ASSESSING CORN QUALITY AND TRANSFORMATION DURING NIXTAMALIZATION: A PHYSICO-CHEMICAL APPROACH

THESIS

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ABSTRACT

Corn is the main component of tortilla chips and must undergo several steps of modification to produce masa dough. Different varieties of corn and the interaction of environmental conditions with post-harvest handling can produce variability in the kernel structure and composition, affecting its functionality in the masa-making process. This study sought to understand how corn composition with processing contributed to the texture of acceptable masa using various rheological and thermal analysis techniques.

TGA analysis illustrated the unique water populations in pericarp, soft endosperm, hard endosperm, and germ during the processing of nixtamalization. Alkaline cooking was the most critical step for increasing the water uptake in all fractions and water became more associated in the pericarp and endosperms. Although germ imbibed water, no increase in water entrapment was displayed following cook. Increase in "freezable water" as observed with DSC also confirmed an increase in bulk water for the four fractions. Soaking only modified the soft endosperm water component to an appreciable degree. Possible thermal shifts between unacceptable and acceptable pericarp and hard endosperm fractions were observed and warranted additional analysis to verify these differences.

Findings from this study narrowed the research focus to the hard endosperm and pericarp fractions in the raw and cook state. Further thermal and rheological analysis was combined with additional testing to characterize the basic composition and material behavior of unacceptable and acceptable corn. Masa adhesion as measured by the TAX.T2 Texture Analyzer, masa moisture quantitated with the Moisture Analyzer, macromolecular composition using near-infrared spectroscopy (NIR), and chemical quantitation of unextractable extensin protein were evaluated. One-way ANOVA ($p < 0.05$) established statistical differences between unacceptable and acceptable corn categories while Soft Independent Modeling of Class Analogy (SIMCA) software using Principle Component Analysis (PCA) determined which factors most contributed to the separation of unacceptable and acceptable corn classes.

Results from additional thermal, rheological, and compositional analysis on acceptable and unacceptable corn displayed statistical differences between the two classes. TGA analysis showed a difference in the water populations of raw hard endosperm and pericarp, which may indicate structural variation. Masa adhesion, % protein, and a shift in the onset temperature of a thermal transition in hard endosperm were other important factors distinguishing classes of different corn quality.

Overall, hard endosperm TGA peak temperature and masa adhesion were the greatest distinguishing factors with % protein playing a minor role in classifying corn functionality for nixtamalization. This investigation suggests that a physico-chemical

approach may further add knowledge to the regarding the basis of corn functionality for nixtamalization.

Dedicated to my parents

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LIST OF ABBREVIATIONS

TGA	Thermogravimetric Analysis
DSC	Differential Scanning Calorimetry
FW	“Freezable Water”
TADD	Tangential Abrasive Dehulling Device
HE	Hard Endosperm
Hyd-Pro	Hydroxyproline
UV-Vis	Ultraviolet-Visible Light
Min	Minute
G	Gram
Sec	Second
ml	Milliliter
F	Force
LVR	Linear Viscoelastic Region
WB	Wet Basis
DWB	Dry Weight Basis
MC	Moisture Content
Inc	Incorporated
HRGP	Hydroxyproline-rich Glycoprotein

DMA	Dynamic Mechanical Analyzer
SIMCA	Soft Independent Modeling of Class Analogies
g-F	Gram-Force
N	Newton

CHAPTER 1

INTRODUCTION

Corn is the main component of tortilla chips and must undergo several complex steps of modification to produce masa dough. Traditional processing involves the heating of corn kernels with lime and water to 185-195°F to loosen the pericarp, quenching, and steeping for 10-12 hours at 40°C prior to washing and grinding between two stone plates to form the dough (Bryant and Hamaker 1997). Masa is defined as a glue-like mixture of dispersed material consisting of partially gelatinized starch, hydrated and hydrolyzed protein, lipids and a network formed due to the interaction of amylose with calcium ions (Sahai and Jackson 2001). Different varieties of corn and the interaction of environmental conditions with post-harvest handling can produce variability in the kernel structure and composition, affecting its functionality in the masa-making process (Jackson and others 1991; Sahai and others 2001).

In food manufacturing, a major concern is the ease of pericarp removal which requires lime and heat in a process called nixtamalization (Flores-Farias and others 2001). Overcooking to achieve this separation can cause the masa to lose its structural integrity and become difficult to sheet. Under-processed masa does not have enough adhesion to form a cohesive dough (Ramirez-Wong and others 1996; Ramirez-Wong and

others 1993). The proportion of hard-to-soft endosperm influences the heat-stability of the kernel in addition to the prevalence of stress-cracks and internal fissures (Jackson and others 1991). Food processors have encountered difficulty with some Ohio-grown varieties of corn and are looking to neighboring states such as Indiana as alternative suppliers of grain leading to significant economic losses to the farmers. Identifying and understanding the factors attributing to quality of various corn hybrids will allow Ohio farmers to change either variety or cultural practices in order to produce a grain that is acceptable for snack food manufacturing.

This research seeks to identify the underlying factors attributing to corn quality, particularly hardness, which is associated with grain quality in industry. Current methods for evaluating this parameter are numerous and show variation between methods. Tests include but are not limited to density measurements, test weight, and extent of kernel breakage (US Grains Council 1999).

Once the scientific basis of hardness is better understood, a more accurate and efficient technique can be developed. With the interaction of environment and plant hybrid playing an important role in kernel composition, corn quality can vary from season to season (Sahai and others 2001). Thus, optimizing processing conditions for nixtamalization, particularly during an off-season, and initially selecting corn exhibiting certain quality-determining characteristics within an acceptable range is key to maintaining a quality product (Ji and others 2004).

Thermal analytical and rheological techniques are particularly suited to investigating the structural composition of corn as it relates to functionality in masa production. Previous work by Neher and others (1973) used TGA to evaluate the state of water in grain as affected by drying and storage conditions. Meanwhile, Gonzales and others (2004) used TGA and DSC to study the structural changes that occur in the pericarp during the nixtamalization process as a function of water evaporation and decomposition of hemicellulose and cellulose. The amount and entrapment of water in tortillas using DSC, TGA and DMA characterized the effect of additives and high-pressure processing on “freezable water” and TGA peak moisture loss (Vittadini and others 2003; Clubbs and others 2007). This study seeks to apply similar principles to monitor the migration and state of water in the kernel during processing, to understand the effect of nixtamalization on the thermal properties of kernel tissue, and to characterize the material behavior of acceptable and unacceptable masa.

CHAPTER 2

STATEMENT OF PROBLEM

Specifications are important for ensuring that incoming ingredients do not cause variability in processing and final product quality. With corn, a variety of factors including environment, cultivar, cultural practices, and seasonal changes can affect kernel characteristics and suitability for masa manufacturing. Difficulty of pericarp removal during cooking and kernels being too “soft,” causing the masa dough to lose structural integrity when machined, are common problems. Current methods for assessing corn quality are not sufficient in assisting to predict these issues so they can be avoided, or in guiding the selection of ideal corn varieties for food use.

Masa is a complex dispersion of starch, protein, and lipid. Understanding the structural and physico-chemical changes that corn undergoes during nixtamalization is fundamental for identifying both the corn composition required for an acceptable product texture and also possible alterations in processing to maximize results. Basic research in this area has not fully elucidated the structure-function relationship of corn and thus warrants further investigation. Such learning could be translated into additional criteria for plant breeders and aid in the development of a rapid method for determining grain quality for masa.

Therefore, the overall objective of this study was to characterize acceptable and unacceptable masa at each major step during nixtamalization using macroscopic and microscopic techniques. It was hypothesized that alterations of specific corn fractions during nixtamalization are predictive of the final masa texture. To investigate this hypothesis, the following aims were identified.

Aim 1. Understanding physico-chemical changes occurring during critical steps in nixtamalization and differentiate acceptable and unacceptable corn using thermal and rheological analysis.

1. Monitor the uptake and distribution of water throughout corn kernel fractions with thermogravimetric analysis.
2. Characterize the physical state of pericarp, hard endosperm, soft endosperm, and germ fractions with differential scanning calorimetry.
3. Describe rheological properties of acceptable and unacceptable masa.

Aim 2. Develop criteria for measuring corn quality during processing by comparing results from above aims to determine which information is most relevant in distinguishing acceptable and unacceptable corn for quality purposes.

CHAPTER 3

LITERATURE REVIEW

3.1. Corn Composition

Corn, a composite of protein, starch and lipid, provides the major ingredient for tortilla chip production. Figure 3.1 displays a cross-section of the pericarp, hard endosperm, soft endosperm, and germ sections in a corn kernel. An understanding of kernel composition is necessary for comprehending the functionality of each component in nixtamalization. The four fractions are discussed in-depth below.

3.1.1 *Pericarp Structure and Processing*

Pericarp removal is one of the primary objectives of nixtamalization. Further understanding of the structure and degradation of the outer coat is important when comparing the ease of pericarp removal with different processing treatments and corn varieties (Flores-Farias and others 2000). The structure of pericarp (Fig 3.2) is theorized to contain a web of long cellulose chains (22%) and heteroxylans (50%) substituted with 60 ferulic acids per polysaccharide which associate to provide secondary structure; protein (4-6%) with dityrosine cross bridges knit the entire network together (Saulnier and others 1995, Saulnier and others 1999, Saulnier and Thibault 2001; Kieliszewski and Lamport 1987; Lasztity 1996).

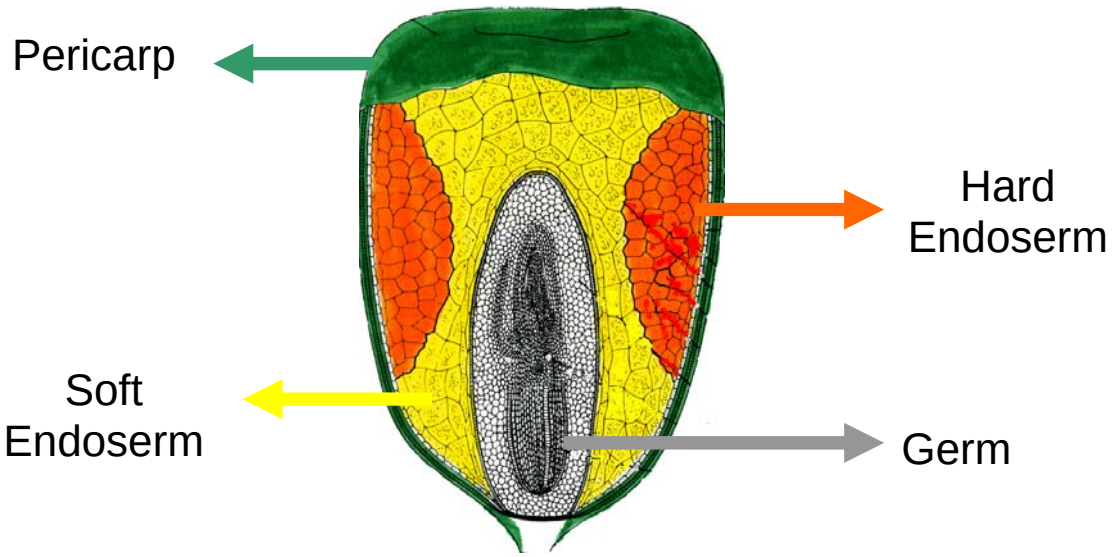


Figure 3.1. Cross-section of Corn Kernel (Hoseney 1998)

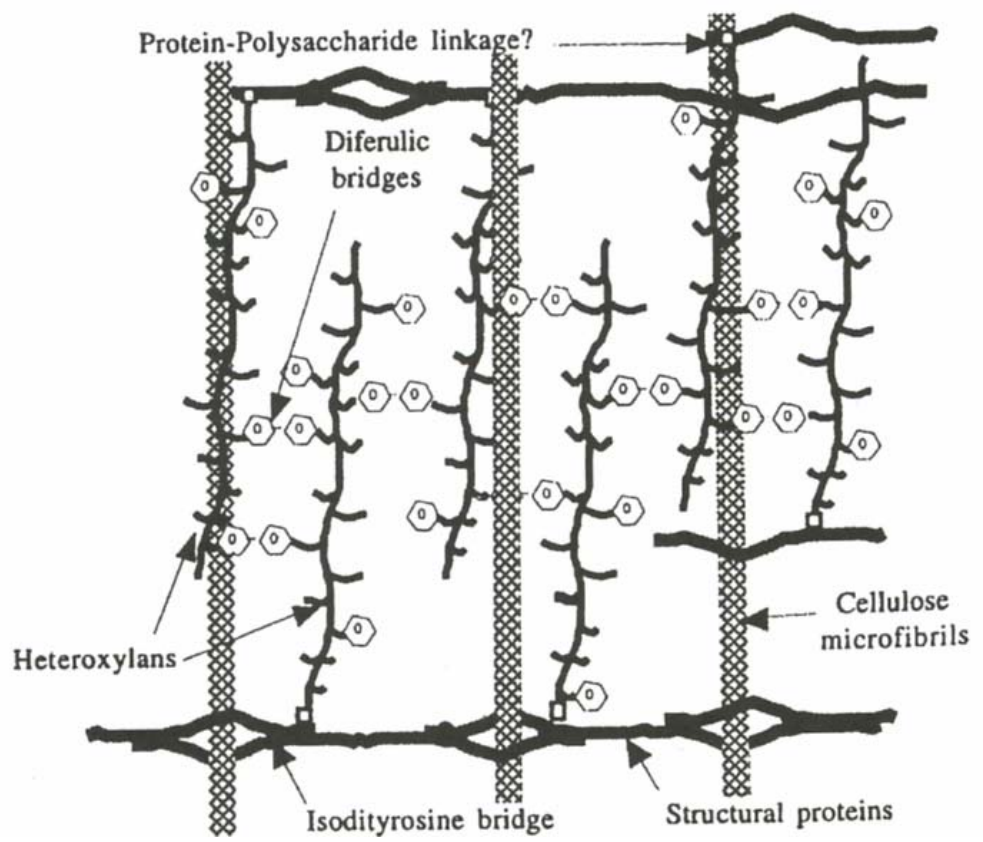


Figure 3.2. Proposed Structure of Pericarp (Saulnier and Thibault 1999)

Structural collapse can be facilitated through various means. Alkaline conditions neutralize acidic groups such as ferulics and partially release heteroxylan sugars and lignan gums into water (Sahai and Jackson 2001; Gonzalez and others 2004; Martinez and others 2001). However, the greatest degree of polysaccharide solubilization occurs when high pH and heat are coupled together, as during traditional nixtamalization (Saulnier and others 1995). The increase in cell wall degradation is attributed to the modification of hydroxyproline-rich glycoproteins (HPRG) which belong to the extensin family. Two of 4-5 pericarp proteins have been categorized as extensins; one of these is noted to have an exceptionally high amount of threonine (25%) as well as hydroxyproline (25%), along with a lesser content of proline, lysine, and serine (Kieliszewski and Lamport 1987; Fritz and others 1991). These highly basic proteins appear as rods and are glycosylated with 35% arabinose by weight (Fritz and others 1991; Kieliszewski and Lamport 1987). The glycosidic-protein bond is alkali-stable and is only disrupted to an appreciable amount by addition of hydrogen fluoride (HF) (Saulnier and others 1995). Instead, the function of alkali and heat during nixtamalization is to sever isodityrosine bonds between proteins (Saulnier and others 1995). An increase in isodityrosine linkages has been associated with greater protein insolublization and hull toughness (Fritz and others 1991). The tyrosine residues are located in the hydrophobic regions of the protein (Stiefel and others 1990) which may better protect them from water.

The HRGP's in cell walls have been well-associated with stress response, physiology, and growth regulation in dicots and algae; developing research on graminaceous monocots such as *Zea mays* has shown similar functionality and biological response (Kieliszewski and Lamport 1987). Extensin appears in pericarp tissue early on

during plant development and increases until cessation of growth (Hood and others 1991b; Stiefel and others 1990). During maturity, the total amount of hydroxyproline detected may decrease or modify itself in the pericarp, but the proportion of insoluble to soluble HRGP increases (Hood and others 1988; Hood and others 1991a; Stiefel and others 1990; Hood and others 1991b). Biochemical and physical differences in pericarp including thickness, bran-to-total kernel weight, and molecular weight heterogeneity of extensin protein have been observed between sweet corn and popcorn (Hood and others 1991b). Extensin is thought to provide resistance to bran degradation from enzymatic attack by pests, microorganisms, and physical wounding (Saulnier and others 1995; Fritz and others 1991). Garcia-Lara and others (2004) negatively associated resistance to maize weevil with quantity of pericarp diferulic acids and HRGPs, pericarp weight to kernel weight ratio, and overall hardness, thus elucidating the link of ferulics and HRGPs to structural integrity and seed preservation.

3.1.2 *Endosperm Structure and Processing*

The endosperm is also the site of several physical and chemical transformations during masa processing. Key changes include denaturation and solubilization of proteins and partial gelatinization of starch (Sahai and Jackson 2001). In this tissue, starch granules are encased in a protein matrix with deposits of protein bodies throughout. Although 10% of endosperm is protein, this portion represents 50-75% of total seed protein (Hamaker and others 1995; Lasztity 1996). Small concentrations of albumins and globulins exist within the cell structure and can be separated from the more prevalent zeins and glutelins based on properties such as solubility and hydrophobicity (Lasztity 1996). Several nomenclature schemes have been proposed (Osborne, Wilson, Laundry-

Moureaux, Esen), but recent classification has grouped proteins based on physiological function and similar amino acid profile (Lawton and Wilson 2003, Lasztity 1996).

Prolamin, the protein storage unit (62-74% in endosperm, 50-60% total), comprises four subsets. Native α-zein is soluble in alcohol while β-zein, γ-zein, and δ-zein require prior reduction of sulfhydryl bonds (Hamaker and others 1995; Dombrink-Kurtzman and Bietz 1993; Coleman and others 1997). During development, β-zein and γ-zein arise first. α-zein is predominant in the later phase, appearing mostly in the interior of the storage body (Dombrink-Kurtzman and Bietz 1993). α-zein is also the most abundant zein at 75-85% and blends polypeptides with varying hydrophobicity and conformation (Hamaker and others 1995). Upon reduction and SDS-PAGE, two major bands are resolved at M_r 19 kDa and 22 kDa (Hamaker and others 1995; Wallace and others 1990). The β-zein comprises 10-15% prolamin with three polypeptides (M_r 14 kDa, 22 kDa, and 24 kDa) while the γ-zein composes 20% of the storage unit (M_r 16 kDa, 27 kDa). This latter protein is also unusually rich in proline and a small concentration of lysine (Hamaker and others 1995; Lasztity 1996). Both β- and γ-zein exhibit similar amino acid sequencing, indicating a closely-related genetic link (Lawton and Wilson 2003). Unique in its lysine and high methionine content, δ-zein contributes the smallest zein segment at 5% (Lawton and Wilson 2003).

The amorphous proteins in the endosperm matrix are collectively referred to as the glutelin fraction. This polydisperse group is distinguished by its structural susceptibility to a combination of alkaline conditions, sodium dodecyl sulfate and reducing agents (Laztitzy 1996; Paulis and Bietz 1998). A basic environment may induce protein modifications to the native structure such as cystine and arginine loss and

glutamine deamination (Lasztity 1996). Likewise, detergents may serve to disrupt hydrogen bonding of aggregates. Finally, reducing agents necessary for extracting glutelin may alter the molecular weight and conformation (Paulis and Bietz 1988). Three types and related functions of sulfide bonding include intramolecular, intermolecular with a polymerizing effect, and intermolecular conferring additional 3-dimensional structure and insolubility (Laztitzy 1996; Paulis and Bietz 1988).

During endosperm expansion, albumins and globulins make up the cytoplamic constituents. Only upon drying does the matrix become insoluble. Recent theory suggests that glutelins are actually globulins that become crosslinked after maturity. Similar amino acid profiling and SDS-PAGE results support this idea. No knowledge of genetic expression is traced to glutelin formation. Due to their complex structure, glutelins are much less understood than other endosperm proteins but contribute a significant structural portion to the endosperm tissue (Shandera and Jackson 2002; Laztitzy 1996; Dierks-Ventling 1981).

Hard and Soft Endosperm

Within the endosperm, soft and hard endosperm categorize the different textures of the tissue (Fig 3.3). A thicker protein body surrounds spherical starch granules in soft endosperm. Under light, the soft endosperm appears opaque because granules are loosely associated in the protein matrix, leaving several voids which scatter light rays. In contrast, hard endosperm protein bodies are more abundant and densely packed (Dombrink-Kurtzman and Bietz, 1993). Resulting cell walls appear thinner, starch granules are polygonal in shape, and tissue is translucent (Hoseney 1998; Figure 3.3).

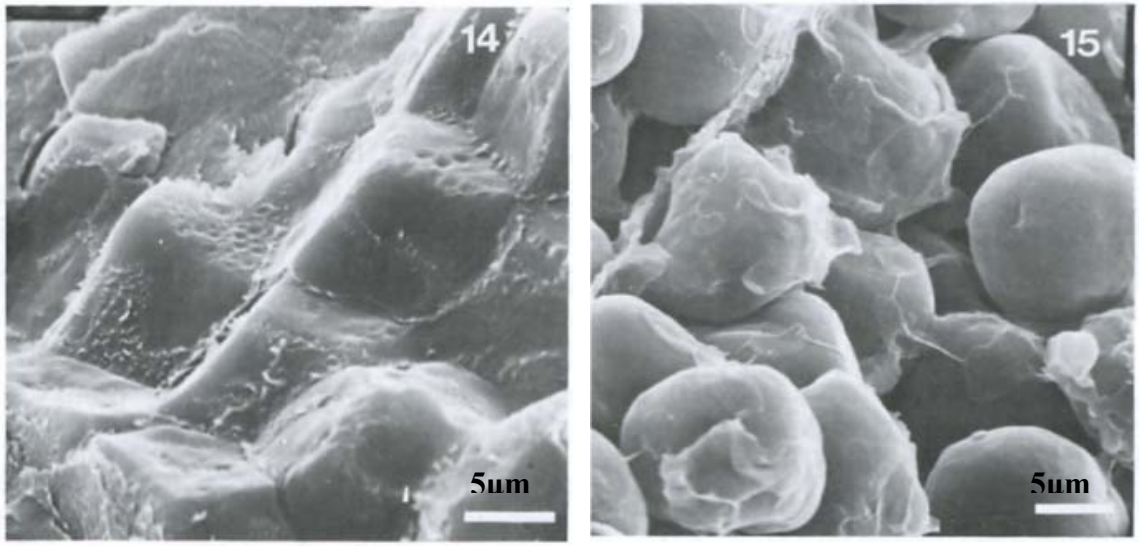


Figure 3.3 Scanning Electron Micrograph of Hard and Soft Endoperm (Hoseney 1998)

The ratio of each fraction contributes to the overall hardness and structural integrity of the kernel. In snack food processing, manufacturers prefer kernels with a higher hard-to-soft endosperm ratio. Such a composition is also preferred for shipping stability, pest resistance, and milling (Hunter and others 2002).

