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Corn Characterization and Development of a Convenient Laboratory Scale Alkaline Cooking Process

Shreya N. Sahasrabudhe
University of Nebraska-Lincoln, shreya.sahasrabudhe12@huskers.unl.edu

CORN CHARACTERIZATION AND DEVELOPMENT OF A CONVENIENT LABORATORY SCALE ALKALINE COOKING PROCESS

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Shreya Narayan Sahasrabudhe

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Shreya N. Sahasrabudhe, M.S

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Advisor: David S. Jackson

Nixtamalized (alkaline cooked) corn (*Zea mays* L.) products are increasing in popularity due to their affordable cost, ease of production, and the diversity of products that can be made using similar unit operations. The nixtamal produced after alkaline cooking depends on the processing parameters used during cooking and steeping, as well as the physicochemical properties of the corn hybrids used. Processors incur high costs in narrowing down hybrids suitable for a given process, or they must be able to adjust cooking conditions to obtain the desired end-product characteristics. Improper processing generates large quantities of waste. Researchers have developed small scale cooking procedures that can mimic industrial nixtamalization process. Many of these methods, however, still require significant quantities of grain, such that the screening processes are expensive. The primary aim of this study was to develop a small scale bench top method with 100 g corn, using simple apparatus that can be used to analyze multiple samples at a time. The method was compared to a previously established 500 g method using range of commercially used cook times, steep times and cook temperatures. Previous studies on relating physico-chemical parameters and nixtamal characteristics have concluded that it is essential to cook corn, at least in small quantities, to understand how corn will process when nixtamalized. The results indicate that the 100 g method can replicate industrial cooking process at a range of processing conditions as the intercept and slopes for

response surface models were not significantly different ($p < 0.05$). The second aim of this study was to understand the effect of physicochemical properties of nine different hybrids, grown in the same season on nixtamal characteristics using the 100 g cooking method. The study found regressions for dry matter loss with thousand kernel weight and kernel calcium content ($r^2 = 0.98$) and for nixtamal moisture with test weight ($r^2 = 0.52$) when corn was cooked for 25 min and steeped for 12 h. Consistent with previous studies, this study found that no one grain parameter can predict all nixtamalized corn properties, confirming the need to cook corn to best understand its potential alkaline cooking performance.

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Abbreviations

AACCI American Association of Cereal Chemists International

AOAC Association of Official Agricultural Chemists

db dry basis

DML Dry matter loss

DSC Differential scanning calorimetry

MVA Micro Viscosity-analyzer

RVA Rapid Viscosity Analyzer

TADD Tangential abrasive dehulling device

w/w Weight by weight

WBT Wisconsin Breakage test

TW Test Weight

Tc Conclusion/end temperature

To Onset temperature

Tp Peak temperature

INTRODUCTION

Corn (*Zea mays* L.) is the most widely produced feed grain in the United States, and it can be processed into a wide range of industrial and food products. Nixtamalization is the process of cooking corn in the presence of lime and water at a temperature of 80-100^0 C. The corn is then soaked for 8-12 h followed by washing. The cooked and steeped product is called nixtamal, which can be processed into masa by grinding. Alkaline cooked products such as tortillas, tortilla chips, tacos, tostadas, enchiladas, nachos, tamales, and others are gaining popularity.

For nixtamalization, corn is procured from different suppliers. These hybrids vary in their physical and chemical properties depending on the season, growth region, and genetics. Nixtamal with 50-51 % moisture is desired for tortillas and 46-48 % moisture for tortilla chips. Processors need to change alkaline processing conditions such as cook time, cook temperature and steep time depending on the type of hybrid, in order to obtain the desired end-product characteristics. In many cases, up to 453 kg (3000 lb.) of corn is screened in a production facility. If routine processing conditions do not yield the desired quality nixtamal, it leads to wastage of raw materials and a problem of waste disposal. Various studies have aimed at reducing the sample size required to screen corn for nixtamalization, as well as attempts to relate alkaline cook quality with inherent physico-chemical properties of corn samples. Pilot plant and lab techniques require relatively large quantities and can analyze only a single sample at a time. Studies on relating kernel properties with cook quality have concluded that it is essential to cook corn, at least in small quantities in order to understand its cooking quality. The development of a small-scale method that can analyze multiple samples at a time and uses very small sample

quantities for analysis, can help processors to analyze new hybrids at a faster pace. Processors can test very small quantities, and even breeding material to determine alkaline cook quality.

CHAPTER 1: LITERATURE REVIEW

1.1. Background

Corn (Maize- *Zea mays* L.) is the third most important crop world-wide, with a total estimated production of 8,96,371,098 metric tons (988.08 US tons) (FAO 2014). Corn, as a species originated in Mexico and spread northward to Canada and southward to Argentina. In 2013, 3.24×10^7 hectares of land was planted in US for corn production (USDA 2013). According to USDA (2013), corn is the primary US feed grain and it accounts for more than 90 % of total feed grain production and use. United States accounts for 32 % of the world production, and is by-far the largest producer of corn (NCGA 2013). Other major producers are China, Brazil, Mexico, Argentina, Central America, and many African countries. According to National Crop Growers Association (2013), an American usually consumes 11.34 kg (25 lb.) of corn annually. The cost of corn, which fluctuates based on numerous market and political factors, was 192.76 USD/metric ton in 2014 (FAO 2014). Corn can be used as a staple food for animal feed and as a raw material for industrial use. It can also be processed directly into a wide variety of food products such as tortillas, porridges, chips, arepas, empanadas, atoles, and polenta. About 12 % of the corn produced is directly consumed (e.g. Corn Chips) or indirectly consumed (e.g. High Fructose Corn Syrup) (McDonough et al. 2001, Lusas and Rooney 2001).

The corn kernel is classified as a caryopsis. It is composed of three main regions: germ, endosperm and pericarp. Knowledge about kernel structure and composition is essential for corn's efficient use for seed production, animal feed or industrial uses, and human food products (Watson et al. 2003, White and Johnson 2003). The germ,

endosperm and pericarp comprise 12 %, 82 % and 6-8 % of the dry kernel weight, respectively. The pericarp protects the kernel and helps prevent water uptake. It consists mainly of ash, fiber, oil, and small quantities of proteins and starch. The germ is mainly composed of oil, sugars, vitamins, protein and minerals. The endosperm is composed of 86-89 % starch and some protein. The aleurone layer is a single layer of cells surrounding the endosperm, and mainly contains oils, proteins, minerals, ash, vitamins, and enzymes. The endosperm can be divided into soft/ floury endosperm and hard/ horny endosperm, based on the proportion of starch and protein and their packing within the kernel. For a dent corn kernel, both types of endosperm coexist, in varying ratios. Corn can be divided into soft and hard varieties depending on the ratio of floury to horny endosperm. In the hard endosperm, starch granules are tightly packed together. The polygonal starch granules are surrounded by a protein matrix. This is also called translucent endosperm, as there are few visible air spaces; light can pass though hard endosperm easily. In the soft endosperm, starch is loosely packed and not tightly surrounded by a protein matrix. Starch granules are spherical and partially covered with a protein matrix. This type of endosperm is opaque because of the presence of visible air pockets which can diffract light in many directions. In dent corn, the peripheral hard endosperm portion has a higher protein content (11-15 %), while the center floury endosperm has a lower amount of protein (4-5 %) and higher amounts of starch Yellow and white dent corn in the US typically contain about 8-10 % protein, 3.5-4 % fat, 1.5-2 % ash, 1.5-2.1 % crude fiber, 1.4-2.0 % soluble sugars, 10-15 % water, and 65-70 % starch The composition can vary depending on genetic and environmental conditions (Hoseney 1994, Lusas and Rooney 2001, Rooney and Suhendro 2001).

1.2. Nixtamalization

Corn nixtamalization is the traditional method developed by ancient Mesoamericans for the alkaline processing of corn into table tortillas. In Mexico, 72 % of the corn produced is used for human food consumption, mainly in the form of tortillas (McDonough et al. 2001, Rooney and Suhendro 2001). The use of corn for tortilla chips and ethnic Mexican foods has increased rapidly in the US, especially since World War II. Their production is also increasing in other countries due to affordable cost, ease of production, feasibility of alkaline cooking, and diversity of products that can be made.

Nixtamalization is often considered as an art more than science. Water, containing 1 % lime is used to cook the grain for 5-50 min. This is followed by steeping for 8-16 h The use of alkali helps to effectively remove the pericarp, increasing the palatability and nutritional value of the corn kernel (McDonough et al. 2001, Rooney and Suhendro 2001). The lime-cooked corn is called nixtamal, which is next water-washed to remove excess lime and pericarp. Cook water combined with the water drained from kernels during washing is called nejayote. The washed nixtamal is then ground using a stone-type mill, sometimes with additional water added, to form a dough called masa. Masa contains about 55 % water, and is a slightly cohesive dough formed as a result of a network of solubilized starch polymers, dispersed uncooked and swollen starch granules, cell fragments, proteins, and lipids (Guzmán et al. 2011). Changes in starch during nixtamalization and grinding determine mechanical properties of masa (Gomez et al. 1992).

Ground masa is further sheeted or form-extruded before processing. It can then be baked to make tortillas, fried to make corn chips, and baked and fried for tortilla chips and taco shells (Gomez et al. 1992).

1.2.1. Nixtamalization Ingredients

Corn, water and lime are the three main ingredients used in making alkaline cooked corn based products.

1.2.1.1. Lime

Food grade lime, such as quicklime, consists mainly of calcium oxide and generally contains less than 5 % magnesium oxide. Most alkaline cooking processes use a 1 % lime to corn ratio (Lusas and Rooney 2001, McDonough et al. 2001). Lime concentration plays an important role in the cooking process; increases in lime concentration cause greater water uptake during cooking and soaking (Laria et al. 2005). These researchers suggested that the reaction rate and hence diffusion rate is lower at low lime concentration, and only when some of the reaction sites have been used, can the alkaline solution diffuse into the pericarp, across to the seed coat and into the aluerone structures. Sefa-Dedeh et al. (2004) reported an increase in moisture and ash content of the cooked kernel with an increase in lime concentration up to 0.5 %, followed by a decrease in moisture as concentration of lime increased to 1 %. The presence of lime in cook/ steep liquid develops an osmotic potential in the grain that causes maize to absorb more water until equilibrium is reached, thus resulting in higher kernel moistures than with water alone.

Lime added during nixtamalization also changes the flavor, texture, aroma, color, shelf life, and nutritional value of tortillas and corn snacks. It weakens the pericarp and aids in its removal, controls microbial activity, and frees up bound B-vitamins making them readily available (Bressani 1990, Serna-Saldivar et al. 1990). In cooked corn, calcium is primarily deposited on the hull followed by germ, and then endosperm. Only a small quantity of the lime used for cooking, is ultimately retained by the corn kernel. The calcium added during cooking, breaks hemicellulose bonds in the cell wall to facilitate pericarp removal (McDonough et al. 2001, Rooney and Suhendro 2001). Degraded pericarp imparts desirable properties to corn tortillas. Dissolved skin becomes a mass of gummy polysaccharides that act like a hydrocolloid, imparting good working qualities to the resulting masa and reduces staling in finished table tortillas (Arnold 2011). Nixtamal that is over-rinsed after steeping, or that is made with corn from which the pericarp has been mechanically removed does not have these characteristics (Arnold 2011). For the production of corn snacks, a thorough washing procedure is used to remove the attached pericarp. Incomplete pericarp removal can lead to dark-colored products, and may affect the grinding and sheeting/forming processes adversely (McDonough et al. 2001, Rooney and Suhendro 2001). Excessive lime concentrations used for cooking increase dry matter loss causing reduced product yield, and increase the cost of processing effluents (Serna-Saldivar et al. 1990, Pflugfelder et al. 1988). Lime is generally not stored for a long period of time before using for alkaline cooking as storage may cause caking (Serna-Saldivar et al. 1993).

1.2.1.2. Water

Water plays an important role during the cooking process, and causes kernels to swell during cooking and steeping processes due to water absorption and diffusion. Kernels are submerged in water during cooking. Generally a 1:3 ratio of corn to water is used for cooking. Lime used for nixtamalization is partially solubilized in water during cooking. Kernels absorb about 28-30 % water during the cooking process and 5-8 % more during the steeping process (McDonough et al. 2001, Rooney and Suhendro 2001). Water absorption during cooking leads to increase in kernel weight and kernels swell to about 1.5 times their original size. The amount of water absorbed depends on the extent of cooking, which is dictated by the end product desired. Laria et al. (2005) studied impact of calcium on water uptake, and reported that the water uptake increases rapidly at the beginning of soaking but as the soaking time increases, moisture gain decreases gradually. Water uptake is a kinetic process which is governed by physical changes in corn components. These changes depend on lime concentration, pericarp thickness and endosperm type (Laria et al. 2005). Nixtamal moisture of 50 % has been reported to give masa that has acceptable plasticity and machinability (Gomez et al. 1989).

1.3. Traditional Nixtamalization Unit Operations

Traditional Industrial scale nixtamalization can be divided into 2 categories, *Nixtamal production* and *Masa production*. Nixtamal production consists mainly of 2 unit operations: 1) cooking and steeping, and 2) washing. To obtain masa, the nixtamal can be ground using stone mills with addition of water. Masa is sheeted followed by cutting, baking or frying depending on the end product desired. Process parameters need to be

adjusted depending on the type of corn and its characteristics (Lusas and Rooney 2001, McDonough et al. 2001).

1.3.1. Cooking and Steeping

Industrial scale cooking of corn is generally conducted at temperatures of around 85-90^0 C for 15-45 min. It is carried out in vertical cookers/ steam kettles with 1.5-3 parts of water and 1 % lime. The capacity of steam kettles and vertical cookers ranges from 136-270 kg and 1,360-2,730 kg respectively (McDonough et al. 2001, Rooney and Suhendro 2001). Temperature control is provided within these systems for consistent heating, and slow agitation is provided to prevent hot spots.

The cooking process has 3 time-temperature phases: rise time, cook time and the time for temperature decrease. Contact of water with raw corn causes limited amount of kernel hydration. Hydration rate is accelerated as the heating process begins. When portions of the kernel reach a temperature of about 65^0 C, any fully hydrated granules start gelatinizing. Limited gelatinization continues until corn temperature declines to 65^0 C or less. However, all portions of the corn kernel are not fully hydrated, hence cooking causes only partial gelatinization. Degree of corn cook depends on various interacting factors, including corn physical characteristics, cook time and temperature, steep time and temperature, lime concentration, corn/ water ratio, and agitation speed. Optimum cooking and steeping times are determined depending on the degree of desired pericarp removal, kernel softening/ gelatinization, water uptake and overall nixtamal appearance (Lusas and Rooney 2001, McDonough et al. 2001). After cooking, the corn sometimes is subjected to a quenching process that involves addition of cold water to the cooked corn.

This step helps to drop the temperature of corn below gelatinization temperature, and avoids overcooking (Jackson et al. 1988, Johnson et al. 2010). After cooking, the nixtamal is steeped by simply allowing the cooked corn to stand in its cook water (with or without additional water addition) for 12-16 h. This can be done in the cooking tank itself or by transferring the cooked corn to another vessel (Gomez et al. 1989, Serna-Saldivar et al. 1990, Serna-Saldivar et al. 1993).

1.3.2. Washing

The nixtamal obtained after steeping is washed using mechanical washers. Typically, water is pumped or sprayed onto rotating barrels containing corn to efficiently remove the pericarp and rinse off lime attached to the kernels. The wash water is drained and pumped into the effluent treatment system. Industrial scale washing is typically done using drum washers. These consist of a conveyor which transfers the cooked kernel onto a rotating perforated cylinder (Lusas and Rooney 2001, McDonough et al. 2001). Water sprayers are located inside the vessel, and the pericarp and lime removed pass though these holes. The nixtamal then passes on a conveyor where excess water is removed. Drained nixtamal is transferred to a hopper, and then into a stone grinder for masa production (Lusas and Rooney 2001, McDonough et al. 2001).

1.4. Effect of Processing Parameters on Nixtamal Quality

Process parameters such as cook time, steep time and cook temperature are known to have an impact on the solubility, thermal and rheological properties of cooked starch (Sahai et al. 1999). Mondragón et al. (2004) reported that an increase in cook time

leads to an increase in the onset and peak temperature, and a decrease in the range and enthalpy of ground nixtamal when measured by Differential scanning calorimetry (DSC). DSC measures the changes in heat flow as starch undergoes thermal transition. Longer cook times lead to a higher amount of starch gelatinization and increased loss of crystallinity, resulting in low enthalpy values when analyzed using DSC (Sahai et al. 1999, Mondragón et al. 2004). Mondragón et al. (2004) also reported that granules most easily gelatinized during cooking are likely to do so at lower temperatures, resulting in higher onset and peak temperatures for the still ungelatinized nixtamal portion. During steeping, as nixtamal is held at sub-gelatinization temperatures for a very long time, polymers are reorganized into more stable configurations which causes an increase in gelatinization temperature. Increase in enthalpy temperature during steeping can thus be an indicator of the extent of steeping (Sahai et al. 1999). RVA parameters like peak viscosity, set-back and final viscosity increase as steep time increases. However, a study by Sahai et al. (1999) found DSC parameters to be more predictive of the degree of cook than RVA parameters.

1.5. Effect of Grain Quality Parameters on Nixtamal Quality

Corn with a rounded crown, smooth dent, hard endosperm, easily removable pericarp, clean bright color, and tolerance to damage during handling is preferred for alkaline cooking. Corn physical and chemical properties are affected by genetics, environment, handling and storage practices (Lusas and Rooney 2001, Rooney and Suhendro 2001). Processing conditions have to be changed depending on the hybrid used to obtain the optimal quality end products (Sahai et al. 1999). Different physico-chemical

tests are conducted to understand, what quality attributes of corn give the best end product for a selected set of processing conditions.

1.5.1. Moisture Content

Corn moisture content determines appropriate storage, handling, and cooking practices that should be followed. Corn above 14-15 % moisture is dried before storage, while corn with 6-10 % moisture is slowly hydrated (Lusas and Rooney 2001, Rooney and Suhendro 2001). Dry corn is brittle, breaks easily during storage, and requires a long time for cook water to penetrate inside the endosperm. High moisture content during storage can causes microbial spoilage.

1.5.2. Test Weight

Test weight is the weight of a quart container filled with grain expressed in pounds per bushel; most US dent corn has a test weight of around 682-772 kg/m^3 (53-60 lb/bu). Kernel size, shape and density all affect kernel's packing ability and thus test weight. Higher test weights indicate that the kernels are well-filled with a medium to high proportion of hard endosperm. Low test weight/ density indicates that the corn is immature or has a high proportion of soft endosperm (Lusas and Rooney 2001, Rooney and Suhendro 2001). Kernels with low test weights thus have a loosely packed endosperm structure that causes high and/or rapid water diffusion during cooking (Sahai et al. 2001). Almeida-Dominguez et al. (1997) reported positive correlation between dry matter loss (DML) and test weight.

1.5.3. Density

The density of dent corn usually ranges between 1.18 to 1.4 g/cm^3. Density is measured by a displacement method using alcohol, helium or nitrogen (Lusas and Rooney 2001, Rooney and Suhendro 2001). Corn with a high amount of hard endosperm generally has high density values, as the structure is tightly packed without the presence of large air cells. Density can be correlated to hardness and kernel texture (Lusas and Rooney 2001, Rooney and Suhendro 2001). Almeida-Dominguez et al. (1997) and Jackson et al (1988) observed a positive correlation between density and DML. Johnson et al. (2010) observed a negative correlation between density and nixtamal moisture.

1.5.4. Floaters

To determine percent floaters, a known quantity of kernels is immersed in a 1.275 specific gravity solution of sodium nitrate ($NaNO_3$). The percentage of floating kernels is counted according to the method outlined by Wichser (1961). Percentage floaters can also be expressed by hardness class. It is a simple and quick method to estimate corn density. It is sometimes used by processors to determine optimum cooking times during alkaline processing. Almeida-Dominguez et al. (1997) and Sahai et al. (2001) reported correlations between DML and hardness class, as measured by floaters.

1.5.5. Tangential Abrasive Dehulling Device (TADD)

The TADD is a device used for hardness measurement based on the amount of material abraded from 40 g of corn when it is subjected to abrasive dehulling for 10 min (Lusas and Rooney 2001, Rooney and Suhendro 2001). Corn with a hard endosperm has

low TADD values, as it is comparatively difficult to abrade. Almeida Dominguez and Rooney (1997) reported a negative correlation between TADD and DML. Johnson et al. (2010) reported positive correlation between TADD and nixtamal moisture.

