, and chewiness can also be obtained from the graph. Unfortunately, these methods have not been standardized, so it is difficult to compare data between different testing facilities (Karim et al, 2000).

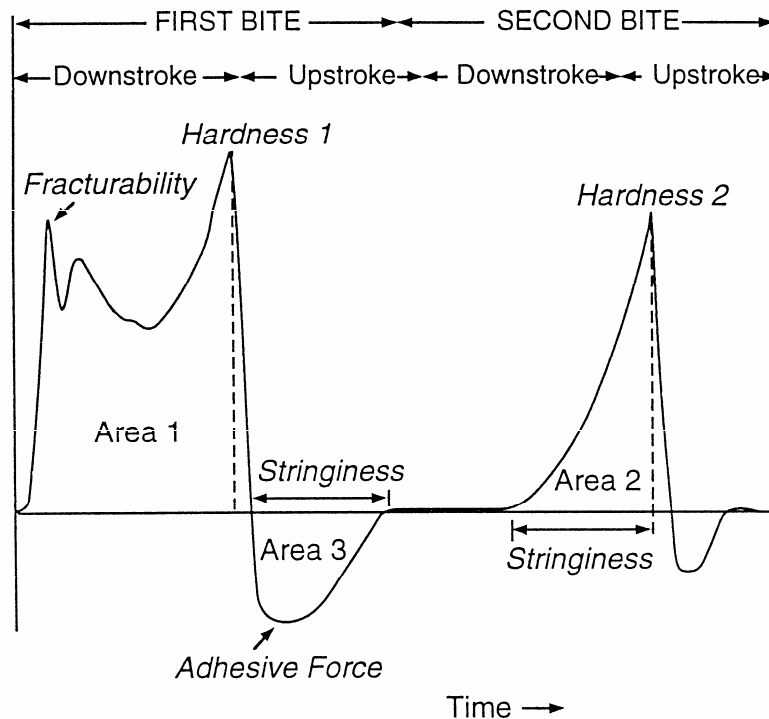


Figure 2.1 Texture Profile Curve Simulating Two Bites Being Taken Out of a Tortilla. The Curve Comes From the Instron Testing Machine (Karim et al, 2000).

Rheological testing has also been used effectively to measure the viscoelastic properties of starch gels. Dynamic oscillatory rheometry is used to measure the changes in a starch gel over time. The advantages of this type of testing are that it is continuous and does not harm the gel. It is also a standardized technique that takes measurements in physical units, meaning that the results can easily be compared between experiments. Dynamic oscillatory rheometry applies a stress to the sample in a repeating pattern, straining the sample. If the strain is in phase with the stress, the sample has a storage modulus (G') which is representative of energy stored in the material. This is demonstrating the elastic (solid-like) properties of the material. If the strain is out of phase with the stress, it is said to have a loss modulus (G'') which is showing the energy lost or viscous (liquid-like) characteristics of the sample. Typically, the storage modulus will be seen in systems that are permanently cross-linked while the loss modulus is seen in systems that are tangled together (Karim et al, 2000).

Stress relaxation techniques can also be used to measure the viscoelasticity of a starch gel or product. Stress relaxation is done by applying a constant strain to a system. In order to maintain this strain, a system will reorganize itself in order to decrease the stress. This change in the amount of stress on the system is measured in the form of a curve seen in Figure 2.2 (Karim et al, 2000).

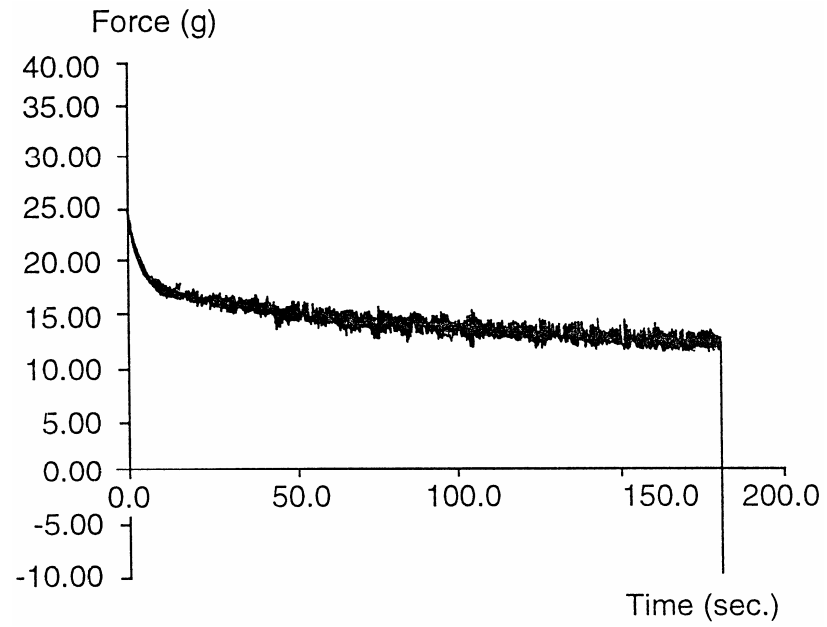


Figure 2.2 Stress Relaxation Curve That is Representative of a Sweet Potato Starch Gel

(Karim et al, 2000).

CHAPTER 3 - Masa

Fresh masa dough is made by boiling corn with calcium hydroxide and then grinding it into a dough. Fresh masa dough can be dried and ground, making masa flour or nixtamalized masa flour. Similar to wheat, there are two different types of corn; hard and soft. Hard corn is typically used for making chips and other fried corn products. When processing hard corn, it is likely that changes in the process time and the amount of lime added will be necessary. Soft corn is a more consistent product and is better to use for corn tortilla production. It has better water absorption and retention capabilities, with a moisture retention rate of 9-12% (Cornell, 1999).

In manufacturing tortillas, two of the most critical factors are the granulation of the masa flour and the amount of water that it will absorb. Masa flour comes in several granulations; fine, fine/medium, medium, medium/coarse, and coarse. The finer the granulation of the masa flour, the better the water absorption will be (Cornell, 1999; Gómez et al, 1991). The optimal absorption level is 1.15 to 1.20 lbs of water for every 1.0 lb of corn masa flour (Trevino and Norton, 2006). A finer masa flour granulation has a greater number of free starch granules. This will increase the viscosity during cooking. A coarser product slows the diffusion of the water through the masa, limiting the swelling of the starch granule. Because the swelling of the starch granule is inhibited, the viscosity develops much more slowly (Gómez et al, 1991). In order to have optimum texture of a finished tortilla, it is also necessary to have a very consistent granulation of masa. The optimum granulation is “4% maximum on a U.S. Standard Sieve #45

and 40% minimum through a U.S. Standard Sieve #100". This granulation will absorb the optimum amount of water (Trevino and Norton, 2006).

The color of the masa is closely related to the thickness of the skin on the corn. If the skin is thick, it can be tightly adhered to the corn, making it more difficult to remove, keeping the yellow color out of the masa flour or dough. The color is also affected by the pH. A more acidic pH will result in a more yellow product. The amount of lime left in the finished product will also have an affect on the flavor of the product, giving it a soapy flavor (Cornell, 1999).

In order to make the highest quality tortilla, it is important to use masa flour that has been properly stored. If it is not going to be used for several months, it is best to store the masa at a temperature just above freezing in a low moisture environment. When it will only be stored for a few days, a 70-75 °F room with low moisture will maintain the masa quality (Cornell, 1999).

Masa manufacturing companies are producing many different types of masa flour to make higher quality tortillas. Masas come in a large variety of granulations with many different additives. Masa flours are being made that have preservatives already in them in order to lengthen the shelf life of a corn tortilla. Higher yield masas are also being made that will retain moisture better over time. Masa flours come in a variety of colors, pH levels, and even added various flavors, such as pinto bean powder (Cornell, 1999).

When producing corn tortillas to be sold in groceries stores, it is important to extend the shelf life beyond the day or two that a simple masa and water formula will attain. Similar to flour tortillas, other additives such as gums, wheat gluten, dough conditioners, leavening agents, and antimicrobials can be used.

Nixtamalization Effects on Starch

Nixtamalization is a process where corn is boiled in a 1-5% lime solution. This process weakens the cell walls and softens the pericarp, making it easier to remove the pericarp. The cell wall in the peripheral endosperm is softened and the starch granules are partially gelatinized and damaged. During this process, corn starch granules are changed both physically and chemically, which has a direct impact on the functional properties of the masa, such as the texture, crispiness, color, flavor, and shelf life (Gómez et al, 1989).

Lime is critical part of the nixtamalization process. In a study done by Gómez et al (1989) it was found that when the corn is boiled in the absence of lime, the pericarp will not separate from the kernel, even if the kernel is severely overcooked. However, if the kernel is undercooked in the presence of lime, it will still separate from the kernel. While the kernel is boiled, the starch granules begin to swell until they fill the entire endosperm, and eventually they will break out of the endosperm. Interestingly enough, after the starch granules break out of the endosperm, they retain the shape of the endosperm (Gómez et al, 1989).

Properties of starch in masa vary depending on the granule size, ratio of amylose to amylopectin, organization within the starch granule, and lipids and phosphate groups within the granule. All of these things are closely linked to the quality of the tortillas that are made from the starch. All of these qualities will change during the nixtamalization process (Toro Vasquez and Gómez-Aldapa, 2001).

The process of turning raw corn into a corn tortilla has several steps that affect the starch in different ways. Raw corn starch granules have a bit of fluorescence around their edges (Gómez et al, 1989), exhibit birefringence (Gómez et al, 1989), and have the A-type starch structure (Gómez et al, 1992). As the raw corn is cooked in lime, the starch granules begin to

swell and partially gelatinize (Gómez et al, 1989, Gómez et al, 1992) which disturbs the crystalline portion of the matrix (Gómez et al, 1992). Once the nixtamal is made, a difference in the starch granules starts to become apparent. The starch granules are now swollen and they are not exhibiting as much birefringence as the raw corn starch. The fluorescence that was visible around the edges has now disappeared (Gómez et al., 1989). While the nixtamal is left to steep, starch begins to recrystallize in order to revert to a state that is more similar to its native form. At this point, the nixtamal is very resistant to breaking down, so has a very consistent viscosity, giving it a peak on the viscogram at 95° (Gómez et al, 1992).

The nixtamal is now ground to make the masa dough. This is a physical process, where the starch granules are ground and exposed to a large amount of shear through the grinding stones (Gómez et al, 1989, Gómez et al, 1992). The grinding process tears the kernels apart and the starch granules are exposed to additional heat and friction. This causes additional gelatinization and damage of the granules (Gómez et al, 1989), and a 4-7% loss in birefringence. The starch granules found in the masa dough are irregularly shaped (Gómez et al, 1989, Gómez et al, 1992). The masa is gaining cohesiveness, which is attributed to the amylose and amylopectin that has leached out of the granules. Throughout the process of turning raw corn into masa dough, the starch granules have lost anywhere from 1-10% of their crystallinity. The texture and handling properties of the masa dough are highly influenced by the cooking and steeping of the corn. Raw or undercooked corn or a steep that is too long or too short can greatly influence the texture of the masa (Gómez et al, 1989).