Work by Dombrink-Kurtzman and Bietz (1993) compared the microstructure of hard and soft endosperm proteins using both reverse-phase high performance liquid chromatography (R/P HPLC) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to demonstrate the relationship of protein composition to texture. Significant differences included tripling of the concentration of α-zein in hard endosperm. Meanwhile, soft endosperm contained double the amount of γ-zein.

Several opaque mutant maize lines have been studied in an effort to relate genetic factors and alteration of protein structure. Geetha and others (1991) researched the *opaque-2* mutation and found that γ-zein concentration and mRNA upregulation was 2-4 times that of wild maize. Total zein was reduced while lysine content increased (Geetha and others 1991). Examination of the *floury-2* mutation also displayed a reduction in zein, a point mutation creating both a 24 kDa α-zein and over expression of a 70 kD chaperone, increased lysine concentration, and disruption in storage body arrangement (Coleman and others 1997). Hunter and others (2002) extended protein analysis to 18 opaque mutants which were bred into the same genetic line. Results were correlated with GeneChip analysis. As a trend, mutants exhibited decreased zein production and higher levels of lysine. Degree of prolamin accumulation varied widely, but at least one gene responsible for zein transcription was modified in each sample. All mutants likewise displayed up-regulation in a group of stress response genes encoding molecular

chaperones, cell wall proteins, and wound/pathogen-activating proteins (Hunter and others 2002). Interestingly, modified *opaque-2* maize displaying a vitreous texture revealed a reduced expression in stress response genes when compared to normal *opaque-2* mutants. Overall, opaque mutants exhibited very different gene expression from the control group as indicated by the up- and down-regulation of numerous gene types; modification of those encoding storage and stress response proteins were common throughout all mutations (Hunter and others 2002).

Starch Composition and Processing

With starch occupying the majority of endosperm, processing changes with this fraction may also have significant influence on masa texture (Boyer and Shannon 2003). Typically, only 20-40% starch is hydrated and loses crystallinity during the cooking stage (Sahai and Jackson 2001; Gomez and others 1992) because the calcium from lime complexes with the amylose to prevent leaching. Endothermic peak difference between raw kernel and nixtamal starch obtained with differential scanning calorimetry indicated that gelatinization did not increase more than 2°C for onset and peak temperatures. Thus, crystallinity did not significantly change until after baking (Robles and others 1988) and this conclusion was verified with polarized light microscopy. Most starch that melts during the cook phase recrystallizes to its native form during steeping (Gomez and others 1992). The intact aleurone layer slows the diffusion of water and lime into the endosperm but the steeping stage allows enough time for the water and lime to migrate throughout the kernel (Rooney and Serna-Saldivar 2003). Surrounding proteins and starch granules may swell, but both maintain most of their structural integrity until grinding (Rojas-Molena and others 2007). During grinding, the cells are disturbed, allowing hydrated

starch and protein to disperse around the ungelatinized endosperm and form a heterogeneous dough (Ramirez-Wong and others 1994).

3.1.3 *Germ Composition and Processing*

Corresponding to 10% of total dry weight, the germ is the source for three-forths of kernel lipids (Weber 1978). Several organelles composed of phospholipids, hemicelluloses, and oleosins provide resevoirs for storing energy-rich triacylglycerides (Stanley and Watson 2003). To anchor this section within the endosperm and pericarp, an amorphous mixture of protein, hemicellulose, and cellose is deposited on the exterior. Although the cementing layer is insoluble and a large fraction of interior components are hydrophobic, the germ has 3.5-3.8 and 4.7-5.3 times the diffusive absorption of opaque and vitreous endosperms (Stanley and Watson 2003). During alkaline cooking, the germ absorbs water and a minor amount of oils may become saponified (Martinez-Flores and others 2006). Grinding reduces the particle size of the germ, but the tissue remains intact as separate pieces; evidence has indicated that the germ does not greatly contribute to masa texture (Vidal-Quintanar and others 2001).

3.2 Factors affecting Masa Texture

Different studies have tried to explain the significant variables factoring into the overall masa texture by evaluating the effects of processing and kernel quality factors. Ramirez-Wong and others (1994) concluded that masa adhesiveness and hardness were due to the degree of cooking time, grinding size, and moisture level with various interactions between these three parameters. In another study, Sahai and others (2001) explored both the kernel attributes and processing aspects by running regression analysis on 5 white corn varieties. These researchers concluded that masa gumminess was most

influenced by cook temperature, steep time, corn calcium content, and Wisconsin breakage test results. Masa adhesion was affected by cook temperature, cook time, steep time, Tangential Abrasive Dehulling Device (TADD) data, and Stenvert hardness values.

Studies targeting the starch fraction give evidence of environmental and varietal influence on maize functional characteristics. Using Differential Scanning Calorimetry (DSC), Ji and others (2004) determined that granule size and shape and amylopectin molecular weight distribution and branch length were significantly different between genetic lines. Gelatinization onset temperature increased with different environmental conditions of some progeny lines while a few genetic lines remained fairly consistent. For food processing, selecting a variety with the least amount of variation in starch and other features would be ideal because incoming material may have less season-dependent fluctuations and processing parameters could remain relatively constant (Ji and others 2004).

In industry, corn hardness is one parameter that grain elevators measure to determine quality. This attribute is defined as the ratio of hard to soft endosperm, with the hard endosperm exhibiting a greater protein content, density, and subsequent strength (Paulsen and others 2003). Alkaline food processors prefer a harder corn because it is less susceptible to postharvest handling damage which can create internal fissures that weaken the structure and increase chances of overcooking (Paulsen and others 2003). Many methods exist for quantifying this characteristic, but each measures a slightly different aspect of hardness. Some raw kernel tests such as the Wisconsin breakage tester and the Stein breakage device measure the mechanical strength by impacting the kernel and measuring the percent kernel breakage. Others such as the thousand kernel weight

and floater test attempt to indirectly measure the hardness on the basis of the hard endosperm's higher density. Only the pycnometer and alcohol displacement test measure the true density by volume displacement (US Grains Council 1999). Because hardness is the main determinant of kernel quality for food processing, understanding the physico-chemical basis of this feature and not just quantitating indirect physical measurments is imperative for selecting an appropriate procedure to monitor the functionality of incoming grains during masa production. Currently, the methods for evaluating corn hardness are very crude.

3.3. Instrumental Analysis

3.3.1. *Texture Evaluation*

As established previously, maintaining a consistent masa texture is important for machinability in tortilla chip production (Ramirez-Wong and others 1993). In the snack food industry, textural evaluation is subjectively performed by a masa processing expert. Quantifying textural attributes with instrumentation and correlating it with processing response would provide objective data and allow comparison for quality purposes. Textural analysis with instruments such as the Instron Texture Analyzer and a rheometer provide means for quantitatively monitoring the mechanical changes in bread and masa (Vodovotz and others 2001). These tools have been used in previous food science research for many applications. Falcone and others (2007) utilized a controlled-strain rotational rheometer to characterize traditional balsamic vinegar for quality purposes. Rheometry was applied to the study of the physical properties in three fat blends with varying ratios of *trans*-fats (Bell and others 2007). Ahmed and Ramaswamy (2007) monitored the physical behavior of meat-based strained baby foods using similar

instrumentation. Some researchers have also used these instruments to quantitate masa texture. Flores-Farias and others (2000) measured masa firmness and adhesiveness with a TA.XT2 Texture Analyzer and rheological analysis to evaluate the effect of large macromolecular components and additives in commercial nixtamalized maize flours. A Rapid Visco Analyzer (RVA) was also employed for describing the pasting properties of corn samples and relating to masa processing (Jackson 2005). Guo and others (1999) employed a stress relaxation test by applying a constant unilateral strain with the TA.XT2 Texture Analyzer, concluding that increasing water content caused the dough to become more viscoelastic or liquid-like based on differences in energy dissipated and stiffness. However, less work on masa has been conducted with dynamic rheology.

Rheology, defined as the study of the deformation and flow of matter, has many measurements to describe a material when a force is applied (Tabilo-Munizaga and Barbosa-Canovas 2004). Initial assumptions are that the material is homogeneous and isotropic (Daubert and Foegeding 2003) which is a challenge considering masa in itself is a heterogeneous dough. The three main factors affecting the compression stress-strain curve are the elastic deformation of the cell wall, the collapse of the cell wall as it buckles under the load, and fracture and increase in density when the cell structure is completely destroyed (Vodovotz and others 2001). Visco-elastic behavior can be elucidated with cscillatory rheology which gives an indication of how the material will perform under processing conditions requiring mechanical changes. The ability of oscillatory rheology to characterize visco-elasticity makes this instrument unique from the other deformation-response instrumentation used to study masa texture.

3.3.2. *Thermal Analysis*

Because corn is a composite of carbohydrates and proteins, techniques common in polymer science research can be employed for understanding the underlying basis for masa's texture and processing performance. Polymers can be classified into one of three categories: crystalline, amorphous, or semi-crystalline. Type and thermal location of transitions microscopically describe the chemical composition of the material and indicate both its thermal history and how it will react macroscopically under various conditions (Roos 1995).

In crystalline material, polymer chains are tightly packed into an organized structure, forming a stiff solid below the melting temperature (T_m). Upon heating, molecules gain vibrational motion, causing the material to increase in volume at T_m. At this critical temperature, molecules overcome enough intermolecular bonding during latent heat to liquefy and change in heat capacity (Roos 1995). In the amorphous phase, molecular chains are randomly dispersed (Liu and others 2006). At low temperature, the substance exhibits increased viscosity and brittleness due to limited range in vibrational motion; however, it is considered a glass, not a solid. At the glass transition temperature (T_g), molecules gradually increase in their rotational and vibrational energy. The T_g is not a set point, but a temperature range at which the material exists in a rubbery or leathery state, allowing for flexibility (Hoseney 1998; Roos 1995). Transitioning from glass to leather, and then to liquid, requires no latent heat. A small change in heat capacity accompanies the T_g. Semi-crystalline substances exhibit characteristics of both amorphous and crystalline materials. Native starch, a significant component of corn endosperm, is an example of a semi-crystalline biopolymer. Processing can alter the

amylopectin form by melting the crystals during cooking and reorganizing again during storage (Hoseney 1998; Liu and others 2006).

Differential Scanning Calorimetry (DSC) operates by measuring the thermal changes, either endothermic or exothermic, in a sample as it is heated in tandem with a reference (Schenck 2003). The difference in temperature between the two thermocouples is calculated as heat flow is absorbed or generated. Narrow endothermic transitions signify crystallized melting and phase state changes in pure materials while broad endothermic peaks show events such as dehydration, polymer melting, and temperature-dependent behavior. Exothermic transitions not resulting in decomposition range from crystallization, to solid-solid phase transitions, chemical reactions, polymerization, and curing of polymers and vary in broadness of peaks. Glass transitions are second-order exothermic reactions that are displayed as step changes in the heat flow as the mobility of amorphous material changes (Schenck 2003).

A drawback to this technique is that the small baseline shift of Tg can be difficult to monitor due to sensitivity. Natural polymers have complex matrices to interpret, particularly in heterogeneous systems where other numerous and overlapping peaks or transitions may overshadow such small transitions (Vodovotz and others 2003). Rapid freezing and rescanning may aid in detecting these small changes (Vodovotz and others 2003).

Thermogravimetric analysis (TGA) monitors weight change in a food system over either temperature or time if performed isothermally (Schenz 2003; Hatakeyama and Zhenhai 1998). The derivative weight loss depicts the rate of change. Basic components

include an electronic microbalance, furnace, temperature programmer, atmospheric controller, and data collector (Hatakeyama and Zhenhai 1998).

Thermal analysis instrumentation such as Differential Scanning Calorimetry (DSC) and Thermogravimetry (TGA) in conjunction with rheological testing can provide many means for quantitatively monitoring the physicochemical and mechanical changes in masa and other foods that relate to material properties. These tools have been used in previous food science research for many applications. Kim and Akoh (2006) utilized DSC to compare the melting and crystallization differences between natural sesame seed oil and enzymatically restructured sesame oil with caprylic acid. The moisture content effect on the thermo-mechanical properties of an oil-egg, albumen-cassava starch biopolymer was studied with Dynamic Mechanical Analysis (DMA) to determine strength, while DSC further helped to explain possible physicochemical causes occurring at various temperatures (Wongsasulak and others 2006). TGA was applied to the study of free and bound water in traditionally and high-pressure processed cheese during ripening (Saldo and others 2002). Much work has also been conducted using thermal analytical methods in the baking and cereal study. Neher and others (1973) applied TGA instrumentation to assess the drying and storing of grains. Nixtamalization changes to the pericarp were evaluated by TGA decomposition data at various processing points (Gonzalez and others 2004) while Vittadin and others (2003) and Clubbs and others (2007) characterized the water in tortillas with different ingredient additions and processing methods by using DSC, TGA, and DMA.

3.3.3 *Near-infrared Reflectance*

In the last decade, the grain industry has adapted the use of near-infrared reflectance (NIR) technology as a rapid, non-destructive means to measure corn composition and resulting quality. Each molecule absorbs near-infrared energy at a unique wavelength (400-2,500 nm) proportional to the concentration in the sample. Specific chemical groups distinguishing each component are targeted; absorption profiles for CH_2, NH, and OH substituents relate to lipid, protein, and water and are established by creating a calibration curve with known standards. Theoretical basis is below (Paulsen and others 2003):

Equation 3.1:

Concentration of chemical = Log (1/Reflectance at specified wavelength)

or Log (1/Transmittance at specified wavelength)

Work by Siska and Hurburgh (1994) employed NIR to predict breakage susceptibility. After assessing protein and starch content, data was correlated with physical quality measurements such as test weight and kernel density. Work by Lee and others (2005) elaborated on the relationship between near-infrared reflectance and various hardness tests with Principle Component Analysis.

CHAPTER 4

MATERIALS AND METHODS

4.1 Masa Ingredients and Processing

The benchtop nixtamalization procedure was based on a large-scale version of a manufacturing process at a snack food plant for tortilla chips. See Figure 4.1 for ingredients, proportions, and process flow. Five batches of acceptable corn, 5 batches of unacceptable corn, and 3 batches of experimental corn were performed using grain from different truck loads received by Wyandot for each batch. Table 4.1 and Table 4.2 list laboratory ingredients and batch amounts used for this study. The nixtamalization process was conducted as described below:

1. Dry corn, distilled water, and lime were added to a 1,000 ml beaker and heated on a hot plate (setting: 7)
2. Contents were manually stirred with a glass rod; come-up time (CUT) to 90°C was 18-22 minutes
3. Sample cooked at 90°C for 20 minutes; periodic manual stirring continued
4. Beaker was removed from hot plate and contents were quenched below 60°C by addition of distilled water; further stirring facilitated heat dissipation (~1 minute)
5. Beaker opening was sealed off with latex glove and soaked for 12 hours in a 40°C oven (Fisher Isotemp Oven 2000 Series, Fair Lawn, NJ)

6. Steep water was decanted off and kernels were rinsed and manually rubbed between fingers until sufficient pericarp removal was achieved by visual inspection.

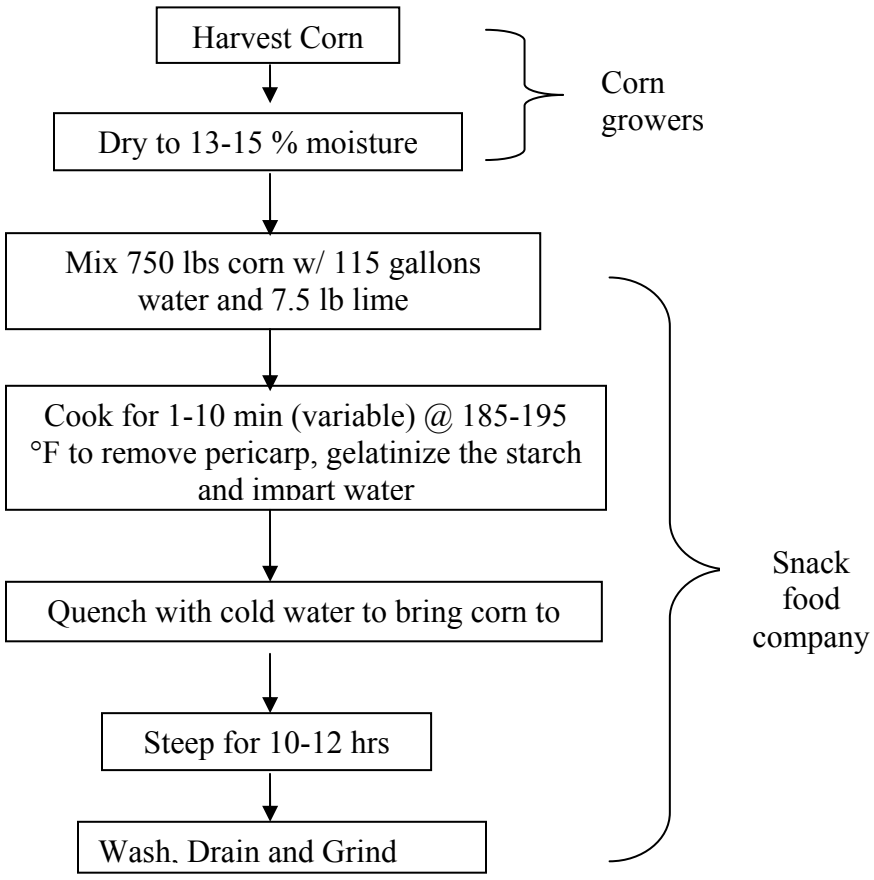


Figure 4.1 Large-scale nixtamalization of masa

7. Cooked kernels were fed through hand-cranked cast iron corn grinder with ceramic composite plates (Porkert, Czech Republic); dough was collected in polyethylene bag

Table 4.1. Ingredients included in masa process

Ingredient	Source	Amount
Yellow Dent Corn	Wyandot, Inc., Marion, OH and Delong Elevator, Kirby, OH	400 grams
Food-Grade Lime (CaOH)	Wyandot, Inc	4 grams
Distilled Water	OSU Parker Food Science and Technology	512 ml; Excess
Tap Water	OSU Parker Food Science and Technology	Excess

Table 4.2. Corn varieties and descriptions used for nixtamalization

Corn Variety	Category	Season
34K77	Unacceptable	2005
34H31	Acceptable	2006, 2007
34M78	Experimental	2007

4.2 Thermal Analysis

4.2.1 *Sample Preparation*

The thermal transitions in the pericarp, soft and hard endosperm, and germ fractions were monitored in the raw kernel and during critical processing points (post-cook and post-soak) to understand the structural functionality of each component and how nixtamalization aides in the development of a cohesive dough. Each portion was separated and comminuted to assure homogeneity of the sample and to prevent thermal gradients. Likewise, separation of the different physiological tissues reduced overlapping first derivative modals in TGA and overlapping transitions in DSC that would occur from a blend of matrices in a sample. Kernel fractions were expeditiously prepared to prevent significant moisture loss before instrumental analysis.

4.2.2 *Thermogravimetric Analysis (TGA)*

Corn fractions were analyzed on a TA Q5000 (TA Instruments, New Castle, Delaware). Monthly instrument calibrations were performed.

Platinum pans were tarred with an aluminum pan insert (Perkin Elmer Life and Analytical Sciences, Boston, MA) to prevent alkaline oxidation of the basket. Approximately 20 g of sample was loaded in the insert and linearly heated 5°C/minute from ambient temperature to 160°C. Assuming all changes in weight at this temperature range were due to water removal (Fessas and Schiraldi 2004), moisture content on a wet basis (wb) was determined by subtracting the final from the initial weight as described in Equation 4.1.

Equation 4.1

$$\%\ \text{moisture content}_{wb} = \frac{(\text{initial sample weight} - \text{final sample weight})}{\text{initial sample weight}} \times 100\%$$

To determine the effect of temperature on moisture loss, the rate of weight change (Δmg/min) over temperature was transformed by calculating the first derivative using Universal Analysis (TA Instruments, New Castle, DE). Materials which require a higher temperature before the peak rate of water removal is achieved, display a greater matrix association with the water molecules.

4.2.3 *Differential Scanning Calorimetry (DSC)*

Thermal transitions were monitored with a differential scanning calorimeter, Model DSC Q100 with Refrigerated Cooling System (TA Instruments, New Castle, DE). Temperature calibration was performed with indium as a standard reference.

Corn fractions (~10 g) were loaded in a large-volume stainless steel pan with rubber o-ring (Perkin Elmer Life and Analytical Science, Boston, MA) and hermetically sealed to prevent moisture loss. Sample pan was positioned on the furnace platform to balance an identically prepared reference pan filled with air on the second furnace platform. Temperature was decreased to -60°C and increased to 180°C by linearly ramping 5°C/min to monitoring material changes in this thermal range.

Universal Analysis software (TA Instruments, New Castle, DE) was used to measure the area underneath the thermal curve and resulting exothermic or endothermic

enthalpies were calculated based upon changes in heat flow (J/g) to the sample. Glass transitions (T_g) were measured as a shift in the baseline of the heat flow (J/g) to the sample due to a change in heat capacity.

Percent "freezable water" (FW), the fraction of loosely entrapped water able to form ice crystals at ~0°C, is computed by determining the sample enthalpy, Q, at ~0°C. It is assumed that the only thermal reaction occurring at this point during temperature ramping is the melting (T_m) of water. The latent heat of fusion for water (λ =334 J/g) also factors into the "freezable water" below in Equation 4.2:

Equation 4.2

$$\text{Amount "Freezable Water" in mg } H_20\text{/mg sample} = \frac{Q \text{ (mJ/ mg sample)}}{\lambda \text{ (mJ/ mg } H_20)}$$

In order to determine the percentage of "freezable water," this amount is compared to the total water obtained from TGA moisture content as revealed in Equation 4.3.