1.5.6. Stenvert Hardness

The Stenvert hardness tester can be used to measure the degree of hardness of a particular sample of corn. The time required to grind the sample is measured, as is the ratio (by weight) of soft to hard endosperm and height of soft and hard endosperm, collected in a tube. These values can then be used to determine the hardness of a given corn sample. Hard corn requires a longer time to grind compared to a soft kernel. Kernel size, shape, pericarp toughness, dent characteristics, proportion of hard to soft endosperm, and moisture content affect the grinding method significantly (Lusas and Rooney 2001, Rooney and Suhendro 2001). Hard corn can tolerate inconsistency in processing and handling conditions. Soft corn is very sensitive to overcooking, hence very difficult to control during processing (Serna-Saldivar et al. 1990). Jackson et al. (1988) also observed that hard corn hybrids are less affected by inconsistency in processing and handling parameters. Soft kernels on the other hand, are more prone to overcooking, if complete removal of pericarp is essential. Sahai et al. (2001) found negative correlation between hardness and DML. However, Pflugfelder et al. (1988) observed positive correlations between hardness and DML.

1.5.7. Rapid Viscosity Analyzer (RVA)

Almeida Dominguez and Rooney (1997) measured RVA viscosity and slope for milled whole corn slurries from soft and hard kernels. They reported intermediate to high slopes for RVA peaks when soft corn was used, and low to intermediate slopes when hard corn was used for cooking. RVA viscosity is a measure of the rate of water uptake or hydration capacity of ground corn particles in the presence of heat and water. Soft kernels have air spaces in the floury endosperm, which cause high rate of water diffusion and hydration yielding high viscosity values when measured by RVA.

1.5.8. Stress Cracks

Improper storage and drying practices can cause stress cracks to form. Stress cracks are measured by placing a known quantity of kernels on a light box and manually counting the presence of fissures. Jackson et al. (1988) reported that stress cracks by themselves are not known to affect cooking quality. However, multiple cracks make the kernels prone to breakage from handling; if broken kernels are cooked they can cause an increase in DML, chemical oxygen demand and thus increased nixtamal moisture during nixtamalization. Large number of stress cracks facilitate water uptake by providing access to the kernel endosperm (Jackson et al. 1988).

1.5.9. Wisconsin Breakage Test (WBT)

WBT measures breakage susceptibility of corn when kernels are subjected to an impact force. Approximately 100 g of corn is fed to the WBT using a hopper. The corn most susceptible to breakage is broken into small pieces due to the impact force. The

collected sample is then sieved on a sieve shaker with US no 3 to separate overs from the intact kernels. Jackson et al. (1988) reported kernels with high breakage susceptibility to have a high nixtamal moisture, DML and water uptake during alkaline cooking. They also reported that WBT can be used as an indicator of corn quality as it quantifies the presence of stress cracks. Sahai et al. (2001) also reported high DML when large numbers of broken kernels were used for cooking.

1.6. Physico-Chemical Changes during Nixtamalization

Alkaline cooking results in water and calcium uptake by the corn kernels. Moisture content of the kernels increases from 10-12 % to 48-50 % (Serna-Saldivar et al. 1990). The alkaline solution degrades and solubilizes cell wall components resulting in partial removal of pericarp, softening of the endosperm structure and partial denaturation of the protein matrix (Serna-Saldivar et al. 1990, Gomez et al. 1992).

1.6.1. Effect of Nixtamalization on Pericarp

The high temperature of the alkaline solution removes the waxy layer that covers the pericarp, and allows the entrance of water and calcium ions into the internal layers of the maize kernel (Gutiérrez-Cortez et al. 2010). Calcium ions are carried by water though the tip-cap, germ and pericarp. At the end of nixtamalization, the pericarp is softened such that it can be removed by rubbing nixtamal between fingers (Gutiérrez-Cortez et al. 2010). Nixtamalization is a mass transfer phenomenon since there is diffusion of water and calcium ions into the kernel during cooking and steeping, depending on the physical state of the pericarp (Gutiérrez-Cortez et al. 2010). Degradation of the cuticle and other

pericarp layers occurs during cooking and steeping. Pericarp breakdown leads to formation of gums, which mostly become a part of the starch continuous phase that holds masa together (Gonzalez et al. 2004). Hemicellulose and cellulose portions of pericarp do not lose integrity during alkaline cooking as they do not degrade until temperatures of 285-340^0 C are reached (Gonzalez et al. 2004). The changes that occur during alkaline cooking include pericarp removal and neutralization of acidic groups in the hemicellulose in lime solution, attachment of calcium to the pericarp, and removal of water soluble hemicellulose and lignin from the pericarp's fiber matrix (Gutiérrez-Cortez et al. 2010).

1.6.2. Effect of Nixtamalization on Starch

Starch gelatinization is the most important physico-chemical event that occurs during the alkaline cooking process. Starch granules undergo limited swelling due to the physical constraints of endosperm cells, and insufficient heat and moisture. Hence, partial gelatinization of starch occurs (Gomez et al. 1992). A small amount of amylose leaches from the granules after they swell. The leached amylose forms a network with other cellular components. Masa can be considered as a network of solubilized starch polymers containing uncooked, swollen and dispersed starch granules (Gomez et al. 1992). Most of the studies on corn alkaline cooking have focused on changes in structural, thermal and rheological properties of starch using X-ray diffraction, Rapid Visco Analyzer, and Differential Scanning Calorimeter (Trejo-González et al. 1982, Robles et al. 1988, Gomez et al. 1992, Sefa-Dedeh et al. 2004, Mendez-Montealvo et al. 2006, Guzmán et al. 2011). RVA is used to measure the viscosity changes in the cooked corn starch slurry as it is heated and cooled with agitation. DSC is used to analyze heat capacity of the starch

as the cooked corn is heated in the presence of excess water. Heat flow increases as starch begins to gelatinize which can be measured in terms of enthalpy. X-ray analysis is used to measure degree of starch crystallinity which is obtained as peaks on the X-ray pattern. Relative crystallinity can be measured using X-ray diffraction analysis to estimate the degree of crystallinity of a cooked starch sample. Scanning electron microscopy (SEM) can be used to observe changes in the starch granule structure as corn is nixtamalized.

1.6.2.1. Rheological Properties

A study by Mendez-Montealvo et al. (2006) on nixtamalized starch using RVA found that masa has a lower maximum peak viscosity than raw corn starch, but the viscosity is obtained at a higher temperature. This is because some of the amylose chains are solubilized during nixtamalization, thus decreasing their contribution to increase in viscosity. The higher peak temperatures are because of starch annealing and also because some starch has already gelatinized which increase the temperature (Gomez et al. 1992). Guzmán et al. (2011) studied the Rotational test to understand the behaviour of raw starch and nixtamalized starch pastes and reported that the low viscosity obtained was mainly because some granules in the endosperm periphery are gelatinized, losing their capacity to produce more compact pastes with higher viscosity. Thus nixtamalization leads to the formation of softer gels because of partial gelatinization during the process. Robles et al. (1988) suggested that starch recrystalization during steeping results in higher melting points and increased pasting temperatures, which reduce starch solubility. Gomez et al. (1992) reported that retrogradation of amylose and amylopectin occurs very

rapidly during the steeping phase. Annealing occurs during steeping as starch is held at sub-gelatinization temperatures in excess water for a long time. This causes re-organization of starch molecules to a more ordered structure. Annealing of starch also causes higher gelatinization temperatures as there is alteration in the starch crystal structure during cooking.

1.6.2.2. Thermal Properties (DSC)

Nixtamalized and raw corn starch studied by Mendez-Montealvo et al. (2006) using DSC showed higher peak temperature in cooked starch than raw corn starch because the Ca^{2+} ions stabilize the starch structure of the nixtamalized sample. Also, starch annealing during steeping leads to the formation of more ordered starch chains which increase the gelatinization transition temperature. However, the study observed was no difference in the range and enthalpy values for starch from raw and nixtamalized samples unlike the study by Mondragón et al. (2004) where a decrease in enthalpy of nixtamal was observed with increase in cook time. Amylose-lipid complexes observed in raw corn starch were not found in nixtamalized starch, as the lipids are saponified during nixtamalization reducing their interaction with amylose (Mendez-Montealvo et al. 2006).

1.6.2.3. Crystallinity by X-ray Analysis

Gomez et al. (1992) found cooking to decrease the intensity of peaks indicating that crystalline starch structure was partially disrupted during cooking. However, the native crystalline starch structure was regained after steeping. Changes in starch crystallinity occurring during cooking is reduced by the annealing phenomenon which

occurs during steeping. Grinding the nixtamal to produce masa does not cause significant changes in starch crystallinity, even though the nixtamal (50 % moisture) is exposed to mechanical shearing and warm temperatures (45-50^0 C).

1.6.3. Effect of Nixtamalization on Proteins

A study using SEM, DSC and SDS-PAGE by Guzmán et al. (2011) on alkaline cooked starch found the presence of polygonal to round shaped granules with adhered protein bodies in all nixtamalized samples. Guzmán et al. (2010) observed that protein polymerization occurred during cooking (30-150 min) and increased with increase in cook time, which may be due to the cross-linking of disulfide bonds. Several groups of bands were observed using SDS-PAGE for all the cooked samples. Presence of these bands indicates that lime promotes calcium-protein (zein-lime) complex formation. These calcium complexes are difficult to disrupt and thus increase protein's thermo-resistance. Thus, calcium increases protein polymerization the by formation of calcium bridges. Increased thermo-resistance was also observed using DSC when extracted protein samples of uncooked corn and nixtamal were compared, as higher denaturation temperatures and enthalpy were obtained for a nixtamalized sample at all cooking times compared to raw corn. It is proposed that zein polymers are formed during nixtamalization which alter the thermal properties of proteins (Guzmán et al. 2010). Guzmán et al. (2011) also studied the structure of granules after cooking with and without the use of lime and observed that for cooked corn with lime, round to polygonal starch granules with adhered protein bodies were obtained. All the samples showed damage on the surface because of heat and alkali presence. Corn cooked without lime had

smoother surface of starch granules compared to corn cooked with lime. Small areas in the corneous endosperm showed the presence of a continuous protein matrix and no protein bodies. Hydration and swelling occurring during the cooking process may have caused the edges of starch granules to disappear (Guzmán et al. 2011). Trejo-González et al. (1982) observed that alkaline cooking alters the molecular weight distribution of proteins as observed by electrophoretic patterns. The study also noted that reduced protein content after nixtamalization is mainly due to changes in the protein solubility. Hydrophobic interactions, protein denaturation and cross-linking of proteins are probably responsible for the changes in the solubility of protein fractions during nixtamalization. Sefa-Dedeh et al. (2004) reported a slight increase in protein content from 8.14 % in raw maize to 8.88 % in 30 min alkaline cooked sample. The protein content increased with increase in lime concentration. The increase in protein content was proposed to be due to a concentration effect observed in nixtamal.

1.6.4. Effect of Nixtamalization on Lipids

There is little information available on role of lipids in nixtamalization. Lipids from the germ are present in the form of free lipids in the continuous phase of masa. The lipid fraction contains partially emulsified lipids, and free lipids that interact with peptides and carbohydrates. Fluorescence by lipids may increase on alkaline cooking, as heat may release or expand more lipid bodies, freeing more material for fluorescence (Lusas and Rooney 2001, McDonough et al. 2001).

1.6.5. Effect of Nixtamalization on Calcium Content

The pericarp of nixtamalized kernels has a high calcium content compared to raw corn. During cooking, water enters the kernel by diffusion though tip cap and fissures in the pericarp and seed coat around the kernel. It can then diffuse across the seed coat and aleurone layer into the germ and endosperm (Laria et al. 2005). Calcium uptake by corn kernels thus takes place according to the calcium ion diffusion process and depends mainly on the cook temperature and steep time (Laria et al. 2005). However, Valderrama-Bravo et al. (2010) suggested that for short steeping times, kernel calcium uptake is governed by accumulation process in the external layers of pericarp and the diffusion process is not relevant. Calcium absorption by kernels is much slower than water absorption. Endosperm and germ calcium content increases with an increase in lime concentration, cook time and steep time (Bressani et al. 2004). Trejo-González et al. (1982) reported that corn kernel calcium uptake during alkaline cooking is due to calcium bound to starch Bressani et al. (2004) reported an increase in calcium content of raw corn from 8.98 mg/100 g to 76.7 mg/100 g during alkaline cooking. Alkaline cooked starch contains about 2.9 times more calcium than raw corn starch (Gomez et al. 1991).

1.7. Nixtamal Moisture

Gomez et al. (1991) suggested that the optimal moisture of 48-50 % for masa was necessary to obtain acceptable plasticity, cohesiveness and machinability. Nixtamal is cooked depending on the end-product application; 50–51 % moisture for table tortillas and 46–48 % moisture for tortilla chips (Serna-Saldivar et al. 1993). Serna-Saldivar et al. (1993) found a high correlation between nixtamal moisture and cook time. Sahai et al.

(2001) reported cook temperature, cook time and steep time to have a significant impact on nixtamal moisture. A longer cook time is required to increase the nixtamal moisture for table tortillas vs tortilla chips/ snacks to obtain softer, more pliable and rollable tortillas (Serna-Saldivar et al. 1993).

1.8. Dry Matter Loss (DML)

Solids in the nejayote and wash-water together comprise the total dry matter lost. Loss of corn DML is economically important to commercial producers as it contributes to the effluent treatment costs and yield loss. Commercial corn DML has been estimated to be 5-14 % for the traditional nixtamalization process (Khan et al. 1982). Pflugfelder et al. (1988) reported that 8-12 % solids are lost during nixtamalization, depending on the type of corn and the processing conditions used. These are distributed between cooking and steeping (2.8-10.7 %) and washing (1.6-2.0 %). Sahai et al. (2000) reported variation of DML between 3.17-9.82 %. They found most of the DML to be present in nejayote (4.12-6.55 %), while DML in wash-water was 1.08-2.23 %.

Dry matter losses in the nejayote are mainly composed of pericarp, starch, protein and germ solubles. Typical composition of waste-water is 75.6 % non-starch polysaccharides (mainly pericarp), 11.6 % starch and 1.4 % proteins. DML increases with increase in cooking time but DML during steeping accounts for most of the loss (Pflugfelder et al. 1988). Sahai et al. (2000) observed that most DML occurred during the first 6-8 h of steeping. Loss of solids is related more to the cook temperature and lime concentration than to cooking and steeping times. They observed using response surface regression model that when cook temperature was increased from 85^0 C to 95^0 C,

keeping other factors constant (30 min cook time, 1 % lime concentration and 8 h steep time), DML increased from 4.31 to 5.25 %. Pflugfelder et al. (1988) reported that DML is affected by corn hybrid as well as processing conditions. Corn with a soft crown and endosperm is more prone to shearing losses compared to corn with a hard crown and endosperm, thus yielding high amount of suspended solids (DML). Cooking of corn at high temperature for a long time with minimal steeping causes higher amount of DML as compared to cooking at a low-temperature and steeping for long periods (Pflugfelder et al. 1988). Corn DML in nejayote is also increased with increase in steep time (Sahai et al. 2000).

1.9. Nixtamalization Studies

Corn processors procure specific corn types from suppliers and farmers depending upon the products they intend to produce. The type of corn received depends on the supplier type, growing season, region, environment, and maize genetic factors. Often the corn procured is subjected to a series of physico-chemical tests before it is selected for alkaline cooking (Sahai et al. 2000). The best way to predict alkaline cooking performance however, is to conduct the cooking process itself (Jackson et al. 1988, Almeida-Dominguez et al. 1997). Studies by Khan et al. (1982), Jackson et al. (1988), Serna-Saldivar et al. (1993), Sahai et al. (1999, 2000, 2001) have attempted to scale down the industrial nixtamalization process. Yglesias et al. (2005) developed a laboratory method for nixtamalization using 500 g of corn. These screening methods however, still require either a large quantity of sample or are expensive to conduct due to special apparatus required.

1.9.1. Pilot Plant Nixtamalization Processes

Sahai et al. (1999) used response surface methodology to study the effect of processing variables like cook time, steep time, initial steep temperature, and cook temperature on the degree of cook measured using RVA and DSC. A Yellow corn variety grown in Nebraska (1997- growth year) was used for all nixtamalization experiments. Sahai et al. (2000) studied the impact of processing parameters on DML and effluent pH during nixtamalization using response surface methodology. White corn grown in Sinaloa region of Mexico (1997- crop year), traditionally grown for tortilla production was used for the study. Sahai et al. (2001) tried to establish empirical correlations between nixtamalization process and product variables (DML, pH, nixtamal moisture and color, masa moisture, color and texture, and tortilla moisture, color and rollability) by conducting nixtamalization experiments on five corn hybrids at ten different processing conditions. White corn samples grown in Sinaloa, Veracruz, Chiapas, Chihuahua, and Jaliso were used for the study. For nixtamalization, 20 or 30 kg corn was cooked in 120 L or 150 L water such that corn: water ratios of 1:5 or 1:6 were obtained. Lime was added in 100:1 corn: lime ratio. A gas-fired horizontal cook steep tank covered with a lid was used for cooking. Lime was added to the water and stirred well. The mixture was preheated before adding corn. Cook time was started when the temperature of corn-water-lime mixture reached the specified cook temperature. Steeping was started by adding boiling water or cold water to the nixtamal depending on the steep temperature desired. Steeping ended with draining the nejayote and rinsing the nixtamal twice with 50 L water.

Serna-Saldivar et al. (1993) developed a small sample nixtamalization method using nylon bags to conduct alkaline cooking of corn in a pilot plant. The method involved cooking of corn with 50 L of water to achieve a 1:3 ratio. Water was heated in a steam kettle to 98^0 C and 166.6 g lime was added and mixed well. Corn samples of 100 g were weighed in small nylon bags, and multiple bags placed in a perforated nylon bag to make a total of 12 kg corn per nixtamalization run. The samples were then cooked for different amounts time at 98^0 C. The cooked corn was steeped for 14-16 h, taken out from the cooking bags, and washed with running water to remove the lime and pericarp attached. It was then blotted on paper towels to dry off excess surface moisture. Dry matter loss and nixtamal moisture were then estimated. The nylon bag technique was found to be simple and effective for evaluating alkaline cooking properties. The authors suggested that the method could be used to analyze a large number of samples in breeding programs. It could also be used by processors to optimize cooking conditions at factory levels.

However, pericarp is stuck in nylon bags, making accurate DML assessment difficult, nylon bags get caught up in hot-spots as they cannot move by agitation provided during cooking resulting in uneven heating in the sample. The system requires use of a special set-up such as large cooking vessel in a pilot plant setting.

1.9.2. Small-Scale Alkaline Cooking Processes

Jackson et al. (1988) cooked thee varieties of corn with different levels of stress cracks using a steam kettle to understand the effect of stress cracked and broken kernels on alkaline cooking. For this method, 2 kg of corn was cooked in 5 L water. A 1 % lime:

water ratio was used. The steam kettle was covered with a Plexiglass cover. The cover had a center hole to allow a commercial-scale Hobart mixer to gently stir the mixture continuously. The steam heating began after pre-weighed corn and lime were added to the water. The mixture boiled in approximately 3-4 min and boiling was continued for 20 min with constant stirring. Once cooking was completed, 2 L quench water was added to the steam kettle. This was followed by steeping the corn for 16 h.

1.9.3. Laboratory Scale Nixtamalization Processes

To reduce the quantity of sample or the requirement to use pilot plant scale cooking tanks, a laboratory scale method was developed by Yglesias et al. (2005), using 500 g of corn with 1:3 corn to water ratio. The objective of this method was to develop a lab scale process that could mimic a wide range of industrial cooking conditions. A response surface design was developed to model the pasting and thermal properties of nixtamal and masa as a function of cook time, cook temperature and steep time. Nixtamal and masa properties such as moisture, DML, pasting, and gelatinization properties were compared for the laboratory scale method and pilot plant study. For the laboratory scale process, 500 g corn was heated on a digital hot-plate with stirrer with 1500 ml water and 5 g lime in a 2000 ml beaker. A wire mesh basket with a 3 cm gap from the lower surface was constructed to place the corn inside the basket. The space was used to place a magnetic stirrer for constant mixing of the lime-water solution and temperature control. The beaker was insulated using polystyrene rings wrapped with electric tape, specifically designed for the process. The nixtamal obtained was washed twice using 700 ml water, and the water was drained and washed. Nixtamal was blotted on paper towels. Nixtamal

moisture was measured by drying washed corn for 72 h in a conventional oven. Dry matter loss was measured by drying wash-water and nejayote for 48 h in a conventional oven. The authors concluded that the laboratory method could be used to mimic industrial nixtamalization over a wide range of processing conditions, and thus can serve as an important tool for researchers and processors. For the pilot plant study, 1:3 ratio of corn: water and 100:1 for corn: lime was used. Alkaline cooking, steeping and washing was conducted similar to the method outlined by Sahai et al. (1999, 2000, 2001).

Previous studies on scaling down the quantity of corn for nixtamalization still require relatively large quantities of corn to be used for screening and are expensive. Hence, opportunities for method improvement would include a reduction in sample size, use of less complex apparatus and analysis of multiple samples at a time.

1.10. References

Almeida Dominguez, H, Suhendro, EL and Rooney, L. 1997. Corn alkaline cooking properties related to grain characteristics and viscosity (RVA). Journal of Food Science 62:516-519.

Arnold, D. 2011. Mesoamerican miracle megapost: tortillas and nixtamalization in: cooking issues- The International Cuilinary Center's Tech' N Stuff Blog.

Bressani, R. 1990. Chemistry, technology, and nutritive value of maize tortillas. Food Reviews International 6:225-264.

