2009

Thermal Characterization of Corn Starch Mutants and Textural Effects on Tortillas

Kim Anne Rohlfing
Iowa State University

Thermal characterization of corn starch mutants and textural effects on tortillas

By

Kimberly Anne Rohlfing

A thesis submitted to the graduate faculty
In partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Pamela J. White, Major Professor
Cheryll A. Reitmeier
Linda M. Pollak

Iowa State University

Ames, Iowa

2009

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Ten parent corn lines, comprised of four mutants (*dull sugary2, amylose-extender sugary2, amylose-extender dull,* and an *amylose-extender* (*ae*) with introgressed Guatemalan (GUAT) germplasm) and six lines with introgressed exotic germplasm backgrounds were crossed with each other to create 20 progeny crosses. The parents and progeny crosses were characterized for % resistant starch (RS), gelatinization, and retrogradation characteristics. The RS was measured from the extracted starch targeting the measurement of RS 2, which is present in ungelatinized starch, by using the Megazyme Resistant Starch kit. The RS values from the 10 parent lines varied from 18.3 % to 52.2 %, and the values from the 20 progeny crosses ranged from 16.6 to 34.0 %. Greater RS in parents was correlated to greater RS in the progeny crosses ($r = 0.63$, $P \leq 0.05$). The Differential Scanning Calorimeter (DSC) was used to measure the gelatinization and retrogradation characteristics of the starches. Peak gelatinization temperature and change in enthalpy were positively correlated to % RS ($r = 0.65$ and $r = 0.67$, $P \leq 0.05$); however, the retrogradation parameters, a measure of RS 3, did not correlate with % RS (RS 2 type). All parents, with the exception of Guat *ae*, and progeny crosses had % RS greater than that of commercial cornstarch (8.9%), but lower than that of a high-amylose standard (50 % apparent amylose, 40.2 % RS). The % RS and onset temperature increased with the addition of the *ae* gene.

Tortillas are a simple food system made from whole corn that has been nixtamalized. A high-amylose, non-*floury* corn type with 55.2% RS, a *floury* corn type with 1% RS, and a 1:1 blend with 28.2% RS were used to make traditional tortillas. Whole corn was nixtamalized

and ground to make masa. The masa was evaluated for pasting properties on a Rapid-Visco Analyser. The high-amylose masa slurry gelatinized only slightly, as noted by a small change in peak viscosity during the 95° C heat treatment. The *floury* masa had the greatest peak viscosity, whereas the blend was intermediate in value. Tortillas were evaluated by an 11-member sensory panel who evaluated the textural attributes of grittiness, moistness, chewiness, rollability, and tearability. The *floury* tortillas were chewier, more rollable, and grittier than the high-amylose tortillas. The blend tortillas were intermediate in most parameters. The cutting force of the high-amylose tortillas, as measured by a texture analyzer, was very low, whereas the blend and *floury* tortillas required more force. Chewiness was correlated to rollability ($r = 0.99$, $P \leq 0.05$). The RS percentage was correlated to rollability ($r = 0.99$), and cutting force ($r = 0.99$). The *floury* and blend tortillas had a firm texture that would be expected when eating a tortilla with a filling. The high-amylose tortillas fell apart with very little force, and would not roll around a filling, making them unsuitable for this use. Although the high-amylose tortillas had increased dietary fiber in the form of RS, it had very poor textural attributes. The blend tortillas retained enough of the textural properties of the *floury* tortilla to make it a suitable product. Understanding the impact of RS on the gelatinization characteristics of starches and the texture of food products will help the food industry understand its impact on food processing, especially processing involving heating.

GENERAL INTRODUCTION

Introduction

Starch is important to the diet, providing the bulk of the calories a person needs daily. Starch can be broken up into a digestible fraction, and a non-digestible fraction. The non-digestible fraction is called dietary fiber (DF), and includes resistant starch (RS). Four types of RS exist. The RS 1 is made resistant by the surrounding food matrix, RS 2 is present in ungelatinized, raw starches, RS 3 is created by retrogradation, and RS 4 is produced through chemical alteration (Englyst et al., 1996). All behave differently during processing, making their measurement difficult. There are several options available for RS measurement, none of which is perfect. The gelatinization characteristics of RS can also be examined by a Differential Scanning Calorimeter (DSC).