Equation 4.3

$$\text{Percentage ``Freezable Water''} = \frac{\text{``Freezable Water'' (mg water/ mg sample)}}{\text{Total Water (mg water/ mg sample)}}$$

4.2.4 *Moisture Analysis*

In order to assess masa moisture content, a Moisture Analyzer with an infrared heating element (Moisture Analyzer Model LJ16, Mettler Toledo, Greifensee, Switzerland) was used. Data not presented show that the Moisture Analyzer was within 2% moisture of TGA results. Masa samples (~1 and ~5 g) were drawn from center of the bulk of masa and added to the tarred aluminum sample pan. After negligible weight change was observed during heating, % moisture content on a wet basis was calculated. Measurement was repeated 3-5 times.

4.3 Texture Analysis

4.3.1 *TAX.T2 Texture Analyzer*

Masa adhesiveness values were obtained using a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK). Method was modified from Adhesive Test 16W-B for tortilla dough which employs the adhesiveness software feature (Stable Micro Systems, Surrey, UK). Acrylic probe TA 11 was attached to the arm. Three grams of freshly ground masa was evenly spread out across the bottom of an aluminum pan (Fisher

Scientific, Fair Lawn, NJ) to standardize area and thickness of masa granules. The pan was centered beneath the probe and braced to the sample platform with masking tape. See Figure 4.2 for setup.

Arm speeds were set at 3.0 mm/sec for force speed and 2.0 mm/s for pre- and post-test speeds. Probe distance was 2.5 mm from platform at start. Probe applied 150 g-F for 10 seconds to the sample and withdrew at 10 mm/sec. Adhesiveness was calculated in g-F exerted on probe by sample at withdrawal.

4.3.2 *Dynamic Rheology*

Masa sample preparation

Masa was formed into a sheet by evenly distributing 36 g of fresh dough into a 2.5 mm gauge stainless steel plate with 100 x 100 mm opening. Metal plate with masa-filled opening was sandwiched between two aluminum foil-wrapped polyethylene plates and compressed at 2,000 pounds per square inch with a Carver press for 2 seconds. Top plate was removed and sample rested 5+ minutes. Masa was covered with a Ziploc bag to reduce moisture loss before instrumental analysis. Samples were cut into circles, 20 mm in diameter. Thickness was ~3.0 mm.

Stress Sweep Test

Dynamic stress sweeping was performed using the AR 2000ex Rheometer (TA Instruments, New Castle, DE) following proper instrumental calibration procedures. A 20 mm stainless steel parallel plate geometry with solvent trap was attached and geometry gap set at 3000 mm. Frequency sweep testing was performed prior to find the linear visco-elastic region; 1 Hz was in this range and thus was fixed at this value. Force was

applied at 5 N +/- 1 N. After 2 minutes of equilibrium, geometry swept from 1 to 1,000 Torque (μ N.m) in continuous oscillation mode at 25°C isothermally.

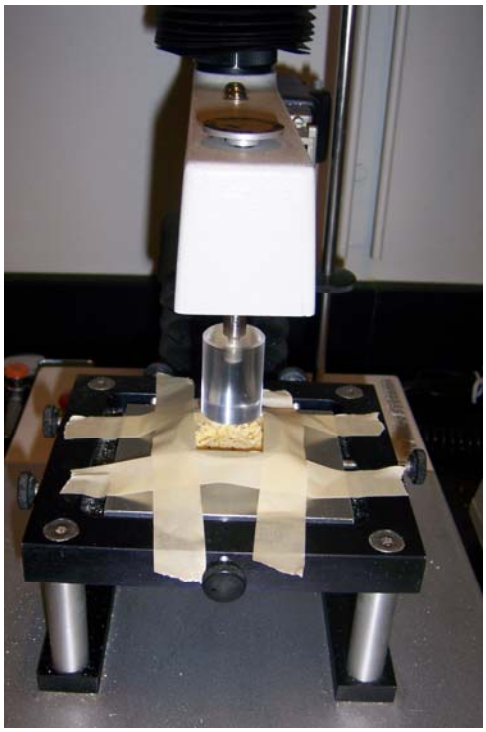


Figure 4.2 TAX.T2 Masa Adhesiveness setup

Creep Test

Similar instrumentation was used for creep analysis but settings were modified. During creep phase, 5 Pa of shear stress was applied to the sample for 30 seconds. A 2 minute recovery phase at 0 Pa stress followed. Testing was performed at 25°C.

4.4 Near-infrared Spectroscopy (NIR)

4.4.1 *Sample Preparation*

Twenty grams of dry kernels were ground in a Cyclone Mill (UDY Corp., Fort Collins, CO) and collected in a polypropylene capped centrifuge tube.

4.4.2 *Instrumental Analysis of Kernel Composition*

Near-infrared spectroscopy was employed using a Foss InfraXact Analyzer (FOSS NIRSystems, Inc, Laurel, MD) to obtain oil, protein and moisture composition of corn. Twenty grams of ground sample was evenly dispersed in a small sample cup with transparent bottom and run on the instrument. Each sample view was scanned 6 times before averaging and compared to two internal reference scans. Wavelength range included 570-1848 nm.

4.5 Extensin Quantification and Qualification

4.5.1 *Extensin Extraction*

Sample preparation

Extensin quantification was performed similar to the method of Garcia-Lara and others (2004) and Hood and others (1991). Five dry kernels of each sample were weighed and submerged in distilled water at 4°C for a minimum of 20 minutes to allow softening of seed. Pericarp was separated from the kernel body using a scalpel and pooled together.

Composite was ground in mortar and pestle with liquid nitrogen to form a fine powder. Sample was collected in a preweighed microcentrifuge tube.

Initial Extraction

A 1 ml aliquot of 4mM sodium bisulfite, 1% potassium acetate (pH 6-7) solution was added to the tube and vortexed for 10 seconds. Samples were centrifuged (Eppendorf Centrifuge 5415D, Vernon Hills, IL) at 10,000•gravity for 6 minutes and bulk of supernatant was removed. Sample was again centrifuged 2 minutes at 13,000•gravity to remove additional supernatant.

Lipid and Cytoplasmic Content Removal

Pellet was washed with 1ml of 0.5% Tergitol, 2 mM sodium bisulfite solution to remove lipid. Tube was centrifuged at 13,000 gravity for 4 minutes; soapy supernatant was discarded. Pellet was washed three times with 1 ml of 2 mM sodium bisulfite to remove cytoplasmic contents.

Solubility Protein Extraction

One milliliter of 0.2 M $CaCl_2$, 4 mM sodium bisulfite (pH 7) solution was added to the pellet and vortexed 1 minute. Samples were sonicated (Branson 2200, Branson, Danbury, CT) in an ice bath for 1.25 hour and centrifuged at 15,000•gravity for 2 minutes. Supernatant was collected while pellet was washed with 0.5 ml of 0.2 M $CaCl_2$, vortexed 1 minute, and centrifuged 2 minutes at 15,000•gravity. After 1 repeated washing, supernatants (3) were pooled, frozen, and lyophilized (Freeze Dry System Lyph Lock 4.5, Labconco, Kansas City, MO) until dry (Figure 4.3).

Figure 4.3 Lyophilization of Pericarp Extensin Extracts

Desalting Protein

A 50 mM ammonium acetate solution (~ pH 7) was degassed for ~1 hour using vacuum pump and 250 ml flask, and sides of flask tapped to remove air bubbles. Bio-Gel P-6DG Desalting Gel was hydrated 30 minutes with excess ammonium acetate buffer and small gel fines were decanted. Additional buffer was added so liquid volume was double the gel volume and contents were degassed again as above. Plastic columns with frit (Poly-Prep Chromatography Columns, Bio-Rad, Hercules, CA) were wetted with 400 ml of ammonium acetate buffer and a 2 ml packed bed was poured. Column medium was kept hydrated until sample addition. Lyophilized protein extract was resolubilized in 500 µl of buffer and added to column. Four milliliters of running buffer was added to top of column to flush sample and eluant was collected in 15 ml polypropylene vials. Protein extract and pellet were frozen and lyophilized until dry.

4.5.2 *Colorimetric Quantitation*

The unextracted protein remaining in the pellet was quantitated using the colorimetric procedure developed by Drodz (1976). Half a milliliter of 6 M HCl was added to the pericarp pellet, sealed with a screw lid, and heated 24 hours at 110°C with an autoclave (SG-120 Scientific Gravity Sterilizer, Steris Amsco Century, Mentor, OH). Norit A (0.05g) and 0.5 ml of 0.5 N HCl was included in the tube, vortexed, and poured into a microeluator. Contents remaining in microcentrifuge tube were washed successively with 0.5 ml aliquots of 0.5 N HCl. Microeluator with sample was centrifuged 10 minutes at 2,000 rpm (IEC HN-SII Centrifuge, Damon/IEC, Needham Heights, MA). An additional 0.5 ml of 0.5 N HCl was added to microeluator and centrifuged as above. The 0.5 ml of 0.5 N HCl washing was again repeated.

Pooled eluants were collected in pre-weighed crucibles and evaporated to dryness with a boiling water bath. Sample was resuspended in 0.2 ml distilled water and 1 drop of 1% phenophtlalein in ethanol solution. Crucible contents were neutralized with 0.5 N NaOH and final volume was adjusted to 1 gram (Figure 4.4).

Oxidation of hydroxyproline required several reagents. An acetate-citrate buffer (pH 6) was first combined with liquid sample and 0.2 ml Chloramine T (10% w/v). After holding mixture 10 minutes at room temperature, 0.2 ml of 70% perchloric acid was included. The final reagent, p-dimethylaminobenzaldehyde (1ml) was dropped into the cocktail. Contents were transferred to glass test tube vials with lids and heated in a water bath at 60°C for 20 minutes (Figure 4.5). After cooling in tap water, sample

Figure 4.4 Crucibles Containing Hydroxyproline Oxidative Colorimetric Cocktail

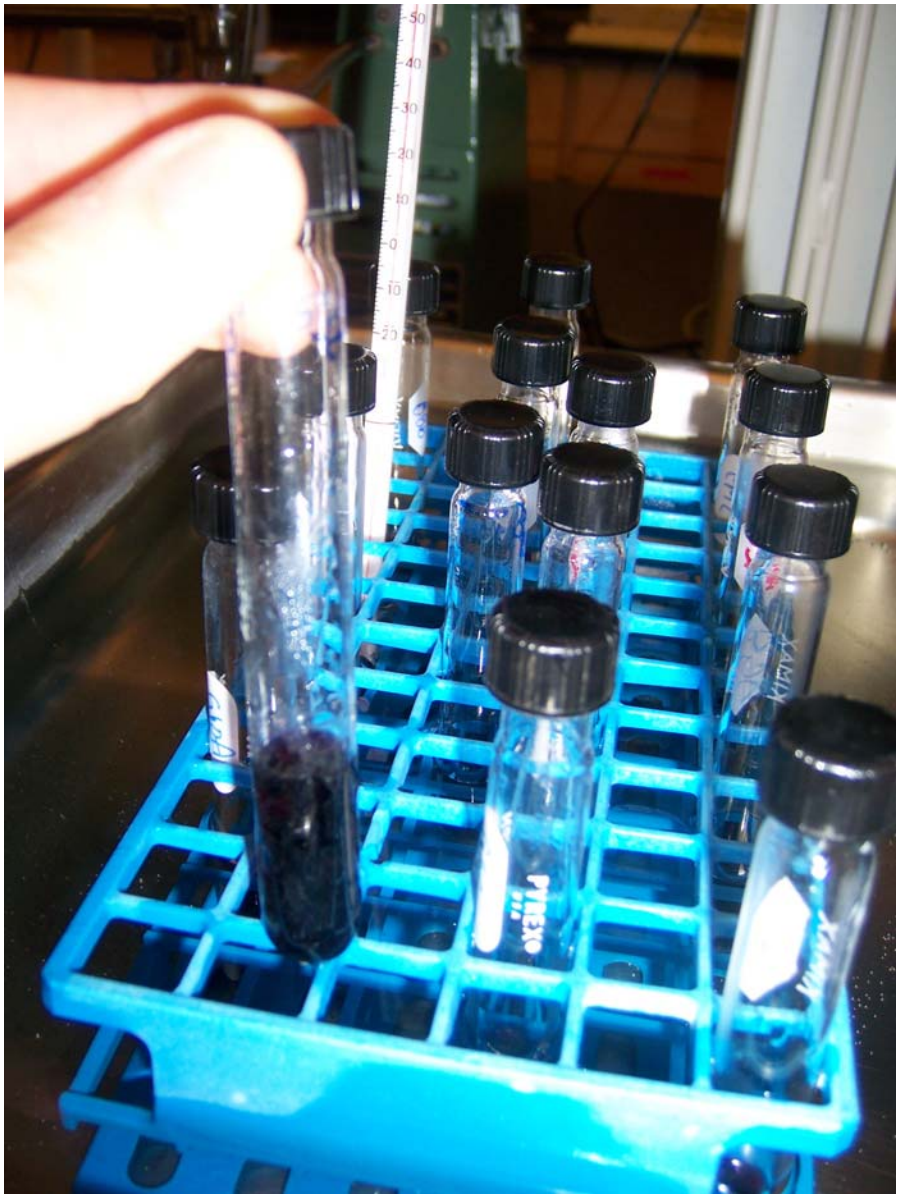


Figure 4.5. Water bath for Hydroxyproline Colorimetric Assay

concentrations were determined at 560 nm using a UV-Vis spectrophotometer (Hewlett Packard 8453 Diode Array Spectrophotometer, Agilent Technologies, Palo Alto, CA). A hydroxyproline standard curve using 2mM stock solution was performed using a pure standard (*trans*-4-hydroxy-D-proline, Sigma Aldrich, St. Louis, MO).

4.6 Statistical Analysis

One-way ANOVA using SPSS software (Chicago, IL) was used to evaluate statistical significance ($p = 0.05$). Additionally, SIMCA (Soft Independent Modeling of Class Analogy), a multi-variate analysis tool using Pirouette software (InfoMextrix, Bothell, WA) was used to assess which measurement/s best separated unacceptable and acceptable categories. Interclass distance of 3 was used as a cut-off for determining a measurement as a contributing component to the model.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Aim 1. Physico-chemical changes occurring during nixtamalization and characterization of acceptable and unacceptable corn using thermal and rheological analysis

5.1.1. *Thermal analysis: Thermogravimetric analysis*

Transforming corn to masa requires several complex steps which include cooking corn at 185-195°F to remove pericarp and steeping in lime for 10-12 hours prior to washing and grinding (Bryant and Hamaker 1997). Because water is an important component in modifying the kernel components (Sahai and others 1999), monitoring its state is essential for understanding what occurs during nixtamalization, the extent of processing, and its effect on masa texture. A benchtop process mimicking large-scale manufacturing in regard to process phases, time, and temperature, was developed as described previously in Materials and Methods. This allowed for close control of testing parameters, immediate instrumental analysis, and reduction of energy and sample waste. Thermogravimetry was employed to characterize the extent of water association with the matrices of pericarp, hard endosperm, soft endosperm, and germ fractions for the raw, cook, and soak state. Trends from Chapter 5.1

were used to narrow the processing steps and corn fractions most relevant to assessing maize functionality for masa in future work.

Figure 5.1- 5.4 display the first derivative weight loss occurring in the germ, soft endosperm, hard endosperm, and germ fractions during processing. The first derivative weight loss explains the rate of change in weight loss over a temperature range, with the peak of the curve indicating the degree of water entrapment and subsequent structure of the matrix. In the germ, cooking altered the water content. Table 5.1 reflects a 41% increase in germ moisture following cooking (8.8% +/- 0.9 versus 49.8% +/- 0.7). Little change in moisture content (46.3% +/- 3.9) accompanied the soak (Tb 5.1).

To assess the state of the water population, the peak of the first derivative was compared in Figure 5.1 and Table 5.2. The germ peak temperature of water removal occurred at 55.2°C +/- 2.5 in raw, 61.3°C +/- 1.3 in cooked, and 59.9°C +/- 0.3 in soaked kernels (Fig 5.1, Tb 5.2). These results demonstrate that although moisture content may have risen during cooking, its degree of association with the matrix only tended to increase slightly.

The high lipid content of germ (31-39% lipid; Weber 1978) may help exclude water from entering the matrix during cooking. During alkaline heating, a small degree of lipid is saponified (Martinez-Flores and others 2006). However, germ contains 19% protein (dwb) (Lawton and Wilson 2003) which may unfold during cooking and loosely trap water. The gluten meal concentrate, predominately sulfur-rich glutelins, has demonstrated water holding and fat emulsifying capabilities (Lasztity 1996). Additionally, albumins compose 1/3 of germ protein (Lawton and Wilson 2003). Both protein fractions may possibly contribute to the swelling of water in germ as

demonstrated in Figure 5.1. Overall, 75% of germ proteins are water- and salt-soluble (Lawton and Wilson 2003). Little change in peak temperature

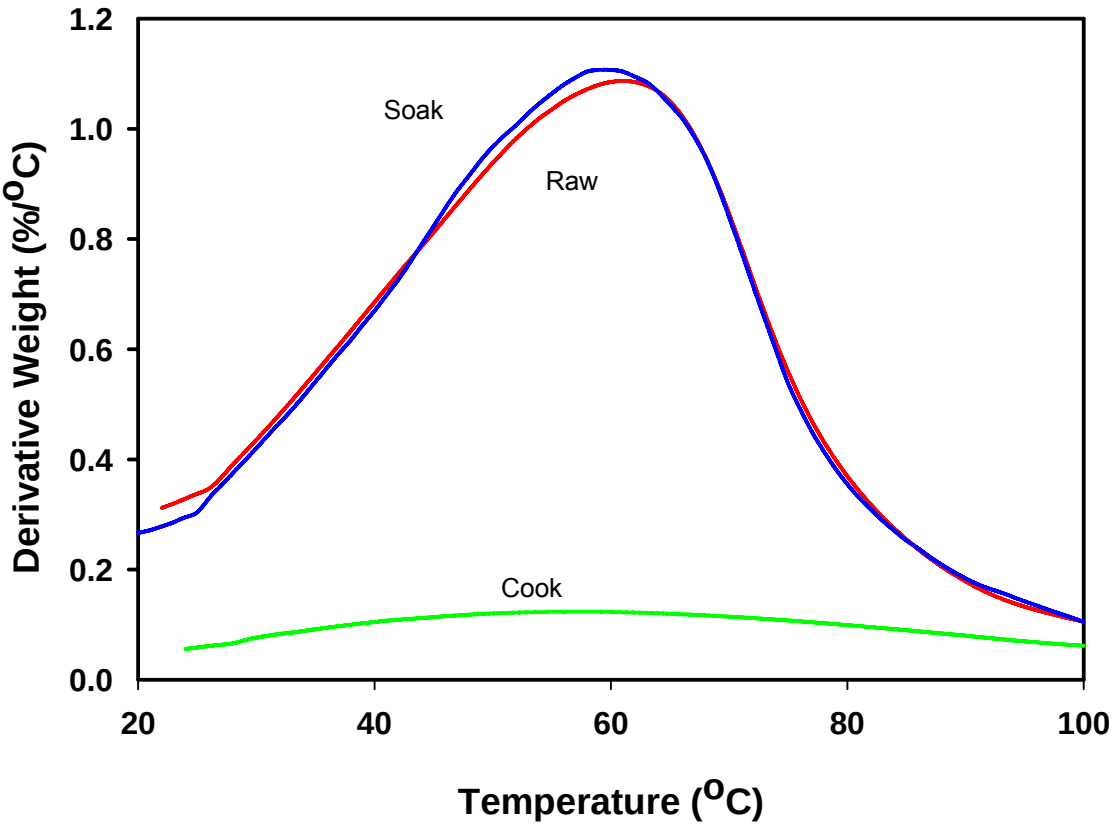


Figure 5.1. TGA First Derivative Weight of Germ for Raw, Cook, and Soak

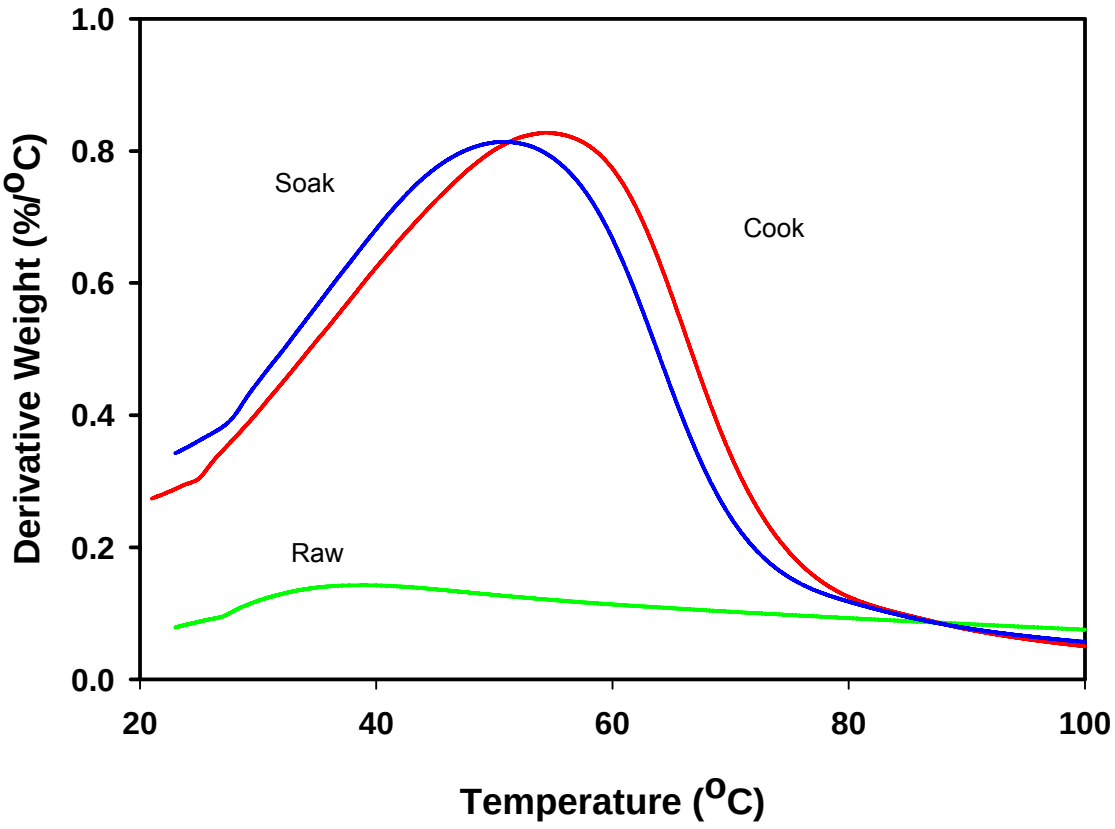


Figure 5.2. TGA First Derivative Weight of Hard Endosperm for Raw, Cook, and Soak