Once the masa dough is made, the masa is formed into tortillas and then they are baked. The tortillas are exposed to temperatures greater than 240 °F which cause some changes to the tortilla on a microstructural level. The tortillas have a limited amount of water and they are only

exposed to heat for a short amount of time, so there is some additional starch gelatinization that happens. The edges of the tortilla are exposed to more extreme heat than the center of the tortilla (Gómez et al, 1989). This causes additional evaporation of the water, preventing starch gelatinization. The edges of the tortilla maintain their birefringence, while the starch in the center of the tortilla gelatinizes and loses its birefringence (Gómez et al, 1992). After the tortilla is baked, it has lost 30-40% of its crystallinity (Gómez et al, 1989). In addition to this change in birefringence, the fluorescence of the granule changes during the baking process. The corneous endosperm fluorescence decreases in intensity while the peripheral endosperm loses its intensity completely (Gomez et al, 1989).

Instead of taking the masa dough and using it to make tortillas, the masa can be dried and ground further to make nixtamalized corn flour (NCF). NCF can then be stored for several months and then rehydrated to make tortillas or corn products. Once the NCF is rehydrated, the dough has very different properties than fresh masa dough. NCF dough is less cohesive and less plastic. NCF dough lacks the protective coating that the partially gelatinized starch, water, and starch polymers form around the kernel (Gómez et al, 1989; Gómez et al, 1990). This coating protects the kernel during kneading.

The amount of damaged starch is represented by the enzyme susceptible starch ratio (ESS). It is important to have the proper amount of ESS, because it helps the masa dough stick together. If the ESS is too low, as there is in undercooked corn, then the masa will not be sticky enough and will not hold together. The opposite is the case in overcooked corn. The ESS is too high, so the masa is very sticky and this could cause problems during production (Gómez et al, 1991). Starch retrogradation starts during the production of NCF due to the heating and the

agitation that happens during the drying and grinding process. Finer NCF has more exposure to the grinding process, causing more retrogradation in finer ground NCF (Gómez et al, 1991).

According to Gómez et al (1991), the crystallinity of the starch granules decreases, due to partial gelatinization during the process of drying out the fresh masa dough. Although there is this change in crystallinity, there is a larger difference between raw corn flour and the fresh masa dough than there is between the fresh masa dough and the NCF. Gomez et al (1991) also found that when the corn was subjected to a lower amount of shear the particles of the NCF would be larger. There would be more starch crystallinity because the granules would be less damaged.

CHAPTER 4 - Tortilla Quality Determination

Corn Tortilla Staling

The major factor in consumer acceptance of tortillas are the texture and the handling properties of the product. Right after baking, tortillas are soft and pliable and can easily be rolled or folded without cracking. As the tortillas begin to stale, they undergo changes that result in a more firm, rigid tortilla that will easily crack when rolled or folded (Limanond et al, 2002). It is estimated that \$1 billion worth of baked goods are thrown away each year due to staling, equaling roughly 3-5% of all baked goods (Hebeda et al, 1990).

Two main ways that the quality of a tortilla can deteriorate over time are by staling or mold growth. Mold growth is easily understood and inhibited by the use of hurdle technology. Staling, however, is a complex and rapid process that starts as soon as the tortillas leave the oven and start to cool down (Limanond et al, 2002). When a tortilla is fresh, it is very soft and very flexible. Only a few hours after baking, corn tortillas have already started to exhibit signs of staling, such as breaking and cracking when rolled (Campas-Baypoli et al, 2002).

Starch is the main component that makes up a tortilla. Most of the textural changes that happen throughout the processing and shelf life of a tortilla are a result of the starch interactions with other components of the tortilla, including itself (Campas-Baypoli et al, 2002). The process of staling involves gelatinization of starch granules, retrogradation of starch, moisture loss over time, and interactions between other ingredients in the tortilla. During staling, the molecules in the tortilla are realigning themselves into a more ordered crystalline structure that results in a harder crumb (Limanond et al, 2002). Specifically, after a tortilla is baked, there are changes in the content of soluble amylose and amylopectin. During baking the starch crystals gelatinize but

only partially disperse in the tortillas. As the crystals gelatinize, amylose leeches out and surrounds the hydrated starch crystals. The amylose polymers start to associate with one another, building an insoluble, rigid network of amylose. Amylopectin polymers slowly associate, forming an insoluble, rigid, amylopectin network. Starch crystal nuclei that are retained after baking also contribute to the retrogradation of the starch (Fernandez et al, 1999).

The staling process moves much more quickly in a tortilla than it does in bread. The enthalpy for bread after 24 hours is similar to that of a 4 hour old flour tortilla. The textural changes that happen in a tortilla can be completely reversed upon heating, while the textural changes in bread can only be partially reversed with heat. There are two main things involved in the staling of bread, starch retrogradation and the migration of moisture from the gluten to the starch. Bread often has dough conditioners and softeners and help with the extension of shelf life. Tortillas have a lower fat content than bread and there is a much higher starch concentration, causing tortillas to stale much faster than bread (Campas-Baypoli et al, 2002).

The process of staling is dependent on storage time and temperature. Campas-Baypoli et al (2002) reported changes in both the apparent amylose content and resistant starch in a flour tortilla. The decrease in apparent amylose content is a result of the rapid starch retrogradation in the tortilla. Most of the decrease in apparent amylose happened in the first 24 hours after baking. This is indicative of the amylose retrogradation happening rapidly and after baking. Resistant starch increases with the starch retrogradation. In the first 2-24 hours after baking, there was a rapid increase in resistant starch. It tapered off until 48 hours, where it increased again until 72 hours. Heat treatments made the starch less susceptible to enzyme hydrolysis by enhancing the interaction of the starch with other tortilla components (Campas-Baypoli et al, 2002).

Campas-Baypoli et al (2002) showed that storing tortillas at refrigerated temperatures promoted starch retrogradation. Tortillas that were stored at a refrigerated temperature had a greater decrease in apparent amylose content after 24 hours of storage. In these same tortillas, when refrigerated for two hours, amylopectin retrogradation happened (Campas-Baypoli et al, 2002). Bueso et al (2006) found that storing a tortilla with no additives at 3 °C, made the texture of the tortilla more brittle and hard than a tortilla stored at either room or freezing temperature. The melting enthalpy (T_m) of the corn tortilla was most affected when the tortilla was refrigerated. Bueso et al (2006) also found that refrigerated tortillas had a higher melting enthalpy, meaning that more amylopectin had recrystallized. The rate of recrystallization showed a bell shaped dependence on the temperature of storage between -20 °C- 21 °C. A tortilla is a semi-crystalline system that ages at a temperature between T_g and T_m . The T_m of a tortilla has a peak at 57 °C (Campas-Baypoli et al, 2002) and the T_g is -23 °C (Limanond et al, 2002). The maximum starch retrogradation happens in a tortilla at 10-13 °C (Bueso et al, 2006; Limanond et al, 2002). Freezing the tortilla is the only way to extend the shelf life without adding other ingredients to the tortilla (Bueso et al, 2006).

In order to delay the staling of a tortilla, adjustments can be made in the product storage temperature, moisture level, and the use of various additives. Staling rate is linear with respect to time, so it is necessary to utilize these other control factors. Maintaining the maximum moisture level while maintaining product integrity will delay the starch retrogradation process. Storing the tortillas at a temperature above room temperature or at a temperature below freezing will delay the recrystallization of the starch (Hebeda et al, 1990). Various additives, such as enzymes, hydrocolloids, and glycerol, can be used to inhibit staling. In order to delay staling, the additives used could be forming complexes with amylose or amylopectin, breaking down the

amylose to prevent formation of large crystals during retrogradation, maintaining moisture in the product, or strengthening the gel to delay its recrystallization (Gudmundsson, 1994).

After nixtamalization of the corn, there are still some crystalline areas remaining. It is thought that these areas promote the recrystallization of the starch molecules by acting as nuclei. Since tortillas are so flat and they have a large amount of surface area, they are prone to losing water through evaporation. These two factors are what cause tortillas to stale so rapidly. Because of this, any substance that can be added to the tortilla to retain water or to inhibit the recrystallization of the starch will delay the staling of the tortilla (Gómez et al 1991).

Measuring Corn Tortilla Staleness

There are several ways to test tortillas for the degree of staleness. A very common way is a subjective test that involves rolling the tortilla around a dowel and observing the cracks in the tortilla. A score is then given to the tortilla, based on the severity of the cracking. This method introduces a lot of variation due to the scores being given by a human observer. Depending on the person executing the test, completely different results could be obtained. While this test is sensitive enough to measure changes over time, it is not sensitive enough to pick up changes that happen only hours after the tortillas are baked. The subjective firmness test measures so many attributes of the tortilla that it is difficult to quantify. It measures the pliability, squeezability, softness, and firmness of the tortilla (Suhendro et al, 1998a).

Another way that tortilla texture can be analyzed is a more objective method using a texture analyzer. This method is called the objective rollability method and is very sensitive to variability in pilot plant produced product, so it will likely pick up subtle changes that can happen over time in corn tortillas. A significant portion of the textural changes in a tortilla happen in the first 24 hours after baking and this method is sensitive enough to analyze for these

differences. The tortilla is placed double baked side down on a flat surface that has a cylindrical dowel attached to it (Figure 4.1). The tortilla is attached to the dowel and the dowel then rolls the tortilla. The result that is obtained is a rollability curve, as seen in Figure 4.2.

The area underneath this curve is the amount of work that is used in order to roll the tortilla around the dowel. The first peak on this curve is the force that it takes to roll the tortilla around the dowel. When commercial tortillas from the same bag were tested there was a low coefficient of variation, suggesting that this test is repeatable and sensitive. When tortillas that were prepared in an uncontrolled laboratory setting were tested, there was a much higher coefficient of variation. This data suggests that the test method is sensitive to normal variability that is found in a pilot plant (Suhendro et al, 1998a). Suhendro (1998a) found that the longer the tortillas are stored, the more work and force are necessary to roll the tortilla around the dowel.