Many grains, including corn, contain RS. The corn kernel has several anatomical parts that contribute to the proximate composition. The endosperm of the corn kernel contains starch and proteins. The starch is the main source of RS inside a corn kernel, while the pericarp contributes to DF. Corn starch is a commonly utilized product in the food industry. Its properties can be modified with traditional plant breeding by using major (naturally occurring mutant genes), or minor (modifying genes) genetic factors. Exotic corn lines may provide unusual traits of interest through modifying genes, including increased RS, for functional foods. High-amylose corn lines provide higher amounts of RS 2 than *normal* corn through a major (mutant) gene; thus crossing these two corn types may increase the RS, provide unique materials for food use, and maintain cooking properties better than high-amylose corn lines used alone.

Consumption of RS provides many health benefits because it functions as a prebiotic, a fermentable substrate for beneficial gut flora. Fermentation produces short-chain fatty acids which alter the colon environment, and provide health-protective effects. Among other things, short-chain fatty acids reduce intestinal pH, increase arteriole blood flow, and may protect against colon cancer by regulating cell turnover.

Addition of RS to the diet can aid in weight control because RS promotes satiety, and has little effect on blood sugar after eating. An ideal food product for RS is the tortilla because it is a simple food system that has been targeted for nutritional improvement by nutritionists and the Mexican government. Tortillas are commonly eaten around the world, but are a staple food in Latin America. Tortillas provide the bulk of the calories in low income parts of the world.

While tortillas have been made for centuries in the household, industry has begun to produce them. These mass produced tortillas differ in texture and, in many ways, are inferior to the traditionally made product. The corn used in tortillas is treated by a process known as nixtimalization, which involves cooking corn in lime solution over heat. To improve the quality of the product, many steps in tortilla processing have been suggested for quality improvements, such as cooking time, steeping time, lime concentration, and storage conditions.

Storage conditions can increase the RS present by forming RS 3 during retrogradation. Tortillas stale rapidly and therefore retrograde quickly. While stale tortillas are higher in RS they also are less desirable because of textural defects. Industry uses anti staling agents to give their tortillas a longer shelf life, but this also decreases the RS formation. Much work

has focused on examining the textural aspects of tortillas by using instrumental analyses, but there have been few studies using human sensory panels. A sensory panel provides valuable information on what affects overall opinions on a food product. Humans can be better experts on food than instruments and can detect smaller differences in some cases. The main drivers for purchase of commercial tortillas have been identified, and some attempts at objective texture methods have been made including rollability, extensibility, and bending measured by a texture analyzer. The understanding of factors impacting tortilla texture could be enhanced by more sensory work with human subjects.

Thesis Organization

This thesis contains a review of literature, and two manuscripts reporting the findings of the research accomplished during my studies. The first paper titled, "Thermal Characteristics of Starch From Corn Mutants With High Amounts of Resistant Starch" is followed by the second paper, "Resistant Starch Effects on Tortilla Texture". Both papers are formatted for publication in *Cereal Chemistry*.

Review of Literature

Starch

Starch is the carbohydrate storage product of plants. Starch is found in cereals (A type starch), tubers (B type starch), and legumes (a mix of A and B type called C type starch). Starch is deposited in insoluble granules. Inside the granule are linear amylose (linked only by alpha 1,4 glycosidic bonds), and the branched amylopectin (linked with alpha 1,4 chains, and alpha 1,6 branches). The fine structure of amylopectin organizes the internal structure of

the starch granule (Zhang et al., 2006b). Starch granules are stable because they are very compact. Native, raw granules are resistant to hydrolysis because enzymes cannot access linkages (Haralampu, 2000).

The addition of water and heat allows the granule to become disorganized. In the process known as gelatinization, the granule swells, and the amylose seeps from the granule. During gelatinization, foods containing starch become softer in texture, making them more palatable. After gelatinization, digestive enzymes can easily access the glycosidic bonds.