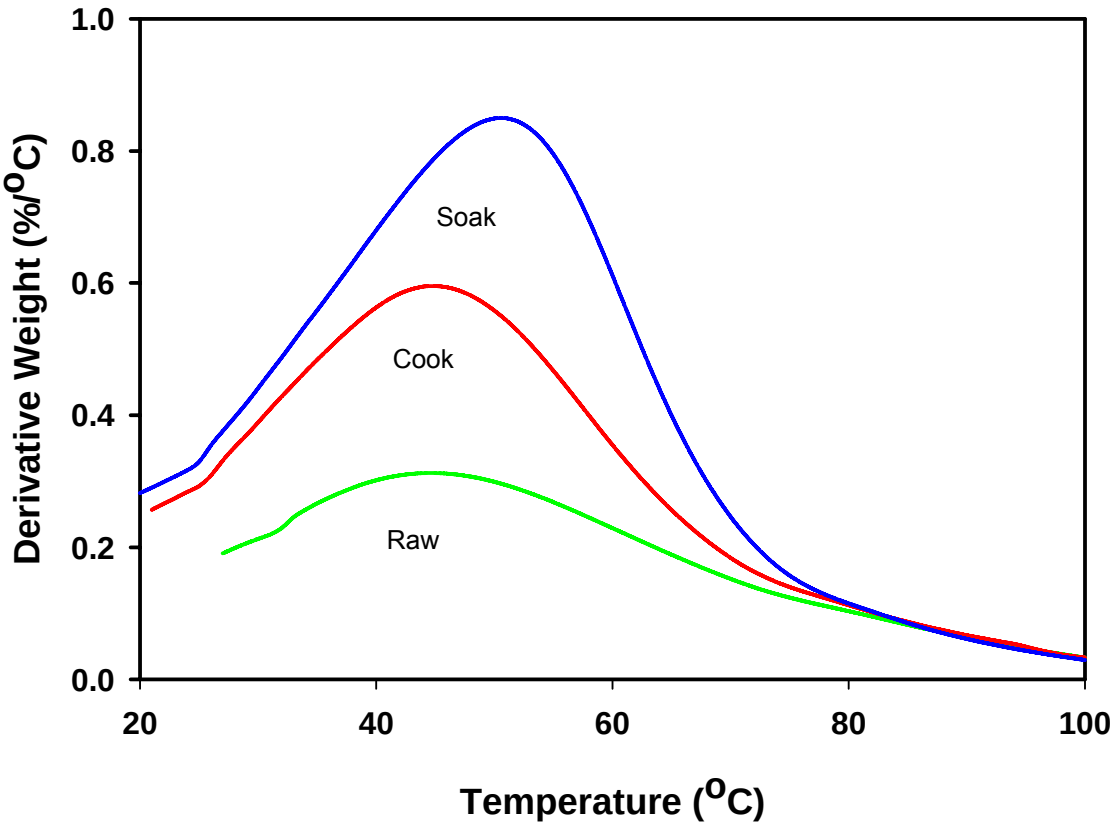


Figure 5.3. TGA First Derivative Weight of Soft Endosperm for Raw, Cook, and Soak

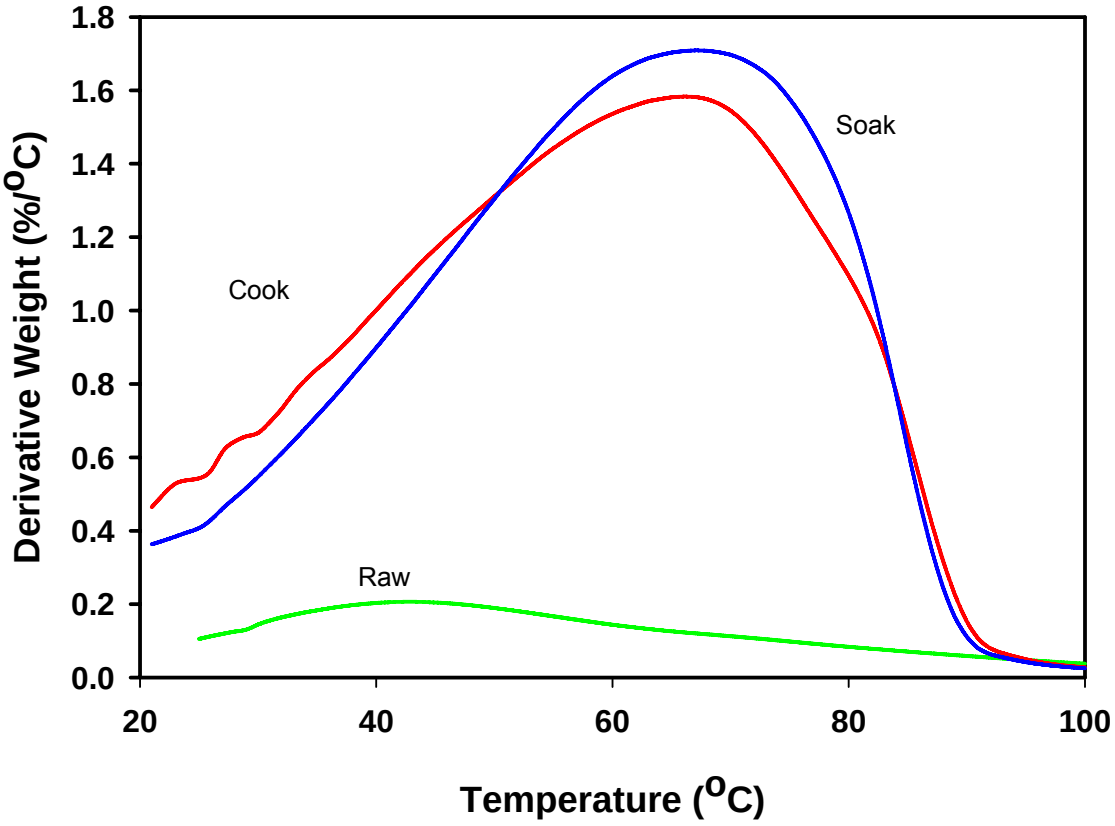


Figure 5.4. TGA First Derivative Weight of Pericarp for Raw, Cook, and Soak

Table 5.1. TGA Moisture for Fractions of Acceptable Corn During Processing

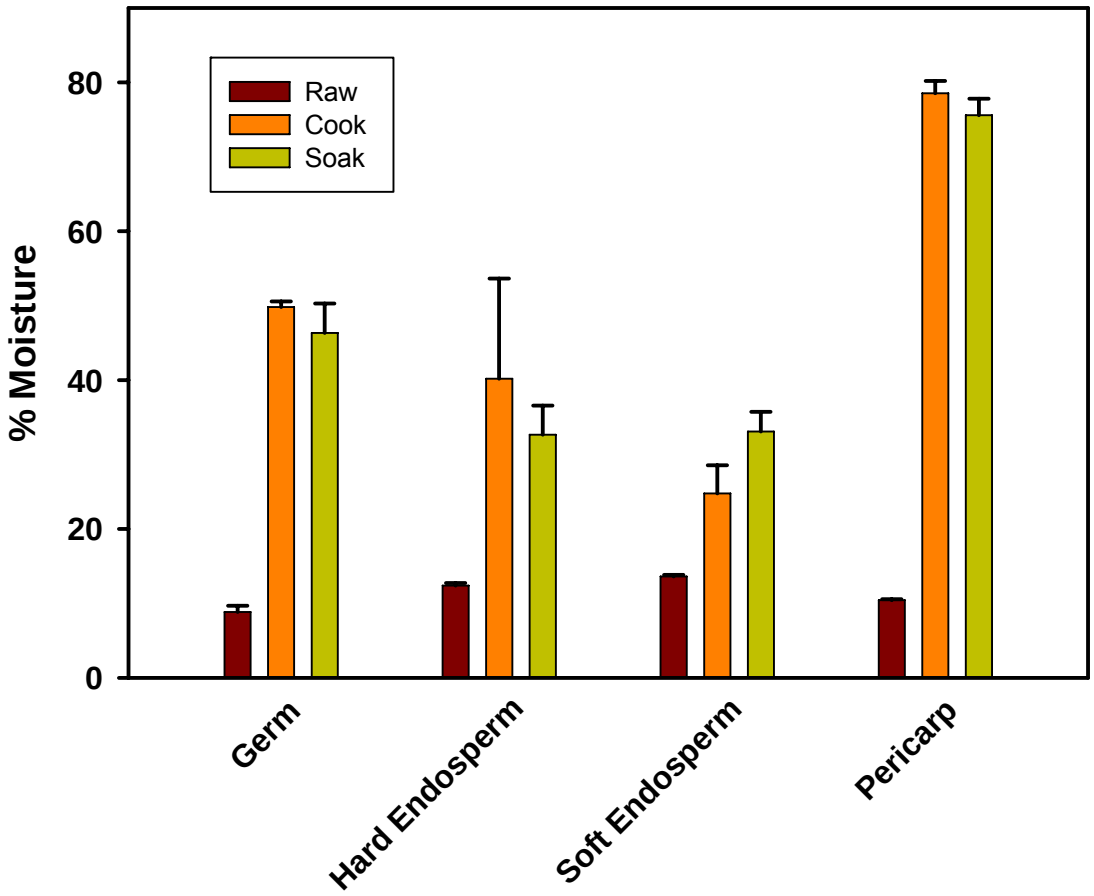
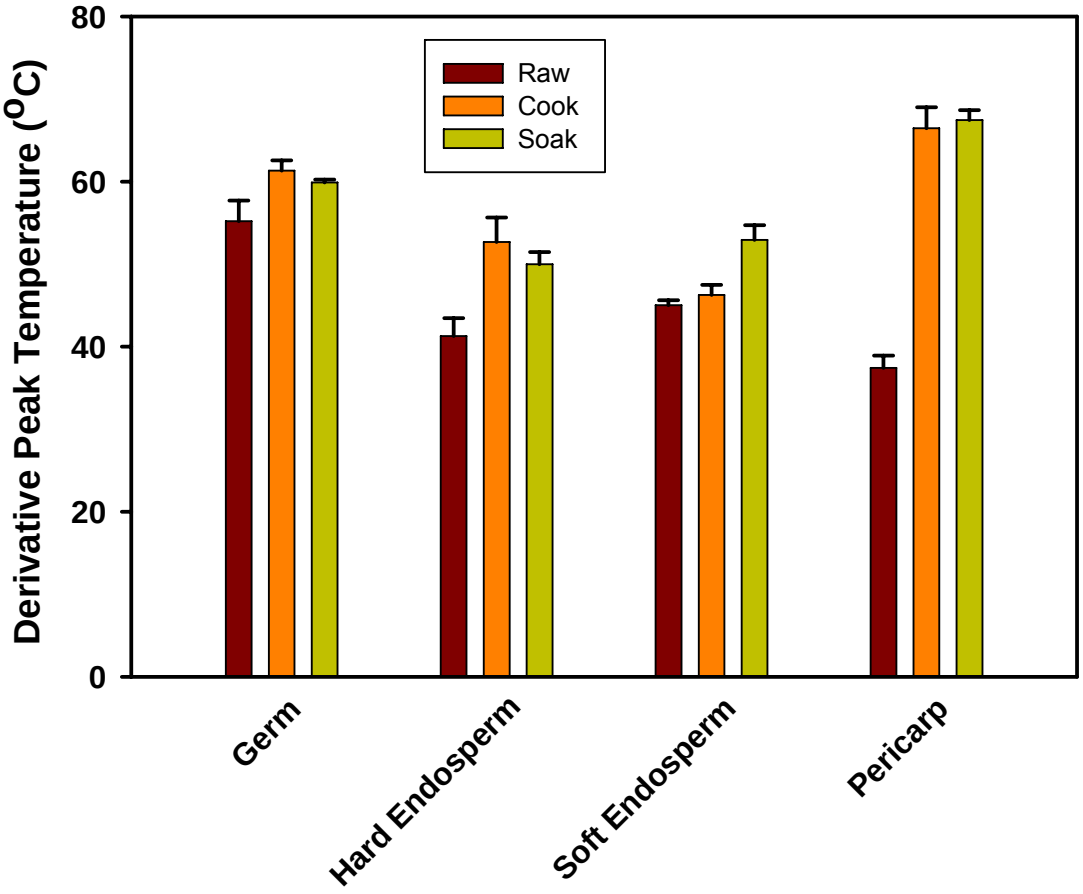


Table 5.2. TGA Derivative Peak Temperature for Fractions of Acceptable Corn During Processing



removal in soaked germ indicates water interaction may not be influenced by this step (Fig 5.1, Tb 5.2) and the fraction may already be fully saturated.

The hard endosperm swelled in moisture content from 12.4% +/- 0.4 in raw to 40.2% +/- 13.5 in cook (Tb 5.1). Likewise, this was accompanied by an 11°C increase in derivative peak temperature (41.3 °C +/- 2.2 versus 52.7°C +/- 3.0), demonstrating a greater degree of entrapment in the matrix (Fig 5.2, Tb 5.2). During alkaline cooking, the denaturation and solubilization of hard endosperm proteins including zeins and glutelins swell with water and facilitate successive permeation and partial gelatinization of the starch granule (Hamaker and others 1995, Sahai and Jackson 2001). Gelatinization occurs at temperatures above ~65°C and in an environment with excess water; both conditions are present during the cook stage of nixtamalization (McDonough and others 2001). In this study, soaking neither seemed to increase the moisture content (32.7% +/- 3.9) nor raise the peak maximum of water loss (50.0°C +/ -3.9; Fig 5.2, Tb. 5.2). Nixtamal is quenched below 65°C before soaking to stop gelatinization, likely accounting for little change in the water component of starch. After cooking, amorphous starch may recrystallize, which would further prevent moisture increase (Mondragon and others 2003). Additionally, the calcium ion in lime complexes with amylose to prevent total gelatinization from occurring (Bryant and Hamaker 1997; Rodriguez and others 1996).

In Table 5.1, the moisture content approximately doubled from raw (MC: 13.6% +/- 0.2) to cook (MC: 24.8 +/- 3.8). Although the water content increased, the TGA peak temperature did not show a difference for the cook phase (raw: 45.0°C +/- 0.6; cook: 46.3°C +/- 1.2) (Fig 5.3, Tb 5.1). The soak step increased the moisture content to 33.1% +/- 2.7 while the TGA peak temperature increased 6°C to 50.0°C +/- 1.8, indicating that

water became more associated during the 12 hour steep (Tb 5.1, Fig 5.2). Because the soft endosperm extends to the interior of the kernel, the long soak may be necessary to allow water to permeate throughout this fraction.

Raw pericarp displayed a moisture content of 10.4% +/- 0.2 and a TGA peak maximum at 37.4 °C +/- 1.5 in Table 5.1 and Figure 5.4. Following the cook, the total moisture increased to 78.5 % +/-1.7 and peak temperature for water removal shifted to 66.5 °C +/-2.6 (Tb 5.1, Fig 5.4). The native pericarp serves the physiological purpose as a protective coat to prevent desiccation and infestation of pests with ~40% of the fraction composed of cellulose (Saulnier and others 1999; Saulnier and Thibault 2001). A variety of gums such as heteroxylan are also interwoven through the fiber and are released as diferulic cross bridges and isodityrosine bonds are cleaved during alkaline cooking (Saulnier and others 1999; Saulnier and Thibault 2001). The structural breakdown increased gum mobility and facilitated interaction with water. Soaking did not affect the moisture content (MC: 75.6%, +/-2.2) or the TGA peak maximum temperature (67.4°C +/- 1.2). Thus, it is likely that cooking is the critical step for modifying pericarp structure and subsequent processing has little effect on this fraction.

The same set of experiments was performed on corn deemed unacceptable by a snack food manufacturing facility. Table 5.3 summarizes the TGA peak temperatures for the four fractions during raw, cook, and soak phases, while Table 5.4 summarizes the moisture content. Similar trends for the effects of processing on water content and derivative peak temperature were observed for unacceptable corn.

Table 5.3. TGA Derivative Peak Temperature for Fractions of Unacceptable Corn During Processing

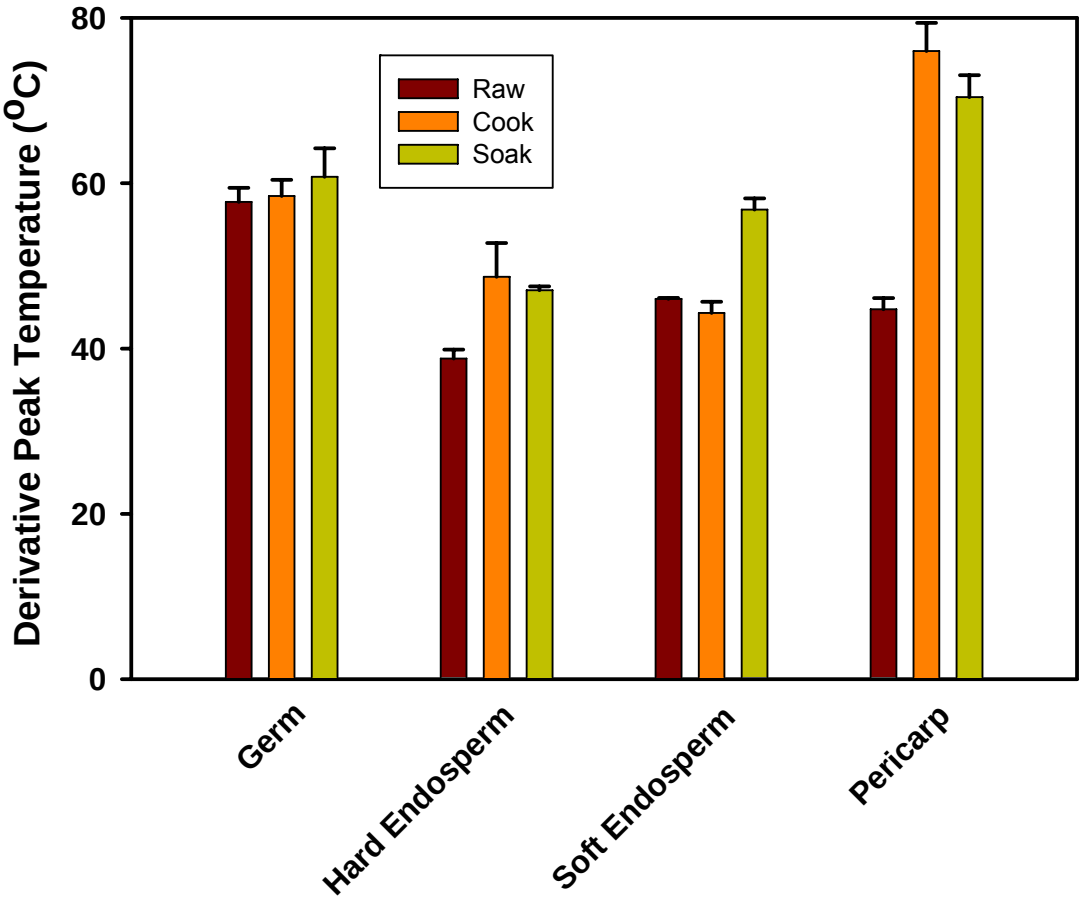
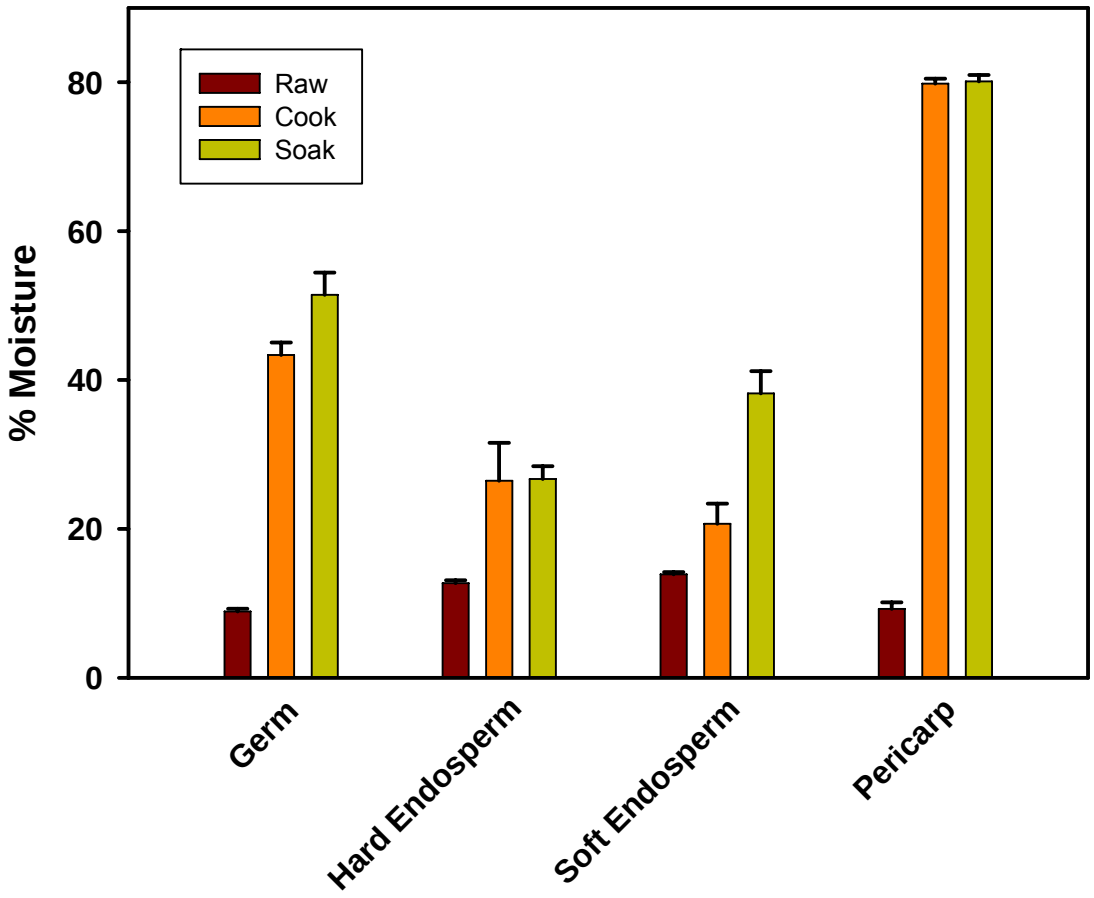


Table 5.4. TGA Moisture for Fractions of Unacceptable Corn During Processing



To distinguish any possible differences in the corn fractions of acceptable and unacceptable corn, the TGA peak temperature (Tb 5.5) and moisture content (Tb 5.6) of the two sample sets were compared to one another. The germ displayed no difference in water association (Tb 5.5) but the moisture content varied in the cook state by ~6% (Tb 5.6).