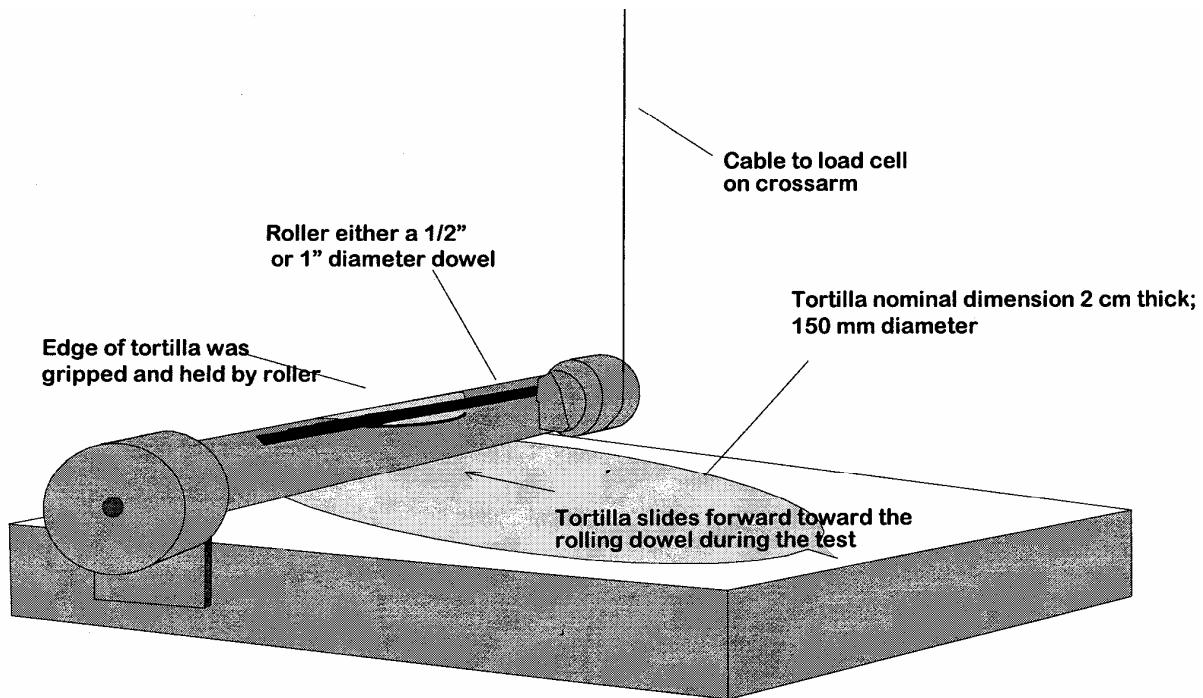


Figure 4.1 Device That Can be Used to Take Objective Measurements of Corn Tortilla Rollability (Suhendro et al, 1998a).

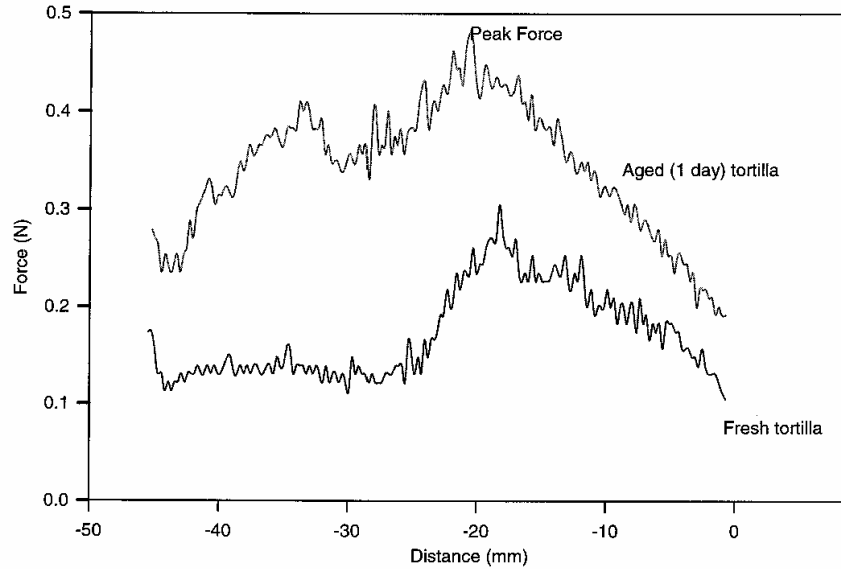


Figure 4.2 Rollability Curve That is Typical for a Fresh and an Aged Corn Tortilla (Suhendro et al, 1998a).

Another objective measuring tool for tortilla quality is a bending technique using a texture analyzer (Figure 4.3). A portion of the tortilla is cut from the center of the tortilla and then clamped horizontally to the texture analyzer platform. A guillotine is held vertically over the tortilla. The guillotine is moved at a constant speed down to the tortilla, putting pressure on the tortilla and bending it to a 40 degree angle. This is a very precise bending technique that does not have a lot of variability. This method can be used to make predictions about the linear region of a material that is viscoelastic. The modulus of deformation, force at 1 mm, work and apparent force can all be measured in this technique. This test was used to show that the older the tortilla is, the more force is necessary to bend them (Suhendro et al, 1998b).

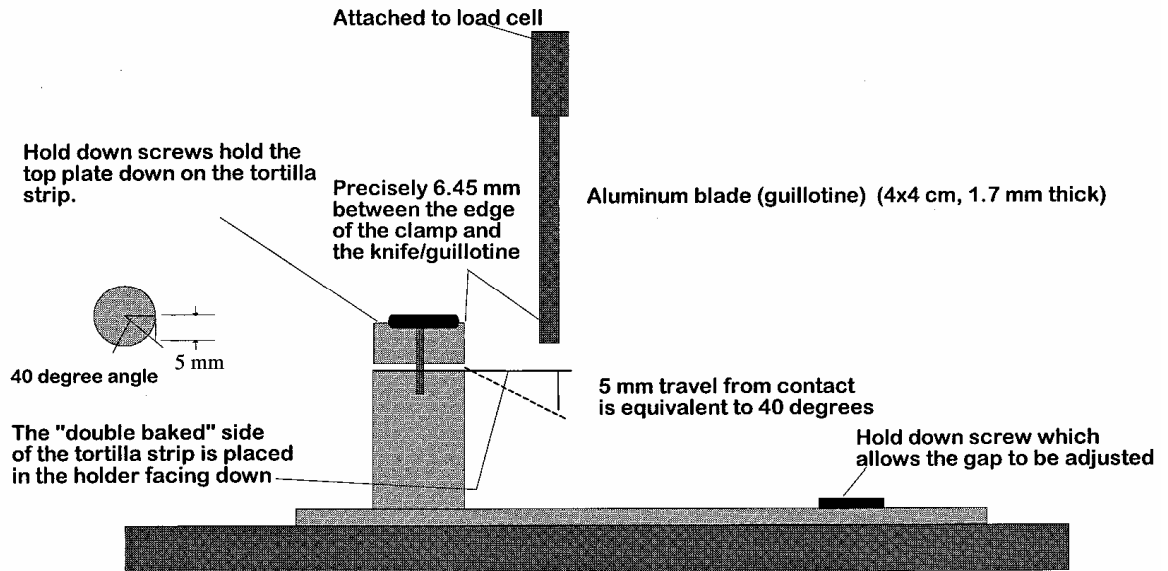


Figure 4.3 Diagram of Device Used to Take Objective Measurements of the Bending Properties of a Tortilla (Suhendro et al, 1998b).

CHAPTER 5 - Preservation Technology

Methods of Preservation

At some point in the shelf life of any product, the quality will begin to deteriorate. There are several physical, chemical, or microbial factors (Table 5.1) that can contribute to this quality loss. Intrinsic factors are either physical or chemical factors that are present within the food item. Microorganisms that have contaminated the food are influenced by these intrinsic factors. Extrinsic factors are things that exist in the environment around the food that will influence microorganisms during storage. Food that is put through various processing steps in order to increase the preservation will have processing factors that will affect the quality of the product over time. Implicit factors are characteristics of the contaminating microorganisms themselves and how they interact with the environment around them. It is difficult to look at any of these factors alone, as they will likely influence one another. The net effects of all of the above factors together may be greater than the expected effect of only one factor (Leistner and Gould, 2002).

Table 5.1 Reasons for a Decrease in the Quality of Food (Leistner and Gould, 2002).

<i>Chemical</i>	<i>Physical</i>	<i>Enzymatic</i>	<i>Microbiological</i>
Oxidative rancidity	Mass transfer, movement of low molecular weight components	Lipolytic rancidity	Growth or presence of infectious microorganisms
Oxidative and reductive discoloration	Loss of crisp textures	Rancidity catalyzed by lipoxygenases	Growth of toxinogenic microorganisms
Nonenzymatic browning	Evaporative loss of flavors	Proteolysis	Growth of spoilage microorganisms
Destruction of nutrients	Freeze- induced structural damage	Enzymic browning	

In order to slow or inhibit the growth of microorganisms, it is necessary to choose preservation techniques (Table 5.2) that “target these major factors that influence microbial growth and survival” (Leistner and Gould, 2002). The majority of methods that are currently used slow the growth of organisms. Low temperature holding (storage), pH reduction, low water activity, preservatives, vacuum packaging, and modified atmosphere packaging are commonly used, but will not actually inactivate microorganisms or prevent contamination by microorganisms. Only a few methods, such as pasteurization and heat sterilization will actually kill the microorganisms. Aseptic processing and packaging can be used in order to prevent microorganisms from accessing the product at all (Leistner and Gould, 2002). There are many different ways to reduce microbial growth, but reduction in storage temperature, water activity, pH, preservatives and packaging technology will be focused on for the purposes of this paper.

Storing food in either a refrigerator or a freezer will significantly slow the growth of many pathogens and spoilage microorganisms. *Clostridium perfringens* and some types of *Clostridium botulinum* cannot grow at temperatures below 12 °C. At temperatures below 0 °C, most pathogens cannot grow, so storage will be safer. Reducing the temperature to freezing has the effect of tying up some of the water into ice crystals, thereby reducing the water activity. Temperatures below -10 °C are low enough to prevent the growth of almost all microorganisms (Leistner and Gould, 2002).

Table 5.2 Food Preservation Technologies (Leistner and Gould, 2002).

<i>Objective</i>	<i>Factor</i>	<i>Mode of Achievement</i>	
Slowing or complete inhibition of microbial growth	Reduced temperature	Chill Distribution and storage Freezing and frozen distribution and storage	
	Reduced water activity/raised osmolality	Drying and Freeze Drying Curing with added salts Conserving with added sugars	
	Decreased Oxygen	Vacuum and nitrogen packaging	
	Increased Carbon Dioxide	Carbon dioxide enriched “controlled atmosphere” storage and “modified atmosphere” packaging	
	Decreased pH Value	Addition of acids Lactic or Acetic fermentation	
	Restriction of Availability of Nutrients	Control of microstructure: compartmentalization of aqueous phases in water-in-oil emulsions	
	Preservatives	Addition of preservatives inorganic (e.g. sulfite, nitrite) organic (e.g. propionate, sorbate, Benzoate, parabens) bacteriocin(e.g. nisin) Antimycotic (e.g. natamycin/pimaricin)	
	Inactivation of microorganisms	Heating	Thermization, to injure heat-sensitive vegetative microorganisms
			Pasteurization, to inactivate heat-sensitive microorganisms
	Restriction of access of microorganisms to food products	Decontamination	Carcass, fruit, and vegetable decontamination (e.g. with steam organic acids, hypochlorite, ozone)
Ingredient Decontamination (e.g. with heat, irradiation)			
Aseptic Processing		Decontamination of packaging materials (e.g. with heat, hydrogen peroxide, irradiation) Thermal processing and packaging without recontamination	

In addition to freezing, the water activity of food can be reduced in a variety of ways.