Bressani, R., Turcios, J. C., Colmenares de Ruiz, A. S. and de Palomo, P. P. 2004. Effect of processing conditions on phytic acid, calcium, iron, and zinc contents of lime-cooked maize. Journal of Agricultural and Food Chemistry 52:1157-1162.

FAO. 2014. Food Agriculture Organization in: hhtp//apps.fao.org: United Nations.

Gomez, M., Lee, J., McDonough, C., Waniska, R. and Rooney, L. 1992. Corn starch changes during tortilla and tortilla chip processing. Cereal Chemistry 69:275-279.

Gomez, M., McDonough, C., Rooney, L. and Waniska, R. 1989. Changes in corn and sorghum during nixtamalization and tortilla baking. Journal of Food Science 54:330-336.

Gomez, M., Waniska, R. and Rooney, L. 1991. Starch characterization of nixtamalized corn flour. Cereal Chemistry 68:578-582.

Gutiérrez-Cortez, E., Rojas-Molina, I., Rojas, A., Arjona, J., Cornejo-Villegas, M., Zepeda-Benítez, Y., Velázquez-Hernández, R., Ibarra-Alvarado, C. and Rodríguez-García, M. 2010. Microstructural changes in the maize kernel pericarp during cooking stage in nixtamalization process. Journal of Cereal Science 51:81-88.

Guzmán, A. Q., Jaramillo Flores, M. E., Feria, J. S., Méndez Montealvo, M. G. and Wang, Y.-J. 2011. Rheological and thermal properties of masa as related to changes in corn protein during nixtamalization. Journal of Cereal Science 53:139-147.

Delcour J.A and Hoseney, R. C. 2010. Structure of cereals in Principles of cereal science and technology, Pages 1-22, Ed 3. American Association of Cereal Chemists International, St. Paul, Minnesota.

Jackson, D., Rooney, L., Kunze, O. and Waniska, R. 1988. Alkaline processing properties of stress-cracked and broken corn (*Zea mays* L.). Cereal Chemistry 65:133-137.

Johnson, W. B., Ratnayake, W. S., Jackson, D. S., Lee, K.-M., Herrman, T. J., Bean, S. R. and Mason, S. C. 2010. Factors affecting the alkaline cooking performance of selected corn and sorghum hybrids. Cereal Chemistry 87:524-531.

Khan, M., DesRosiers, M., Rooney, L., Morgan, R. and Sweat, V. 1982. Corn tortillas: evaluations of cooking procedures. Cereal Chemistry 59:274-284.

Laria, J., Meza, E., Mondragon, M., Silva, R. and Peña, J. 2005. Comparison of overall water uptake by corn kernel with and without dissolved calcium hydroxide at room temperature. Journal of Food Engineering 67:451-456.

McDonough, C., Gomez, M., Rooney, L. and Serna-Saldivar, S. 2001. Chapter 4: Alkaline-cooked corn products. Pages 72- , Snack Foods Processing, E. Lusas and L. Rooney, eds. Technomic Publishing Co.: Lancaster, PA.

Mondragón, M., Bello-Pérez, L. A., Agama-Acevedo, E., Betancur-Ancona, D. and Peña, J. L. 2004. Effect of cooking time, steeping and lime concentration on starch gelatinization of corn during nixtamalization. Starch 56:248-253.

NCGA. 2013. World of corn. Unlimites possibilities in: National Corn Growers Association. www.ncga.com: Chesterfield, MO.

Pflugfelder, R., Rooney, L. and Waniska, R. 1988. Dry matter losses in commercial corn masa production. Cereal Chemistry 65:127-132.

Rooney, L. and Suhendro, E. 2001. Food quality of corn. Pages 39-71, Snack Foods Processing, EW Lusas and LW Rooney (eds.). Technomic Publishing Co., Inc., Lancaster.

Sahai, D., Mua, J., Surjewan, I., Buendia, M., Rowe, M. and Jackson, D. 1999. Assessing Degree of Cook During Corn Nixtamalization: Impact of Processing Variables 1. Cereal Chemistry 76:850-854.

Sahai, D., Mua, J., Surjewan, I., Buendia, M., Rowe, M. and Jackson, D. 2001. alkaline processing (nixtamalization) of white mexican corn hybrids for tortilla production: significance of corn physicochemical characteristics and process conditions. Cereal Chemistry 78:116-120.

Sahai, D., Surjewan, I., Mua, J., Buendia, M., Rowe, M. and Jackson, D. 2000. Dry matter loss during nixtamalization of a white corn hybrid: Impact of processing parameters. Cereal Chemistry 77:254-258.

Sefa-Dedeh, S., Cornelius, B., Sakyi-Dawson, E. and Afoakwa, E. O. 2004. Effect of nixtamalization on the chemical and functional properties of maize. Food Chemistry 86:317-324.

Serna-Saldivar, S., Gomez, M., Almeida-Dominguez, H, Islas-Rubio, A. and Rooney, L. 1993. A method to evaluate the lime-cooking properties of corn (*Zea mays*). Cereal Chemistry 70:762-762.

Serna-Saldivar, S., Gomez, M. and Rooney, L. 1990. Technology, chemistry, and nutritional value of alkaline-cooked corn products. Advances in Cereal Science and Technology (USA).

Trejo-González, A., Feria-Morales, A. and Wild-Altamirano, C. 1982. The role of lime in the alkaline treatment of corn for tortilla preparation. Advances in Chemistry Series 198:245-263.

USDA. 2013. United States Department of Agriculture Economic Research Division. www.usda.gov: Washington, DC.

Valderrama-Bravo, C., Rojas-Molina, A., Gutiérrez-Cortez, E., Rojas-Molina, I., Oaxaca-Luna, A., De la Rosa-Rincón, E. and Rodríguez-García, M. 2010. Mechanism of calcium uptake in corn kernels during the traditional nixtamalization process: Diffusion, accumulation and percolation. Journal of Food Engineering 98:126-132.

Watson, S., White, P. and Johnson, L. 2003. Description, development, structure, and composition of the corn kernel in Corn: Chemistry and Technology, Pages 69-106, Ed 2, American Association of Cereal Chemists, St. Paul, Minnesota

Wichser, W. 1961. The world of corn processing. Am. Miller Process 89:29-31.

Yglesias, R., Parkhurst, A. M. and Jackson, D. S. 2005. Development of laboratory techniques to mimic industrial-scale nixtamalization. Cereal Chemistry 82:695-701.

CHAPTER 2: ASSESSMENT OF CORN QUALITY FOR NIXTAMALIZATION: DEVELOPMENT OF A BENCH-TOP COOKING METHOD

Abstract

A convenient small-scale laboratory method, that can be used to simultaneously analyze multiple samples, was developed to rapidly assess suitability of corn for nixtamalization. This new 100 g method was based on an existing 500 g laboratory nixtamalization procedure that has been shown to mimic industrial scale nixtamalization. The 500 g and 100 g methods were compared by evaluating nixtamal moisture, DML, degree of pericarp removal, and gelatinization properties of cooked corn. The heating and cooling profile of the 500 g method was obtained by measuring the temperature changes every 30 s during cooking and steeping. A new 100 g method was developed using this profile. Nixtamalization was conducted using a 1:4 corn to water ratio, with 1% lime (corn weight basis). A Response Surface Central Composite Design was used to model a wide range of processing conditions for both the methods. Nixtamalization was conducted at selected ranges of cook temperature (80 - 95^0 C), cook time (3 - 40 min) and steep time (2 - 12 h). Parameter estimates and response surfaces for nixtamal moisture, DML, pericarp removal, pH, total starch and pasting and gelatinization properties were compared and models were fit. The response surface models developed were not significantly different for nixtamal moisture, DML, and gelatinization enthalpy ($p < 0.05$, $r^2 > 0.7$); there was an overlap of the 90 % Bonferroni confidence intervals. The bench-top 100 g nixtamalization process can successfully mimic the 500 g method over a wide range of processing conditions. Food corn breeders, wholesalers or tortilla processors may use this technique to screen new hybrids for acceptability.

2.1. Introduction

Alkaline cooking, also known as nixtamalization, transforms maize (*Zea mays* L.) into tortillas and related products, and has been practiced for centuries. For nixtamalization of corn, the whole kernels are placed in large vats at 85-100^0 C for 10-40 min and then steeped for 12-16 h.

Corn processors procure hybrids from different farmers or suppliers. Corn for alkaline cooking generally is sought as hybrids with bright white or yellow color, 11-15 % moisture, 720-772 kg/m^3 (56-60 lb/bu) bulk density, no presence of foreign material, maximum 5-10 % broken/damaged kernels, less than 30 % stress cracks, uniform kernel shape and size, less than 0.1 % dead germ, and aflatoxins and fumonisins less than 20 ppb and 4 ppm respectively (FGIS 2013). Hybrids that are candidates for nixtamalization are tested for physico-chemical properties. Hybrids potentially acceptable for alkaline cooking are identified, and further evaluated.

The suitability of nixtamalized corn products often depends on the cooking and processing quality of corn kernels (Gomez et al. 1989). The best method to determine the quality of a particular hybrid is by cooking the hybrid itself (Jackson et al. 1988, Almeida-Dominiguez et al. 1988). Processors test the quality of corn for end use applications by cooking a large batch, sometimes in excess of 453.6 kg (3000 lb.) of corn in the plant, and obtaining results for nixtamal and masa moisture. High cost is incurred if the standard processing conditions are not suitable for a hybrid, as corn is wasted, there is generation of waste stream solids, an unsatisfactory end-product is produced, and the other suitable candidates have to be tested. Various studies have been conducted to reduce the scale of industrial nixtamalization for testing: Jackson et al. (1988) studied the

effect of stress cracks on nixtamalization by cooking 2 kg of corn in 5 L water and 1 % lime inside a kettle. The kettle was covered with Plexiglass and had a center hole to place a commercial-scale Hobart mixer for continuous gentle mixing. The study was conducted using 3 samples with different levels of stress cracks cooked under mild and harsh cooking conditions, with a process similar to industrial scale cooking. Serna-Saldivar et al. (1993) developed a pilot plant method using 50 L water and 166.6 g lime with 100 g corn samples in small perforated nylon bags, to make a total of 12 kg corn in a large nylon bag. Alkaline cooking processes were conducted on eleven hybrids with contrasting physical properties. Sahai et al. (1999, 2000, 2001) developed a pilot plant method using corn and water in 1:5 to 1:6 ratios, cooked in a gas-fired horizontal cook steep tank. The study analyzed five corn hybrids grown in different regions of Mexico, cooking each hybrid at ten processing conditions, and found empirical relations between nixtamal quality, process variables and grain quality parameters. The pilot techniques used previously have been found to mimic industrial nixtamalization, and are considered as simple and effective techniques to test the cooking quality of corn (Serna-Saldivar et al. 1993). While these methods require less corn than for a commercial-size processing run, the pilot plant methods still require relatively large quantities (2-30 kg per sample) to be used as screening techniques. This testing also required access to a pilot plant processing facility. The nylon bag method is subject to the presence of hot spots, because the bags cannot move, and can be caught in the hot spots in the cooking vessel. In a large scale process where corn is directly inside the vessel, continuous or periodic agitation is provided. This causes movement of kernels in different potions of the vessel, even if hot spots are created. Besides space, these techniques require relatively expensive equipment

such as large stainless steel vessels for cooking. For the nylon bag method, although small quantity of corn is requires, the pericarp removed is stuck to the surface of nylon bags making analysis of DML difficult.

Yglesias et al. (2005) developed a laboratory scale method using 500 g of corn in-order to scale down the pilot-plant process. The 500 g method was compared for nixtamal and masa characteristics to the pilot plant studies by Sahai et al. (1999, 2000, 2001). A range of commercially used processing conditions of cook time, steep time and cook temperature were tested for the two methods. Response surface models indicated that the nixtamal and masa produced using the two methods had similar characteristics in terms of moisture, DML, pasting and thermal properties. This method could thus mimic pilot-plant technique and thus near-industrial scale nixtamalization over a range of processing conditions. The authors suggested the use of this method as a screening tool for processors to test the potential processing characteristics of hybrids. The 500 g method, however, still requires a relatively large sample size (especially if intended for use with breeders samples), it is relatively difficult to run, requires expensive apparatus, and was originally designed to analyze only one sample at a time. Hence, the aim of this study was to develop a high-throughput, small-scale method, based on the 500 g method, which can mimic a wide range of industrial scale cooking conditions. Ideally such a method would use fewer resources, have a simple set-up, and could be used for screening a larger number of corn samples at a time.

Several studies, including those by Almeida Dominguez and Rooney (1997), Sahai et al. (1999, 2001) tried to predict changes in post-nixtamalization thermal properties and starch pasting characteristics based on the physical characteristics of raw

corn. Sahai et al. (1999, 2001) suggested that processing conditions, such as lime concentration, cooking temperature, extent of cooking and steeping, influence the textural characteristics of masa and nixtamalized products. Pflugfelder et al. (1988) and Serna-Saldivar et al. (1993) reported that, to obtain optimal end products, cooking conditions have to be adjusted depending on the type of corn as well as the specifically intended end product. Cook time, cook temperature and steep time are considered as the main factors which affect nixtamal quality when measured in terms of nixtamal moisture, dry matter loss, degree of pericarp removal, pasting, and gelatinization properties (Sahai et al. 2001). In order to be applicable, testing methods must be capable of accommodating various cooking times, steeping times and cooking temperatures used in industrial production. These factors should be selected such that the effect of a range of likely industrial nixtamalization conditions can be understood.

2.2. Materials and Methods

Corn (*Zea mays* L.) grown in Nebraska, USA and harvested during 2013 was used for all nixtamalization experiments. After harvesting, the corn was dried in bins at 15-20^0 C and 55-75 % relative humidity, with ambient forced air circulation. It was then packed in 22.6 kg (50 lb.) paper bags and stored at ambient temperature for a month. Corn was subsequently stored at 2-5^0 C. These hybrids were cultivated primarily for nixtamalization, i.e., tortilla and tortilla chips production. Small amounts of sample 1 kg (~2 lb.) were equilibrated to room temperature (25^0 C) for 24 h before physical characterization and nixtamalization experiments.

2.2.1. Corn Characterization

Test weight was measured in pounds/ bushel and expressed as (kg/m^3) according to (AACCI Method 55-10 AACCI 2000). Thousand kernel weight was calculated by weighing 100 manually counted kernels as described by Sahai et al. (2001). The Wisconsin Breakage Test (WBT) (Model: 9/84, Cargill Grain Research Lab, MN, USA) was conducted using the described method by Paulsen and Hill (1985). Kernel stress cracks were measured by examining 100 whole kernels on a light box for the presence of cracks and dividing them into 6 categories as described by Thompson and Foster (1963). Hardness was evaluated using the Stenvert Hardness Test according to Pomeranz et al. (1986), with modifications as follows: A Kinematica Polymix, PX-MFC 90D Microhammer Mill (Glenmills Inc., Clifton, NJ, USA) equipped with a 2 mm screen was used to grind samples at 3600 rpm. Height of soft endosperm, total ground material, time to grind, lowest rpm at maximum grinding power and weight of material recovered over a 425 µm sieve were measured. Apparent corn density was measured using Floaters Test. The percentage of buoyant kernels immersed in a 31.3° Baume solution of sodium nitrate ($NaNO_3$) maintained at 60° C, corresponding to a specific gravity of 1.275, was measured according to the method described by Peplinski et al. (1989). The Hardness class was determined by using data from the Floaters test and the moisture content of the sample as described by Wichser (1961). Whole corn kernel density was measured using a Gas Multi-pycnometer (Quantachome, MVP 05034, Boyton Beach, FL, USA) according to the method by Pomeranz et al. (1986), Anonymous (2003), Serna-Saldivar (2012a). Kernel amylose content was measured by the dual-wavelength iodine binding method outlined by Zhu et al. (2008). Amylose corn standards of 10, 20, 30, 50, and 80 (w/w,

d.b), also prepared according to the method outlined by Zhu et al. (2008), were used to create a standard curve: (differential absorbance vs. % amylose.)

2.2.2. Five Hundred Gram Nixtamalization Process

The method outlined by Yglesias et al. (2005) was used to cook 500 g of corn in a 1: 3 corn to water ratio. The total cost for analysis of 12 samples at a time in 2 batches was 14,775 USD. Water (1500 ml) was heated in a 2000 ml beaker with 5 g food-grade lime $Ca(OH)_2$ (Vitacal ^{TM}, Mississippi Lime Company, St. Louis, MO, USA). A wire mesh basket of 1/4 inch (0.64 cm) mesh size, 17.5 cm height and with a diameter of 11 cm, was constructed and placed in the beaker such that the kernels stayed ~ 3 cm from the beaker lower surface. A stirring bar was placed in this space. Temperatures were monitored using a probe dipped into the cooking solution and connected to a digital hot plate/ stirrer (Model HS30, Torrey Pines Scientific, and Solano Beach, CA, USA) (Appendix A). To efficiently maintain the programmed cooling rate during the steeping step, eight polystyrene rings were constructed (2 cm thickness, 18 cm external diameter, and 14 cm internal diameter), wrapped with electric tape, and placed around the beaker for insulation. Once cooking was complete, the steeping phase was started, and was ended by draining the nejayote. Nixtamal was rinsed twice using 700 ml distilled water, drained and small amounts of samples were collected for nixtamal moisture analysis. Nixtamal samples ~ 100 g were collected and flash frozen in liquid nitrogen. The nixtamal samples were then freeze dried at 21^0 C and 0.22 mbar vacuum pressure using a bench top freeze drier (FreeZone 4.5L, Labconco Co., Kansas City, MO., USA) for approximately 72 h, and stored at - 4^0 C until further analysis.

2.2.3. One Hundred Gram Nixtamalization Process

Based on the previous published method (Yglesias et al. 2005), a scaled down process deigned to conduct cooking quality tests with 100 g raw corn was established. The total cost for analysis of 12 samples at a time in 2 batches was 7,622 USD. Water (400 ml) and 1 g food-grade lime $Ca(OH)_2$ (Vitacal ^{TM}, Mississippi Lime Company, St. Louis, MO, USA) were mixed in a 1000 ml glass beaker. Same ratios of corn, water and lime as the 500 g method were used. Aluminum baskets with round holes of 1/8 inch (0.32 cm) diameter, basket height of 14.5 cm, 10 cm diameter and a spacing of ~ 3 cm above the beaker surface were placed in the solution, and pre-weighed corn (100 g) was added. A stirrer was placed in this 3 cm space for uniform solution mixing. The aluminum baskets were obtained from National Manufacturers (Division of TMCO, Inc., Lincoln, NE, USA). Hot-plates (Super Nuova, Model: SP135935 Thermo Fischer Scientific Inc., 81 Wyman street, MA, USA) were used to control temperatures during the cooking experiments. The hot plates accommodate four beakers at a time (Appendix B). Temperatures were monitored by inserting a probe into the cooking solution, and connected to the digital hot-plate during the cooking step. The hot plates were set to obtain a similar profile to the 500 g method. To maintain a constant heating rate during cooking, and a constant cooling rate during steeping, the beakers were insulated with aluminum foils on top and side surfaces. Once the cooking step was complete, the beakers were removed from the hot plates, and the cooked corn was washed with 200 ml water. The washed corn was then freeze dried in the same way as that for the 500 g method.

2.2.4. Nixtamalization Time-Temperature Profile

Changes in temperature during the cooking and steeping period were recorded for the 100 g and 500 g methods by placing a data logger (Model TDF1, ThermoWorks, Lindon, UT, USA) inside the beaker, and recording temperature changes every 30 s. The data obtained was plotted (Temperature vs time) using excel to compare the cooking and steeping profiles for nixtamal using the 100 g and 500 g methods at all the processing conditions. Similar temperature profiles indicate that the two methods exposed the grain to similar thermal inputs.

2.2.5. Experimental Design

The nixtamalization process was developed after preliminary tests using selected time, temperature and lime-level combinations. The experimental design parameters were fixed as cook time, cook temperature and steep time. To conduct nixtamalization over a wide range of processing conditions used commercially, a Response Surface Central Composite Design (CCD) with thee quantitative factors (cook temperature, cook time and steep time) similar to the design by Yglesias et al. (2005), was generated using Design Expert software (Stat Ease, Version 7.1.3, East Hennepin, Minneapolis, MN, USA). The cook temperature was varied from 80^0 C to 95^0 C, cook time from 3 min to 40 min and steep time from 2 h to 12 h; a total of 34 trials (6 central + 8 factorial + 6 axial) were run. All axial and factorial points were replicated and run under both, the 500 g and 100 g methods, as shown in Table 2.1. Similar response surfaces and equations for the 100 g and 500 g method would indicate similar degree of cook using the two methods. It would

thus mean that the 100 g method could replicate the 500 g, and hence near-industrial scale nixtamalization over a wide range of processing conditions.

2.2.6. Compositional Analysis

Protein, crude fiber, fat, ash and total starch content of raw corn and freeze-dried nixtamal were determined by Ward Laboratories (Kearney, NE, USA) using AOAC approved methods (AOAC 2000). Percentage ash was determined using AOAC 923.03, calcium and phosphorous using AOAC 984.27 and 985.01, fat using AOAC 922.06, protein by Dumas method AOAC 968.06 and 992.15, and starch using ethanolic extraction AOAC 996.11.