The hard endosperm in acceptable and unacceptable samples demonstrated little difference in TGA peak temperature during raw, cook, and soak stages except for a ~2°C shift in the former sample at post-soak (Tb 5.7). The average of repeated moisture content measurements was ~14% different (Tb 5.8) between acceptable and unacceptable cooked samples, but overlapped due to high variability in the acceptable corn (SD: 13.5). In addition, the soak step of the acceptable corn produced a 6% higher moisture content (Tb 5.8).

The results in Table 5.9 indicate that both water entrapment and moisture content in the unacceptable soft endosperm does not diverge much from acceptable corn until the soaking step. During the latter phase, the peak temperature shifts ~4°C higher in the former sample (unacceptable: 56.8°C +/- 1.3; acceptable: 52.9°C +/- 1.8 while the latter displays a slightly higher percent moisture at 5% MC difference (Tb 5.9, Tb 5.10).
Of all fractions, the pericarp exhibited the most disparity between the two corn quality categories. The TGA peak temperature for the unacceptable pericarp was 3°C higher in the raw fraction, although the percent moisture content was less than 1% different (Tb 5.11, Tb 5.12). However, following the cook, the unacceptable bran shifted ~10°C higher

Table 5.5. TGA Peak Temperature for Germ During Processing

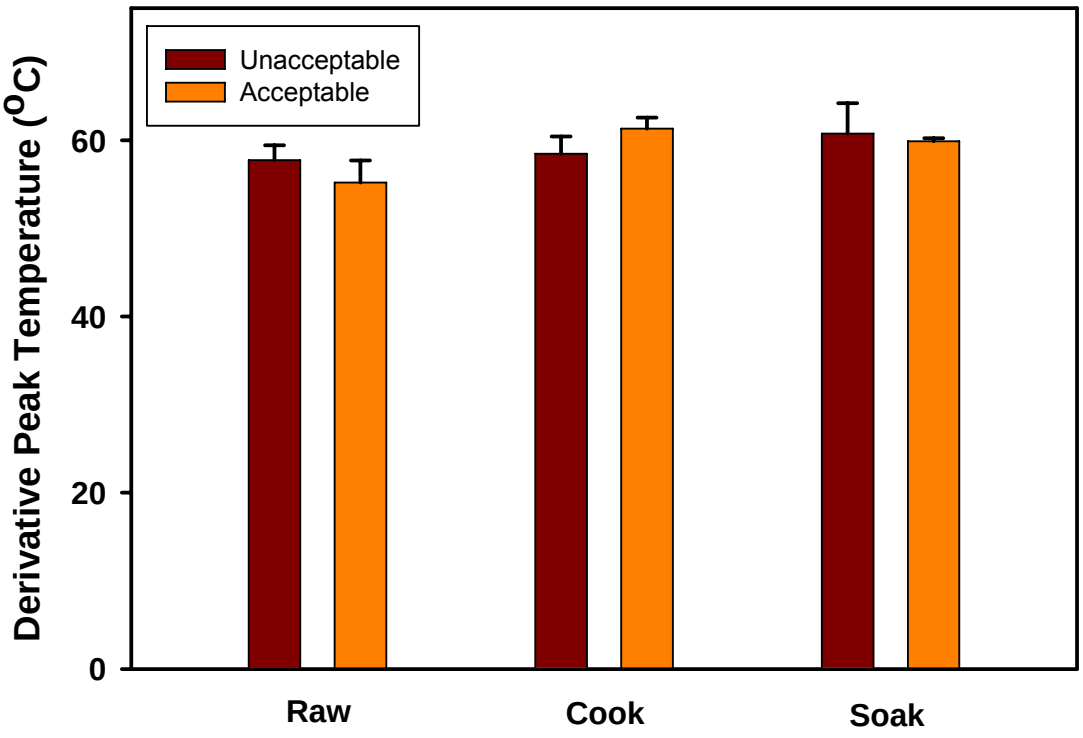


Table 5.6. TGA Moisture for Germ During Processing

Table 5.7. TGA Peak Temperature for Hard Endosperm During Processing

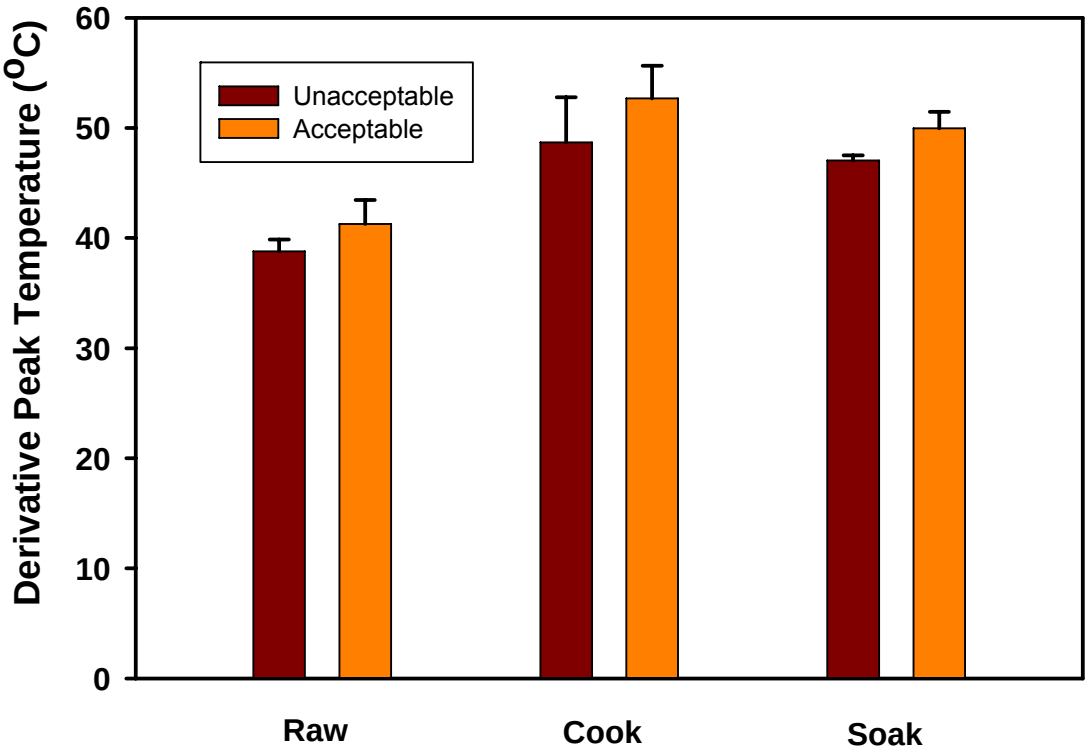


Table 5.8. TGA Moisture for Hard Endosperm During Processing

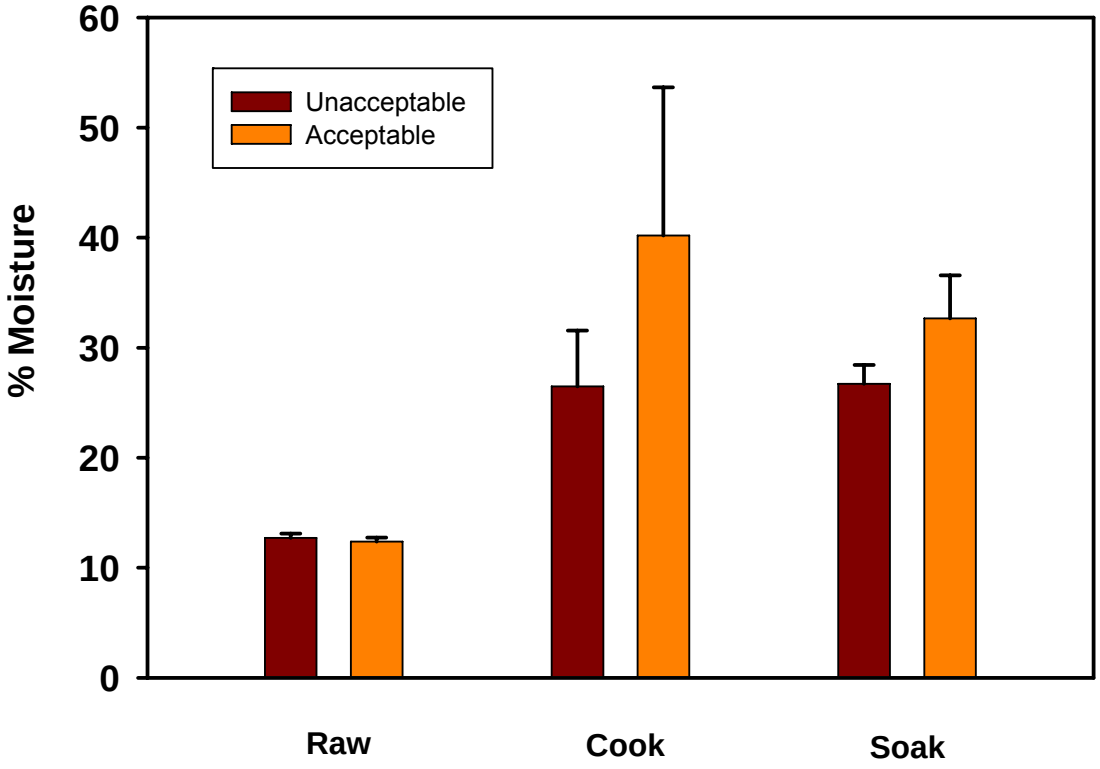


Table 5.9. TGA Peak Temperature for Soft Endosperm During Processing

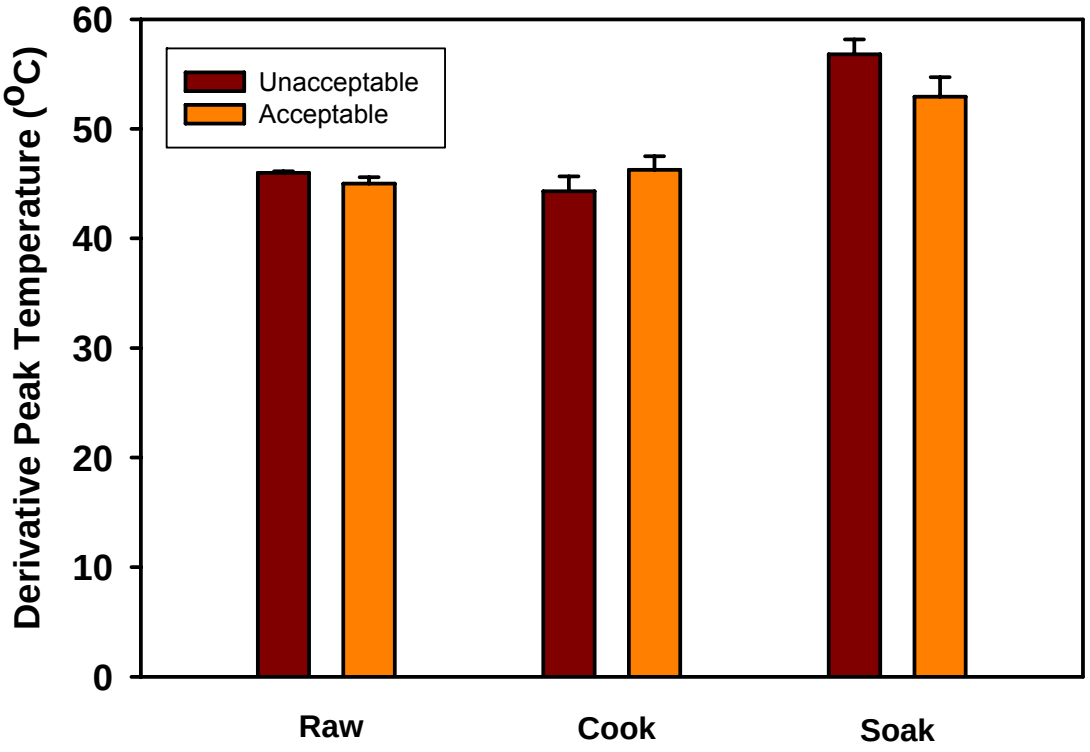


Table 5.10. TGA Moisture for Soft Endosperm During Processing

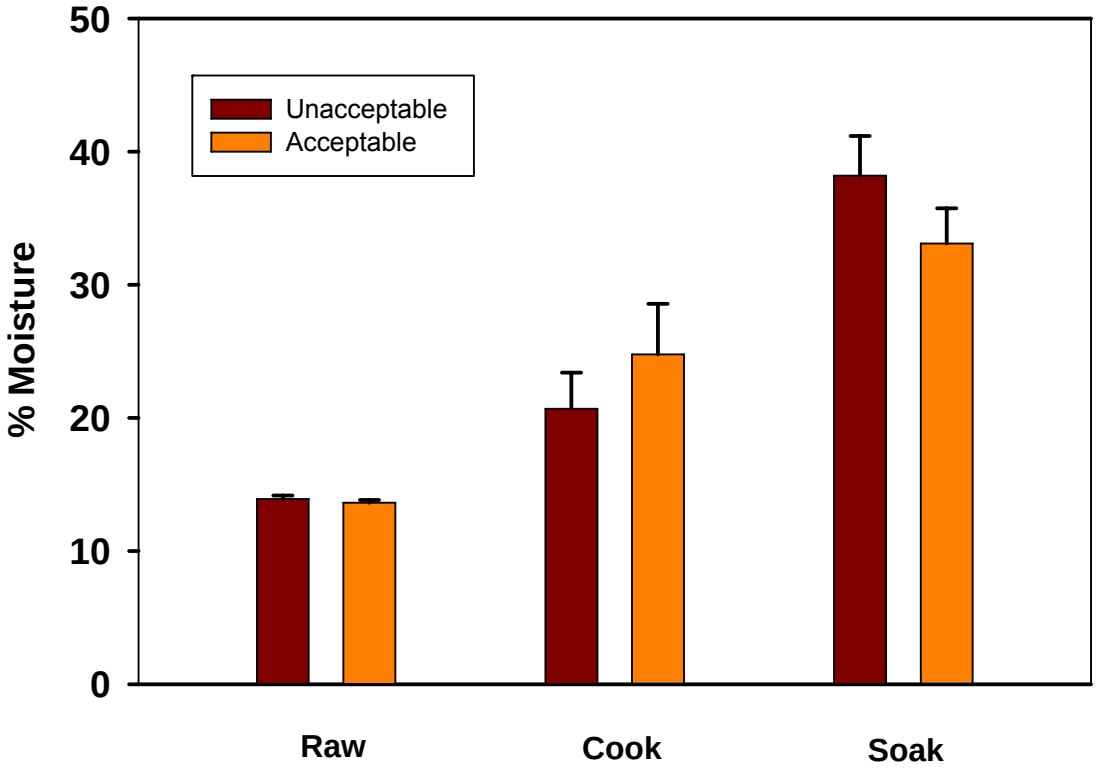


Table 5.11. TGA Peak Temperature for Pericarp During Processing

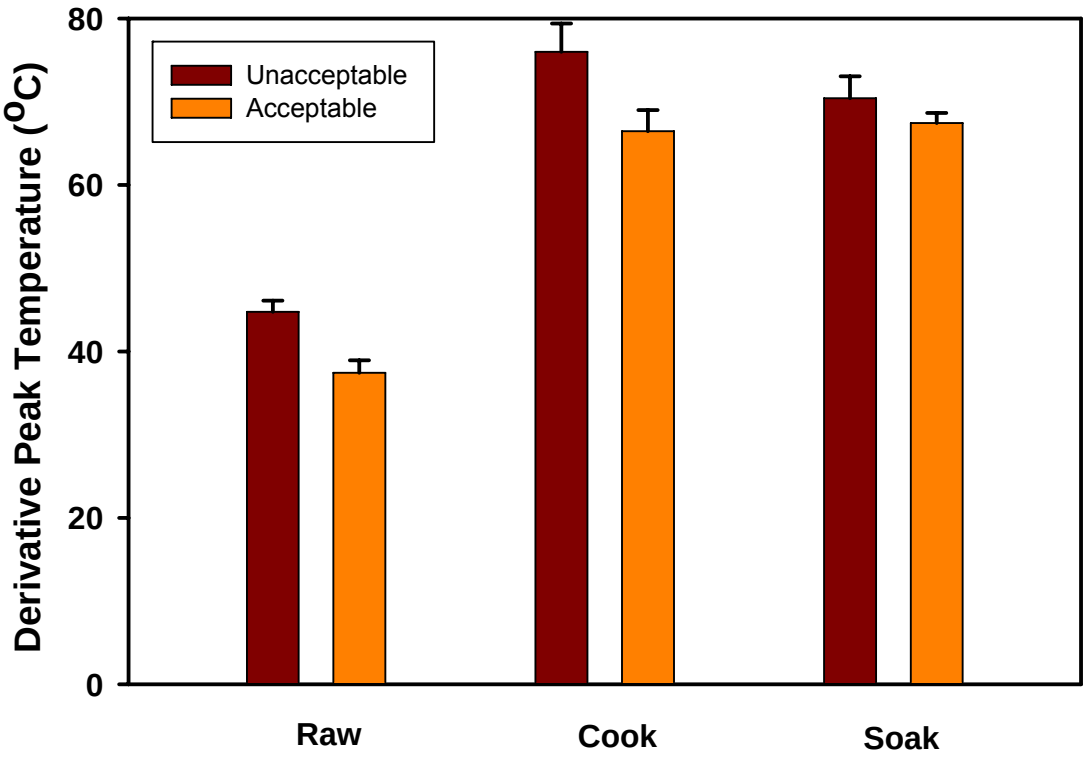
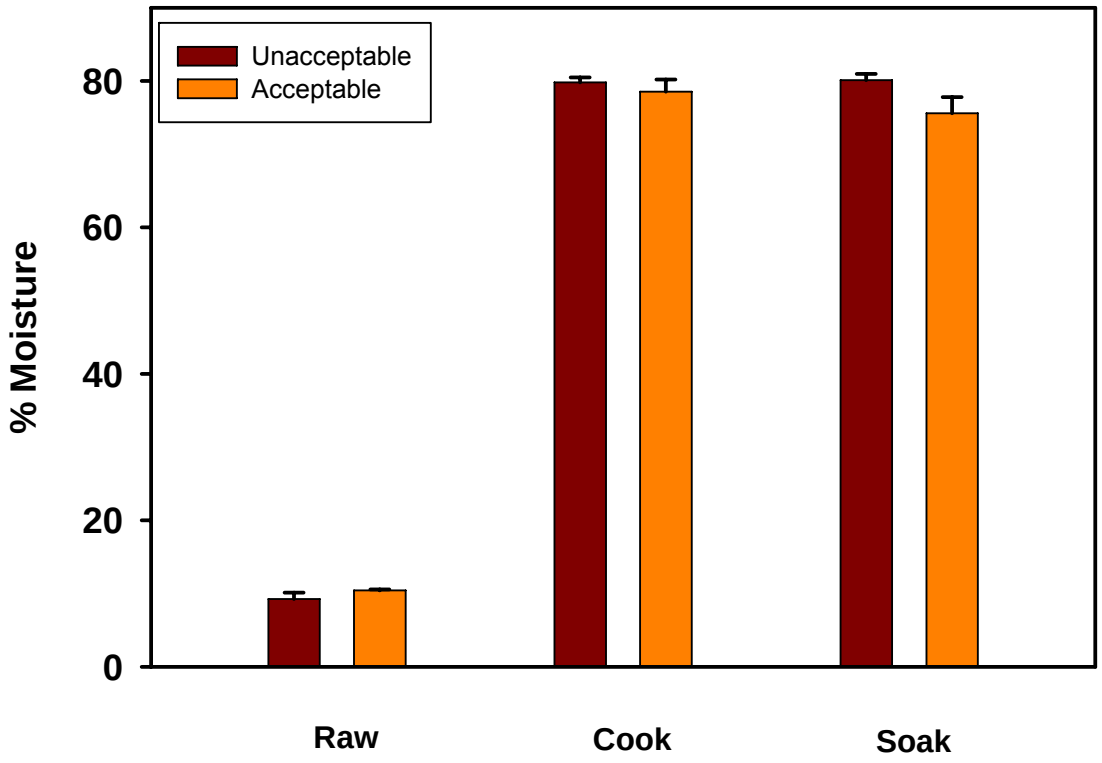


Table 5.12. Average TGA Moisture for Pericarp During Processing



compared to the acceptable in TGA peak temperature; both were similar in moisture content. As mentioned previously, this stage seemed to be the most important processing step for altering the pericarp. Thus, the cook step would naturally accentuate small variations between samples. After the soak step, the disparity narrowed for the TGA peak maximum with the peak of water removal from the unacceptable bran occurring at 3°C higher temperatures (Tb 5.11). Moisture content was also ~5% more in unacceptable corn (Tb 5.12).

5.1.2 *Thermal analysis: Differential Scanning Calorimetry*

To assess effects of processing on kernel structure and distinguish between acceptable and unacceptable corn material properties, the kernel was divided into germ, hard endosperm, soft endosperm, and pericarp fractions for differential scanning calorimetry analysis. Figure 5.5 represents the thermal transitions occurring at raw, cook, and soak steps for acceptable and unacceptable corn. Cooking increased the germ "freezable water" at ~0.0°C which overlapped the endothermic peak occurring at -20°C to 0°C in the raw state. No other major change in thermal properties was observed (Fig 5.5). This is in accordance with TGA data which displayed a large increase in moisture content following cook, but little change in the state of the water throughout processing. Figure 5.6 reveals the thermal transitions of acceptable and unacceptable hard endosperm from raw to soak. The cooked freezable water greatly increased as evidenced by the appearance of an exothermic peak at ~O°C after cooking (Fig 5.6, Tb 5.13). Little appreciable change occurred in enthalpy and broadening of the endothermic peak at ~0°C from cook to soak, indicating that the amount of loosely associated water is not further affected by soaking (Tb 5.13).