The degree of polymerization (DP) is the number of monosaccharide units a starch source contains. The designation can be used to separate carbohydrates into general groups: sugars, or monosaccharides (DP 1) and disaccharides (DP 2); oligosaccharides (DP 3-10), and polysaccharides (DP 10+). Sugars, except for lactose in lactose-intolerant individuals, are easily absorbed, and increase the human insulin response in the blood. Oligosaccharides, such as malto-oligoasccharides, are digestible, but other types, such as fructooligosaccharides and galactooligosacchrides, are poorly digested, and can be fermented in the large intestine. Polysaccharides may be digestible (starch) or indigestible (resistant starch and non-starch polysaccharides).

Digestion begins when enzymes attack the pores on the surface of the granule. As digestion continues, the pores enlarge, and digestion continues into the granule center, creating channels (Zhang et al., 2006a). Starch is digested mainly by α-amylase, glucoamylase, and sucrose-isomaltase in the small intestine, which results in free glucose. The α-amylase determines the rate of digestion. Glucoamylase acts on the intermediates from α-amylase

digestion, clearing them to keep the reaction uninhibited (Zhang et al., 2006a). The glucose is absorbed and used for energy.

The rate of digestion can impact the calorie content of starch products. If a starch source is not fully digested by the time it reaches the large intestine, it will become a fermentable carbohydrate for natural flora. The actual rate of polysaccharide digestion and absorption is based on the rate of stomach emptying, and the rate of diffusion of released sugars from the food bolus, which depends on the carbohydrate type, biological origin, and processing conditions (Englyst et al., 2007). Rapidly digestible starch (RDS) is fully digested in 20 minutes, and provides 4 kcal/g (Seifter et al., 2005). Digestion completed between 20 and 120 minutes is termed slowly digestible starch (SDS), and will provide partial calories to the diet. Anything still in the large intestine after 120 minutes is termed resistant starch (RS), and further reduces the calories ingested (Englyst et al., 2003).

The level of SDS is parabolically related to the weight ratio of short chains to long chains of amylopectin (Zhang et al., 2008). Both amylose and amylopectin are present in SDS, but the amylopectin provides the majority of the molecules, as does cornstarch. It is hypothesized that SDS characteristics come from the alternating crystalline and amorphous regions within the granule. Amylose content or structure does not have an effect on SDS, but does affect RS content (Zhang et al., 2006b). The slowed digestion is the result of amylopectin's branches, because amyloglucosidase slows as it nears a branch location (Zhang et al., 2008). A greater proportion of high-branch-density amylopectin (many short chains with a lot of branching) increases the amount of SDS; this is accomplished by chemical modification (Zhang et al., 2008). Because of the compact arrangement, the enzymes have difficulty

binding to the amylopectin chain at enough α-amylase binding sites to achieve rapid digestion. While the time limit for SDS has been set at two hours, the rate is sustainable past this time, making the line between SDS and RS hazy (Zhang et al., 2006a).

Undigestable starch types

Undigestable starch is generally referred to as dietary fiber (DF). The term was first used in 1953 by Hipsley (DeVries et al., 1999), and is defined by the American Association of Cereal Chemists (AACC) as, "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plants substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation (AACC, 2001)." A daily reference value (DRI) has not been set for DF; however, a recommended total DF intake has been set at 25g/day for adult females, and 38g/day for adult males (American Dietetic Association, 2008), or 14 g/1000 cal consumed (United States Department of Agriculture, 2005). Many Americans are not meeting these recommendations.

There are multiple sources of DF, including fruits, vegetables, and whole grains. Examples include lignin, cellulose, polysaccharides, and oligosaccharides, all of which are complex, long-chain carbohydrates not fully digested by human digestive enzymes. The measurement of DF has evolved from the initial method of separating fiber into neutral detergent fiber and acid detergent fiber. This method underestimated many components we now recognize as

fiber (Robertson and Horvath, 1993). This was corrected with the total dietary fiber method currently used that includes almost all fiber components (AOAC 985.29/AACC 32-05).