Moisture content of raw corn and nixtamal blotted with paper towels was determined using the AACCI approved method 44-15A (AACCI 2000). This was done to analyze if the degree of cook was similar for the 100 g and 500 g methods. Nejayote pH was measured with a pH meter (Orion SA-520, Boston, MA, USA). Data was collected in triplicates and averages reported. The whole corn samples were ground to a powder using a Micro-hammer mill (Kinematica Polymix, PX-MFC 90D) with a 2 mm screen at 3600 rpm prior to characterization of compositional characteristics and percent amylose content.

2.2.7. Process Waste Analysis

Degree of nixtamal pericarp removal was estimated by staining kernels with May-Greenwald solution according to a method outlined by Serna-Saldivar et al. (1991). The stained kernels were subjectively evaluated on a 0 to 5 scale (0: Pericarp completely

removed to 5: Kernel fully covered with pericarp). Twenty (20) kernels were stained for each experiment, and were divided into classes by physically counting them. The percent staining values were also obtained according to the method by Serna-Saldivar et al. (1991) and were compared for the 100 g and 500 g methods at all the treatment combinations. Similar values were an indication that the efficiency of pericarp removal was similar for the 100 g and 500 g methods over a wide range of processing conditions. DML was calculated by determining total solids in nejayote and wash water. Total weight of nejayote and wash water were measured by drying the water in aluminum pans for 48 h at 103^0 C in a conventional oven. The weight of dry matter obtained was then back calculated to obtain total losses as described by Sahai et al. (2000).

2.2.8. Pasting Properties

Pasting properties as a partial assessment of the degree of cook, were measured for ground raw corn and freeze-dried nixtamal using a Micro Visco Analyzer (MVA) (Brabender Instruments, Hackensack, NJ). Samples were prepared and analyzed according to the method of Mariotti et al. (2005). The analysis was performed using 15 g sample and 100 ml water (adjusted to 14 % moisture level). The Standard 1 heating profile was used for all the samples which is holding at 30^0 C for 1 min, followed by heating to 95^0 C and holding for 5 min, cooling to 50^0 C and holding for 1 min. MVA parameters peak viscosity, peak temperature, shear thinning viscosity, set-back viscosity, final viscosity, and pasting temperature were analyzed as defined by Wang and Ratnayake (2014), and compared for the two methods. Similar pasting properties of nixtamal processed using the two methods would indicate a similar degree of cook.

2.2.9. Gelatinization Properties

Gelatinization properties, as an assessment of the thermal properties of ground raw corn, freeze-dried nixtamal and masa, were evaluated using Differential Scanning Calorimetry (DSC). The sample were tested in a Perkin Elmer Pyris 1 DSC, and the scanned samples were analyzed using Pyris version 3.50 software (Perkin-Elmer Co., Norwalk, Conn., USA). Samples (10 µg) were prepared in excess water (56 µl) according to the method described by Ratnayake et al. (2009). The heating profile was to hold at 25^0 C for 1 min and heat from 25^0 C to 125^0 C at 10^0 C per minute. DSC parameters of onset temperature (To), peak temperature (Tp), transition conclusion temperature (Tc), range, and enthalpy were determined for all the samples.

2.2.10. Statistical Analysis

Central Composite Design Response Surfaces were analyzed using SAS Proc Mixed (v 9.4. SAS Institute, Cary, NC, USA) as outlined by Yglesias et al. (2005). A second order model was fit for the responses, including the linear interaction terms. Non-significant terms ($p > 0.05$) were dropped and the lack-of-fit tests were conducted to check the fit of the model. Bonferroni confidence intervals were built around the parameter estimates. The alpha level was adjusted for the three parameters such that a 90 % Bonferroni confidence interval was obtained similar to the study by Yglesias et al. (2005). These intervals were then compared for the 100 g and 500 g methods for all response variables which contained significant terms. Overlapping intervals indicated that the estimates were not significantly different for the two methods. Surface plots were built using Design Expert software (Stat Ease, Version 7.1.3, East Hennepin,

Minneapolis, MN, USA). The effect of cook temperature, cook time and steep time on the degree of cook using both the cooking methods were modeled using response surfaces. Similar response surface would indicate that these factors have the same effect within the range of processing parameters studied for both the methods. If the response variable could not be explained with any predictor model, or if there was a significant lack of fit, the mean response model was considered to be the best estimate. For these variables, the responses were also compared using JMP (Version 10.0, SAS Ins. Inc., Cary, NC, USA) to understand if the responses were significantly different by Tukey LSD at alpha = 0.05.

2.3. Results and Discussion

2.3.1. Corn Characterization

The composition and physico-chemical properties of the corn samples used for nixtamalization were measured. The results are summarized in Table 2.2. Given that the sample tested had relatively low levels of stress cracks, hard endosperm, high test weight and density, moisture of around 14 %, high protein content indicating a packed structure, and a starch content of around 72 %, the sample represented a typical nixtamalization-suitable sample.

2.3.2. Pericarp Removal, pH and Total Starch

The nixtamal obtained after cooking using the 100 g and 500 g methods was tested for degree of pericarp removal, pH of the wash water and total starch. The results are summarized in Table 2.3. Mean values of pericarp removal of 24 % were obtained

according to the method by Serna-Saldivar at al. (1991). Nejayote pH of 11 and percentage total starch content of 81 % was observed for both the methods. Since a single variety was processed at different conditions, the same percentage total starch after cooking is an indication that the degree of pericarp removal is similar for both the methods. Similar values for these nixtamal quality parameters for the two methods at all the treatment conditions used indicate similar nixtamal quality of the sample treated with both the methods. There was no significant effect ($p \leq 0.05$) of cook temperature, cook time and steep time on these thee parameters for the processing conditions selected. Also, the values for these responses were not significantly different when Tukey LSD means were compared at alpha = 0.05.

Yglesias et al. (2005) observed no effect of cooking conditions on pH, nixtamal pericarp removal and total starch content. Serna-Saldivar et al. (1991) observed that degree of pericarp removal is affected by the corn hybrid as well as location. Trejo-González et al. (1982) reported a decrease in pH as more calcium is absorbed by corn during cooking. Sahai et al. (2001) found poor correlations ($p < 0.05$, $r^2 = 0.43$) between wash water pH and alkaline cooking parameters (cook temperature and steep time). Since there was only one hybrid and the same water-lime ratios were used in this study, the no significant difference in nejayote pH and degree of pericarp removal observed for both the methods was as expected.

2.3.3. Nixtamal Moisture

Nixtamal moisture plays an important role in determining the final properties of the product. Optimum cook time and steep time are determined by subjectively evaluating nixtamal moisture (Gomez et al. 1991, Serna-Saldivar 2012b). The effect of process variables was similar for the 100 g and 500 g method. Nixtamal moisture in the range of 45.06 % to 52.24 % was obtained using the 500 g method and 45 % to 52.94 %, using the 100 g method. The range of values for nixtamal moisture was similar for the two methods and there was a linear effect of all 3 process variables: cook temperature, cook time, and steep time as described in equation (1).

$$\hat{Y} = \beta 0 + \beta 1\ (\text{cook time}) + \beta 2\ (\text{steep time}) + \beta 3\ (\text{cook temperature}) \quad (1)$$

Where β0 is the intercept and β1, β2, β3 are the linear slopes for each of the process variables. There was no significant slope difference between the 100 g and 500 g methods when 90 % Bonferroni confidence intervals were compared (Fig. 2.1.A). The response surfaces using the two methods were similar. At a fixed steep time of 7 h, there was a linear increase in nixtamal moisture with an increase in both cook temperature and cook time for both the methods (Fig. 2.2). The response surface and confidence interval comparison for the 100 g and 500 g methods indicate that corn cooked with the two methods had the same post cook characteristics, when measured in terms of nixtamal moisture.

For the 100 g method, ($p < 0.0001$, $r^2 = 0.76$, lack of fit = 0.16):

$$\text{Nixtamal moisture} = 11.47 + 0.211 * \text{steep time} + 0.100 * \text{cook time} + 0.377 * \text{cook temperature} \quad (2)$$

For the 500 g method, ($p < 0.0001$, $r^2 = 0.76$, lack of fit = 0.31):

*Nixtamal moisture = 8.940 + 0.173 * steep time + 0.090 * cook time + 0.416 * cook temperature* (3)

Serna-Saldivar et al. (1993) observed a high overall relationship ($p < 0.01$, $r = 0.7$) between cook time and nixtamal moisture and concluded that the cook time can be adjusted depending on the nixtamal moisture desired. Sahai et al. (2001) noted that nixtamal moisture increased with increase in cook temperature, cook time and steep time.

The results obtained for both the methods were consistent with previous studies. Increases in cook time and temperature causes more absorption of water by the corn kernels leading to higher nixtamal moisture values. The 100 g method was able to mimic the nixtamalization characteristics of the 500 g method for the hybrid used in this study, and can be used as a predictor for nixtamal moisture.

2.3.4. Dry-Matter Loss (DML)

DML is the estimate of the amount of dry matter removed from the kernel during the cooking and washing steps. The processing variables used had a similar effect on DML for the 100 g and 500 g methods when the response surfaces and equations were compared. DML values varied from 3.78 to 5.47 g using the 500 g method and from 3.62 to 5.61 g using the 100 g method. The range of values for DML were similar for the two methods. Equation 4 summarizes the effect of processing on DML for both the methods.

$$\hat{Y} = \beta 0 - \beta 1 (\text{steep time}) + \beta 2 (\text{cook time}) + \beta 3 (\text{cook temperature}) + \beta 4 (\text{steep time}) * (\text{cook time}) + \beta 5 (\text{cook time}) * (\text{cook temperature}) * - \beta 6 (\text{cook temperature})^2 \quad (4)$$

Where β0 is the intercept and β1, β2 and β3 are the linear slopes for steep time, cook time and cook temperature respectively, β4 is the linear slope for interaction effect between cook time and steep time, β5 is the linear slope for interaction effect between cook time and cook temperature, and β6 is the quadratic slope for cook temperature.

For the 100 g method, ($p < 0.0001$, $r^2 = 0.92$, lack of fit = 0.60):

$$DML = -29.29 - 0.061 * \text{steep time} + 0.085 * \text{cook temperature} + 0.065 * \text{cook time} + 3.25 (\text{steep time} * \text{cook time}) + 7.49 * (\text{cook time} * \text{cook temperature}) - 5.34 * (\text{cook temperature})^2 \quad (5)$$

For the 500 g method, ($p < 0.0001$, $r^2 = 0.83$, lack of fit = 0.06):

$$DML = -36.406 + 0.078 * \text{steep time} + 0.91 * \text{cook temperature} + 0.202 * \text{cook time} + 2.46 (\text{steep time} * \text{cook time}) + 2.38 * (\text{cook time} * \text{cook temperature}) - 9.3 * (\text{steep time})^2 - 5.02 * (\text{cook temperature})^2 \quad (6)$$

For a steep time of 7 h, there was an increase in DML with increasing cook time and cook temperature for both the methods (Fig. 2.3). DML increased almost linearly with an increase in cook time for the 500 g method; for the 100 g method, DML almost remained constant with increasing cook times at a very low cook temperature (83 ^{0}C) and decreased with increasing cook temperature at a very low cook time (10.5 min). The response surface of the 100 g method however, mimics that of the 500 g method with cook temperature > 85 ^{0}C and cook time > 15 min. As the common expectation would be

that DML increases with increases in cook temperature; the DML model for the 100 g method suggests that the 100 g procedure might not produce valid DML values at very low cook temperatures (< 85 ^{0}C) and cook times (< 15 min). The 100 g method was not sensitive enough to detect changes in DML at very low cook time and cook temperature.

Although steep time had a negative quadratic effect for the 500 g method, it was balanced by a positive linear effect of steep time. Thus, indicating that for both the methods, steep time impacts DML. A quadratic effect of cook temperature and a linear effect of cook time was observed using both the methods. Also, no significant slope differences were observed when 90 % Bonferroni confidence intervals were compared for 100 g and 500 g methods (Fig 2.1..B). The high r^2 values and low p-values indicate the model fit well for both the methods. Slight differences observed in the response surface could be due to the small sample size used, which likely increased variability. The 100 g method, like the 500 g method can be used as a screening technique to assess the degree of cook in terms of DML.

Pflugfelder et al. (1988) reported that the main loss in dry matter occurred during the steeping period. However, Sahai et al. (2000) noted that cook temperature and lime concentration during nixtamalization were primary factors affecting DML ($p < 0.0002$, lack of fit $p = 0.1325$, $r^2 = 0.72$). Steep time and cook time were of secondary importance. Although we did not test lime concentration variable, the quadratic relationship with cook temperature observed for both the methods is consistent with the study by Sahai et al. (2000). Yglesias et al. (2005) also observed linear and quadratic effect of cook temperature ($p < 0.001$, lack of fit $p = 0.8059$, $r^2 = 0.74$) with 500 g and pilot plant nixtamalization methods.

2.3.5. Nixtamalization Time-Temperature Profile

The time temperature profile obtained by recording temperatures every 30 s was used to compare the thermal changes occurring in the 100 g and 500 g methods. Continuous monitoring of temperature helped to ensure that the experimental design conditions were achieved and maintained throughout the process. The 100 g method has a similar profile to the 500 g method.

Fig. 2.4 represents the time-temperature profile for the center points in the designs, cook temperature: 87.5 ^{0} C, steep time: 7 h and cook time: 21.5 min. Four distinct thermal phases were observed from the temperature profiles. The results were similar to Yglesias et al. (2005): 1) linear drop in temperature when corn kernels were added to the pre-heated water-lime solution (- 3.5 ± 0.2 ^{0} C/min), 2) linear increase in temperature to reach the desired cook temperature (+ 1.82 ± 0.4^{0} C), 3) a nearly steady temperature state during cooking period (± 3^{0} C), 4) exponential decline in temperature during the steeping period (- 0.14 ± 0.02^{0} C). Thus, the 100 g method was successfully able to mimic the thermal profile observed using the 500 g method, and hence the near-industrial scale pilot plant nixtamalization process. The 100 g method would have a similar effect on corn cook properties as for the industrial scale cooked corn.

2.3.6. MVA Analysis

Pasting properties of the nixtamal cooked under different conditions was studied to understand the effect on starch gelatinization. There was no significant effect of the processing conditions used on peak viscosity, setback and final viscosity, peak temperature, and pasting temperature of the samples using either method for cooking.

There was a significant lack of fit for both the methods, hence mean models were fit. Sahai et al. (1999) observed significant effect of steep time on peak viscosity, setback and final viscosity ($p < 0.005$, $r^2 = 0.63$) and no effect on peak temperature ($p < 0.2025$, mean model). RVA peak time also had a significant linear relationship with the processing parameters ($p < 0.0006$, $r^2 = 0.57$, lack of fit, $p = 0.2481$). However, they concluded that RVA peaks, though significant, were less predictive of degree of cook by nixtamalization than DSC enthalpy values. Yglesias et al. (2005) found no effect of cooking properties on peak, setback and final viscosity, but a significant effect of cook time and cook temperature on peak temperature. Gomez et al. (1989) observed restricted swelling and gelatinization of starch granules during nixtamalization as they are tightly locked within endosperm cells. Gomez et al. (1992) reported partial gelatinization of starch to occur during nixtamalization as a result of the physical constraints of endosperm cells and insufficient heat and moisture levels reached during cooking and steeping. The main reason for not observing any effect on pasting properties could be because, nixtamalization did not cause a wide enough range in gelatinization of starch using any of the cook conditions in the study. Since all the samples were already cooked prior to MVA analysis, very subtle differences were recorded in their rate of gelatinization as well as gelatinization viscosities and temperatures; no significant effect could be modeled. Overlapping values were obtained for peak temperature (~94^0 C), peak viscosity (~210 μ), breakdown viscosity (~176 μ), and final viscosity (~331 μ) (Table 2.3). Also, individual properties were not significantly different ($p > 0.05$) when compared using Tukey LSD means at alpha = 0.05.

2.3.7. DSC Enthalpy

Nixtamal DSC enthalpy was affected significantly by steep time and cook time using both the methods for cooking. DSC enthalpy ranged from 6.53 to 11.58 J/kg using the 500 g method for cooking, and from 6.37 to 10.98 J/kg using the 100 g method. The range of values for enthalpy were similar for the two methods. There was a quadratic effect of steep time and linear effect of cook time as described in equation 7.

$$\hat{Y} = \beta 0 + \beta 1\,(\text{cook time}) + \beta 2\,(\text{steep time}) - \beta 3\,(\text{cook temperature}) + \beta 4\,(\text{cook time}) * (\text{steep time}) - \beta 5\,(\text{steep time})^2 \quad (7)$$

Where β0 is the intercept and β1, β2 and β3 are the linear slopes for cook time, steep time and cook temperature respectively, β4 is the linear slope for interaction effect between cook time and steep time and β5 is the quadratic slope for steep time.

For the 100 g method ($p < 0.0001$, $r^2 = 0.70$, lack of fit = 0.08);

$$\text{Enthalpy} = 10.86 + 1.05 * \text{steep time} + 0.078 * \text{cook time} - 0.065 * \text{cook temperature} + 0.018 * (\text{steep time} * \text{cook time}) - 0.083 * (\text{steep time})^2 \quad (8)$$

For the 500 g method, ($p < 0.0001$, $r^2 = 0.82$, lack of fit = 0.11);

$$\text{Enthalpy} = 7.91 + 1.317 * \text{steep time} + 0.034 * \text{cook time} - 0.054 * \text{cook temperature} - 0.074 * (\text{steep time})^2 \quad (9)$$

For a cook temperature of 87.5^0 C, there was an increase in enthalpy with an increasing cook time and steep time for both the methods (Fig. 2.5). For the 500 g method, enthalpy increased linearly with an increase in cook time at a very low steep time (4 h). For the 100 g method enthalpy almost remained constant with increasing cook

times at a very low steep time (4 h) and decreased with increasing steep time at a very low cook time (10.5 min). The 100 g method is not likely sensitive enough at low cook time and steep time to properly model the effect of alkaline cooking on DSC enthalpy. The 100 g method, however, mimics the 500 g method in producing a quadratic increase in enthalpy with steep time and a linear increase with cook time when the steep time is > 5.51 h and the cook time is > 15 min.

A quadratic effect of steep time indicates that it has maximum influence on nixtamal enthalpy. The 100 g method is not very sensitive at low cook time and seep time to detect changes in enthalpy. When response surfaces were compared, keeping cook time constant at 21.5 min, enthalpy linearly decreased with increase in cook temperature and quadratically decreased with steep time for both the methods (Fig. 2.6). No significant slope differences were observed when Bonferroni confidence intervals were compared for the 100 g and 500 g methods (Fig. 2.1.C). High r^2 and low p-values were obtained when the same equations were fit for the 100 g and 500 g methods, which indicate their similarity. DSC parameters like nixtamal onset temperature, peak temperature and end temperature were not significantly different when slopes were compared for the 100g and 500g method (data not shown). Similar gelatinization properties for the 100 g and 500 g method indicate that the 100 g method can be used to predict the thermal properties of cooked corn using DSC.

Sahai et al. (1999) observed DSC enthalpy is primarily influenced by steep time ($p < 0.001$, $r^2 = 0.82$, lack of fit $p = 0.2428$) and an increase in enthalpy with an increase in steep time. Starch remains at temperatures just below gelatinization for almost 5 h during steeping, thus having a maximum effect on thermal properties of starch. Hence,

DSC enthalpy values can be used as an indicator of the extent of steeping. Yglesias et al. (2005) also noted that nixtamal enthalpy was primarily affected by steep time. The results observed using the 100 g and 500 g method are consistent with these studies.

2.4. Study Limitations

The study was conducted on one hybrid in order to determine if the process would mimic the previously studied 500 g method over a wide range of processing conditions. A total number of 34 runs were tested for this assessment. If adopted in a commercial environment, additional hybrid and multi-year testing would be advisable. Also, experimental variation in cook temperature for the 100 g method, compared to the methods using more corn, may have accounted for some of the major differences seen between the 100 g and 500 g methods.

2.5. Conclusion

Nixtamal moisture, dry matter loss and DSC enthalpy were significantly affected using the selected ranges of cook temperature, cook time and steep time. The models fitted using the 100 g and 500 g methods for these parameters were comparable, and the response surfaces produced for various nixtamal quality parameters were similar. There were some differences observed between the 100 g and 500 g methods, but they were mainly at low cook time and steep time (For gelatinization properties when steep time was 4 h and cook time 10.5 min and for DML when cook temperature 80^0 C and cook time 10.5 min). The variability may be because of the very small sample size used. Regardless, for important nixtamalization parameter of nixtamal moisture and DML, the

response surfaces obtained using the 100 g method were not different from those using the 500 g method. The cost of analyzing 12 samples at a time in 2 batches using the 100 g method is half of that for the 500 g method (Appendix C). The 100 g method successfully mimics the 500 g method over a wide range of processing conditions and can thus be used as an analytical tool by corn processors to screen corn hybrids.