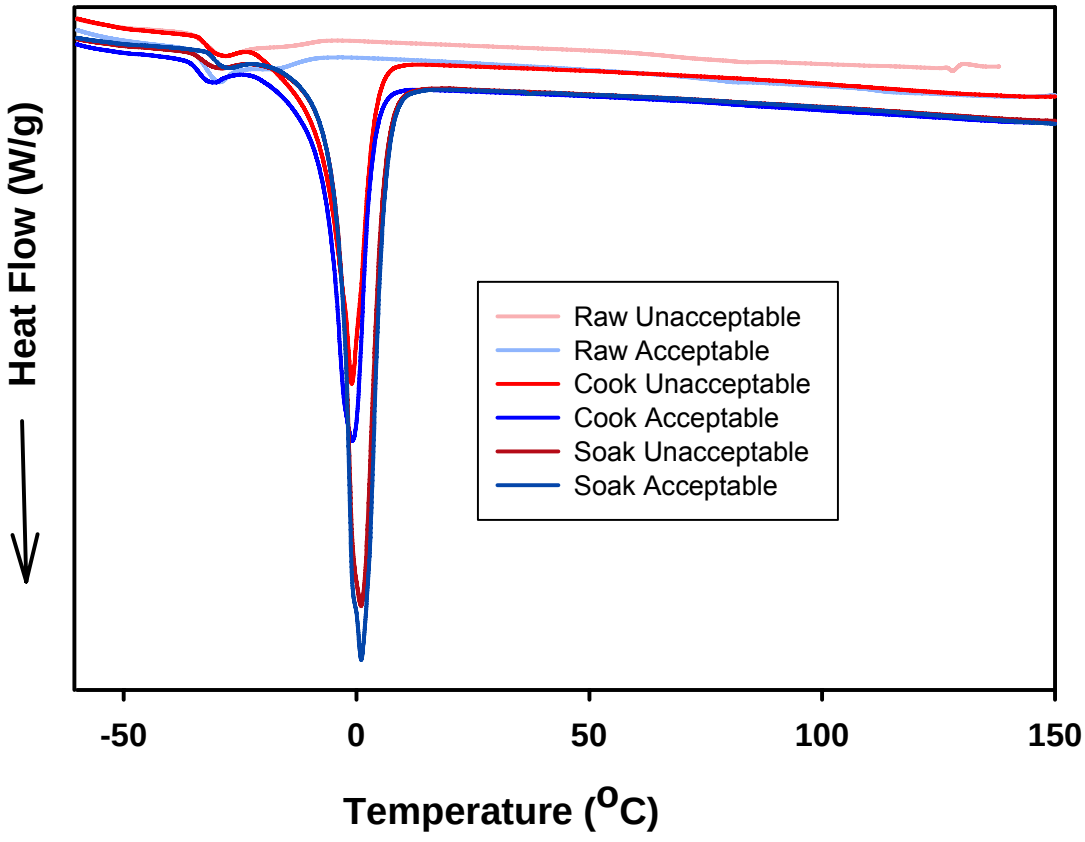


Figure 5.5. DSC Thermogram of Unacceptable and Acceptable Germ from -55°C to 150°C

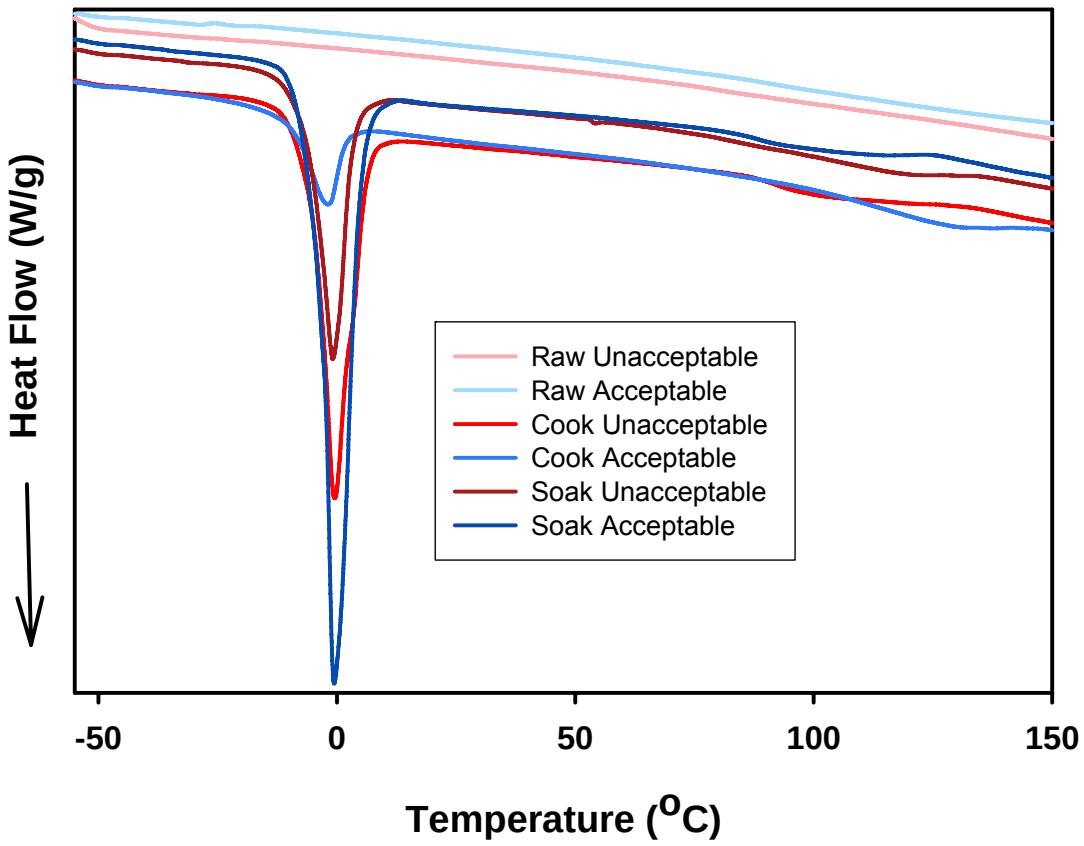


Figure 5.6. DSC Thermogram of Unacceptable and Acceptable Hard Endosperm from -55°C to 150°C

Table 5.13. Enthalpy Values, Peak Onset, and Peak End for Freezable Water Component in Kernel Fractions During Processing

Sample	Enthalpy at ~O°C (J/g)	SD	Peak Onset (°C)	SD	Peak End (°C)	SD
Raw Germ	0.0	*	N/A	*	N/A	*
Cook Germ	125.9	15.7	-5.5	2.7	-0.4	0.8
Soak Germ	141.5	38.1	-3.2	0.7	0.8	1.1
Raw Hard Endosperm	0.0	*	N/A	*	N/A	*
Cook Hard Endosperm	32.4	29.6	-5.6	5.1	-0.4	1.8
Soak Hard Endosperm	68.6	34.9	-2.3	0.5	-1.2	0.2
Raw Soft Endosperm	0.0	*	N/A	*	N/A	*
Cook Soft Endosperm	52.2	81.5	-7.4	9.5	-1.1	3.7
Soak Soft Endosperm	64.5	29.5	-1.8	0.1	0.7	0.9
Raw Pericarp	0.0	*	N/A	*	N/A	*
Cook Pericarp	242.8	4.8	-2.6	0.2	0.6	0.2
Soak Pericarp	254.2	13.7	-2.5	0.1	1.1	1.0

Figure 5.7 displays a transitional difference at -35°C to -25°C. In the raw state, this transition begins at ~ -28°C and a second transition begins at -20°C for both acceptable and unacceptable hard endosperm (Figure 5.7). Upon processing, a slight variation in the ~ -30°C transitional shift between acceptable and unacceptable soak hard endosperm can be observed (Fig 5.8). Comparing the unacceptable cook and soak hard endosperm in Figure 5.9, it seems the broadened transition was not apparent during the cook phase, so soaking may have assisted this enlargement or plasticization of this transition. However, soaking does not change the state of water to a great degree due to consistency in enthalpy values at ~0°C and peak onset and peak end temperatures (Tb 5.13).

At 30°C to 45°C, another important endothermic peak occurs (Fig 5.10). This is less apparent in the raw, but is accentuated by subsequent cooking and soaking (Fig 5.10) as exhibited by a larger enthalpic peak.

In Figure 5.10, the endothermic peak between 35°C and 55°C in unacceptable raw pericarp may have shifted 10°C lower in acceptable raw hard endosperm. However, it is difficult to confirm that both endothermic peaks are representing the same transition. This thermal range will be important to analyze with future replications to confirm if this phenomena is repeatable.

The DSC thermogram for soft endosperm is exhibited in Figure 5.11. No observable endothermic peak is found at ~O°C in raw hard endosperm (Fig 5.11., Tb 5.13). Following the cooking process, a modest thermal peak (43.4 J/g +/- 29.6) relating to “freezable water” was apparent and also following soak (68.6 J/g +/- 34.9) (Fig 5.11). Also apparent is the emphasis of the transition at 75-85°C following soaking in both

acceptable and unacceptable samples (Fig 5.11). Figure 5.11 presents another transition at 40°C.

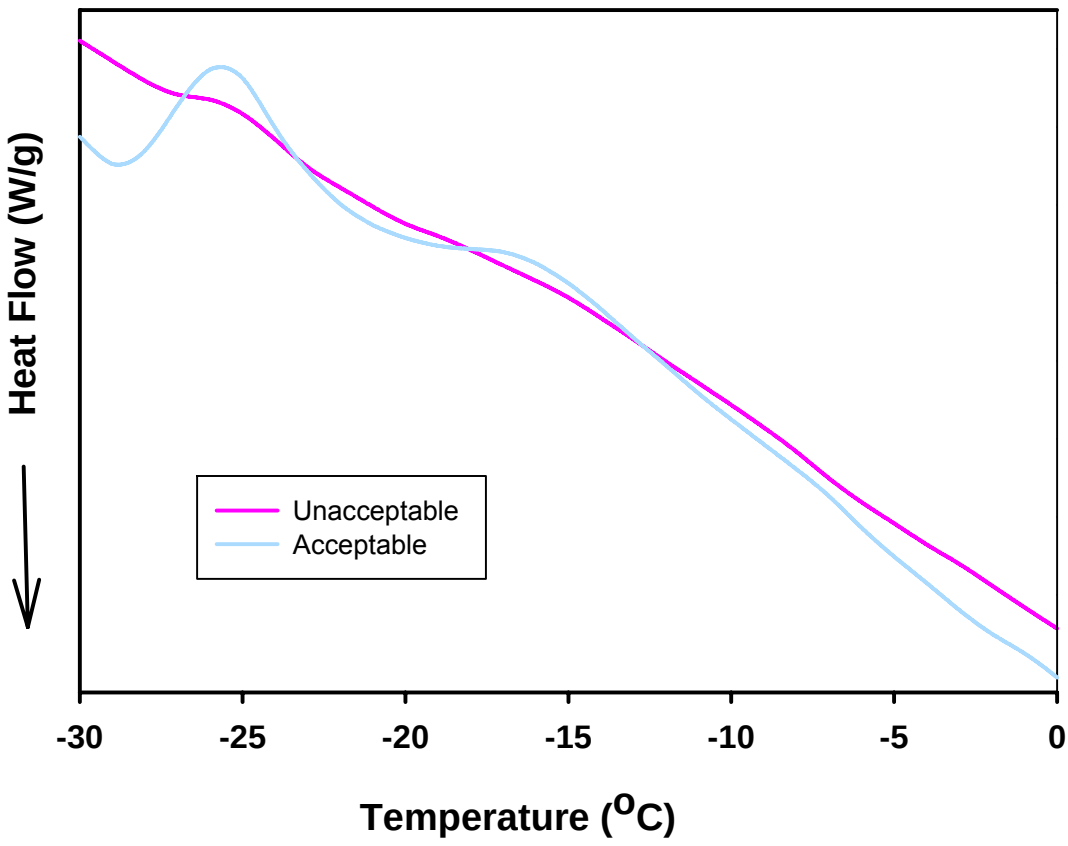


Figure 5.7. DSC Thermogram of Raw Hard Endosperm from -30 to 0°C

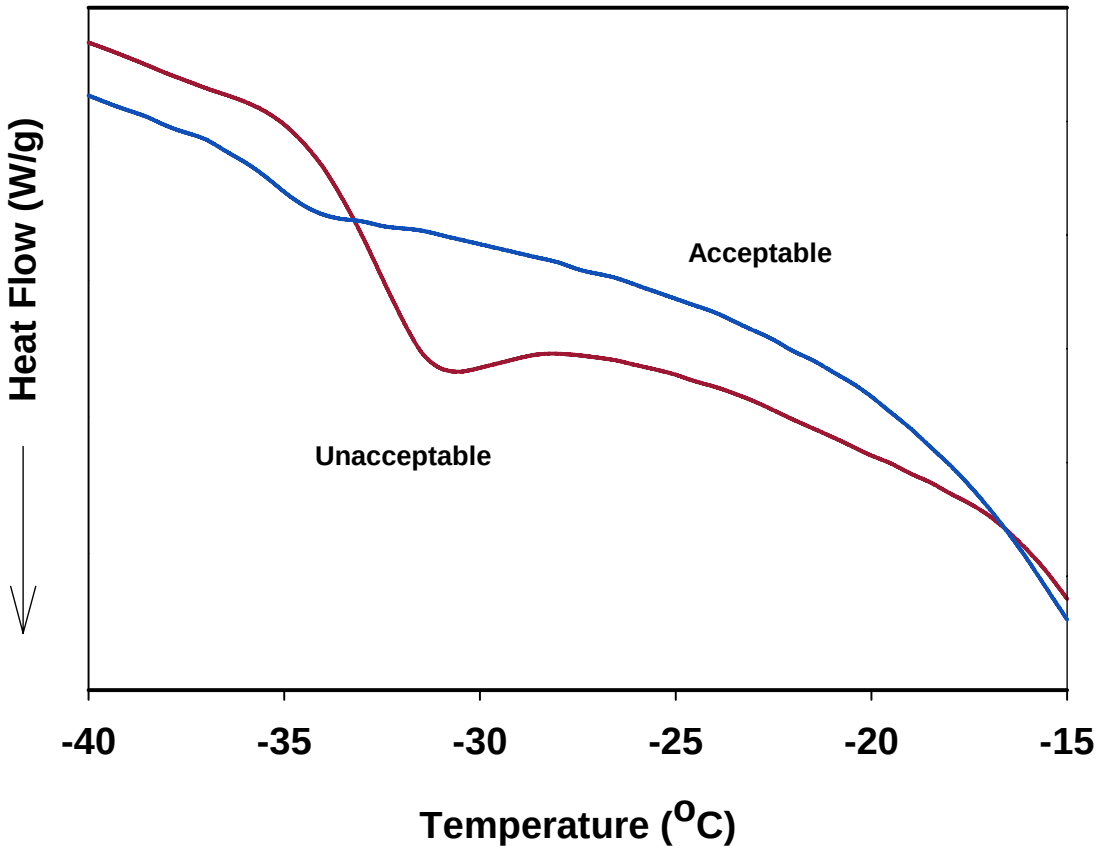


Figure 5.8. DSC Thermogram of Soak Hard Endosperm from -40 to -15°C

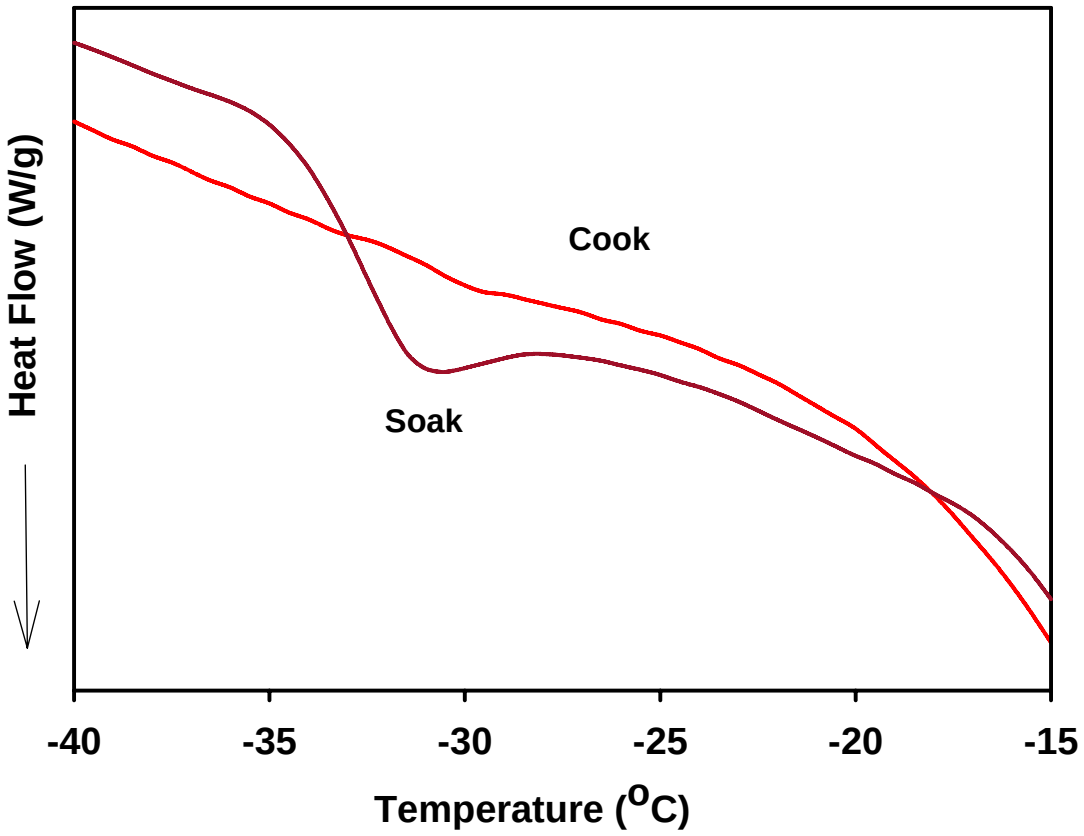


Figure 5.9. DSC Thermogram of Unacceptable Hard Endosperm During Processing

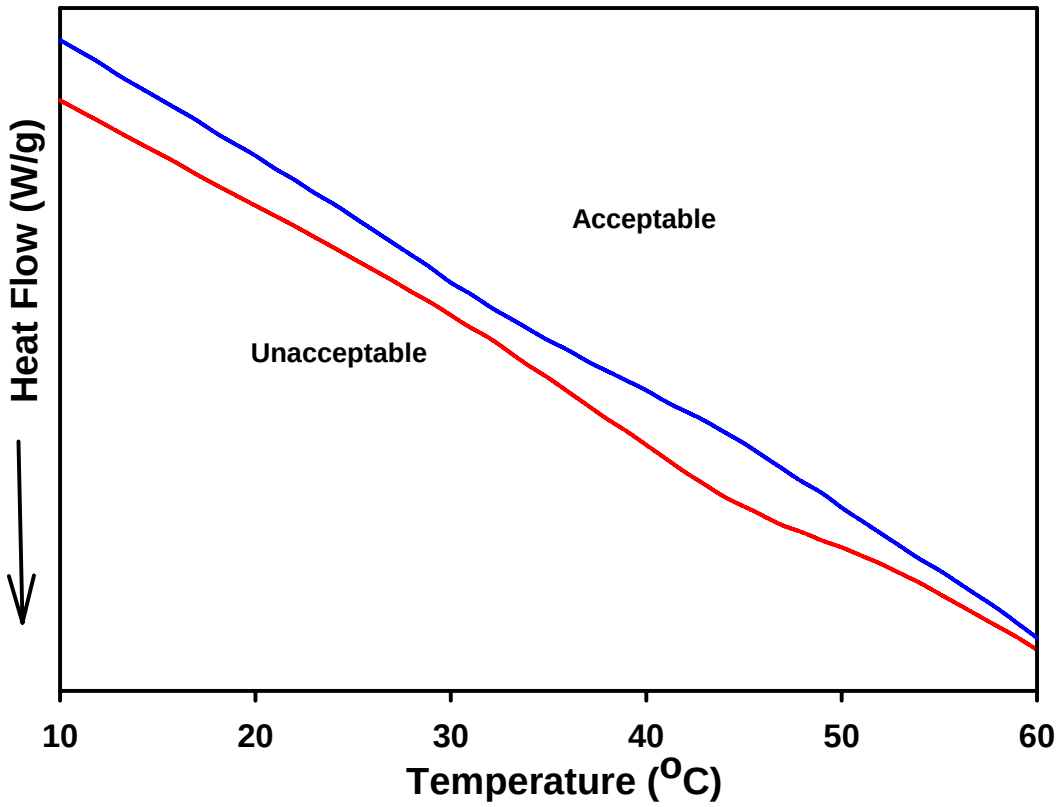


Figure 5.10. DSC Thermogram of Raw Hard Endosperm from 10°C to 60°C

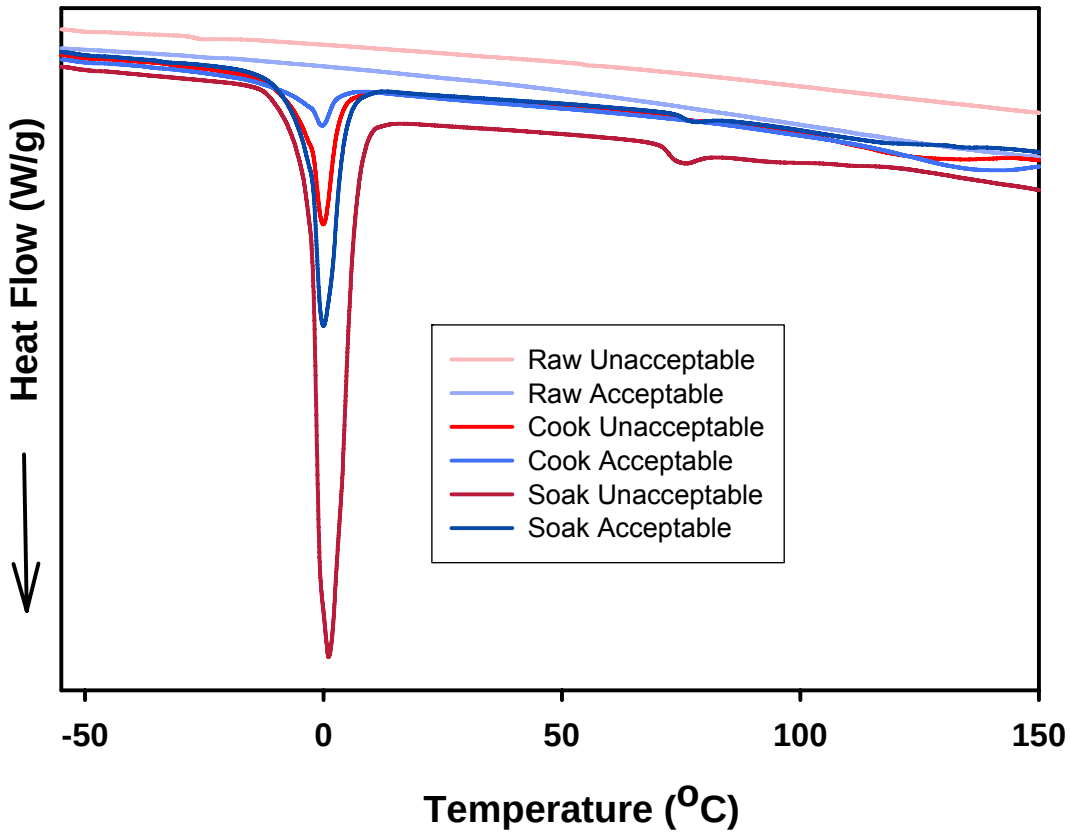


Figure 5.11. DSC Thermogram of Unacceptable and Acceptable Soft Endosperm from -55°C to 150°C

45°C in the raw soft endosperm. This endothermic transition is shifted to slightly lower temperatures in the unacceptable soft endosperm.