Food can be cured with salt, or sugar, or dried. In order to reduce the risk of food poisoning

bacteria, the water activity needs to be reduced to only 0.86 in an aerobic environment and 0.91

in an anaerobic environment. Once the water activity is below 0.86, the main concern is with yeasts and molds which are more tolerant of lower water activity than bacteria. The lower limit of yeasts and molds is 0.6, so many foods are formulated with a water activity as low as 0.3 to minimize any possibility of yeast or mold growth (Leistner and Gould, 2002).

Lowering the pH of the product is often very useful in preventing the growth of food poisoning microorganisms. *Clostridium botulinum* is unable to grow at a pH below 4.5. Other organisms that cause food poisoning cannot grow below a pH of 4.2. A lower pH aids in the thermal processing of foods, decreasing the amount of thermal processing necessary in low acid foods (Leistner and Gould, 2002).

Preservatives are often used to extend the shelf life of processed foods. There is a trend in today's marketplace to move away from chemical preservatives toward "natural" products. Consumers are showing an interest in foods that are less processed with fewer chemicals in them. Preservatives are effective at the prevention in growth of microorganisms. Preservatives are more effective at a lower pH, so they are often used in conjunction with lowering the pH of the product. Weak organic acids, such as sorbic, benzoic, and propionic, and inorganic acids, such as sulfite and nitrite, are often used (Leistner and Gould, 2002).

Another way to slow growth of microorganisms is the use of either vacuum or modified atmosphere packaging. Vacuum packaging will remove all air from the package. Either of these types of packing will first remove the oxygen from the package, thus making an anaerobic environment. Carbon dioxide is often used in modified atmosphere packaging because it will limit the growth of microbes and is very effective when combined with refrigeration. Removing oxygen from the package is very beneficial for products that are susceptible to oxidative rancidity (Leistner and Gould, 2002).

Hurdle Technology

Hurdle technology is the use of multiple preservation techniques in order to optimize the safety and quality of a processed food while preserving the food for a desired amount of time. Figure 5.1 shows some examples of how the hurdle effect works. The second example shows a situation where water activity and preservatives are the main hurdles that the microorganisms need to overcome. Since the microbes cannot overcome these hurdles to grow, the food is microbiologically safe to consume. If only one of these hurdles was used at the same level that it is in this scenario, the food would not be safe, but when used together these five hurdles are able to work in conjunction with one another in order to maintain food safety (Leistner and Gould, 2002). The fourth example in this figure shows an instance where the product could have been contaminated along the way. In this situation, the microbial load was greater than expected, so the microorganisms were able to overtake the hurdles and this food would not be safe to consume. In some cases, the hurdles could change in the food during storage. This is demonstrated in example number 7. This example shows what happens in foods where the preservative effect is decreased over time. The final example on this figure demonstrates the synergistic effect of hurdles. When multiple hurdles are used, the effect of all of the hurdles is increased, making a more effective system to keep food safe and stable (Leistner and Gould, 2002).

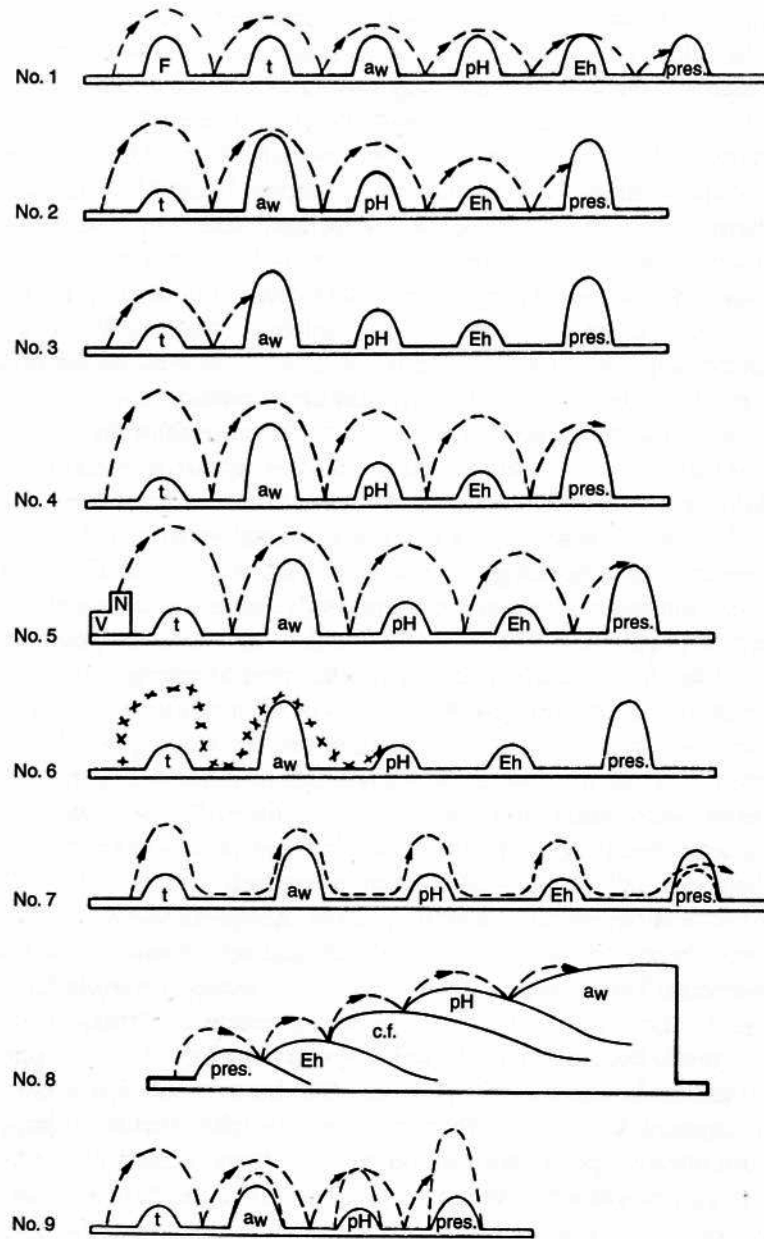


Figure 5.1 Examples of Hurdle Technology (F= Heating, t= Chilling, aw= Water Activity, Eh= Redox Potential, pres= Preservatives, V= Vitamins, N= Nutrients, c.f.= Competitive Flora) (Leistner and Gould, 2002).

These hurdles work together in a synergistic way, rather than additive. This synergy that will be found within the hurdles is due to an interruption in the homeostasis of the microorganisms, preventing them from multiplying. Homeostasis refers to “the tendency of microorganisms to maintain a stable and balanced internal environment” (Stauffer, 2002). Hurdle technology takes advantage of this homeostasis by interrupting several mechanisms involved in this balance (Stauffer, 2002). In working with hurdle technology, it is also important to ensure that the different hurdles are being used at the correct levels. If the hurdle intensity is too high or too low, it could be damaging to the system (see Figure 5.2) (Leistner, 1994).

An important factor in using hurdle technology is that it helps to maintain the eating quality of processed foods. If only a reduction in pH was used to prevent the growth of microorganisms, products would be very sour and unpalatable. In using this technology, it makes it much easier to make foods that will be accepted by consumers. As hurdle technology has been studied and better understood, it has even made it possible to improve the eating quality of processed foods through its use (Leistner, 1994).

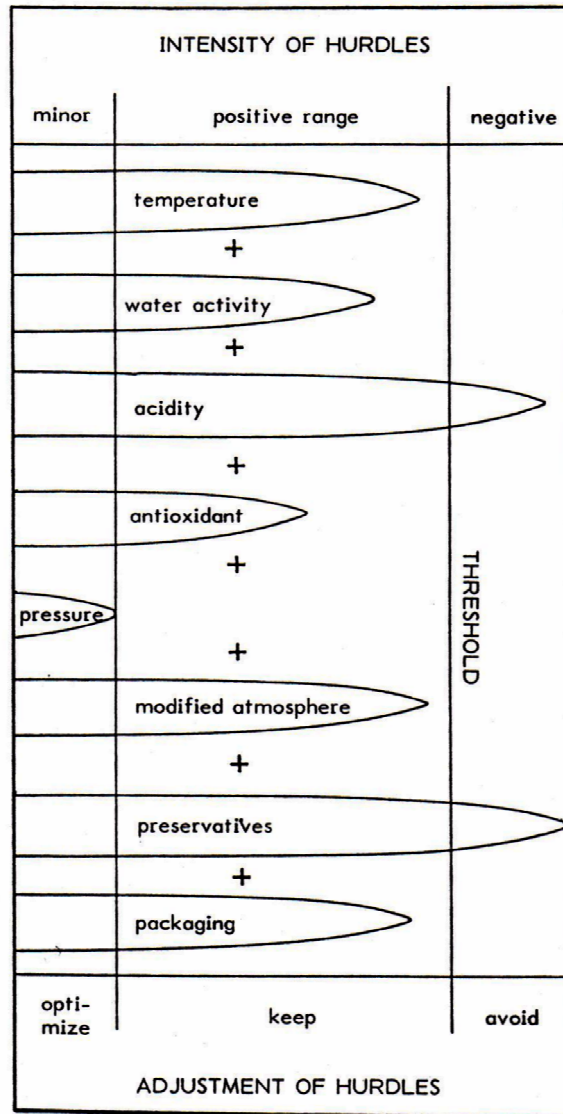


Figure 5.2 Food Quality Hurdles that Could Also be Safety Hurdles (Leistner, 1994).

Application of Hurdle Technology to Corn Tortillas

When choosing which hurdles to use, it has been suggested to take an integrated approach to designing the product, including both Good Manufacturing Practices (GMP's) and Hazard Analysis and Critical Control Points (HACCP) (Table 5.3). Leistner (1994) has come up with a set of steps to follow in order to aid in this process. These steps set a clear outline to

ensure that the food produced is safe and stable. The most common hurdles that are used in food preservation are the reduction of water activity, reducing the storage temperature, lowered pH, and the use of chemical preservatives (Leistner and Gould, 2002).

Tortillas are typically produced either for refrigerated or ambient temperature storage. Refrigerated temperatures will reduce mold and bacteria growth, but will not be enough to ensure that the product remains safe throughout its shelf life. Regardless of storage temperature, pH and water activity will be lowered and preservatives will be used. With a lower storage temperature, pH or water activity might be a little higher and there may be fewer preservatives necessary.