2.6. Acknowledgements

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2.7. References

AACC. 2000. Approved Methods of the American Association of Cereal Chemists, 10th AACC.

Almeida Dominguez, H, Suhendro, EL and Rooney, L. 1997. Corn alkaline cooking properties related to grain characteristics and viscosity (RVA). Journal of Food Science 62:516-519.

Anonymous. 2003. Quantachome- Particle size technology operating manual in: Quantachome instruments- Multipycnometer. Quantachome Instruments: 1900 Corporate drive, Boyton Beach, FL, USA 33426.

AOAC. 2000. Association of Analytical Communities. AOAC International: Rockville, MD, USA.

FGIS. 2013. Federal grain inspection service, 06/26/2013. http://www.gipsa.usda.gov/fgis/fgis.aspx: Washigton DC.

Gomez, M., Lee, J., McDonough, C., Waniska, R. and Rooney, L. 1992. Corn starch changes during tortilla and tortilla chip processing. Cereal Chemistry 69:275-279.

Gomez, M., McDonough, C., Rooney, L. and Waniska, R. 1989. Changes in corn and sorghum during nixtamalization and tortilla baking. Journal of Food Science 54:330-336.

Gomez, M., Waniska, R. and Rooney, L. 1991. Starch characterization of nixtamalized corn flour. Cereal Chemistry 68:578-582.

Jackson, D., Rooney, L., Kunze, O. and Waniska, R. 1988. Alkaline processing properties of stress-cracked and broken corn (*Zea mays* L.). Cereal Chemistry 65:133-137.

Johnson, W. B., Ratnayake, W. S., Jackson, D. S., Lee, K.-M., Herrman, T. J., Bean, S. R. and Mason, S. C. 2010. Factors affecting the alkaline cooking performance of selected corn and sorghum hybrids. Cereal Chemistry 87:524-531.

Khan, M., DesRosiers, M., Rooney, L., Morgan, R. and Sweat, V. 1982. Corn tortillas: evaluations of cooking procedures. Cereal Chemistry 59:274-284.

Mariotti, M., Zardi, M., Lucisano, M. and Pagani, M. A. 2005. Influence of the heating rate on the pasting properties of various flours. Starch 57:564-572.

Paulsen, M. and Hill, L. 1985. Corn quality factors affecting dry milling performance. Journal of Agricultural Engineering Research 31:255-263.

Peplinski, A., Paulsen, M., Anderson, R. and Kwolek, W. 1989. Physical, chemical, and dry-milling characteristics of corn hybrids from various genotypes. Cereal chemistry (USA), 66.2:117-120.

Pflugfelder, R., Rooney, L. and Waniska, R. 1988. Dry matter losses in commercial corn masa production. Cereal Chemistry 65:127-132.

Pomeranz, Y., Hall, G., Czuchajowska, Z. and Lai, F. 1986. Test weight, hardness, and breakage susceptibility of yellow dent corn hybrids. Cereal Chemistry 63:349-351.

Ratnayake, W. S., Otani, C. and Jackson, D. S. 2009. DSC enthalpy transitions during starch gelatinization in excess water, dilute sodium chloride and dilute sucrose solutions. Journal of the Science of Food and Agriculture 89:2156-2164.

Sahai, D., Mua, J., Surjewan, I., Buendia, M., Rowe, M. and Jackson, D. 1999. Assessing degree of cook during corn nixtamalization: impact of processing variables. Cereal Chemistry 76:850-854.

Sahai, D., Mua, J., Surjewan, I., Buendia, M., Rowe, M. and Jackson, D. 2001. Alkaline processing (nixtamalization) of white mexican corn hybrids for tortilla production: significance of corn physicochemical characteristics and process conditions. Cereal Chemistry 78:116-120.

Sahai, D., Surjewan, I., Mua, J., Buendia, M., Rowe, M. and Jackson, D. 2000. Dry matter loss during nixtamalization of a white corn hybrid: Impact of processing parameters. Cereal Chemistry 77:254-258.

Serna-Saldivar, S., Almeida-Dominguez, H, Gomez, M., Bockholt, A. and Rooney, L. 1991. Method to evaluate ease of pericarp removal on lime-cooked corn kernels. Crop Science 31:842-844.

Serna-Saldivar, S., Gomez, M., Almeida-Dominguez, H, Islas-Rubio, A. and Rooney, L. 1993. A method to evaluate the lime-cooking properties of corn (*Zea mays*). Cereal Chemistry 70:762-762.

Serna-Saldivar, S. O. 2012. Physical and morphological properties of cereal grains, Pages 1-20 in Cereal Grains: Laboratory Reference and Procedures Manual. CRC Press. Taylor and Francis group, Boca Raton, FL.

Serna-Saldivar, S. O. 2012, Production of maize tortillas and lime cooked products, pages 179-195 in Cereal Grains: Laboratory Reference and Procedures Manual. CRC Press. Taylor and Francis group, Boca Raton, FL.

Thompson, R. A. and Foster, G. H 1963. Stress cracks and breakage in artificially dried corn, Pages 1-35, Volume 651 of USDA Marketing Research Report, Agriculture

Marketing Services, US.

Trejo-González, A., Feria-Morales, A. and Wild-Altamirano, C. 1982. The role of lime in the alkaline treatment of corn for tortilla preparation. Advances in Chemistry Series 198:245-263.

Wang, H and Ratnayake, W. S. 2014. Physicochemical and thermal properties of Phaseolus *vulgaris* l. var. great northern bean starch Journal of Food Science. 79.3:C295-C300.

Wichser, W. 1961. The world of corn processing. Am. Miller Process 89:29-31.

Yglesias, R., Parkhurst, A. M. and Jackson, D. S. 2005. Development of laboratory techniques to mimic industrial-scale nixtamalization. Cereal Chemistry 82:695-701.

Zhu, T., Jackson, D. S., Wehling, R. L. and Geera, B. 2008. Comparison of amylose determination methods and the development of a dual wavelength iodine binding technique. Cereal Chemistry 85(1):51-58

Table 2.1: Levels of Factors Used in the Response Surface Central Composite Design for Corn Nixtamalization

Factors	Units	Levels [a]			
		-1 Level	+1 Level	-alpha	+alpha
Steep time	h	4.03	9.97	2	12
Cook time	min	10.50	32.50	3	40
Cook temperature	°C	83.04	91.96	80	95

[a] Each numeric factor is varied over 5 levels : ± α (axial points), ± 1 (factorial points), and center point.
Lowest and highest values for – alpha and + alpha were defined and - 1 level and + 1 level obtained by setting α = 1.68.

Table 2.2: Proximate composition and physico-chemical characteristics of yellow corn (NE-Y hybrid, crop year 2013) used in nixtamalization study

Constituents (%) [a]	
Starch	71.97 ± 0.76
Protein	7.53 ± 0.06
Total dietary fiber	15.17 ± 0.21
Fat	4.40 ± 0.20
Ash	1.40 ± 0.02
Calcium	0.49 ± 0.01
Phosphorous	30.67 ± 0.24
Moisture	14.62 ± 0.06
Physical Characteristics [b]	
Test weight (kg/m^3)	841.8 ± 3.22
Thousand kernel weight (g)	316.35 ± 3.95
Breakage (WBT) (%)[c]	41.65 ± 3.08
Stress cracks (%)[d]	11.33 ± 0.01
Stenvert hardness (%)[e]	64.39 ± 0.52
Floaters (%)	94.00 ± 1.00
Hardness index	Hard
Density (Pycnometer) (g/cc)	1.29 ± 0.01

[a] Results based on a 100 % db sample weight

[b] Mean and standard deviation of results in triplicates

[c] Ratio of kernels recovered over a US no. 3 sieve after shaking for 90 s to total weight of sample

[d] % Stress cracks = (1 * single crack) + (2 * double cracks) + (3 * multiple cracks)

[e] Stenvert hardness % corresponds to weight recovered over 425 µm divided by total weight of the ground sample; other Stenvert data not shown

Table 2.3: Mean and standard deviation values for non-significant parameters [b] (pericarp removal, Nejayote pH, Nixtamal total starch, MVA properties) measured by nixtamalization experiments

Variable	100 g method	500 g method
Nixtamal pericarp removal (%)	25.67 ± 2.02	23.45 ± 3.87
Nejayote pH	11.24 ± 0.12	11.09 ± 0.18
Nixtamal total starch (%) [a]	81.44 ± 1.12	81.89 ± 1.34
Nixtamal peak temperature (^{0} C)	94.84 ± 1.64	94.76 ± 1.56
Nixtamal peak viscosity (μ)	210.06 ± 19.32	211.08 ± 18.85
Nixtamal breakdown viscosity (μ)	176.79 ± 12.56	176.16 ± 13.78
Nixtamal final viscosity (μ)	331.29 ± 14.52	331.87 ± 15.89

[a] Results based on a 100 % db sample weight

[b] Models were not significant using Design Expert or there was a significant lack of fit for the model. Hence, mean models were analyzed. The variables were also compared using Tukey LSD at 0.05 significance level, and no differences were found. Hence, a mean value is stated.

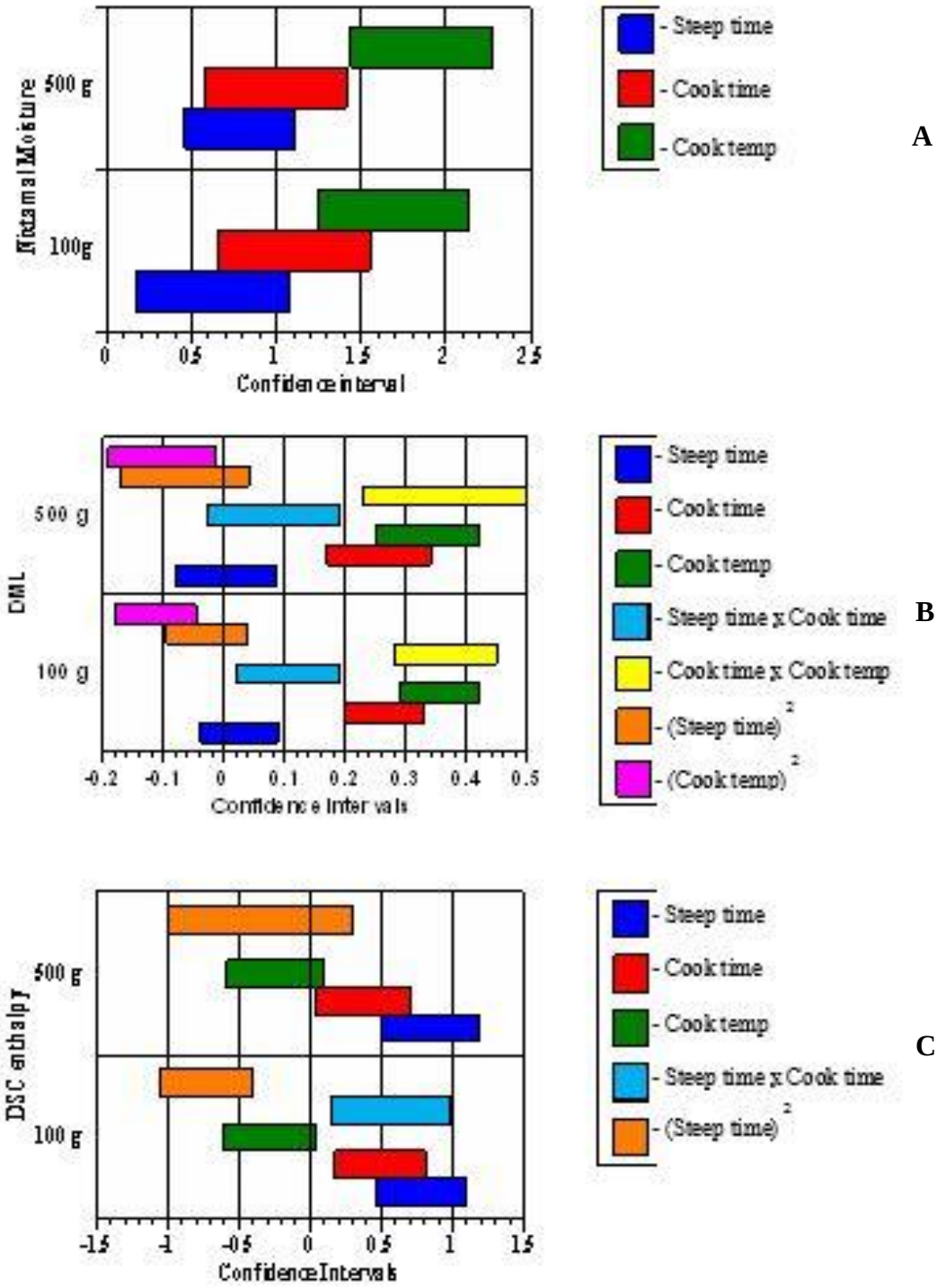
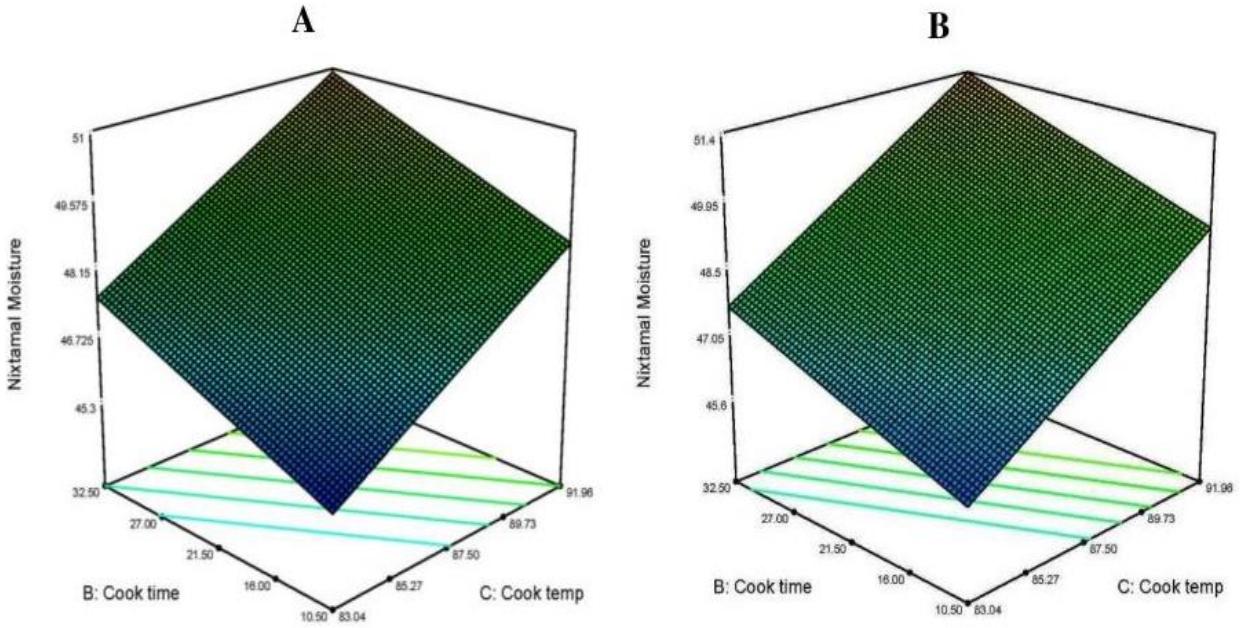
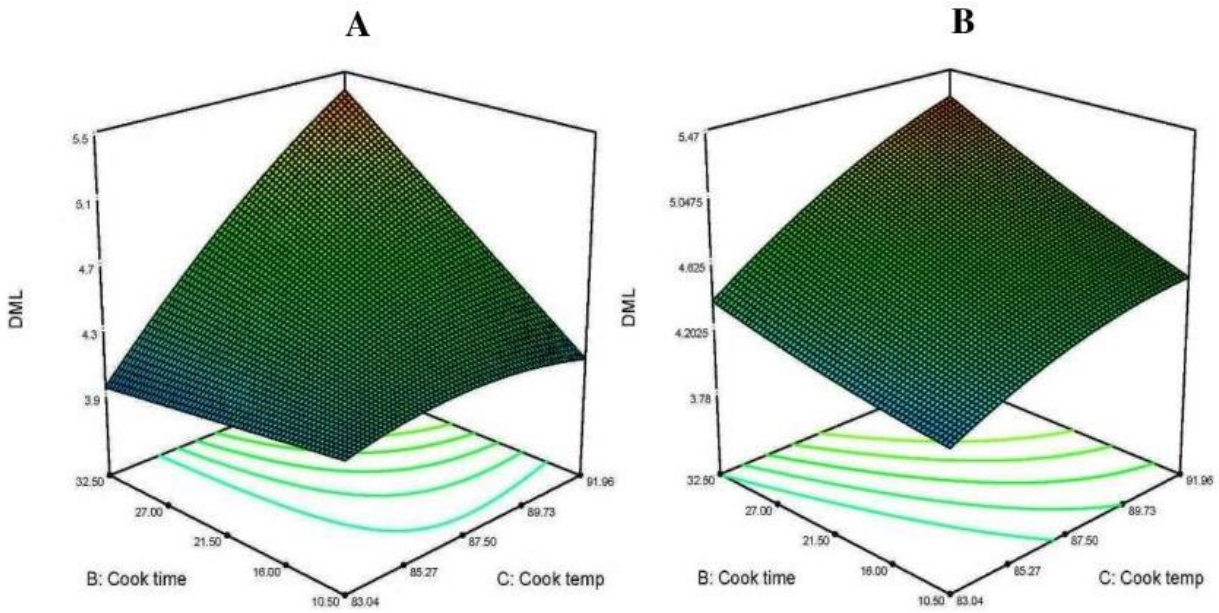


Fig 2.1: Bonferroni confidence intervals (90 %) for A) nixtamal moisture, B) dry matter loss and C) DSC enthalpy for nixtamal using the 100 g and 500 g methods for cooking.
Overlapping bars with the same color indicate no significant difference for the 100 g and 500 g methods.



($p < 0.0001$, $r^2 = 0.76$, lack of fit = 0.16) ($p < 0.0001$, $r^2 = 0.76$, lack of fit = 0.31)

Fig 2.2: 100 g (**A**) and 500 g (**B**) predicted values for nixtamal moisture (%) as a function of cook time (min) and cook temperature (^{0}C), holding steeping time constant at 7 h.



($p < 0.0001$, $r^2 = 0.92$, lack of fit = 0.60) ($p < 0.0001$, $r^2 = 0.83$, lack of fit = 0.06)

Fig 2.3: 100 g method (**A**) and 500 g method (**B**) predicted values for dry matter loss as a function of cook time (min) and cook temperature (^{0}C), holding steep time constant at 7 h.