The DSC thermogram for pericarp is shown in Figure 5.12. Again, both the cook and soak show a rise in freezable water at ~0°C which is congruent with TGA data

recording a two-fold increase in moisture content (Tb 5.12). Although the TGA peak temperature likewise increased (Tb 5.4) after cooking, a portion of this gain in water was still free enough to partition into ice at ~O°C.

Figure 5.13 highlights a shift in two transition pericarps between -50°C to -20°C from raw to cook. The acceptable and unacceptable cook pericarps display slightly different thermograms (Fig 5.14) in which the acceptable pericarp is shifted to the lower temperature by ~5-10°C. The higher transition in unacceptable pericarp suggests that it is more structurally rigid in the unacceptable pericarp following cook. Because unacceptable corn must be cooked longer to remove the outer coat, possibly the material in the bran is more chemically and thus mechanically resistant to alteration during normal processing conditions. Further experiments are needed to confirm that this thermal transition is legitimately different in acceptable and unacceptable corn. If so, possibly this transition may be associated with the extent of alkaline processing needed to break down the bran.

Figure 5.15. demonstrates that both acceptable and unacceptable soak pericarps show little disparity in this transition as the initial and end endothermic temperatures for acceptable pericarp are similar (Tb 5.14). Possibly after several processing steps, the components from acceptable and unacceptable corns represented at this thermal transition are physically identical.

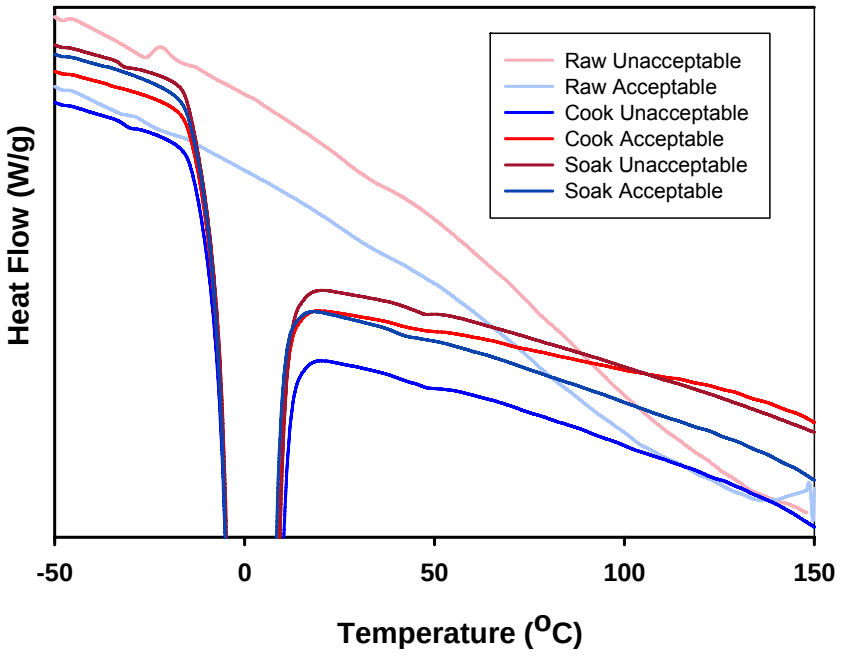


Figure 5.12. DSC Thermogram of Unacceptable and Acceptable Pericarp from -50°C to 150°C

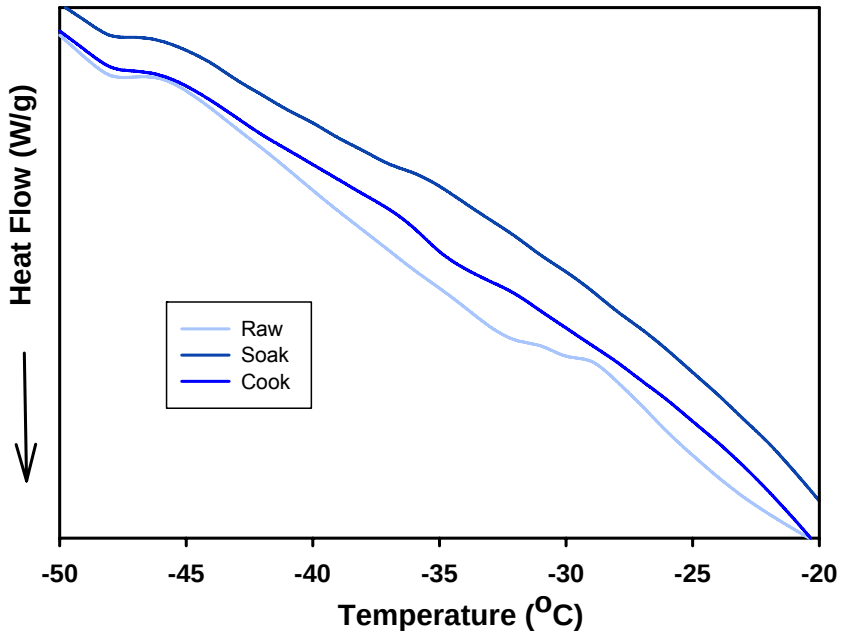


Figure 5.13. DSC Thermogram of Acceptable Pericarp from -50°C to -20°C

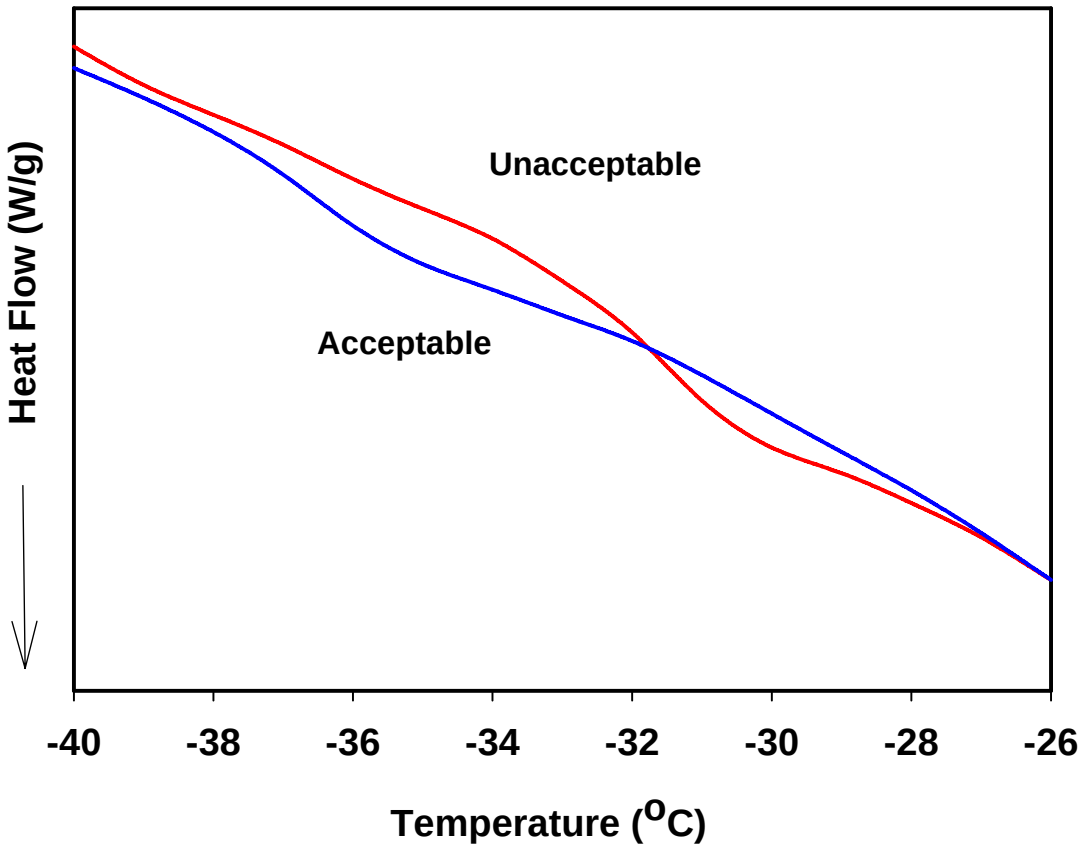


Figure 5.14. DSC Thermogram of Cook Pericarp During from -40°C to -26°C

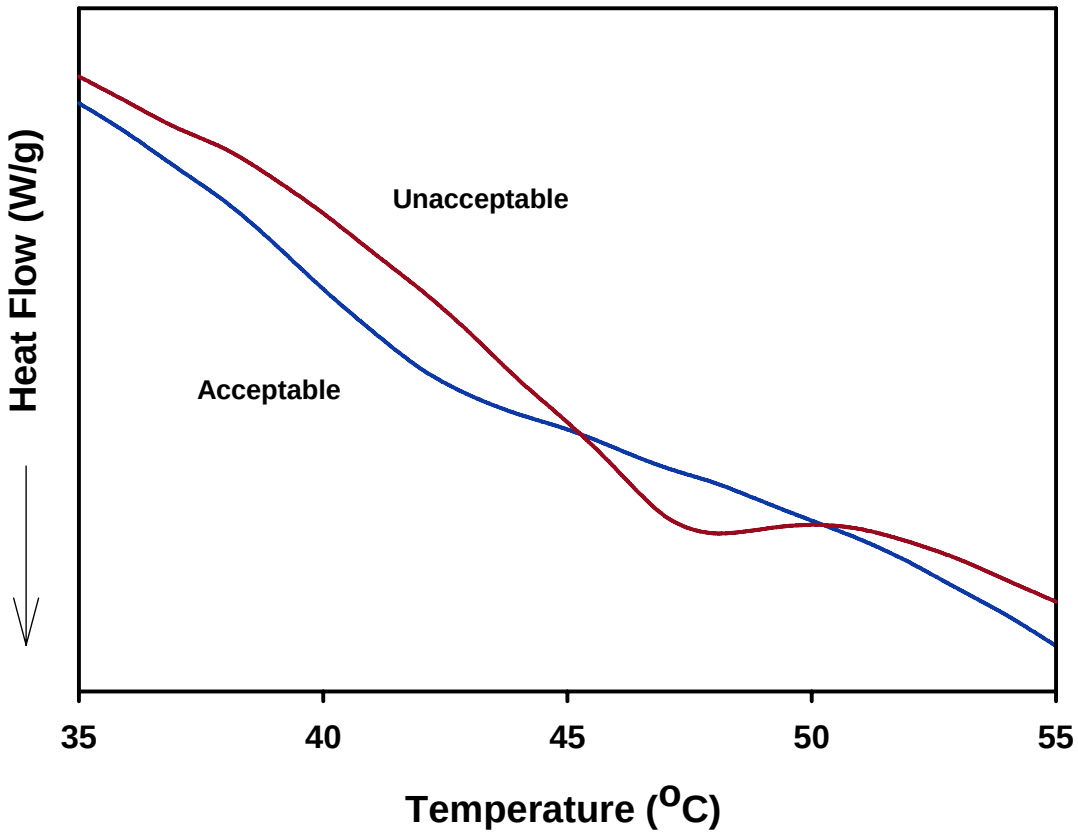


Figure 5.15. DSC Thermogram of Unacceptable and Acceptable Soak Pericarp from 35°C to 55°C

Table 5.14. Enthalpy and Peak Onset and Peak End Temperature for Exothermic Transition at 40°C to 55°C

Sample	Enthalpy (J/g)	SD	Peak Onset (°C)	SD	Peak End (°C)	SD
Unacceptable Soak Pericarp	0.2	0.2	43.5	1.8	47.6	0.5
Acceptable Soak Pericarp	0.2	0.4	40.0	4.0	44.8	2.8

5.1.3 *Rheological Analysis: Creep Testing*

To objectively characterize the structural composition of acceptable and unacceptable masa, a creep test was conducted. Results from Figure 5.16 indicate that unacceptable masa demonstrated less recovery when compared to acceptable masa. Physically, masa should have a certain degree of visco-elasticity to allow proper machinability (Guo 1999). Rheological analysis may thus be helpful in distinguishing the textural attribute that contributes to successful processing for tortilla chips.

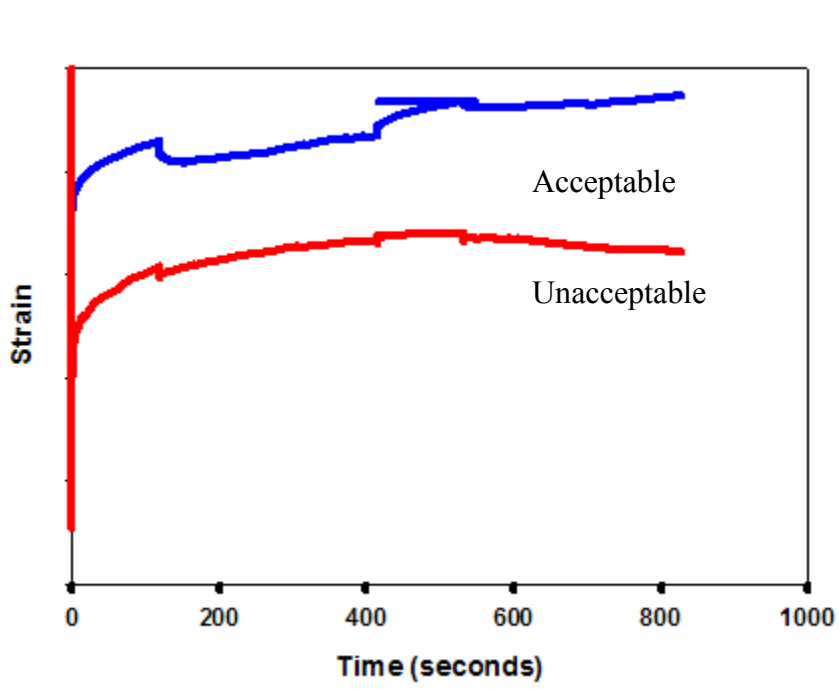


Figure 5.16. Creep data of masa produced from acceptable and unacceptable corn

5.2 Aim 2. Compare physico-chemical measurements of grain and determine which factors are most critical for predicting corn quality for nixtamalization purposes

5.2.1 Textural and Moisture Composition of Masa

A common problem in the snack food industry occurs during the sheeting of masa; dough is too adhesive and remains on rollers due to overcooking the starch (Ramirez-Wong and others 1993). In some cases, processors may purposely extend cooking because of difficult-to-remove pericarps which can lead to difficulties by hanging onto wire cutters if not removed (Sahai and Jackson 2001). Other factors influencing the extent of cooking include presence of stress cracks and overall kernel hardness (Jackson and others 1988, Paulsen and others 2003). In this study, a snack food company categorized acceptable and unacceptable corn by determining the overall ease of sheeting using typical large-scale nixtamalization process and equipment. The results in Table 5.15 characterize the textural properties of benchtop-produced masa from acceptable and unacceptable corn and an additional corn variety in consideration for snack food processing. Because water uptake is a major phenomenon that occurs during nixtamalization, measuring the moisture content of masa should give an indication of the extent of processing. The ideal moisture range is 40-50% (Paulsen and others 2003). All three MC values (41.96-45.11%) fell within this region, but only the experimental corn was significantly different from the other two ($p < 0.05$). Because the overall goal was to distinguish acceptable from unacceptable corn, masa moisture content seems to be a less relevant factor (Tb 5.15**)**.

However, adhesion values were significantly different ($p < 0.05$) for unacceptable masa in comparison to acceptable and experimental masa. Because stickiness during

Table 5.15 Masa Texture and Moisture Composition

Corn Sample	Masa Moisture Content (wb)	Adhesion (g-F)	Yield Stress (1x105 Pa)
Acceptable	43.88 [ab]	7.74 [a]	4.72 [a]
Experimental	41.96 [a]	8.30 [a]	7.20 [b]
Unacceptable	45.11 [b]	21.85 [b]	3.42 [a]

([a], [b] are significantly different at 95% α-level)

processing was the main distinguishing factor at the snack food plant, it was expected that the unacceptable masa would exhibit markedly different textural characteristics. The texture analysis results utilizing the TAX.T2 results in Table 5.15 quantifies the degree of adhesion that currently is subjectively evaluated in industry.

Rheological data on yield stress does not distinguish between unacceptable and acceptable masa, but does separate experimental as different ($p < 0.05$). Again, this statistical variation does not assist in quality assessment. Although rheological data is extremely useful for describing the behavior of food materials, the yield value obtained by oscillatory stress sweeping only defines one aspect of the material's physical structure. In this case, the uni-axial compression-tension of the TAX.T2 seems to be better suited for describing the masa processing quality. Figure 5.17 display creep deformation and

recovery of masa. Much variation exists within the categories, so it is difficult to definitively make a conclusion, the best examples of each category are represented. Generally, masa experiences a small degree of irreversible deformation when shear is applied, as evidenced by partial recovery in samples (Fig 5.17). Thus, the masa, regardless of category, retains some visco-elastic properties, but is partially altered through mechanical changes.

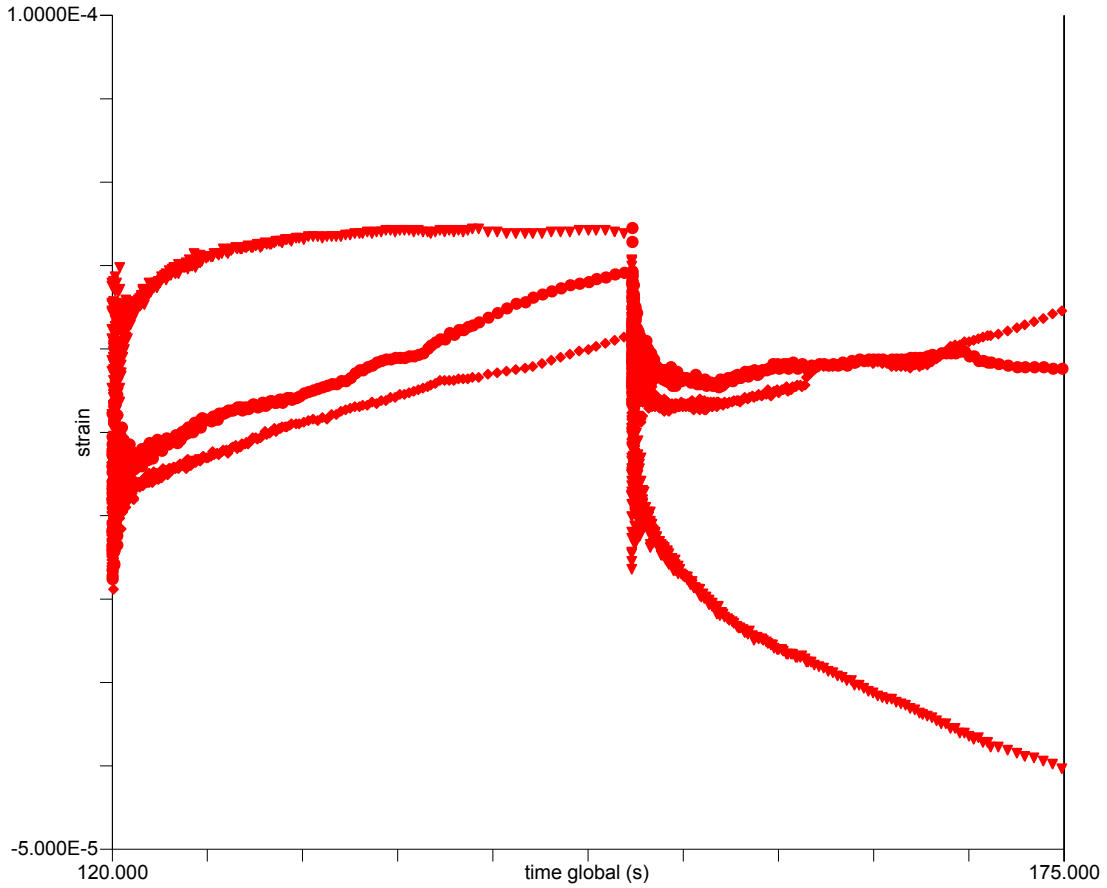


Figure 5.17 Creep Deformation and Recovery in Acceptable, Unacceptable, and Experimental Masa

As NIR technology is gaining popularity in the grain industry for assessing kernel composition, it was important to demonstrate whether this tool was useful for distinguishing acceptable and unacceptable corn (Paulsen and others 2003). Oil demonstrated a significant difference for experimental corn, ($p < 0.05$), but did not discriminate between acceptable and unacceptable. Protein content was significantly lower ($p < 0.05$) in unacceptable corn (Tb 5.16).