In tortillas, glycerol has been identified as a substance that competes for water, reducing the available water in the tortillas. In doing this glycerol lowers the water activity, making it more difficult for bacteria to grow. Glycerol has been shown to reduce the “freezable” water content of baked goods (Vittadini et al, 2004). Freezable water is the water present in the product that can freeze and form ice crystals that can be detected using differential scanning calorimetry. If there is a large amount of freezable water present in the tortilla, it can lead to chemical reactions that promote staling and permit microbial growth. Glycerol is capable of binding to water, decreasing the freezable water while maintaining the product integrity. Figure 5.3 shows that when glycerol is added at 1.5% and salt at 0.4%, the freezable water in the tortillas is reduced by 16% (Vittadini et al, 2004).

Table 5.3 Suggested Steps to Take When Developing a Food Product, Incorporating HACCP, GMP, Predictive Microbiology, and Hurdle Technology (Leistner and Gould, 2002).

1. For a modified or new food product the desired sensoric properties and shelf life are tentatively defined.
2. A feasible technology for the production of the food is outlined.
3. The food is manufactured, first at laboratory or pilot-plant scale, according to this technology, and the resulting product is analyzed for pH, a_w , preservatives, or other inhibitory factors. Temperatures for heating (if intended) and storage as well as the anticipated shelf life are defined.
4. For preliminary microbial stability testing of the food product, predictive microbiology is employed.
5. The product is then challenged with relevant food poisoning and spoilage microorganisms, using somewhat higher inocula and storage temperatures than would be “normal” for the food.
6. If appropriated, the hurdles in the product are modified, taking multitarget preservation and the sensoric and nutritional quality of the food (i.e. total quality) into consideration.
7. The food is again challenged with relevant microorganisms, and if necessary the hurdles in the food are modified again. Predictive microbiology for assessing the safety of the food is helpful at this stage too.
8. After the established hurdles of the modified or new foods are exactly defined, including tolerances, the methods for monitoring the process are agreed on. Preferable physical or sensorical methods for monitoring should be used.
9. The designed food should now be produced under industrial conditions, because the adequacy for a scale-up of the proposed manufacturing process must be validated.
10. If for an industrial process the CCPs and their monitoring are established, the manufacturing process might be controlled by HACCP. If HACCP seems inappropriate, guidelines for the application of manufacturing control by quantitative GMP must be defined.

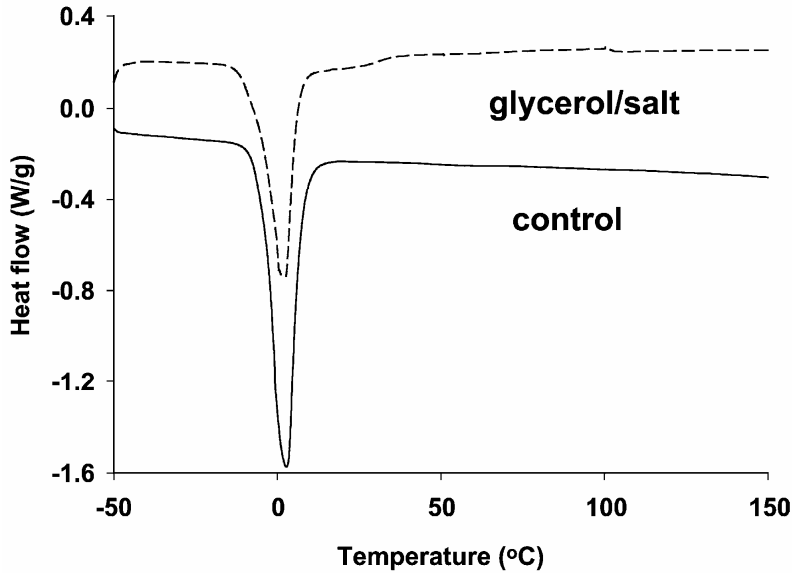


Figure 5.3 DSC Thermograms Comparing Corn tortillas with Glycerol and Salt to a Control. Control had 44% Freezable Water and the Glycerol/Salt Tortilla had 28% (Vittadini et al, 2004).

Preservatives are more effective at a lower pH, so an acidifying agent may be used. Fumaric acid is commonly used, but phosphoric acid can be used as well. Propionic acid, methyl or propyl paraben, potassium sorbate, calcium or sodium propionate, and benzoic acid are all preservatives that could be used to prevent mold growth. The preservative that is used depends on the final product pH (Rolow, 2002).

Almost all preservatives are based on organic acids and are most effective in their undissociated form. At a lower pH, the compounds remain intact, allowing them to prevent the growth of mold and bacteria. Table 5.4 shows the level of dissociation of propionic and sorbic acids at a given pH. In order to have 50% of propionic acid undissociated, the pH of the product needs to be 4.8-4.9. The closer the product gets to this pH, the more preserving action there will be from the propionic acid. Using the same level of preservative and decreasing the pH from 5.2 to 5.0 can increase the shelf life of a corn tortilla from 8 days to greater than 20 days (Rolow,

2002). Various acids can be used to lower the pH of the tortilla. Fumaric and phosphoric are the most common, but vinegar, monocalcium phosphate, and citric acid can all be used to bring the tortilla into the ideal pH range of 5.5 to 6.5. Reducing the pH will have some adverse effects on the flavor and appearance of the tortilla. The tortilla will not brown as well and will have a lighter color and a sour flavor. If the pH is too high, the tortilla will have a darker color and a soapy mouth feel and the preservatives will not be as effective (Stockwell, 2000).

Table 5.4 Relationship Between pH Value and Dissociation of Organic Acids (Rolow, 2002).

Undissociated Acid (%)	pH – Propionic Acid	pH - Sorbic Acid
99	2.87	2.75
95	3.59	3.47
90	3.92	3.80
80	4.27	4.15
70	4.50	4.38
60	4.69	4.57
50	4.87 (pKa)	4.75 (pKa)
40	5.05	4.93
30	5.24	5.12
20	5.47	5.35
10	5.82	5.70
1	6.87	6.75
0.5	7.17	7.05

Preservatives can be used in either dry or liquid form. Dry preservatives are made by reacting the organic acid with a base in order to form a salt. One example of this is calcium propionate. It is made by reacting calcium hydroxide with propionic acid. Excess calcium must be added to ensure that the acid is converted to a dry powder and to aid in the water solubility of the preservative. Potassium sorbate is made in a similar way, reacting potassium hydroxide with sorbic acid and adding excess potassium. The excess cations that are present make the preservative ineffective at a pH higher than 6 (Rolow, 2002). Liquid preservatives are corrosive

and need to be neutralized with calcium, sodium, or potassium ions, according to regulations by the Department of Transportation. The pH is adjusted to be in the range of 4 to 6. The salt will not precipitate out in this range and the acid is associated, therefore maintaining the effectiveness of the preservative. In a liquid preservative 80% of the ingredient could be the active acid (Rolow, 2002).

If a preservative is added in the dry form, it is added in with the dry ingredients and mixed. Water is then added to the mixer. The dry preservative will then need to compete with other dry ingredients in order to dissolve and disperse. A liquid preservative can be added in with the water, allowing it to evenly disperse in the product. This is a process that can be automated (Rolow, 2002).

There are two different types of corn tortillas, fresh masa dough or nixtamalized corn and masa flour, and different preservatives are required for each type. The first type, nixtamalized corn tortillas have been made with corn that has been cooked in calcium hydroxide (lime) and then rinsed to remove excess lime. The rinsed corn is then stone ground, forming masa dough. This dough is then used to produce corn tortillas. In order to produce masa flour tortillas, the masa dough is dried and then ground to a flour with a 10% moisture content. The masa flour is then mixed with water and other additives in order to make tortilla dough. Making masa flour allows for a more consistent process, giving masa flour tortillas a more consistent pH than masa dough tortillas (Rolow, 2002). Given the variation in pH between the different types of tortillas, different preservatives will be needed in order to maintain safety and stability of the tortilla.

Table 5.5 Properties of Common Antimicrobial Agents (Rolow, 2002).

Agent (Related Organic Acid)	Effective Against:			Effective pH (pKa)	Water Soluble
	Mold	Yeast	Bacteria		
Calcium or Sodium Propionate (Propionic Acid)	X		X	4.9	X
Potassium Sorbate (Sorbic Acid)	X	X		4.8	X
Sodium Diacetate (Acetic Acid)	X		X	4.8	X
Sodium Benzoate (Benzoic Acid)	X	X		4.2	X
Methyl or Propyl Paraben	X	X	X	4 to 9	

Nixtamalized corn tortillas are often at a higher pH than masa flour tortillas. In the process of cooking the corn in lime, the final pH is 7. At times, the lime may not be thoroughly rinsed off the corn, resulting in an even higher pH. Phosphoric acid used in conjunction with propionic acid and either methyl or propyl paraben is an effective way of preserving masa dough. Propionic acid and methyl or propyl paraben are the active preservatives while phosphoric acid is used to lower the pH of the dough. Because the dough is already made when the preservative is added, it is best to use a liquid preservative. A dry powder would not easily hydrate and disperse throughout the dough, rendering it ineffective. Methyl or propyl paraben are effective in a pH range of 4 to 9 (Table 5.5) and they will inhibit the growth of mold, yeast, and bacteria. Propionic acid is effective against mold and some bacteria and is most effective at a pH of 4.9 (Rolow, 2002). If only one of these preservatives were to be used it would need to be used at a higher level. As the levels of the preservatives increase, the consumer is more likely to taste

them. There is a level that the consumer can taste the preservative and the acceptance of the product will decrease.

In the preparation of nixtamalized corn tortillas, preservatives are often added at the stones step and then the masa dough is mixed. This helps to ensure even distribution of the preservative. Figure 5.4 shows how critical the mixing step is. When the preservative is added at the stones, the pH is 5.6 or greater. After the dough is mixed in a mixer for ten minutes the pH drops to less than 5.5. Having a more consistent pH is critical to the success of the preservatives in the system (Rolow, 2002).