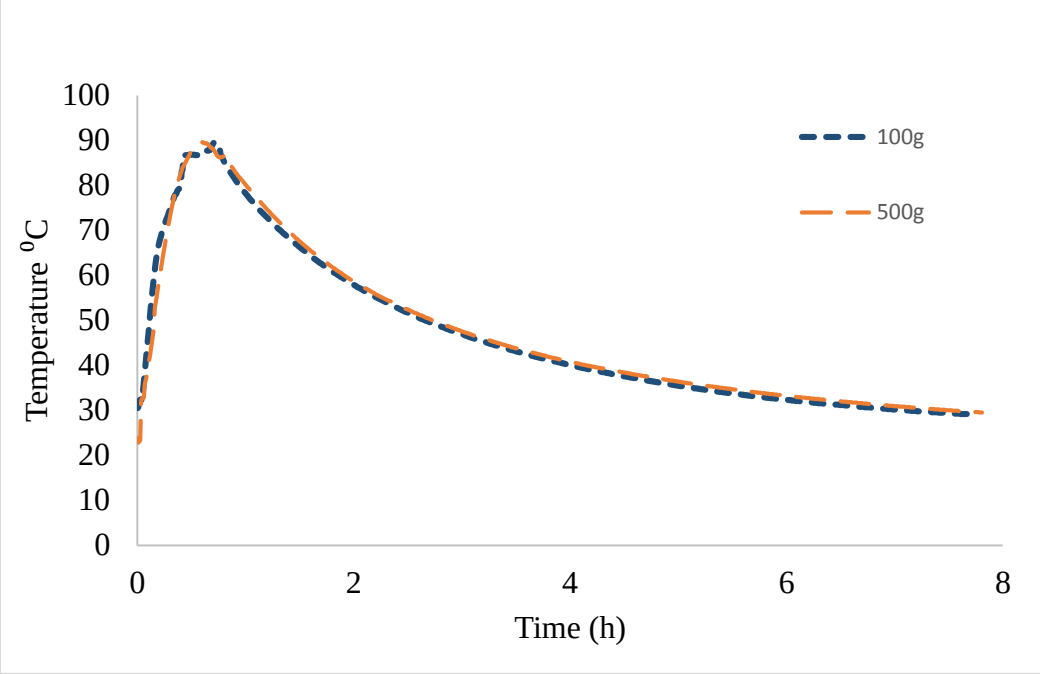
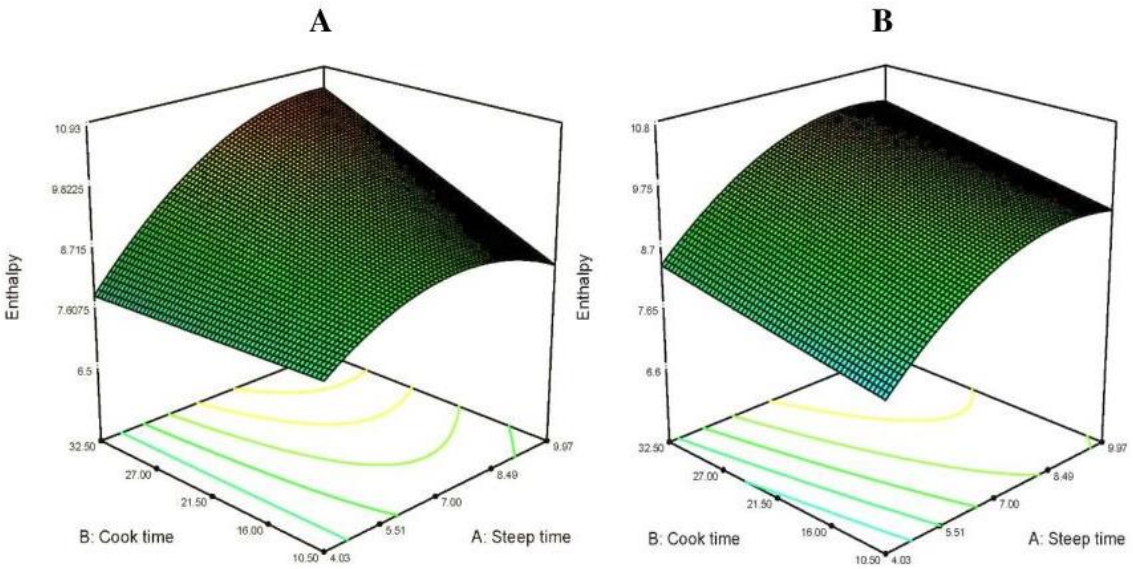
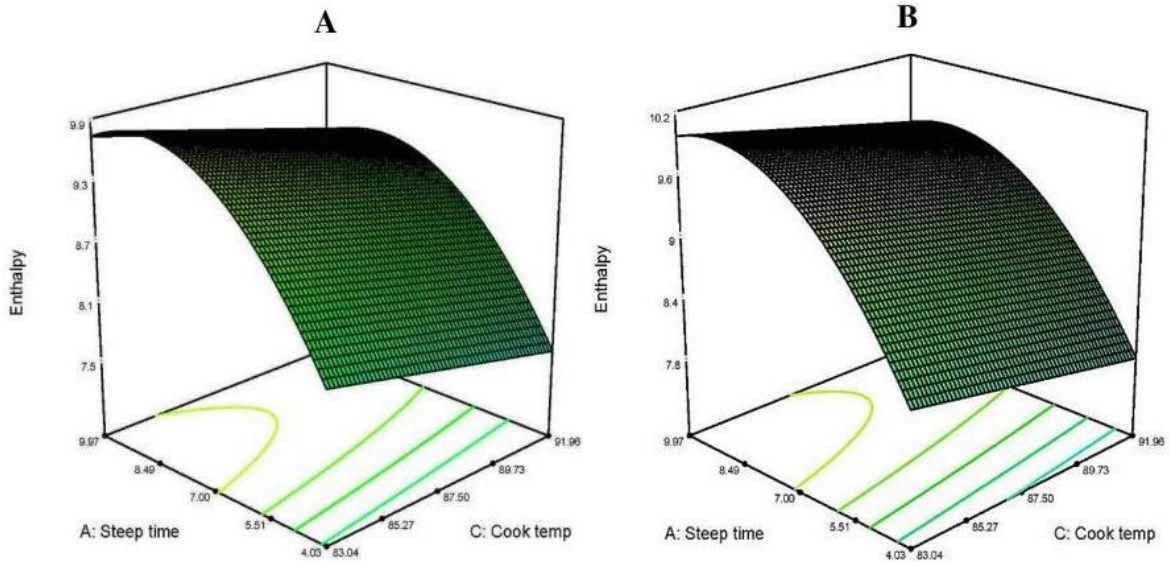


Fig 2.4: Representative time-temperature profile for the 100 g method and 500 g nixtamalization methods at the center point (steep time-7 h, cook time-21.5 min and cook temperature- 87.5^0 C) measured with a data logger at every 30 s intervals.



($p < 0.0001$, $r^2 = 0.70$, lack of fit = 0.08) ($p < 0.0001$, $r^2 = 0.82$, lack of fit = 0.11)

Fig 2.5: 100 g method (**A**) and 500 g method (**B**) predicted values for DSC enthalpy as a function of cook time (min) and steep time (min), holding cook temperature constant at 87.5^0 C.



($p < 0.0001$, $r^2 = 0.70$, lack of fit = 0.08) ($p < 0.0001$, $r^2 = 0.82$, lack of fit = 0.11)

Fig 2.6: 100 g method (**A**) and 500 g method (**B**) predicted values for DSC enthalpy as a function of steep time (min) and cook temperature (^{0}C), holding cook time constant at 21.5 min.

CHAPTER 3: EFFECT OF CORN PHYSICOCHEMICAL PROPERTIES ON NIXTAMAL MOISTURE AND DRY MATTER LOSS USING A BENCH-TOP NIXTAMALIZATION METHOD

Abstract

Corn with a rounded kernel, smooth dent, high proportion of hard endosperm, easily removable pericarp, clean bright color, and tolerance to breakage during handling is desirable for alkaline processing. Processors rely on experience and/or near full scale production testing for selecting hybrids with optimum characteristics for alkaline cooking. Previous studies have attempted to establish relationships between corn cooking properties and raw kernel physico-chemical properties; no single property has been found to accurately predict alkaline cooking performance. The aim of this study was to use a small-scale bench top method to determine if correlations can be established between physico-chemical properties and process-related parameters of corn using 9 hybrids grown during the 2013 crop year. Cook quality was determined in terms of nixtamal moisture and dry matter loss. Multivariate analysis was performed to obtain correlations between raw corn physical and chemical properties, and regressions with nixtamal moisture and DML. No significant correlation was found with DML or nixtamal moisture when corn was cooked for 3 min. When corn was cooked for 25 min, however, DML was related to thousand kernel weight and uncooked kernel calcium content ($p < 0.05$, $r^2 = 0.98$), and nixtamal moisture was related to test weight ($p < 0.05$, $r^2 = 0.52$). Consistent with previous studies, it remains important to nixtamalize corn, at least in small batches, to understand its likely industrial performance. The prototype bench-top method is useful for screening.

3.1. Introduction

Lime cooked products such as tortilla chips, corn chips and table tortillas have gained popularity world-wide. Corn grown in the United States varies in kernel chemical composition and physical characteristics (Peplinski et al. 1992). Corn properties are affected by genetics, environment, harvesting, storage, and handling practices (Almeida Dominguez and Rooney 1997). Corn physical and chemical characteristics impact the nixtamalization process, and resulting product qualities. Nixtamalization process parameters such as cook time, cook temperature and steep time are adjusted by processors depending on the corn sample used in order to obtain the desired end product quality.

Almeida Dominguez and Rooney (1997) studied the effect of different physico-chemical properties on alkaline cooking performance. Percent floaters, hardness by TADD, thousand kernel weight, density by multipycnometer, RVA viscosity slope and peak values were found to predict optimum cook time. Dry matter loss could be predicted by TADD index, density, test weight, and RVA viscosity slope and peak values. RVA viscosity could be correlated with hardness class and alkaline cooking properties. The study nevertheless concluded that alkaline cooking is a complex mechanism and has contributions of endosperm hardness, kernel size and kernel density. Hence, no individual kernel properties can predict alkaline cooking performance, they all contribute to the cooking mechanism. Soft kernels have air spaces which can cause faster rate of water diffusion (Almeida Dominguez and Rooney 1997). This causes high hydration rates and gelatinization when measured by RVA. Thus, techniques like RVA which measure

individual kernel properties such as hydration capacity and surface area can be used as potential techniques to predict alkaline cooking quality.

Jackson et al. (1998) studied the alkaline cooking performance of stress-cracked and broken kernels. Broken kernels cause increased dry matter loss and high nixtamal moisture. Presence of stress cracks had minimal effect on nixtamal properties, but caused broken kernel formation. They found Wisconsin Breakage Test to be a good measure of corn sample susceptibility to kernel breakage. Pflugfelder et al. (1988) observed high dry matter loss when soft corn was cooked using parameters designed for hard corn. Soft corn kernels have short optimum cooking times, small percentages of corneous endosperm and high amount of floaters. These kernels absorb a large amount of water during steeping. Martinez Herrera and Lachance (1979) found that intermediate to hard corn kernels have fewer floaters, high density and test weight. Harder corn requires longer cooking to produce nixtamal with similar hardness characteristics. Shandera et al. (1997) studied the effect of eight dent maize hybrids grown in different locations in Nebraska, USA on nixtamal quality. The authors grouped physical tests by principal component analysis to obtain relevant tests which can help to explain the process variability. Most physical tests showed variability associated with hybrid and location. They found strong correlation between nixtamal moisture and hardness (r = - 0.852) as well as percent floaters (r = 0.795).

Johnson et al. (2010) studied the impact of physical attributes on alkaline cooking performance using 100 dent corn samples consisting of 70 hybrids grown in Illinois, Indiana, Iowa, Kansas, Kentucky, and Nebraska, USA. The physical characteristics were grouped on the basis of their principal component score. Alkaline cooking was conducted

according to the nylon bag method by Serna-Saldivar et al. (1991). Nixtamal moisture was correlated to TADD, kernel moisture, starch and protein content ($p < 0.05$, $r^2 = 0.64$). The study however concluded that laboratory nixtamalization is still the best method for predicting industrial nixtamalization performance. Individual characterization tests can be used for screening.

Sahai et al. (2001) tried to establish non-empirical relationships between nixtamalization process variables, and grain quality parameters to predict nixtamalized product properties. The study used 5 corn hybrids grown in Sinaloa, Veracruz, Chiapas, Chihuahua, Jalisco, and Mexico primarily for traditional nixtamalization and tortilla production. Each hybrid was cooked using 10 different processing conditions and a response-surface model was developed to study these relationships. For alkaline cooking, 30 kg of corn was cooked on a pilot-plant scale with 150 kg water and 300 g lime on a gas-fired horizontal cook steep tank covered with a lid. Dry matter loss during nixtamalization was correlated ($p < 0.05$, $r^2 = 0.79$) to cook time, steep time, cook temperature, percentage breakage value, and hardness class. Nixtamal moisture was correlated ($p < 0.05$, $r^2 = 0.78$) to cook time, steep time, cook temperature, 1000 kernel weight, and calcium content of the kernel. Dry matter loss was significantly dependent ($p < 0.05$, $r^2 = 0.60$) on corn cook temperature, cook time, steep time, percent floaters, and thousand kernel weight. However, a poor correlation was established between wash water pH and processing variables ($p < 0.05$, $r^2 = 0.43$). The study concluded that nixtamal product properties are affected mainly by process variables, which can be adjusted depending on corn hybrid. Kernel composition and hardness class also play important

roles in influencing nixtamal characteristics and processing outputs. The results for these studies are summarized in Table 3.1

When nixtamalization is performed on a commercial scale, there is less opportunity to fully and reproducibly replicate trials. Sahai et al. (2001), however, used fairly large sample quantities, replicated in a response surface design, to obtain relationships between cooking properties and grain parameters. They used small enough (30 kg) batches however, to study diverse hybrids using a wide range of processing conditions. Pilot plant studies by Jackson (1988), Serna-Saldivar (1993) and Johnson et al. (2010) have also tried to establish these relationships, but by using smaller sample quantities. Even the most comprehensive studies however, have concluded that effective tests to rapidly and quantitatively predict alkaline cooking quality using kernel physico-chemical parameters are not possible. Thus, it is essential to test the suitability of corn by performing at-least a small-scale or pilot plant nixtamalization test to understand which corn samples give desired quality end products.

The aim of this study was to use a prototype laboratory scale alkaline cooking method to establish and confirm the relationship between corn physico-chemical properties and nixtamal quality. Although these relationships are unlikely to be sufficient to predict nixtamalization characteristics, consistency with previous studies would help affirm the applicability of the small scale processing method. In order to test the potential relationships between corn properties and nixtamal quality, as measured in a prototype 100 g nixtamalization system, nine food-grade corn hybrids were selected for corn testing.

3.2. Materials and Methods

3.2.1. Corn Samples

Nine samples of yellow corn (*Zea mays* L.) grown in Illinois and Nebraska, USA and harvested during the 2013 crop season (coded as A, B, C, D, E, F, G, H, and I) were used for the physical characterization and nixtamalization experiments. The samples were obtained in pails of 22.67 kg (50 lb.) and stored in a refrigerator (2^0 C).

3.2.2. Corn Characterization

Each corn sample was characterized according to tests described by Sahai et al. (2001). Corn samples (≈ 500 ± 20 g) were removed from each pail. The samples were then equilibrated at room temperature for 24 h. The kernels were shaken on a sieve shaker (Model: SS-15, Gilson company Inc., Ohio, USA) for 90 s using a 12/64" round hole dockage sieve (Seedburo Equipment company, Des plains, IL, USA) to remove any foreign material and broken kernels.

3.2.2.1. Composition Analysis

Uncooked corn moistures were determined using a two-stage method for samples containing more than 13 % moisture, as described by (AACCI Approved method 44-15A, AACCI 2000). Raw corn samples were sent to Ward Laboratories (Kearney, NE, USA) for protein, TDF, fat, ash and total starch analysis as described by AOAC approved methods (AOAC 2000). Percentage ash was determined using AOAC 923.03, calcium and phosphorous using AOAC 984.27 and 985.01, fat using AOAC 922.06, protein by

Dumas method- AOAC 968.06 and 992.15, and starch using ethanolic extraction- AOAC 996.11.

3.2.2.2 Test Weight and Thousand Kernel Weight

Test weight was measured in pounds/ bushel and expressed as (kg/m^3) according to AACC Method 55-10 (AACCI 2000). Thousand kernel weight was obtained by manually counting 100 randomly selected whole corn kernels, weighing up to an accuracy of 0.01 g, and multiplying by a factor of 10 as described by Sahai et al. (2001).

3.2.2.3. Breakage Susceptibility

The Wisconsin Breakage Susceptibility test was conducted according to Paulsen and Hill (1985). The sample was passed through a WBT machine (Model: 9/84, Cargill Grain Research Lab, MN, 55406) using a vibratory feeder for 30 s. The collected sample was passed through a sieve shaker (Model: SS-15, Gilson company Inc., Ohio, USA) for 90 s using a US No. 4 sieve, and the overs weighed. The percentage of broken kernels was calculated using equation 1.

$$\% \text{ Breakage} = \frac{(\text{Original sample weight-Weight of overs})*100}{\text{Original sample weight}} \quad (1)$$

3.2.2.4. Stress Cracks and Fissures

Stress cracks were measured by placing 100 whole kernels on a light box and visually counting them. The kernels were divided into one of the 6 classes: no cracks, single crack, double cracks, multiple cracks, checked / crazed and broken as described by Thompson and Foster (1963).

3.2.2.5. Hardness Index

The Stenvert Hardness Test was conducted by grinding a 20 g corn sample using a Kinematica Polymix, PX-MFC 90 D Micro Hammer Mill (Glenmills Inc., Clifton, NJ) equipped with a 2 mm screen at 3600 rpm according to the method described by Pomeranz et al. (1986). The milled corn was collected in a 20 cm recovery tube. The total height and soft endosperm height of the material collected in the tube were measured using a ruler. The time required to collect 20 g of corn, reduced hammer mill rpm at maximum grinding power, and the quantity of hard endosperm recovered over a 425 µm sieve were also measured.

3.2.2.6. Percent Floaters

The Floaters Test was performed according to Wischer (1961) and Peplinski (1989). A 31.3° Baume solution of sodium nitrate ($NaNO_3$) was prepared at a constant temperature of 60° C corresponding to a specific gravity of 1.275 g/cm^3. The number of floating kernels was counted. Percent floaters were determined according to equation 2. Percent floaters obtained were divided into 3 hardness classes as described by Wischer

(1961). Corn with more than 79 % floaters is soft, less than 47 % floaters is hard, and corn with 47 % to 79 % floaters is considered of average hardness.

$$\% \text{ floating kernels} = \frac{\text{Number of floating kernels}}{\text{Total number of kernels}} \quad (2)$$

3.2.2.7. True Density

True density of whole corn kernels was determined using a Gas multi-pycnometer (Quantachrome, MVP 05034, Boyton Beach, FL) according to the method described in the Quantachrome manual (Anonymous 2003, Pomeranz et al. 1984). A stainless steel calibration sphere (Part number 75212, Ref: Part number 01500- Large) with a volume of 56.5592 cm^3 was used for calibration.

3.2.2.8. Percent Amylose

Amylose content of ground samples was measured by the dual-wavelength iodine binding method outlined by Zhu et al. (2008). Amylose corn standards of 10, 20, 30, 50, and 80 (w/w, d.b.), also prepared according to the method by Zhu et al. (2008), were used to create a differential absorbance vs. % amylose standard curve.

3.2.3. Prototype Laboratory Nixtamalization

A laboratory scale nixtamalization process developed by scaling down to 100 g and modifying Yglesias et al.'s (2005) 500 g method was used to conduct nixtamalization experiments. Consistent with previous research, the laboratory scale nixtamalization process occurs in 4 main stages that included, cooking, quenching, steeping and washing steps.

3.2.3.1. Cooking

Corn and water in the ratio of 1:4 were used for the cooking process. The initial temperature of water was maintained at 25 ± 2^0 C. To this water, 2.4 g of food grade calcium hydroxide (Vitacal ^{TM}, Mississippi Lime Company, St. Louis, MO, USA) was added. The lime solution was stirred on a hotplate for 30 s. Aluminum baskets with round holes of 1/8 inch (0.32 cm) mesh size, basket height 14.5 cm, 10 cm diameter and a spacing of 3 cm above the beaker surface were obtained from National Manufactures (Division of TMCO Inc., Lincoln, NE, USA). A stirrer was placed in the space between the beaker bottom and the basket to ensure uniform solution mixing and appropriate temperature control. The aluminum baskets were placed in the beakers with the lime-water solution and 100 g pre-weighed corn was added. The beakers were then wrapped with aluminum foil, which was designed to act as insulation during the cooking and steeping processes (Appendix B). Hot-plates (Super Nuova, Model: SP135935 Thermo Fischer Scientific Inc., MA, USA) were pre-heated to 60° C, prior to placing the beakers on the hot-plates. The corn was then cooked at 94° C (water temperature) for 3 min or 20 min. Continuous agitation was maintained throughout the process.

3.2.3.2. Quenching

After completion of the cooking step, the beakers were immediately removed from the hot-plates and the aluminum foil insulation unwrapped. The mixture was quenched by adding 180 ml of cold water (25 ± 2° C), followed by stirring with a glass rod so that the final temperature was about 63 ± 2° C.

3.2.3.3. Steeping

After quenching, the samples were covered again with aluminum foil and placed in a water bath (49° C) for a total of 12 h. The temperature and pH of steep liquor was noted before placing in the water bath

3.2.3.4. Washing

A washing process was developed to maximize pericarp removal from the kernels, similar to the set-up described by Mistry and Eckhoff (1992) (Appendix D). The kernels were washed using a 4 inch (10.16 cm) soft white nylon bristle brush (Drill Brush, Useful Products LLC, Marcy, NY, USA) attached to a motor (Caframo A-210, Cole-Parmer Ins. Co., Barrington, IL, USA). The brush was rotated at 100 rpm. Aluminum baskets with round holes similar to those used for cooking but with a mesh size of 1/4 inch (0.64 cm), basket height 17.5 cm, and with a diameter of 11 cm, were made for the washing step. The round holes of the baskets allowed the detached pericarp to drain. The baskets were placed in a 2000 ml glass beaker and the kernels were washed with water pumped at 70 rpm by using a Caframo pump (Caframo 7553-70, Cole-Parmer Ins. Co., Barrington, IL, USA) attached to a Master flex speed controller (Cole-Parmer, Barrington, IL, USA). The water was pumped from a tube at a constant flow rate of 13.8 cc/s. The combined motion of brush abrasion and flowing water was used to remove pericarp loosened by cooking and steeping processes. The corn was washed 3 times with cook water and once with 800 ml fresh water. Pericarp attached to the basket was cleaned by pumping 500 ml clean water through the basket. The washed kernels were blotted with paper towels to remove the surface moisture. The washed kernels were flash frozen

in liquid nitrogen followed by freeze drying at 0.22 mbar vacuum using a bench top freeze drier (FreeZone 4.5L, Labconco Co., Kansas City, MO., USA) for 72 h to achieve a moisture content of 3 ± 2 % at the end of freeze-drying, and stored at - 4° C until further analysis.

3.2.4. Nixtamal Moisture

The washed kernels were blotted with paper towels and dried in the oven at 103° C for 72 h and moisture was calculated by a two-stage method (AACC Approved method 44-15A, AACCI 2000).