Based on total protein, unacceptable corn would contain 86.4% and 90% of the protein in acceptable and experimental samples. Direct density measurement and related testing attempts to estimate the hard-to-soft endosperm ratio, with higher proportions more desirable for snack food processing. Hard endosperm contains thicker protein surrounding the starch granules and it is thought that protein content may relate to this (U.S. Grains Council 1999). Because amount of hard and soft endosperms were not directly measured, it is only suggested that total protein differences may account for variation in hard endosperm: soft endosperm. Although the distribution of the protein can not be ascertained from NIR, results in Table 5.16 indicate that the amount of protein may play a role in masa structure. Research conducted by Jackson and Sahai (2001) support the importance of protein alteration in masa production. However, the challenge is to determine the % protein required for a food processor to establish for corn quality. Certainly more analysis with diverse sampling is needed to further verify the importance of % protein and also develop such a rubric.

Table 5.16. Corn Composition based on Near-infrared Spectroscopy

Corn Sample	Oil (%)	Protein (%)	Kernel Moisture (%)
Acceptable	2.84 [a]	6.72 [a]	12.60
Experimental	2.55 [b]	7.07 [a]	12.55
Unacceptable	2.83 [a]	6.11 [b]	12.04

([a], [b] are significantly different at 95% α-level)

The final measurement gathered from NIR analysis was total moisture (Tb 5.16). Moisture content is a routine measurement for grain elevators with moisture content maximum set at 15% to reduce mold growth during storage (Paulsen and others 2003). Amount of moisture has been shown to affect the mechanical properties of corn (Shelef and Mohsenin 1969). However, the amount of water in raw corn kernels was essentially identical ($p < 0.05$) and thus was not a major attributing factor to masa quality.

As mentioned above, one of the reasons for overcooking masa is to compensate for hard-to-remove pericarp (Ramirez-Wong and others 1993). Extensin has been implicated as a structural pericarp protein (Fritz and others 1991; Kieliszewski and Lamport 1987).

Table 5.17 demonstrates that no significant difference in hydroxyproline concentration existed between all three corn samples. Thus, the concentration of extensin protein, which is proportional to the amount of hydroxyproline, is not different. However, this measurement only relates to the protein concentration in dry matter. Data in Table 5.17

does not establish whether pericarp thickness is different between sample populations, which could factor into increased resistance to alkaline cooking.

Table 5.17 Concentration of Hydroxyproline in Pericarp

Corn Sample	µg Hydroxyproline/ mg Dry Pericarp
Acceptable	0.85
Experimental	0.98
Unacceptable	0.92

Although Table 5.16 established similar moisture content in the whole kernel, TGA results in Table 5.18 characterized the water populations within the hard endosperm and pericarp fractions. Raw hard endosperm peak temperatures varied widely between acceptable (39.3°C), experimental (11.0°C), and unacceptable samples (60.4°C) (Tb 5.18). However, due to one sample outlier in the acceptable and unacceptable fractions, this was not recorded as significantly different at the 95% confidence interval.

However, Principle Component Analysis (PCA) using SIMCA (Soft Independent Modeling of Class Analog**y)** found that of all dependent variables tested in this study, the raw hard endosperm peak temperature was the largest contributing factor for successfully

separating unacceptable and acceptable corn (Figure 5.18). Figure 5.18 shows that hard endosperm peak temperature (Tb 5.18) and adhesion show large differences between categories (Tb 5.19) for class separation. This combination of variables is statistically significant for PCA because interclass differences are 34.3, well above the cut-off interclass difference of 3 (Table 5.19). Experimental data was not included in the PCA analysis.

Table 5.18. TGA Data for Peak Temperature and Moisture Content Relating to Hard Endosperm and Pericarp During Processing

Corn Sample	Raw Hard Endosperm Peak Temperature (^{o}C)	Raw Hard Endosperm Moisture (%)	Raw Pericarp Peak Temperature(^{o}C)	Raw Pericarp Moisture (%)	Cook Hard Endosperm Peak Temperature(^{o}C)	Cook Hard Endosperm Moisture (%)	Cook Pericarp Peak Temperature (^{o}C)	Cook Pericarp Moisture (%)
Acceptable	39.3	12.71 [a]	41.67 [a]	11.05 [a]	42.03 [a]	22.45 [a b]	68.09	80.08
Experimental	55.0	10.98 [a b]	44.27 [a b]	9.60 [a]	41.35 [a]	19.23 [a]	68.00	76.02
Unacceptable	60.4	10.84 [b]	50.95 [b]	7.10 [b]	45.11 [b]	24.43 [b]	70.82	78.16

([a], [b] are significantly different at 95% α-level)

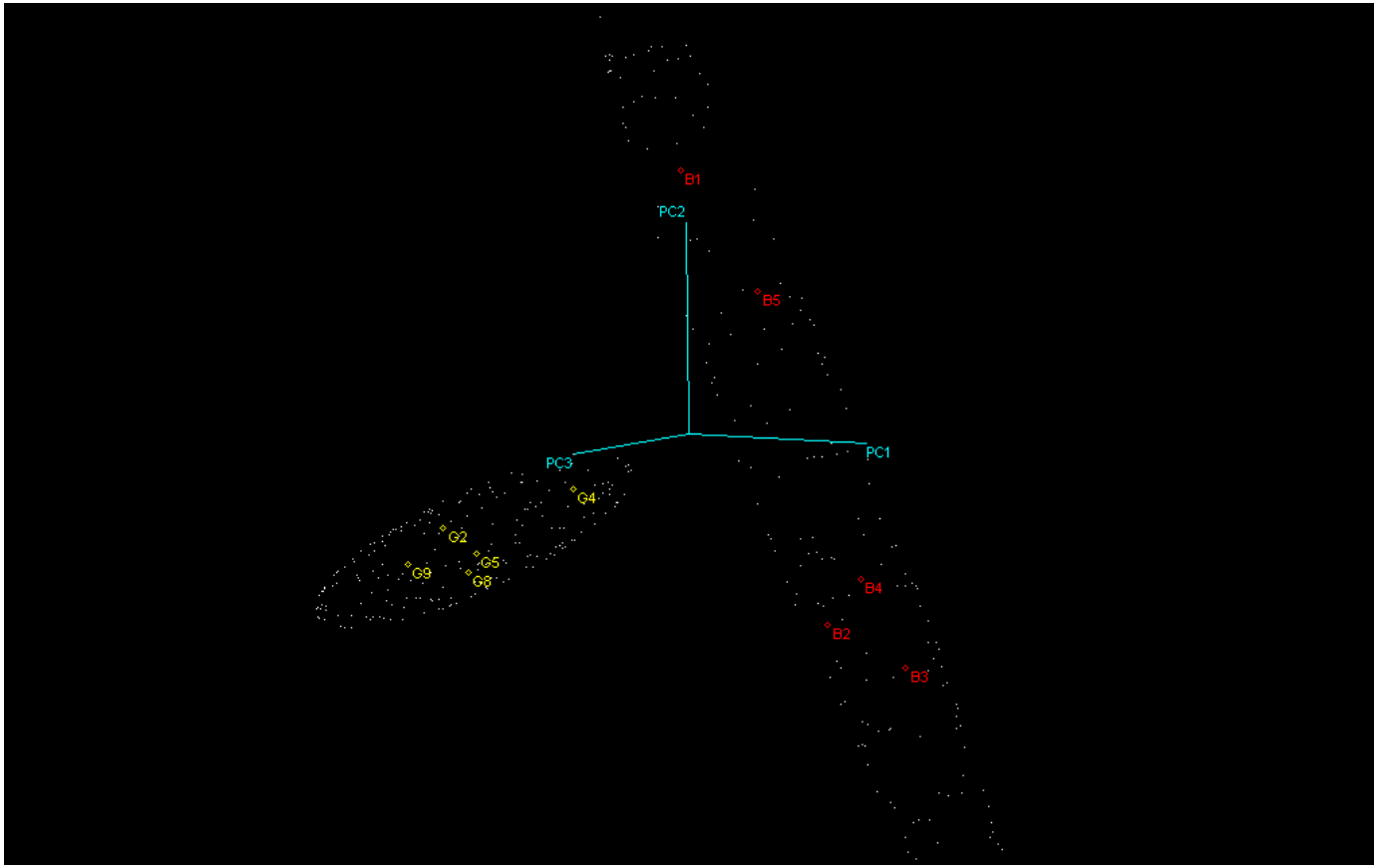


Figure 5.18 SIMCA Spatial Representation of Class Separation Based Upon Multivariate Analysis of Hard Endosperm Peak Temperature, Masa Adhesion, % Protein, and Kernel Moisture

Table 5.19. SIMCA Interclass Distances Between Acceptable and Unacceptable Variables

	Acceptable	Unacceptable
Acceptable	0.000000	34.301620
Unacceptable	34.301620	0.000000

Table 5.18 shows that the amount of moisture in hard endosperm fractions is significantly lower ($p < 0.05$), by 2%, in unacceptable grain. Such information demonstrates that moisture may be allocated differently in the corn fractions. Likewise, if hard endosperm peak temperature is truly a critical factor, the water that is present is more strongly associated in unacceptable corn as evidenced by higher peak temperatures for water removal (39.3°C versus 60.4°C). This information can be interpreted in several ways. The different entrapment of water may indicate a difference in the structural composition of the hard endosperm that affects how it interacts with the liquid component. This structural variation may be inherent during plant growth. However, post-harvest conditions, especially artificial drying, may alter kernel structure. If too high of heat is applied during drying of corn, stress fractures may develop in the hard endosperm and consequently may affect moisture distribution (Jackson and others 1988). To rule out this scenario, evaluation of stress cracks in samples should be conducted.

Following cooking, hard endosperm in unacceptable kernels likewise demonstrated a statistically higher ($p < 0.05$) peak temperature for water loss, although this TGA difference was only ~3°C higher. It is interesting to note that the hard endosperm moisture content was statistically similar between unacceptable and acceptable hard endosperm, but TGA results suggest the water may be associated differently (Tb 5.18). This may help explain the stickiness in the resulting masa. Although the masa moisture content was not statistically different between samples (Tb 5.16), the state of the water in the masa matrix may affect the stickiness. TGA data on masa would be interesting to evaluate, although a ~20 mg or less sample may not be representative of the heterogeneous dough. Possibly the unacceptable hard endosperm in Table 5.18 was more gelatinized after cooking, which would have strengthened the fraction's interaction with water.

Raw pericarp peak temperatures also indicate that unacceptable corn is statistically different from acceptable kernels, but not experimental. Although pericarp contains relatively low moisture, the little water that is present is more entrapped because it required ~9°C higher temperature to reach peak water loss (Tb 5.18). The unacceptable pericarp also contains significantly ($p<0.05$) less moisture overall (7.10%) when compared to the acceptable (11.05%) and experimental (9.6%) pericarp. Again, the TGA data may show small differences in the pericarp structure and storage (Tb 5.18). However, little statistically difference in TGA pericarp peak temperature and moisture exists between any of the corn categories after cooking. Results suggest that although small structural differences may be present in the raw state, cooking may transform pericarp into structurally similar materials (Tb 5.18). Overall, TGA analysis was

successful in uncovering differences in the water state and composition between acceptable and unacceptable corn.

DSC data is displayed for 5 transitions occurring in raw hard endosperm in Table 5.20. The only significant difference ($p < 0.05$) of note occurred in transition 4 at the onset. Acceptable hard endosperm began this transformation at 32°C while this was shifted 10°C lower in unacceptable pericarp. A difference in the location of thermal transitions indicates that the structure of the hard endosperm may be different between acceptable and unacceptable hard endosperms. This is supported by earlier TGA data in regard to water entrapment (Tb 5.18). The shift of a thermal transition to a lower temperature in unacceptable hard endosperm is especially interesting to note; glass transitions shift to lower temperatures if more water is present because water acts as a plasticizer. However, the unacceptable hard endosperm contains statistically less moisture (Tb 5.18).

Table 5.20. DSC Thermal Transitions in Hard Endosperm

Corn Sample	Exothermic Transition 1: Onset (°C)	Exothermic Enthalpy (J/g)	Exothermic Transition 1: Peak (°C)	Exothermic Transition 2: Onset (°C)	Exothermic Enthalpy (J/g)	Exothermic Transition 2: Peak (°C)	Water Endothermic Transition 3: Onset (°C)
Acceptable	-28.31 [a]	0.1 [a]	-24.8 [a]	-19.1 [a]	0.1 [a]	-15.2 [a]	-3.9 [a]
Experimental	-27.42 [a]	0.4 [a]	-23.6 [a]	-18.6 [a]	0.1 [a]	-13.8 [a]	-5.5 [b]
Unacceptable	-29.18 [a]	0.3 [a]	-24.1 [a]	-20.4 [a]	0.2 [a]	-15.74 [a]	-2.7 [a]

Corn Sample	Water Endothermic Enthalpy (J/g)	Endothermic Transition 4: Onset (°C)	Endothermic Enthalpy (J/g)	Endothermic Transition 4: Peak (°C)	Endothermic Transition 5: Onset (°C)	Endothermic Enthalpy (J/g)	Endothermic Transition 5: Peak (°C)
Acceptable	0.01 [a]	32.0 [a]	0.1 [a]	38.2 [a]	49.7 [a]	0.16 [a]	56.9 [a]
Experimental	0.00 [a b]	28.5 [a b]	0.1 [a]	36.4 [a]	50.9 [a]	0.01 [a]	53.9 [a]
Unacceptable	0.00 [b]	22.2 [b]	0.1 [a]	31.1 [a]	50.7 [a]	0.01 [a]	55.3 [a]

([a], [b] are significantly different at 95% α-level)

CHAPTER 6

CONCLUSIONS

Aim 1. Understanding physico-chemical changes during critical steps in nixtamalization and differentiating acceptable and unacceptable corn using thermal and rheological analysis

A variety of thermal and rheological instrumentation was used to characterize the water population and material behavior of four corn kernel fractions and masa during nixtamalization. TGA was used to illustrate the increase in water uptake and degree of water entrapment of each material. In the raw state, germ and pericarp displayed both the lowest moisture content and also the highest degree of water association.

Upon cooking, all four fractions greatly increased in moisture content, indicating the importance of alkaline heating in kernel transformation. Although the germ and pericarp ranked higher in moisture content following cooking, water was not entrapped any differently for the germ compared to its raw state. The pericarp, however, experienced greater water association which was evidenced by an increase in TGA peak temperature. This interaction was likely due to the solubilization of heteroxylan and lignan components. Increase in water entrapment for both endosperms was attributed to gelatinization.

Soaking caused little increase in moisture content except for the soft endosperm which likewise saw an increase in moisture association. Thus, it can be assumed that the main role of soaking was to continue transformation of the soft endosperm. Because soaking occurred at 40°C, below gelatinization temperature, and for an extended time (12 hr), diffusion may have been the primary mode of action for affecting water population.

When evaluating the effects of nixtamalization on unacceptable corn, similar trends were experienced in regard to moisture movement and matrix interaction. Between acceptable and unacceptable kernels, one note of difference was the 6°C shift upward in TGA peak temperature for unacceptable soak soft endosperm. Because soaking tended to alter the soft endosperm to a greater degree than other fractions, it is reasonable that kernel differences would further be accentuated after this step. Overall, the pericarp of acceptable and unacceptable corn showed great distinction with higher TGA peak temperatures in both raw and cook state for unacceptable pericarp. Soaking pericarp reduced the disparity in water entrapment between the two classes.

After evaluating DSC data, a few conclusions regarding processing can be made. Freezable water increased in all corn fractions following cooking, but did not increase greatly following soak. This is supported by little change in TGA MC and peak values for corresponding fractions. Transitions that may possibly show differences in thermal location between acceptable and unacceptable corn were found in the hard endosperm at ~ -30°C and also at ~30°C. Pericarp displayed possible variations between corn types at -35°C and at 30°C. To confirm these differences in thermal behavior of unacceptable and acceptable pericarp and hard endosperm, additional replication is needed.

With a greater understanding of the physicochemical changes occurring in the nixtamalized corn kernel, further investigation should focus on the pericarp and hard endosperm fractions at raw and cook states. Both of these tissues displayed possible shifts in DSC transitions between acceptable and unacceptable fractions and cooking was indicated as the critical step for kernel transformation with regard to modifying water population (DSC and TGA).

Creep testing also showed promise in characterizing masa with different textural attributes. Acceptable masa tended to exhibit greater recovery, indicating more visco-elasticity of the dough.

Aim 2. Assessing critical factors for measuring corn quality as it relates to acceptable masa production

To evaluate the structural composition distinguishing acceptable corn from unacceptable corn, a combination of instrumental techniques was employed to determine which measurement/s was the best quality indicator. Although experimental samples may have exhibited values significantly different from both acceptable and unacceptable samples, only those results showing a statistical difference between the latter two categories were considered worthy of noting.

Because the suitability of grain is based on the resulting machinability during masa sheeting, an objective method to quantitatively characterize the textural properties of the dough was a key objective. The four masa measurements were moisture content, adhesion, yield stress, and creep testing. Of these factors, adhesion values obtained with the TAX.T2 Texture Analyzer were significantly higher in unacceptable masa, which

numerically represented different degrees of stickiness as experienced by snack food manufacturers. Masa moisture content was within the 40-50% acceptable range for all three samples and did not show statistical difference. Dynamic rheology including yield stress determined from stress sweeping and creep testing did not show any definitive difference between acceptable and unacceptable. Creep data exhibited large variability in sampling, but some results indicated that masa displayed a certain degree of visco-elasticity as observed in partial recovery following creep deformation. Possible experimental error may include sample variability and slippage. Overall, adhesiveness testing best distinguished acceptable and unacceptable masa and was the second highest principle component in separating classes.

Due to increasing implementation of NIR spectroscopy in corn commodity evaluation, this technology was investigated to ascertain its relevance in classifying acceptable and unacceptable corn for snack food production. Oil and moisture composition were similar between acceptable and unacceptable sample categories. However, % protein was statistically lower in unacceptable kernels at 6.11% compared to 6.72% and 7.07% in acceptable and experimental samples. SIMCA analysis assigned this factor as a minor principle component in separating corn classes. Because the protein component of corn has been implicated in the formation of masa with proper textural attributes, it is not surprising that this one component displayed some variation. The amount of protein present may indicate ratio of hard-to-soft endosperm in kernels, but density and direct measurement testing would be necessary to support this hypothesis. Likewise, determining at what % concentration corn protein produces unmachinable

masa would require further study with multiple replications before a standard was established.

In addition to masa texture, one concern the food processor conveyed was the difficulty in removing the pericarp in unacceptable corn. This problem led to overcooking the batch of corn. Because the protein extensin has been associated with pericarp structural integrity, its concentration in the seed coat was quantified by extracting the protein. Extensin was then degraded into its base amino acids and hydroxyproline concentration was quantified with a colorimetric method. No significant difference in hydroxyproline concentration based on the dry weight of the pericarp existed. However, this does not rule out the possibility that pericarp of unacceptable and acceptable kernels differ in thickness.

TGA results described the water content and state for both pericarp and hard endosperm fractions at raw and cook steps. Although no significance was found in the raw hard endosperm peak temperature due to outliers skewing the data, the peak temperature in acceptable corn was at 39.3°C while the unacceptable displayed a peak temperature at 60.4°C. SIMCA software compared all the measurements in this study with PCA and concluded that hard endosperm peak temperature was the largest principle component in separating unacceptable and acceptable samples. Thus, although not statistically significant when analyzed using a One-way ANOVA and Tukey's Post-Hoc Test, this measurement may still be a critical key in characterizing corn quality. Higher peak temperature values in unacceptable hard endosperm demonstrated that water was more entrapped in the matrix and thus indicated possible differences in structural

properties of the kernels. Moisture content was also significantly lower in unacceptable hard endosperm. The raw pericarp fraction was also significantly higher in peak temperature and contained a lower moisture content. Again, possible differences in structure may affect the state of water in the matrix. Whether this is due to environmental conditions in the field or post-harvest practices, is unknown. Assessing the amount of stress cracks may give indication to the sample history regarding drying temperature abuse.

At the cook stage, only the hard endosperm demonstrated a statistical significance of a 3°C peak increase for unacceptable hard endosperm, indicating a slightly higher degree of association. Cook hard endosperm moisture content, cook pericarp peak temperature, and cook pericarp moisture did not show significant difference between acceptable and unacceptable corn. Cooking may cause pericarp structure to become more similar.

DSC data displayed 4-5 major thermal transitions in the raw hard endosperm. However, the only statistically significant transition to note was a shift from 32°C onset in acceptable corn to 22°C onset in unacceptable corn. This 10°C difference may indicate that the structure of the unacceptable hard endosperm is structurally unique from the acceptable hard endosperm. With TGA data reporting moisture content as statistically lower in unacceptable hard endosperm, it is interesting to observe that the thermal onset of a transition was decreased because less water would result in less plasticization. Thermal transitions were also observed in pericarp, but no valuable difference was observed.

Overall, many physico-chemical methods were able to distinguish between acceptable and unacceptable corn based on One-way ANOVA statistics. However, SIMCA software was able to distinguish which factors were the strongest determinants of corn quality by their ability to classify acceptable and unacceptable corn. Hard endosperm TGA peak temperature for the raw kernel and masa adhesion were the two most important components. Meanwhile, % protein provided a secondary factor to distinguish corn quality.

Future Work

In order to increase the significance of the hard endosperm peak temperature, further replicates may be conducted.

Because SIMCA analysis denoted hard endosperm peak temperature as the overarching principle component for corn quality, further understanding of the structural differences in this component would be interesting to characterize.

1. Measure kernel density or the hard-to-soft endosperm ratio which may indicate the relative amount of hard endosperm present
2. Characterize proteins in hard endosperm and whole kernel using gel electrophoresis in an effort to establish the chemical microstructure
3. Calculate prevalence of stress cracks in hard endosperm to diagnose potential damage caused by high-heat drying

If % protein determined from NIR is to be used as a quality measure for grain, further studies comparing masa quality with protein content need to be conducted on a wide range of kernel protein compositions.

Pericarp thickness can be calculated on kernels to see if this factor is related to acceptable and unacceptable corn classification.

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