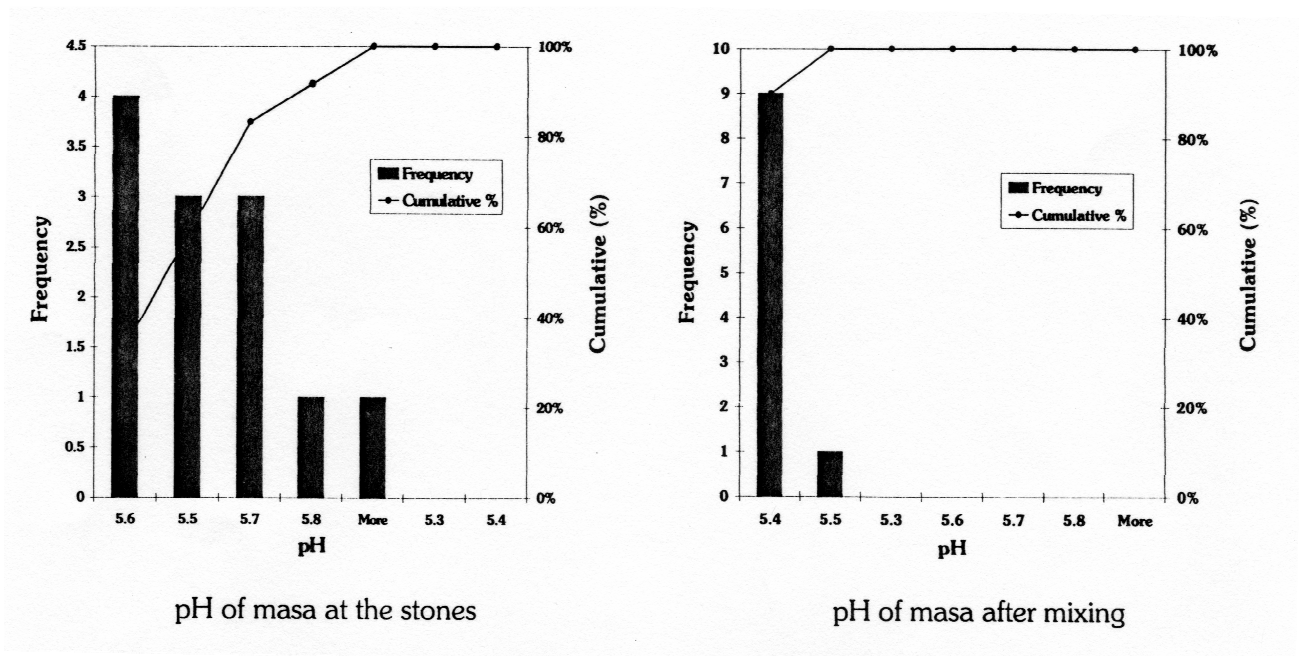


Figure 5.4 Comparing the pH of Masa When Preservatives are Added Before and After the Mixing Step (Rolow, 2002).

The production of masa flour is a controlled process, producing a more consistent pH. Since masa flour is a dry ingredient and commercially produced, it is readily available with preservatives and acidifiers. Fumaric or phosphoric acids are used as acidifiers with preservatives such as sodium propionate, potassium sorbate, or benzoic acid. Sometimes preserved masa flour will contain calcium or sodium propionate and an additional preservative in order to lengthen the shelf life of the tortilla. If the masa flour does not come with preservatives, they can be added in the dry form in the mixer or in liquid form when the water is added. If the liquid form is used, it will be very similar to what is used in nixtamalized corn tortillas. This blend is more acidic than may be necessary for a masa flour tortilla. Masa flour tortillas have a more consistent and lower pH than nixtamalized corn tortillas, so it could be possible to use a less acidic preservative. A blend of sodium propionate and methyl or propyl paraben or potassium sorbate would be less acidic and would likely be effective. Using this blend, it is possible that the preservative taste could be weaker, thus increasing the consumer acceptance of the product (Rolow, 2002).

CHAPTER 6 - Antistaling Agents

Hydrocolloids

Gums are commonly used in the commercial preparation of corn tortillas. They are used to bind water and to retain moisture in the tortilla. They can prevent the edges of the tortilla from cracking and increase the dough strength while improving flexibility of the tortilla. They can also help to prevent stickiness in the dough, making it easier to process (Stockwell, 2000). In addition to slowing staling, gums are thought to create a new, more flexible structure in the tortilla. Used by themselves, gums can create a rubbery texture in the tortilla that some consumers do not like (Bueso et al, 2004).

Carboxymethylcellulose (CMC) is a molecule that is a long linear chain. It is both water soluble and anionic. It has been found to work synergistically with other hydrocolloids and other tortilla additives. CMC competes with other ingredients in the tortilla for water helping it to keep moisture in baked goods (Glicksman, 1986). Since CMC is bound to the water, the starch granules are not able to gelatinize. Without gelatinization, the starch cannot go through retrogradation. CMC and other hydrocolloids have also been found to inhibit the recrystallization of starch (Yau et al, 1994).

When used alone at 0.25- 0.5%, CMC has been found to improve the freeze thaw stability (Bueso et al, 2006), puffing (Yau et al, 1994), rollability over 4 days (Yau et al, 1994), increase the machinability (ability to be processed smoothly) of masa (Bueso et al, 2004), and the extensibility of the tortilla (Bueso et al, 2004). The increase in the masa's machinability is very helpful because it increases the yield of the dough. By making the masa run through the system better, there are less tortillas that are thrown out due to production line complications. The

extensibility increase is demonstrated by an increase in the rupture distance as compared to a control tortilla (Bueso et al, 2004). CMC competes with other ingredients for water and the molecules form “entangled amorphous matrix around hydrated nixtamalized corn flour particles.” This matrix is able to increase the cohesiveness and the flexibility of the masa (Bueso et al, 2004).

When CMC was used in conjunction with gluten, Yau et al (1994) found that there was still an improvement in the puffing of the tortilla. This is due to the network that is created by either the gluten or the gum. The network retains the steam in the tortilla, causing it to puff. They also found that there was better moisture retention after 7 days in the tortillas prepared with gluten and/or CMC. When gluten was added at a 2% level and CMC at 0.5%, the tortillas maintained storage stability for an additional 1 to 2 days over just gluten or CMC alone. When sorbitol was added at 3% to the formula with CMC and gluten, the tortillas lasted for 12 days, more than any other combination (Yau et al, 1994).

The combination of CMC and amylase has proven to be effective as well. When 0.25% CMC was used in conjunction with 1650 activity units of maltogenic amylase, the pliability of a tortilla stored at either room or refrigerated temperatures was increased (Bueso et al, 2006). When CMC was used at 0.25% with 275-825 maltogenic amylase novo units, the melting enthalpy of amylopectin decreased significantly. After 14 days of storage, the tortillas were softer than the control. This combination was superior to any other combination tested by Bueso et al (2004). CMC and maltogenic amylase work together to delay starch recrystallization and to create a matrix around the starch granules that is more flexible (Bueso et al, 2006).

Xanthan, guar, and hydroxypropymethylcellulose (HPMC) have all been tested to see how they affect the shelf life of a corn tortilla. In a study done by Arambula et al (1999)

xanthan, guar, HPMC, and CMC were all added to corn masa either before or after extrusion. It was found that adding the gums before extrusion provided a better texture, even though they were effective when added at either time. The dehydration curve of the masa was then reviewed. When compared to control, the masa that had any of the gums had a slower dehydration rate (Figure 6.1). While all of the masas with gums dehydrated at a similar rate, the one that had xanthan gum lost the least amount of water (Arambula et al, 1999).

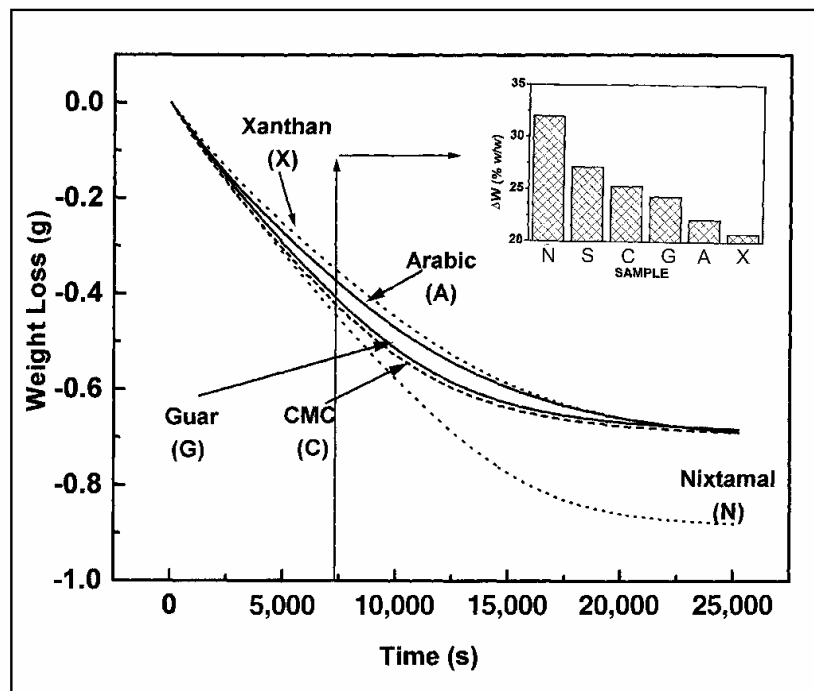


Figure 6.1 Masa Dehydration curve. When CMC, Xanthan, Arabic, or Guar Gums are Added to the Masa Before Processing it Dehydrates at a Slower Rate (Arambula et al, 1999).

The higher the gum concentration, the less water is lost during storage. Hydrocolloids form a network within themselves that does not allow water to migrate as easily, which delays

the retrogradation of starch (Roman-Brito et al, 2007). With an increase in gum concentration, the distance that the tortilla can extend without rupturing is increased, meaning that the extensibility of the tortilla is increased compared to the control. In a tortilla without gums, the longer it is stored, the greater the modulus of deformation. With gums added to the tortilla, the work that is needed to maintain the modulus of deformation decreases. Addition of gums also lessens the force that is necessary to roll the tortilla, showing that the pliability of the tortilla has increased (Roman-Brito et al, 2007).

Enzymes

Since the staling of a corn tortilla has a linear relationship with time, it is important to find ways to retard the starch retrogradation process. Research (Campas-Baypoli, 2002; Bueso et al, 2006, Limanond et al, 2002) has shown that the only way to inhibit retrogradation is to freeze tortillas, it is necessary to find other ingredients that can inhibit retrogradation at room or refrigerated temperature. In order to prolong the freshness of the tortilla, it is also important to find ways to retain moisture in the tortilla.

Enzymes are commonly used in baking applications in order to reduce staling and delay starch retrogradation. Various enzymes such as cereal α -amylases, fungal α -amylases, bacterial α -amylases, and intermediate stability enzymes have been tested for extension of shelf life in tortillas. Alpha-amylases are a family of enzymes that break down starch polymers by cleaving the internal α -D-1,4 bonds of starch, and oligosaccharides. Maltogenic amylases have three characteristics that separate them from α -amylases. First, maltogenic amylases cleave both α -D-1, 4 and α -D-1, 6 leaving maltose. They also hydrolyze the starch leaving oligosaccharides that have DP 3-6. And finally, they are able to cleave acarbose, which is a competitive inhibitor of α -amylases (Ucles, 2003). Two theories have been proposed as to how amylase works to inhibit

the staling process. It is suggested the enzymes attach to the amylopectin chain, reducing the tendency of the amylopectin to go through starch retrogradation. The other idea is that the oligosaccharides that are present after the amylases break down the starch are the compounds that are doing the inhibition of retrogradation (Ucles, 2003).