3.2.5. Dry Matter Loss (DML)

The water drained from the washing step was collected in pre-weighed aluminum pans and dried in a forced air oven at 103° C for 48 h. The amount of dry matter lost from the kernels was calculated as described by Sahai et al. (2000). The degree of pericarp removed from the washed kernels was assessed by staining the kernels with May-Greenwald solution according to the method outlined by Serna- Saldivar et al. (1991). Photographic images of the stained kernels were used for comparison (Appendix D).

3.2.6. Experimental Design and Statistical Analysis

Nine corn hybrids were cooked at two cook times, 3 min and 25 min. Multivariate analysis was conducted using SAS Proc Mixed (v 9.4, SAS Institute, Macy, NY, USA) to establish correlations between the different grain physical and chemical parameters. Correlations with $p < 0.05$ and $r^2 > 0.6$ have been reported. The factors which were

significant in the correlation matrix were analyzed using stepwise regression analysis. Multiple linear regression equations that best described the changes in nixtamal moisture and DML, with change in the independent variables (grain physical parameters and compositional characteristics) were built for DML and nixtamal moisture at two different cook times: 3 min and 25 min using SAS (v 9.4, SAS Institute, Macy, NY, USA) as outlined by Sahai et al. (2001). Regression equations have been reported for variables with $r^2 > 0.5$. The variables which had the greatest effect are listed first for each equation.

3.3. Results and Discussion

3.3.1. Corn Characterization

The proximate composition of each corn sample is given in Table 3.2. The physical characterization test results are summarized in Table 3.3. All samples had a protein content between 7.53 - 9.57 % and a fat content between 3.0 - 5.8 %. Sample G had the highest protein, fat and ash content. Sample A had the lowest protein and F had the lowest fat and moisture content. Sample H showed the highest calcium content. Sample test weight is a measure of the bulk density, but factors including grain history, moisture content, kernel shape, mechanical treatment, percentage and type of broken kernels, and foreign materials affect test weight (Pomeranz et al. 1986). All samples had a bulk density between 841-944 kg/m^3 (65.34-73.34 lb/bu). Sample A had the lowest test weight (much lower than the others) and sample C had the highest test weight. Thousand kernel weight of the samples varied from 290-401 g. Hybrid I had a relatively high thousand kernel weight compared to the other samples.

The Wisconsin Breakage Test (WBT) is a measure of the susceptibility of a kernel to damage and breakage when subjected to a strong centrifugal force. It can be related to the presence of stress cracks (Jackson et al. 1988) and also to the hardness of a sample, as a hard kernel (unless extraordinarily hard and brittle) is less susceptible to breakage. It can be used as an estimate of damage during shipping and handling (Sahai et al. 2001). Samples I, G and H had low breakage values of 8.2, 10.9 and 13.2 % respectively. Samples A and D had high breakage values of 41.7 and 38.6 % respectively. Presence of stress-cracks increases susceptibility of a kernel to breakage when measured using WBT (Jackson et al. 1988). However, sample F had the highest amount of stress cracks (61 %) but a relatively low breakage value (22.6 %) compared to sample A, and sample A had very few stress cracks (4.33 %), but showed the highest breakage value (41.7 %) when measured using WBT. The high breakage for sample A may be due to very low test weight and low Stenvert Hardness value for this sample. Sample D had a high amount of stress cracks (27.67 %) and high WBT (38.6 %) value.

The Stenvert Hardness Test is a measure of kernel hardness. The samples used had harness levels varying between 33 - 46 %. Samples I and H had the highest hardness ratio (height of soft to hard endosperm). Samples A and C had the lowest hardness when measured by Stenvert. Sample I also had the highest thousand kernel weight.

The Floaters test is another measure of apparent density of corn kernels. Sample C had the lowest percentage floaters (40 %), indicating higher density compared to the other samples. C also had the highest bulk density (944 kg/m^3). Samples A, B and E had around 93 % floaters. Sample F and H had the highest percent floaters of around 96 %, indicating that they are softest varieties. These samples were less dense compared to the

other samples analyzed. When the Pycnometer was used as a measure of true density, all the samples had densities between 1.27 - 1.32 g/cm^3.

Sahai et al. (2001) observed no consistent relationship between corn characterization test and corn hybrid, indicating each test measures different physical characteristics. Test weight showed a strong positive correlation with thousand kernel weight ($p = 0.026$, $r^2 = 0.73$) and a strong negative correlation with the WBT ($p = 0.026$, $r^2 = 0.73$). Thousand kernel weight and the WBT had a very strong negative correlation ($p < 0.001$, $r^2 = 0.89$). Also, hardness characteristics measured by Stenvert were negatively related to the WBT ($p = 0.036$, $r^2 = 0.69$). Wisconsin breakage susceptibility increased with increase in stress cracks ($p < 0.001$, $r^2 = 0.89$). Within the sample set, kernels with high hardness ratios had a high density because of the compact packing of starch and proteins in the endosperm. Also, their breakage susceptibility was low when measured in terms of the WBT.

Kernel physical properties measured could also be correlated to the compositional characteristics. Higher protein content typically implies a hard kernel, hence it has a lower breakage susceptibility. There was a strong negative correlation between starch and protein content ($p = 0.009$, $r^2 = 0.80$) and a positive correlation between starch content and moisture ($p = 0.038$, $r^2 = 0.69$). Starch and protein are known to be inversely related for a kernel (Hoseney 1994). Increases in starch can increase the hygroscopicity of the kernel, giving high moisture values (Hoseney 1994). There was also a negative correlation between the WBT and phosphorous content ($p = 0.038$, $r^2 = 0.69$) and a weak correlation of starch content with thousand kernel weight ($p = 0.01$, $r^2 = 0.65$) and test weight ($p = 0.01$, $r^2 = 0.58$). Higher starch content can make the kernel structure loose,

decreasing its weight. Pomeranz et al. (1986) found correlations between density and test weight. They also found that test weight was related to many hardness parameters. Jackson et al. (1988) reported an increase in WBT with increases in stress cracks. They concluded that breakage susceptibility was related to alkaline cooking performance (Table 3.3). Our study also shows correlations between WBT and density, Stenvert hardness characteristics, as well as hybrid composition characteristics.

3.3.2. Nixtamal Moisture

There was no relationship between nixtamal moisture and physical properties when corn was cooked for 3 min. When the nixtamal moisture was measured after 25 min cooking, it could be estimated using regression equation 3 ($p < 0.05$, $r^2 = 0.52$):

$$\textit{Nixtamal moisture (\%)} = 81.89 - (0.51 * \textit{test weight}) \quad (3)$$

Nixtamal moisture is an important parameter which influences final product quality. Nixtamal moisture increased with decreases in kernel test weight when corn was cooked for 25 min. This study indicates that it is necessary to cook corn to understand the alkaline cooking properties of different hybrids. Serna-Saldivar et al. (1993) mentioned that nixtamal moistures should be controlled depending on the final product application, 50-51 % moisture is desired for tortillas and 46-48 % moisture is required for tortilla chips. Jackson et al. (1998) found increases in nixtamal moisture when broken kernels were used for cooking. They also found no significant relationships between DML and nixtamal moisture. Sahai et al. (2001) found positive correlations between nixtamal moisture and cook temperature, cook time and steep time, and negative correlation with

thousand kernel weight, and uncooked kernel calcium content ($p < 0.05$, $r^2 = 0.78$) (Table 3.3). The 100 g prototype bench-top method was able to differentiate between varieties in terms of nixtamal moisture when longer cook times were used. Also, the nixtamal moisture values were similar to those obtained using commercial scale nixtamalization at a tortilla manufacturing facility (Unpublished data, 2014) (Fig. 3.1). This indicates a similarity when kernels using the 100 g method were compared with commercial-scale nixtamalization data. Thus, the method can be used as a screening tool to understand the nixtamal quality of hybrids.

3.3.3. Nixtamalization DML

Correlations were established that relate nixtamalization DML to corn sample physical and chemical properties. There was no relationship established with physical properties when DML of the nixtamal cooked for 3 min was analyzed. When the dry matter loss was measured after 25 min cooking, DML could be estimated using regression equation 4 ($p < 0.05$, $r^2 = 0.98$):

*DML (%) = 19.73 – (0.017 * thousand kernel weight) + (0.146 * uncooked kernel calcium content)* (4)

The cook time of 3 min may not be sufficient to produce a significant effect on the DML of the different hybrids analyzed. For the short cooking time, the impact of variability within replicates was greater than when longer cooking times were used. This large variability within samples may be the reason the model could not be developed to predict physical properties and DML for the short cooking times. The 100 g prototype

bench-top method with cook time of 25 min (and perhaps less) can be used to differentiate between different hybrids based on the DML obtained.

Pflugfelder et al. (1988) studied dry matter loss in corn hybrids and concluded that the commercial process causes 8 - 12.5 % loss of dry matter, depending on kernel characteristics and processing conditions. Loss of starch, lipids and proteins is increased by using soft or damaged kernels, or by harsh conditions during cooking. However, no strong correlations were found. Serna-Saldivar et al. (1993) studied the effect of processing parameters and grain characteristics on nixtamal quality. These researchers found pericarp removal ($p = 0.08$, $r = 0.6$) and grain hardness ($p < 0.05$, $r = 0.6$) to be correlated to dry matter loss. However, in our study, no correlations were found between hardness characteristics and DML. Jackson et al. (1988) found increases in DML with a higher number of visually checked or cracked kernels. The study also found correlations between density and dry matter loss ($p < 0.05$, $r = 0.7$). They also observed that WBT was a good measure of the breakage susceptibility, and hence cook quality ($p < 0.05$, $r = 0.9$). Sahai et al. (2001) also found positive correlations between DML and cook temperature, cook time, steep time and WBT, and negative correlation with hardness index ($p < 0.05$, $r^2 = 0.79$). Serna-Saldivar et al. (1991) found poor correlations between grain hardness and pericarp removal ($r = 0.32$). They also found poor correlations between pericarp removal and thousand kernel weight, test weight and kernel density (Table 3.3). In our study, we found strong correlation between thousand kernel weight and DML, but not with WBT and hardness properties. Pflugfelder et al. (1988) did not establish any correlations between grain parameters and DML when two commercial scale methods were compared. Jackson et al. (1988) and Pflugfelder et al. (1988) were

able to obtain some correlations between kernel properties and nixtamal quality. These studies used moderate number of samples. But, the samples used did not vary greatly in their physical characteristics. Sahai et al. (2001) also found impact of corn composition and hardness on alkaline cooking performance when 5 hybrids at were cooked for a response surface experiment using 10 different processing conditions. However, nixtamalization process parameters had a greater impact on nixtamal characteristics than kernel physico-chemical properties.

The current study used 9 hybrids and some correlations were established, however, the samples did not vary greatly in their physical properties, harvest season and growth region. Almeida Dominguez and Rooney (1997) found that no one physical property could be used to predict alkaline cooking performance by study of 14 different cultivars with different physical properties. Jackson et al. (1988) studied nixtamalization performance of kernels with different level of stress cracks/ breakage and reported the use of WBT as a technique to understand nixtamal characteristics. Pflugfelder et al. (1988) studied effect of kernel characteristics on DML and found differences in DML when soft and hard kernels were cooked using the same processing conditions. Almeida Dominguez and Rooney (1997) studied the effect of different physico-chemical properties on nixtamalization performance and found RVA to be an important tool to understand cook quality of kernels. Sahai et al. (2001) studied several hybrids and a wide range of cook temperature/ lots of replicates/ large sample size and indicated that relationships can be established between nixtamal qualities and certain processing parameters. Processing conditions however, need to be optimized for similar hybrids to reduce variability between them. Hence, it is essential to conduct alkaline cooking

process, at-least on a small scale to understand the impact of processing on cooking quality when a new hybrid is cooked.

3.4. Conclusion

When a new hybrid is received by processors, they must evaluate the sample to determine its suitability for alkaline cooking. Most processors, however, rely on previous experience in order to determine if the sample meets alkaline cooking quality standards. Adjusting processing parameters based on hybrid used is difficult and requires prior knowledge about the corn properties before alkaline cooking. Studies have been conducted by Jackson et al. (1988), Pflugfelder et al. (1988), Almeida Dominguez and Rooney (1997), Rooney and Suhendro (1999), Serna-Saldivar et al. (1993), and Sahai et al. (2001), in an effort to predict nixtamal characteristics in terms of physical and chemical properties as well as processing parameters. However, no strong correlations have been found. These studies have concluded that it is essential to conduct the nixtamalization process itself to understand corn cook quality. In our study, with the use of 9 hybrids to assess correlations between corn physico-chemical properties and cook quality, we found significant correlations among breakage, hardness and raw kernel densities. Correlations were also established between dry matter loss and nixtamal moisture with physico-chemical properties of raw corn, but only when corn was cooked for longer times. The small scale method produced similar results to previous studies confirming that it is essential to cook corn at least in small quantities to understand its nixtamal chatacteristics. Compared to near commercial scale cooking, or even moderate-

sized pilot plant testing, the 100 g method can help to screen hybrids at a faster rate using fewer resources.

3.5. Acknowledgements

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3.6. References

AACC. 2000. Approved Methods of the American Association of Cereal Chemists, 10th AACC.

Almeida Dominguez, H, Suhendro, EL and Rooney, L. 1997. Corn alkaline cooking properties related to grain characteristics and viscosity (RVA). Journal of Food Science 62:516-519.

Anonymous. 2003. Quantachome- Particle size technology Operating Manual in: Quantachome Instruments- Multipycnometer. Quantachome Instruments: 1900 Corporate drive, Boyton Beach, FL, USA 33426.

AOAC. 2000. Assosciation of Analytical Communities. AOAC International: Rockville, MD, USA.

Delcour J.A and Hoseney, R. C. 2010. Structure of cereals in Principles of cereal science and technology, Pages 1-22, Ed 3. American Association of Cereal Chemists (AACC), International, St. Paul, Minnesota.

Jackson, D., Rooney, L., Kunze, O. and Waniska, R. 1988. Alkaline processing properties of stress-cracked and broken corn (*Zea mays* L.). Cereal Chemistry 65:133-137.

Johnson, W. B., Ratnayake, W. S., Jackson, D. S., Lee, K.-M., Herrman, T. J., Bean, S. R. and Mason, S. C. 2010. Factors affecting the alkaline cooking performance of selected corn and sorghum hybrids. Cereal Chemistry 87:524-531.

Martinez Herrera, M. L. and Lachance, P. A. 1979. Corn (*Zea mays*) kernel hardness as an index of the alkaline cooking time for tortilla preparation. Journal of Food Science 44:377-380.

Mistry, A. and Eckhoff, S. 1992. Alkali debranning of corn to obtain corn bran. Cereal Chemistry 69:202-205.

Paulsen, M. and Hill, L. 1985. Corn quality factors affecting dry milling performance. Journal of Agricultural Engineering Research 31:255-263.

Peplinski, A., Paulsen, M., Anderson, R. and Kwolek, W. 1989. Physical, chemical, and dry-milling characteristics of corn hybrids from various genotypes. Cereal Chemistry. 66.2:117-120.

Peplinski, A., Paulsen, M. and Bouzaher, A. 1992. Physical, chemical, and dry-milling properties of corn of varying density and breakage susceptibility. Cereal Chemistry. 69.4 (1992): 397-400.

Pflugfelder, R., Rooney, L. and Waniska, R. 1988. Dry matter losses in commercial corn masa production. Cereal Chemistry 65:127-132.

Pomeranz, Y., Hall, G., Czuchajowska, Z. and Lai, F. 1986. Test weight, hardness, and breakage susceptibility of yellow dent corn hybrids. Cereal Chemistry 63:349-351.

Rooney, L. and Suhendro, E. 1999. Perspectives on nixtamalization (alkaline cooking) of maize for tortillas and snacks. Cereal Foods World.

Sahai, D., Mua, J., Surjewan, I., Buendia, M., Rowe, M. and Jackson, D. 2001. Alkaline processing (nixtamalization) of white mexican corn hybrids for tortilla production: significance of corn physicochemical characteristics and process conditions. Cereal Chemistry 78:116-120.

Sahai, D., Surjewan, I., Mua, J., Buendia, M., Rowe, M. and Jackson, D. 2000. Dry matter loss during nixtamalization of a white corn hybrid: Impact of processing parameters. Cereal Chemistry 77:254-258.

Serna-Saldivar, S., Almeida-Dominguez, H, Gomez, M., Bockholt, A. and Rooney, L. 1991. Method to evaluate ease of pericarp removal on lime-cooked corn kernels. Crop Science 31:842-844.

Serna-Saldivar, S., Gomez, M., Almeida-Dominguez, H, Islas-Rubio, A. and Rooney, L. 1993. A method to evaluate the lime-cooking properties of corn (*Zea mays*). Cereal Chemistry 70:762-762.

Shandera, D. L., D. S. Jackson, and B. E. Johnson. 1997. Quality factors impacting processing of maize dent hybrids. *Maydica* 42.3: 281-289.

Thompson, R. A. and Foster, G. H 1963. Stress cracks and breakage in artificially dried corn, Pages 1-35, Volume 631 of USDA Marketing Research Report, Agriculture Marketing Services, US

Wichser, W. 1961. The world of corn processing. Am. Miller Process 89:29-31.

Yglesias, R., Parkhurst, A. M. and Jackson, D. S. 2005. Development of laboratory techniques to mimic industrial-scale nixtamalization. Cereal Chemistry 82:695-701.

Zhu, T., Jackson, D. S., Wehling, R. L. and Geera, B. 2008. Comparison of amylose determination methods and the development of a dual wavelength iodine binding technique. Cereal Chemistry 85(1):51-58

Table 3.1: Summary of studies on correlation between kernel physico-chemical properties and cook quality

Study	Properties studied												
	TW	1000 kernel weight	Stenvert hardness	Floaters test	Pyc ρ	TADD	WBT	Stress cracks	Pericarp removal	Proximate analysis	RVA	DML	Nixtamal moisture
Sahai et al. (2001)	√ Calcium (-)	√ Ash (+)	√ TADD (-) Fiber (+)	√ WBT (-)	x	√	√ Fat (+)	x	x	√	x	√ Cook temp, Cook time, Steep time & WBT (+) Hardness (-)	√ Cook temp, Cook time & Steep time (+) Kernel Calcium content, 1000 kernel weight (-)
Serna Saldivar et al. (1993)	√ Floaters (-)	√ ρ and Protein (+)	x	√ TADD & Moisture (+), ρ (-)	√	√ TW (-)	x	x	√	x	x	√ Cook time, Pericarp removal & Hardness (+)	√ Cook time & Hardness (%) (+)
Jackson et al. (1988)	√	√		x	√	√	√ Stress cracks (+)	√	√	√	x	√ Steep time, Steep temp, WBT & ρ (+), Pericarp removal (-)	√ Stress cracks, Breakage and DML (+)
Pflugfelder et al. (1988)	√	√	x	x	x	√	x	√	x	√	√	√ Steep time, Steep temperature, Hardness & Pericarp removal (+)	x
Johnson et al. (2010)	√	x	√	√ TADD (+)	√	√	x	x	x	√	√	x	√ TADD & Kernel moisture (+), Starch, Protein & Pyc ρ (-)
Almeida Dominguez and Rooney (1997)	√ Floaters, TADD & RVA (-), ρ (+)	√	√	√	√ TADD, Floaters & RVA (-)	√	x	x	x	√	√ TADD & Floaters (+)	√ Cook time, TW & ρ (+) TADD, Floaters, & RVA (-)	x
Shandera et al. (1997)	√		√	√		√				√		√ WBT (+)	√ TADD, Hardness & Floaters (+) TW & Protein (-)

TW- Test weight, ρ- Density, Pyc- Pycnometer
√: Parameter included in study, x: Parameter not included in study
+ : Positive correlation between parameters, - : Negative correlation between parameters

Table 3.2: Corn composition characteristics of nine (unprocessed corn hybrids) [a]

Constituents [b]	A	B	C	D	E	F	G	H	I
Starch	71.97 (0.76)	66.50 (0.53)	69.17 (0.56)	70.73 (0.68)	72.83 (0.67)	68.53 (0.78)	67.27 (0.56)	70.07 (2.39)	67.03 (0.32)
Protein	7.53 (0.06)	9.27 (0.15)	8.30 (0.09)	8.83 (0.25)	7.70 (0.14)	8.20 (0.26)	9.57 (0.06)	9.07 (0.31)	9.50 (0.10)
TDF [c]	15.17 (0.21)	12.23 (0.65)	11.33 (0.36)	12.93 (0.52)	12.90 (0.52)	19.23 (0.12)	18.10 (0.63)	15.87 (0.41)	17.90 (0.44)
Fat	4.40 (0.20)	3.70 (0.12)	4.33 (0.06)	4.27 (0.12)	3.6 (0.07)	3.00 (0.00)	5.80 (0.00)	3.17 (0.06)	3.63 (0.16)
Ash	1.40 (0.02)	1.42 (0.02)	1.35 (0.01)	1.45 (0.08)	1.44 (0.04)	1.45 (0.04)	1.79 (0.03)	1.41 (0.01)	1.51 (0.12)
Calcium	0.005 (0.00)	0.003 (0.00)	0.003 (0.00)	0.004 (0.00)	0.003 (0.00)	0.004 (0.00)	0.004 (0.00)	0.009 (0.00)	0.003 (0.00)
Phosphorous	0.31 (0.00)	0.28 (0.01)	0.31 (0.01)	0.32 (0.20)	0.31 (0.07)	0.31 (0.27)	0.40 (0.07)	0.35 (0.06)	0.34 (0.15)
Moisture	14.62 (0.06)	13.27 (0.06)	13.97 (0.04)	14.22 (0.09)	15.67 (0.15)	12.7 (0.02)	14.3 (0.13)	14.4 (0.1)	14.11 (0.03)
% Amylose	27.44 (0.2)	26.2 (0.0)	21.54 (0.7)	26.78 (0.4)	20.87 (0.5)	32.50 (0.0)	30.86 (0.5)	27.75 (0.3)	27.35 (0.5)