Alpha-amylases are used to inhibit the retrogradation of amylopectin. When used in bread, α -amylases do not reduce the firmness immediately after baking. It is not until 7 days after baking the bread that a significant reduction in the firmness is seen. When the control bread is fresh, it does not exhibit any birefringence, but over time, the birefringence increases. The bread that was made with α -amylase had birefringence when it was fresh, but the amount of birefringence did not increase with time (Hug-Iten et al, 2001).

According to Hebeda et al (1990), cereal α -amylases, such as barley malt will not extend the shelf life of a tortilla. Fungal α -amylases have an optimal operating temperature of 50-55 °C, so they are inactivated in the oven before the starch even gelatinizes. This does not allow them to have any effect on the amylopectin during starch retrogradation. They can be protected somewhat by mixing them with a sugar solution, which will protect the amylase during baking (Hebeda et al, 1990).

Bacterial α -amylases are not inactivated in the oven, so they remain active in the tortilla during storage. The bacterial α -amylase will continue to break down the starch which will eventually result in a product that is sticky and gummy. The stickiness of the product can be reduced by using a combination of enzymes (Hebeda et al, 1990). Bacterial α -amylases work by attacking the links in the amorphous regions of the starch and breaking them down. This allows the crystals to move around more easily, providing a structure that is not as rigid. (Dragsdorf and Varriano-Marsto, 1980).

Intermediate stability enzymes have an optimal operating temperature of 65-75°C, which allows them to remain active in the oven after the starch has gelatinized, but they are inactivated at the higher temperatures of baking (Hebeda et al, 1990). Bueso et al (2005) suggested that a maltogenic amylase tested by Boyle and Hebeda (1990) would be ideal for use in corn tortillas. It shortens the amylopectin chains which reduces the starch retrogradation. This maltogenic amylase is found to work at 60% of its maximum activity at room temperature, but still works well for corn tortillas due to the short rest and baking time for corn tortillas. It would have time to act during the rest period and would then be inactivated during baking so the product would not be gummy (Bueso et al, 2005).

Glycerol

Glycerol is a clear, colorless, syrupy liquid that can be used in bakery products in order to extend their shelf life. It has been used in both bread products and tortillas, increasing the pliability. Glycerol behaves as a plasticizer in bakery products, increasing the pliability during storage. (Pouplin et al, 1999) In wheat tortillas, glycerol has had a significant effect on the rollability over time. When glycerol was added to wheat tortillas at 4%, the tortillas were able to be rolled without breaking for an additional two to four days (Clubbs et al, 2005). When adding glycerol and salt to the tortilla, the breaking strain is reduced in the gels. The glycerol binds with the water around the chains of starch which stabilizes the gel, possibly slowing the retrogradation and creating a more homogenous structure and pliable product (Clubbs et al, 2005). Since glycerol is binding to the water, it is also keeping the water in the tortilla, maintaining moisture over time (Clubbs et al, 2008).

In another study (Clubbs et al, 2008), glycerol was used at 4% and salt was added at 1% in order to mask the flavor. This study compared the effectiveness of glycerol and salt to that of

carboxymethylcellulose. It found that a combination of glycerol and salt retained elasticity in the tortilla during storage better than carboxymethylcellulose. It is hypothesized that this is due to glycerol helping to create a more homogenous product and its water binding capabilities (Clubbs et al, 2008).

Emulsifiers and Lipids

Emulsifiers are “substances which reduce the surface tension between two immiscible phases at their interface, allowing them to become miscible” (Igoe and Hui, 2001). In a bakery application, the emulsifier will react with the starch in the product. This will soften the crumb of the product by delaying the crystallization of the starch. This will also result in the product holding moisture for a longer period of time, delaying staling of the product. Mono- and di-glycerides are common emulsifiers that are used to complex with the dough (Hebeda et al, 1990, Stockwell, 2000). The retention of moisture will also help with extending the pliability of the tortillas for a longer shelf life (Stockwell, 2000).

Solorio et al (1994) found that when emulsifiers were used, the corn tortillas were softer. In another study (Twillmann and White 1988) the addition of monoglycerides produced a softer tortilla than the control. This study also found that different monoglycerides had different effects throughout storage. One of the monoglycerides used, monostearin, had a stronger effect at the beginning of storage while monomyristin tended to have a stronger effect in a longer storage time (Twillman and White, 1988). This study suggests that using multiply monoglycerides could aid in maintaining a consistent shelf life. According to Twillman and White (1988), this could be due to the abilities of different monoglycerides to complex with amylose or amylopectin. Amylose recrystallizes at the beginning of storage, so monoglycerides that complex with amylose would be more effective early on. Monoglycerides that complex with amylopectin

would be more helpful later in storage when amylopectin is recrystallizing (Twillman and White, 1988).

Emulsifiers work with shortening to prevent the swelling of the starch granules during the baking process. With a reduction in the amount of starch granules that have swollen, not as many of the starch molecules will be solubilized. This means that there will be less surface area available to link to the gluten. With the water, emulsifiers and gluten are able to plasticize the gluten which decreases the firmness of bread (Martin et al, 1991).

In wheat tortillas, fat shortens the flour texture and helps to lubricate the dough during processing. In the final product, fat increases the flexibility, taste, and texture. Most manufacturers used solid shortening, but liquid can be used as well. Solid shortening is more efficient when it is used with mono- and di-glycerides. Solid shortening seems to provide a better eating quality of a tortilla. It is thought that it is due to the higher melting point and that more oil is suspended in the shortening solids (Stockwell, 2000).

Gluten

In wheat flour tortillas gluten is necessary to build up the structure of the dough. This structure is what provides both the viscosity and elasticity in the dough. The gluten also helps in gas retention in the dough (Stockwell, 2000). According to Boyle and Hebeda (1990), gluten plays a role in the staling of bread. The rate that bread stales is correlated with the quality of the protein in the flour. After the bread is baked, there is an interaction between starch and gluten that takes place in the bread. Due to gelatinization of the starch, the elastic modulus of the gluten-starch matrix increases. During baking, there are links formed between the starch and the gluten. More of these links will make the bread more firm. These linkages are formed through hydrogen bonding and can be reversed upon heating the bread (Boyle and Hebeda, 1990).

While gluten can play a negative role in staling it is also helps to make a tortilla with a longer shelf life. Tortillas that have a higher protein content will have a longer shelf life. Vital wheat gluten, gliaden, and glutenin can all play a role in extending the flexibility of a tortilla throughout storage (Boyle and Hebeda, 1990). In corn flour tortillas, the gluten has multiple functions. It contributes to the softening of the tortilla to extend the shelf life and it aids in the brown spots that are formed during baking, making the tortilla visually more appealing to the consumer (Boyle and Hebeda, 1990). When used at 2% or more gluten imparts a “wheaty” flavor to the tortilla. Used at this level, it is still not enough to inhibit the retrogradation of starch as effectively as CMC does at 0.5%. (Bueso et al, 2005)

Alone, gluten has the ability to have a small effect on the shelf life of a corn tortilla. When used with other additives, the quality of the tortillas will increase and the shelf life will be longer. For example, Yau et al (1994) studied a variety of ingredients, including gums, proteins, and starch both individually and in combination. Table 6.1 shows that after seven days most of the tortillas prepared with only one additive were stiff and unrollable, even if they had been more pliable than control on day one. After seven days, tortillas prepared with a combination of 0.5% carboxymethylcellulose, 2% gluten, and 3% sorbitol were still pliable (Yau et al, 1994).

Table 6.1 Effect of Additives on the Pliability of Tortillas After Storage of 1 and 7 days.

(Yau et al, 1994)

Treatment	Tortilla Pliability*	
	1 Day	7 Day
Control	1	1
Protein		
Gluten (0.5, 1, 3%)	2	1
Gluten (1.5, 2%)	2	2
Whey (0.5, 1, 1.5, 2%)	1	1
Whey, denatured (0.5, 1, 1.5, 2%)	1	1
Casein (0.5,1,1.5,2,3%)	1	1
Starch		
Crosslinked-stabilized	1	1
Native Corn (3, 10%)	1	1
Hydrocolloids		
CMC** (0.3%)	2	1
CMC (0.5,1%)	2	2
HPMC *** (0.3, 0.5, 1%)	2	1
Xanthan gum (0,3%)	2	1
Xanthan gum (0.5, 1%)	2	2
Guar gum (0.3, 0.5, 1%)	2	1
Carrageenan (0.3, 0.5, 1%)	2	1
Konjac flour (0.3, 0.5, 1%)	2	1
Polyol		
Sorbitol (3, 7%)	3	3
Combinations		
0.5% CMC + 2% gluten	2	2
0.5% HPMC + 2% gluten	2	2
0.5% CMC + 2% gluten + 3% sorbitol	3	3
2 % gluten + 3% sorbitol	3	3
2% gluten + 0.5% SSL	3	3
2% gluten + 1% SSL	3	3

* Based on a scale of 1-3 where 1= stiff, 2= moderately pliable, 3= pliable

**Sodium carboxymethylcellulose

***Hydroxypropyl methylcellulose

Transglutamase has been unsuccessfully tested in efforts to lengthen the shelf life of the tortilla. It forms covalent bonds between lysine and glutamine, hydrolyzes glutamine to glutamate, and incorporates amine groups. The cross links that are formed make the dough tougher and so it is not as extensible. Tortillas made with transglutamase had similar properties to control such as the diameter, pH, moisture, specific volume, and opacity. Tortillas made with protease instead of transglutamase had a larger diameter, lower moisture content, and they were more opaque. Protease actually decreases the shelf life of a tortilla. This gluten network that is formed is a key factor in how flexible the tortilla is (Alviola et al 2008).

CHAPTER 7 - Summary

As consumer interest in Mexican food grows, so does the interest in consuming corn tortillas. In order to meet the consumer need of a convenient product that has an acceptable taste and texture, it is necessary to use preservatives and various additives to extend the shelf life of corn tortillas. Preservatives are necessary to prevent the growth of mold while other additives are used to maintain texture by holding moisture in the tortilla and delaying or preventing starch retrogradation. The frequency of using hydrocolloids, gluten, and enzymes either alone or in concert is increasing significantly. Based on current research, the shelf life of a corn tortilla can be extended through a combination of CMC (0.5%), maltogenic amylase (1650 Activity Units), sorbitol (3%), and glycerol (4%).

Recently, the development of a shelf stable corn tortilla has become more interesting to research groups. More work has been done studying the effects of various hydrocolloids and the role of enzymes in delaying the retrogradation of starch. It has also been well established that retrogradation of starch will happen more quickly at refrigerated temperatures. The role of gluten in the staling of flour tortillas and its influence on texture in a corn tortilla is not currently well understood. There is also limited research on gluten used in conjunction with amylase. Further study on this could provide some valuable information on how to extend the shelf life of a corn tortilla.

References

AIB International. (2008) Tortilla Statistics and Trends.

<https://www.aibonline.org/resources/statistics/tortillas.html> (Accessed November, 2008).

Alviola, J.N.; Waniska, R.D.; and Rooney, L.W. (2008) Role of Gluten in Flour Tortilla Staling.

Cereal Chem. 85(3):295-300.

Arambula, V.G.; Mauricio, S.R.A; Figueroa, C.J.D; Gonzalez-Hernandez ,J.; and Ordorica,

F.C.A. (1999) Corn Masa and Tortillas from Extruded Instant Corn Flour Containing

Hydrocolloids and Lime. J. Food Sci. 64(1):120-124.

Boyle, P.J. and Hebeda, R.E. (1990) Antistaling Enzyme for Baked Goods. Food Technol.

44(6):129.

Buelon, A.; Colonna, P.; Planchot, V.; and Ball, S. (1998) Starch Granules: Structure and

Biosynthesis. Int. J. Biol Macromol. 23(2):85-112.

Bueso, F.J.; Rooney, L.W.; Waniska, R.D.; and Silva, L. (2004) Combining Maltogenic

Amylase with CMC or Wheat Gluten to Prevent Amylopectin Recrystallization and

Delay Corn Tortilla Staling. Cereal Chem. 81(5):654-659.

Bueso, Francisco J.; Waniska, Ralph D.; Moreira, Rosana; Seetharaman, Koushik; and Rooney,

Lloyd W. (2006) Effect of Temperature on Texture of Corn Tortilla With and Without

Antistaling Agents. Cereal Chem. 83(4): 348-353.

Campas-Baypoli, O. N.; Rosas-Burgos, E. C.;Torres -Chavez, P.I.; Ramirez-Wong, B.; and

Serna-Saldivar, S. O. (2002) Physicochemical Changes of Starch in Maize Tortillas

During Storage at Room and Refrigeration Temperatures. Starch/Starke. 54(8): 358-363.

- Clubbs, E.A.; Vittadini, E.; Shelhammer, T.H., and Vodovotz, Y. (2004) Effect of High Pressure Processing and Addition of Glycerol and Salt on the Properties of Water in Corn Tortillas. *J. Cereal Sci.* 39(1):109-117.
- Clubbs, E.A.; Vittadini, E.; Shelhammer, T.H.; and Vodovotz, Y. (2005) Changes in the Mechanical Properties of Corn Tortillas Due to the Addition of Glycerol and Salt and Selective High Pressure Treatments. *Inn. Food Sci. and Emer. Tech.* 6(3): 304-309.
- Clubbs, E.A., Vittadini, E., Shelhammer, T.H., and Vodovotz, Y. (2008) Effects of Storage on the Physio-Chemical Properties of Corn Tortillas Prepared with Glycerol and Salt. *J. Cereal Sci.* 47(2):162-171.
- Cornell, Mari. Simple Sophistication. (1999) *Baking and Snack.* 18(8): 63-68
- Dragsdorf, R.D. and Varriano-Marston, E. (1980) Bread Staling: X-ray Diffraction Studies on Bread Supplemented with Alpha-Amylases from Different Sources. *Cereal Chem.* 57(5):310-314.
- Ehli, Barbara. (1986) Appeal of Tortillas Taps Ethnic Heritage, While Equipment has Roots in Baking. *Milling and Baking News.* 65(25):BE52-BE57.
- Fernandez, Deborah A.; Waniska, Ralph D.; and Rooney, Lloyd W. (1999) Changes in Starch Properties of Corn Tortillas During Storage. *Starch/Starke.* 51(4): 136-140.
- Glicksman, M. (1986) *Food Hydrocolloids.* CRC Press, Inc. Boca Raton, Florida.
- Gómez, M. H.; Lee J. K.; McDonough, C. M.; Waniska R.D., and Rooney, L.W. (1992) Corn Starch Changes During Tortilla and Tortilla Chip Processing. *Cereal Chem.* 69(3): 275-279.
- Gómez, M.H.; McDonough, C.M.; Waniska, R.D.; and Rooney, L.W. (1989) Changes in Corn and Sorghum During Nixtamalization and Tortilla Baking. *J. Food Sci.* 54(2): 330-336.

- Gómez, M. H.; Waniska R.D.; and Rooney, L.W. (1990) Effects of Nixtamalization and Grinding Conditions on Starch in Masa. *Starch/Starke*. 42(12): 475-482.
- Gómez, M. H.; Waniska R.D.; and Rooney, L.W. (1991) Starch Characterization of Nixtamalized Corn Flour. *Cereal Chem*. 68(6):578-582.
- Gudmundsson, M. (1994) Retrogradation of Starch and the Role of its Components. *Termochimica Acta*. 246(2):329-341.
- Hebeda, R.E.; Bowles, L.K.; and Teague, M. (1990) Developments in Enzymes for Retarding Staling of Baked Goods. *Cereal Foods World*. 35(5):453-457.
- Hug-Iten, S.; Escher, F.; and Conde-Petit, B. (2001) Structural Properties of Starch in Bread and Bread Model Systems: Influence of Anti-Staling Alpha-Amylase. *Cereal Chem*. 78(4): 421-428
- Igoe, R.S. and Hui, Y.H. (2001) *Dictionary of Food Ingredients: Fourth Edition*. Aspen Publishers. Gathersburg, MD.
- Imberty, A.; Chanzy, H.; Perez, S. and Buelon, A.; and Tran, V. (1988) The Double-helical Nature of the Crystalline Part of A-Starch. *J. Mol. Biol*. 201(2):365-378.
- Karim, A. A., Norziah, M. H., and Seow, C. C. (2000) Methods for the Study of Starch Retrogradation. *Food Chem*. 71(1): 9-36.
- Leistner, L. (1994) Further Developments in the Utilization of Hurdle Technology for Food Preservation. *J. Food Eng*. 22(1-4): 421-432.
- Leistner, L. and Gould, G.W. (2002) *Hurdle Technologies: Combination Treatments for Food Stability, Safety and Quality*. Kluwer Academic/Plenum Publishers. New York.

- Limanond, B.; Castell-Perez, M.E.; and Moreira, R.G. (1999) Effect of Time and Storage Conditions on the Rheological Properties of Masa for Corn Tortillas. *Lebensmittel-Wissenschaft und-Technologie*. 32(6): 344-348.
- Limanond, B., Castell-Perez, M.E.; and Moreira, R.G. (2002) Modeling Kinetics of Corn Tortilla Staling Using Stress Relaxation Data. *J. Food Eng.* 53(3): 237-247.
- Martin, M.L., Zeleznak, K.J., and Hosney, R.C. (1991) A Mechanism of Bread Firming. I. Role of Starch Swelling. *Cereal Chem.* 68(5):498-503
- Miles, M.J.; Morris, V.J.; Orford, P.D.; and Ring, S.G. (1985) The Roles of Amylose and Amylopectin in the Gelation and Retrogradation of Starch. *Carb. Res.* 135(2):271-281.
- Pacyniak, Bernard. (1996) Advancing the State of the Art. *Snack Food.* 85(1):31-35.
- Pouplin, M.; Redl, A.; and Gontard, N. (1999) Glass Transition of Wheat Gluten Plasticized with Water, Glycerol, or Sorbitol. *J. Agric. Food Chem.* 47(2):539-543.
- Ring, S.G.; Colonna, P.; F'Anson, K.J.; Kalichevsky, M.T.; Miles, M.J.; Morris, V.J.; and Orford, P.D. (1987) The Gelation and Crystallization of Amylopectin. *Carb. Res.* 162(2):277-293.
- Rolow, A. M. (2002) Preservatives and Their Applications in Flour and Corn Tortillas. Technical Bulletin No. 24. AIB International, Manhattan, KS.
- Roman-Brito, J.A.; Agama-Acevedo, E.; Mendez-Montealvo, G.; and Bello-Perez, L. (2007) Textural Studies of Stored Corn Tortillas with Added Xanthan Gum. *Cereal Chem.* 84(5):502-505.

- Solorio, H.A.; Mallory, S.L.; and Sigel, J.D. (1994) Effects of Emulsifiers, Alpha Amylase, and Hydrocolloids on Textural Properties and Shelf Life Stability of Corn Tortillas. *Cereal Foods World*. 39(8): 627.
- Stauffer, J.E. (2002) Hurdle Technology. *Cereal Foods World*. 47 (4):154-155.
- Stockwell, Amy C. (2000) Wrap it up- Tortilla Style. *Baking and Snack*. 22(7): 47-48, 50-53.
- Suhendro, E.L.; Almeida-Dominguez, H.D.; Rooney, L.W.; and Waniska, R.D. (1998a) Objective Rollability Method for Corn Tortilla Texture Measurement. *Cereal Chem*. 75(3):320-324.
- Suhendro, E.L.; Almeida-Dominguez, H.D.; Rooney, L.W.; and Waniska, R.D. (1998b) Tortilla Bending Technique: An Objective Method for Corn Tortilla Texture Measurement. *Cereal Chem*. 75(6):854-858.
- Toro-Vasquez, J.F. and Gómez,-Aldapa, C.A. (2001) Chemical and Physiochemical Properties of Maize Starch After Industrial Nixtamalization. *Cereal Chem*. 78(5): 543-550.
- Tortilla Industry Association (TIA). 2008. Tortilla Talk. http://www.tortilla-info.com/consumers/tortilla_talk/tortilla_talk.htm#history (Accessed November 2008).
- Trevino, E and Norton, R.C. (2006) Nixtamalized Corn Tortillas. Technical Bulletin No. 28. AIB International. Manhattan, KS.
- Twillman, T.J. and White, P.J. (1988) Influence of Monoglycerides on the Textural Shelf Life and Dough Rheology of Corn Tortillas. *Cereal Chem*. 65(3):253-257.
- Ucles, F.J.B. (2003) Antistaling Properties of Amyloses, Wheat Gluten, and CMC on Corn Tortilla. PhD Dissertation. Texas A&M University. College Station, TX.

- Vittadini, E.; Clubbs, E.; Shellhammer, T.H.; and Vodovotz, Y. (2004) Effects of High Pressure Processing and Addition of Glycerol and Salt on the Properties of Water in Corn Tortillas. *J. Cereal Sci.* 39(1): 109-117.
- Wu, H.; Sarko, A. (1978) The Double Helical Molecular Structure of Crystalline B-Amylose. *Carb. Res.* 256(1):129-137.
- Yau, J.C.; Waniska, R.D. and Rooney, L.W. (1994) Effects of Food Additives on Storage Stability of Corn Tortillas. *Cereal Foods World.* 39(5): 396-402.