[a] Values in parenthesis indicate standard deviations
[b] All values are percentages of constituents present
[c] Total dietary fiber

Table 3.3: Physical characteristics of nine unprocessed corn hybrids [a]

Characteristics	A	B	C	D	E	F	G	H	I
Test Weight (kg/m^3)	841 (1.1)	911 (1.5)	944 (2.0)	889 (1.2)	907 (1.1)	923 (1.3)	935 (1.9)	905 (2.1)	936 (1.1)
1000 Kernel Wt (g)	316 (3.9)	349 (5.8)	362 (7.5)	290 (10.0)	334 (9.8)	342 (4.6)	380 (5.3)	359 (1.6)	401 (7.1)
WBT (%) [b]	41.7 (3.1)	32.3 (2.2)	24.9 (3.0)	38.6 (1.4)	33.3 (2.2)	22.6 (1.6)	10.9 (0.8)	13.2 (1.9)	8.2 (0.6)
Stress Cracks (%)	4.3 (0.6)	5.0 (1.0)	4.0 (1.0)	27.7 (2.3)	1.3 (0.6)	61.0 (8.2)	7.3 (0.6)	38.3 (6.5)	2.67 (1.5)
Stenvert Hardness (%)	35.2 (1.8)	37.5 (1.1)	33.9 (2.1)	41.1 (1.5)	40.3 (1.0)	44.4 (1.5)	43.6 (2.0)	44.7 (1.0)	46.7 (1.5)
% Floaters	94.0 (1.0)	93.7 (0.6)	40.0 (2.0)	86.3 (2.6)	91.3 (1.6)	98.3 (0.6)	78.7 (0.6)	98.7 (0.6)	84.7 (2.6)
True Density (g/cm^3)	1.29 (0.00)	1.27 (0.00)	1.32 (0.01)	1.29 (0.01)	1.30 (0.05)	1.29 (0.00)	1.30 (0.01)	1.28 (0.00)	1.30 (0.00)

[a] Values in parenthesis indicate standard deviations
[b] Wisconsin Breakage test

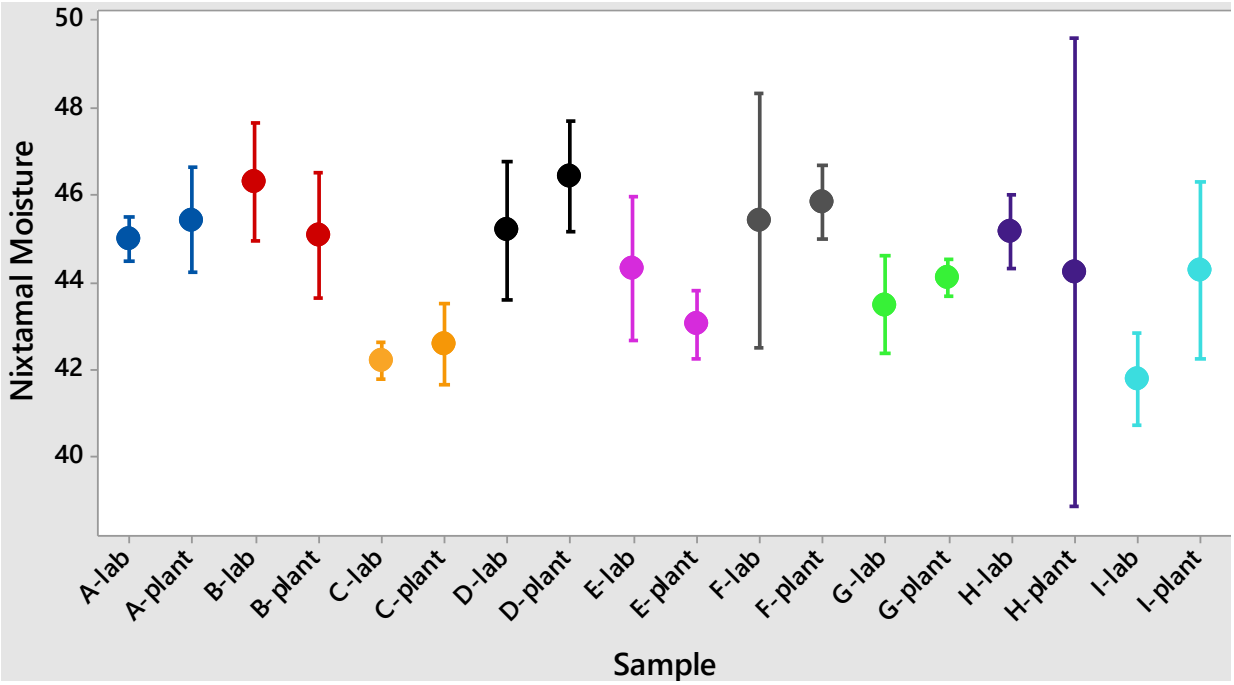


Fig 3.1: Comparison between commercial scale alkaline cooking data and bench-top 100 g method data for nixtamal moisture for 3 min cook time
Plant- Nixtamalization performed at Industrial scale (3 moisture values obtained by drawing multiples samples from the same cooked batch)
Lab- Nixtamalization performed using 100 g alkaline cooking method
Interval plots with the same color indicate the same sample compared for the 2 methods

OVERALL SUMMARY

The main aim of this study was to develop a small-scale nixtamalization process that is less complicated than other methods, is reproducible and can be used to analyze multiple samples at a time. Most processors assess the cooking quality of corn every time they procure a new hybrid. Past studies have tried to scale down the nixtamalization process as well as reduce nixtamalization times. These methods however, still require a large sample size or are expensive due to needing specialized apparatus.

A 100 g laboratory scale technique was developed based on a previously studied 500 g method. The 500 g method had found similar results to pilot plant techniques at a range of nixtamalization conditions of cook time, cook temperature and steep time, and thus was concluded to be similar to industrial nixtamalization. The analysis of response surfaces and equations for nixtamal moisture, dry matter loss, gelatinization and pasting properties indicate that the methods produced similar nixtamal quality characteristics. A range of processing parameters (steep time, cook time and cook temperature) were used in the study. Similarity in the responses over the range of processing parameters used indicates that the 100 g method mimics pilot plant nixtamalization over a range of processing conditions used commercially.

Corn attributes can vary based on the season, region of growth, as well as genetic and environmental conditions. Previous studies have concluded that individual kernel properties cannot predict total alkaline cooking performance, but that several kernel properties have some influence on the cooking mechanism. Adjusting processing parameters based on hybrid can be difficult and requires knowledge about corn properties prior to cooking. Correlations between cooking quality and grain quality parameters can

help processors to identify what physical and/or chemical attributes of a kernel yield desired nixtamal qualities. The physico-chemical properties of different hybrids were measured and samples were nixtamalized using the 100 g method. Test weight, hardness and Wisconsin breakage are correlated to each other. Nixtamalization using the 100 g method indicated that some physical properties such as test weight, Wisconsin breakage, 1000 kernel weight, and kernel calcium content impact nixtamal characteristics. No strong correlations were established. Thus, it is confirmed that it is essential to cook corn, at least on a small scale, to understand its impact on cooking performance.

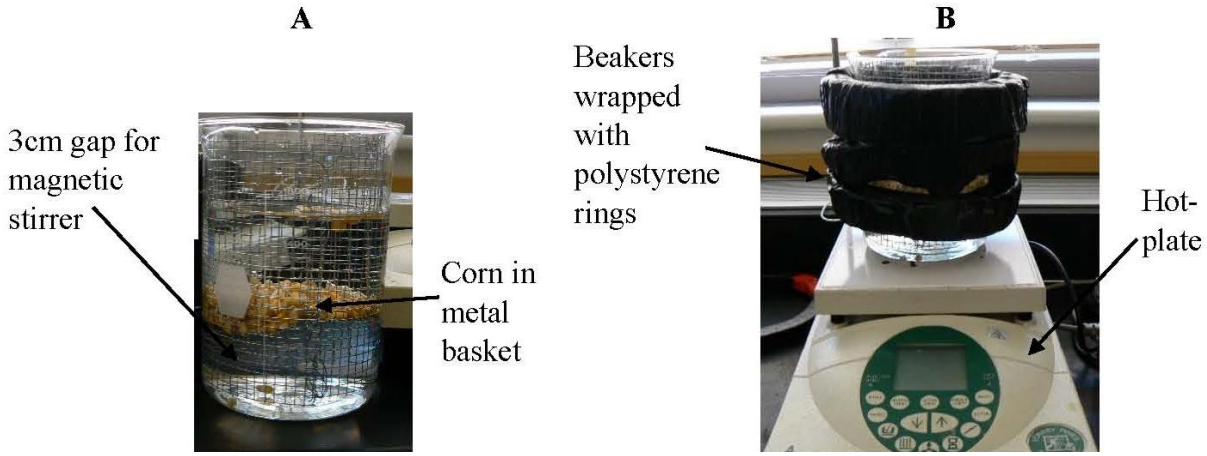
With the use of the 100 g method, processors can quickly and effectively screen various hybrids of samples to evaluate their alkaline cooking performance. This can lead to reduction in water and labor costs, effluent treatment and down time if commercial facilities must be used to test the nixtamalization potential of corn samples. The 100 g method can thus be used as a screening technique for corn with different characteristics, in order to predict nixtamalization performance.

APPENDIX

APPENDIX A: Cooking assembly for 500 g nixtamalization method

A) Beaker with lime-water solution, wire basket with 3 cm gap from the surface

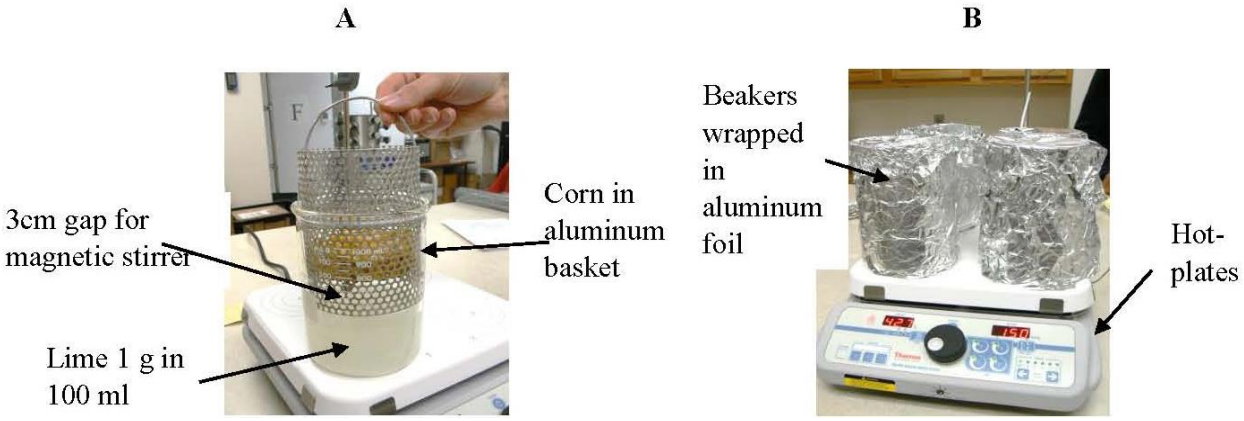
B) Beaker with lime-water solution and corn, wrapped with polystyrene rings and placed on hot-plate for cooking



APPENDIX B: Cooking assembly for 100 g nixtamalization method

A) Beaker with lime-water solution, aluminum basket with 3 cm gap from the surface

B) Beaker with lime-water solution and corn, wrapped with aluminum foil and placed on hot-plate for cooking

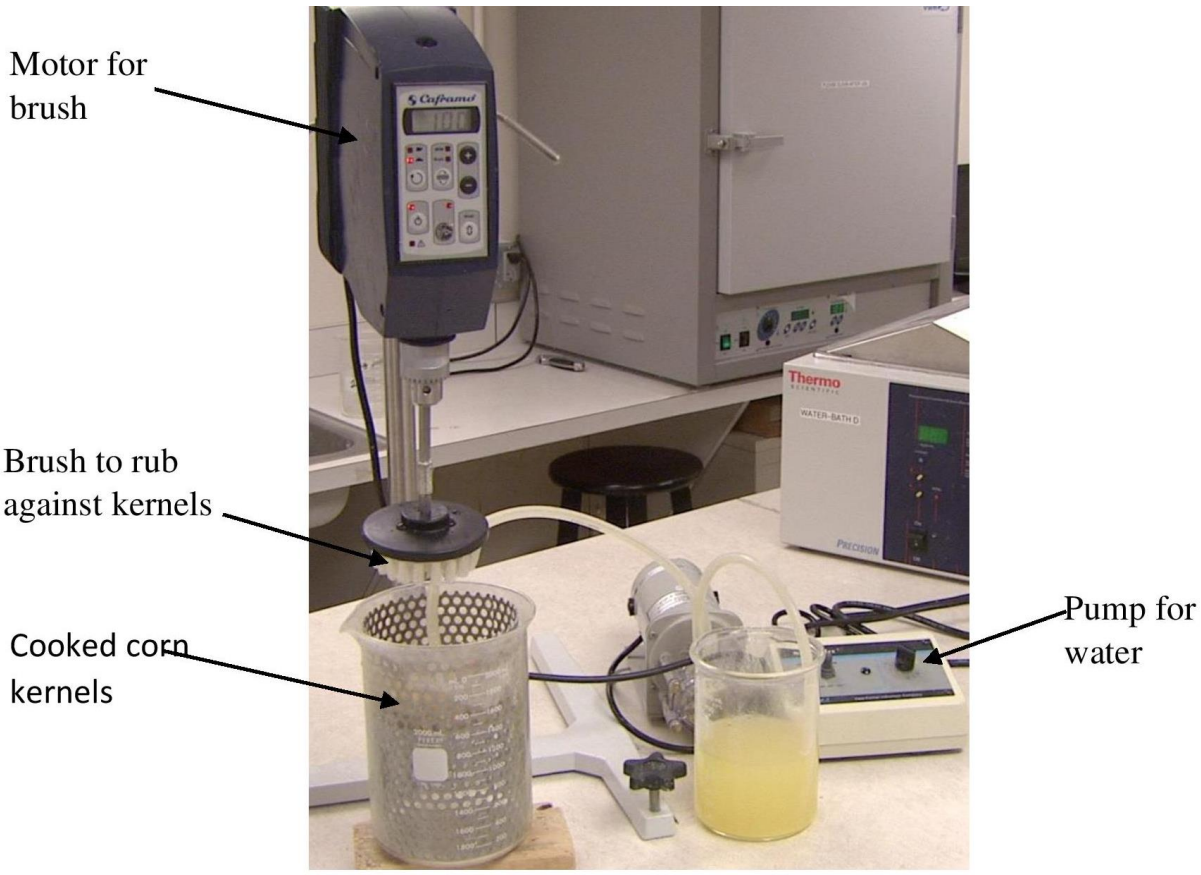


APPENDIX C: Equipment and variable costs for running the 100 g and 500 g methods

Equipment	Costs	500 g method-Cost (USD)	100 g method-Cost (USD)
Hot-plate	$ 900 (500 g)/ plate $ 1896 (100g)/ plate	10,800	5,688
Beakers	$ 21/ 2000 ml beaker (500 g) $ 11/ 1000 ml beaker (100 g)	252	132
Basket	50/ basket	600	600
Insulation	30/ beaker (500 g), 10 / beaker (100 g)	360	120
Data logger	138/ unit	1,656	552
Sub-total (Fixed costs)		13,668	7,092
Lime	$ 87.5 /kg	11	2
Labor	$ 20 / h + 30% benefits	1,040	520
Electricity	0.068/ Kilowatthour	50	10
Sub-total (Variable costs) [1]		1,107	532
Total cost		$ 14,775	$ 7,622

[1] Variable cost estimate for 12 samples in 2 batches

APPENDIX D: Apparatus designed at UNL for washing step after alkaline cooking. A brush to rub against kernel surface and pump for spraying water to remove pericarp loosened by abrasion



APPENDIX E: Pericarp staining images of samples cooked for 3 min and 25 min. Green color indicates presence of pericarp on the kernel, yellow means completely removed pericarp

APPENDIX F: Correlations matrix for corn kernel physical and chemical properties and alkaline cook quality parameters

Property	1000 kernel wt	TW	WBT	Pyc density	Percent floaters	Stenvert hardness	Kernel moisture	TDF	Fat	Protein	Ca	P	Ash	Starch	DML 3 min cook	DML 25 min cook	Nixtamal moisture 3 min cook	Nixtamal moisture 25 min cook
1000 kernel wt	1.00	0.73	-0.89	0.13	-0.29	0.42	-0.12	0.44	0.01	0.57	-0.26	0.46	0.41	-0.65	-0.30	-0.67	-0.56	-0.70
TW	0.73	1.00	-0.73	0.16	-0.50	0.35	-0.30	0.16	0.00	0.54	-0.60	0.32	0.31	-0.63	-0.13	-0.84	-0.50	-0.71
WBT	-0.89	-0.73	1.00	-0.10	0.15	-0.70	0.17	-0.62	0.07	-0.67	0.07	-0.69	-0.51	0.60	-0.12	0.48	0.48	0.64
Pyc density	0.13	0.16	-0.10	1.00	-0.44	-0.05	0.08	0.22	-0.04	-0.52	0.18	0.03	-0.19	0.30	0.22	0.08	-0.49	-0.24
Percent floaters	-0.29	-0.50	0.15	-0.44	1.00	0.46	0.02	0.37	-0.34	-0.04	0.28	-0.09	0.04	0.17	0.20	0.41	0.63	0.68
Stenvert hardness	0.42	0.35	-0.70	-0.05	0.46	1.00	-0.11	0.76	-0.25	0.54	0.13	0.57	0.48	-0.31	0.37	-0.11	-0.16	-0.17
Kernel moisture	-0.12	-0.30	0.17	0.08	0.02	-0.11	1.00	-0.30	0.52	-0.31	0.11	0.16	0.04	0.69	0.01	0.12	-0.27	-0.18
TDF	0.44	0.16	-0.62	0.22	0.37	0.76	-0.30	1.00	-0.13	0.27	0.52	0.59	0.56	-0.33	0.31	0.18	-0.07	0.06
Fat	0.01	0.00	0.07	-0.04	-0.34	-0.25	0.52	-0.13	1.00	0.03	0.18	0.46	0.64	0.11	-0.24	0.00	-0.31	-0.22
Protein	0.57	0.54	-0.67	-0.52	-0.04	0.54	-0.31	0.27	0.03	1.00	-0.20	0.52	0.55	-0.80	-0.12	-0.47	-0.28	-0.51
Ca	-0.26	-0.60	0.07	0.18	0.28	0.13	0.11	0.52	0.18	-0.20	1.00	0.45	0.28	0.31	0.51	0.88	0.13	0.38
P	0.46	0.32	-0.69	0.03	-0.09	0.57	0.16	0.59	0.46	0.52	0.45	1.00	0.82	-0.22	0.40	0.05	-0.40	-0.41
Ash	0.41	0.31	-0.51	-0.19	0.04	0.48	0.04	0.56	0.64	0.55	0.28	0.82	1.00	-0.41	-0.02	-0.09	-0.24	-0.21
Starch	-0.65	-0.63	0.60	0.30	0.17	-0.31	0.69	-0.33	0.11	-0.80	0.31	-0.22	-0.41	1.00	0.38	0.57	0.18	0.37
DML 3 min cook	-0.30	-0.13	-0.12	0.22	0.20	0.37	0.01	0.31	-0.24	-0.12	0.51	0.40	-0.02	0.38	1.00	0.55	0.19	0.20
DML 25 min cook	-0.67	-0.84	0.48	0.08	0.41	-0.11	0.12	0.18	0.00	-0.47	0.88	0.05	-0.09	0.57	0.55	1.00	0.42	0.66
Nixtamal moisture 3 min cook	-0.56	-0.50	0.48	-0.49	0.63	-0.16	-0.27	-0.07	-0.31	-0.28	0.13	-0.40	-0.24	0.18	0.19	0.42	1.00	0.90
Nixtamal moisture 25 min cook	-0.70	-0.71	0.64	-0.24	0.68	-0.17	-0.18	0.06	-0.22	-0.51	0.38	-0.41	-0.21	0.37	0.20	0.66	0.90	1.00