Whole grains are a good source of DF, and are defined by the AACC International Board of Directors (1999) as, "consist[ing] of the intact, ground, cracked, or flaked caryopsis, whose principal anatomical components—the starchy endosperm, germ and bran—are present in the same relative proportions as they exist in the intact caryopsis." Whole grains are beneficial not only because they are high in DF, but also because they contain phytosterols and folates (Harris and Smith, 2006). Phytosterols lower cholesterol, and reduce risk of heart disease (Ostlund et al., 2003). Folate is important for cell growth, DNA synthesis, prevention of anemia, and prevention of neural tube birth defects (Gregory, 2004).

Whole grains are not common to the Western diet; refined flours and simple sugars are preferred. Milling removes much of the DF components (bran) leaving only the starchy endosperm which is higher in energy, gives a smoother texture, and blander taste. Refined flour is also more shelf stable than whole-wheat flour because the germ has been removed making it more oxidatively stable. The 2005 Dietary Guidelines for Americans suggest that men and woman consume whole grains for half of their total grain intake a day (U.S. Department of Agriculture, 2005). In recent years, more attention has been paid to consumption of whole grains, but not all plant sources are equal in terms of DF. Corn has an approximate 7.3 g of DF per 100 g of whole corn per 100-g serving (Salovaara et al., 2007).

It is possible to separate DF into soluble and insoluble fractions. Soluble fiber increases the viscosity of the digestive mucosa, which slows the enzyme/food interaction, and therefore slows energy release (Young et al., 2005). Incorporation of DF into the diet allows a person

to eat more but consume less energy, which is of great interest for overweight and obese individuals.

Soluble fibers reach the proximal colon where they are fermented. Little soluble fiber is left undigested to be expelled in the feces. Soluble fibers have more opportunities for food use because they will mix into solution, creating less disruption to the food matrix. Examples are pectins, gums, mucilages, and some hemicelluloses.

Insoluble fibers include celluloses, lignin, most hemicelluloses, and RS. These pass farther through the colon, to the distal colon, and are slowly fermented. Their indigestibility comes mainly from the human lack of necessary enzymes, or complex structures that prevents enzyme access. They are expelled in the feces, and are a better bulking agent than soluble fibers (Young et al., 2005).

A newer area of DF research is that of resistant starch (RS), which is made of the same glycosidic bonds as digestible starch, but because of various factors, is inaccessible to enzymes, and is only partly digested. Some of the functional properties of refined flour may be retained in flours containing some RS, such as smooth texture and bland taste. These properties help high-RS products overcome many of the perceived negatives of DF held by consumers because it tastes like refined grains, but is still low in energy value.

Resistant starch

The proper way to measure DF is to use the AOAC 985.29/AACC 32-05, but this method does not encompass RS in its measurement of DF. Englyst et al. (1996) coined the term RS after observing that not all components that are resistant to digestion are measured in DF.

They created a classification that divided RS into four groups: RS 1 is physically inaccessible to digestive enzymes because of the surrounding food matrix; RS 2 is present in raw, ungelatinized cereal grains; RS 3 is created by retrogradation; and RS 4 is created by chemical means (Englyst, 1996). All RS types differ in their response to heat treatment, processing, and storage, making them vary in their ideal applications.

The RS 1 content of a food may be altered by various processing steps including grinding, mixing, heating, and storage. Any physical disruption has the greatest effects on RS 1 because the physical surroundings are what impart resistance. It is very hard to measure RS 1 because measurement requires extraction and removal of the surrounding material, which may affect the structures that made the starch inaccessible in the first place. This type of RS has potential in many food uses, but RS degradation and formation need to be tracked at each stage of production to determine if the end product has appreciable levels of RS left for consumption.

Humans do not normally consume RS 2; most starch is first gelatinized, with a few exceptions (raw bananas and potatoes). This type of RS has potential as a food ingredient, but new products may need to be developed to truly capitalize on RS 2's ability to reduce calories. This product would incorporate ungelatinized starch, without the side effects of poor flavor and texture. The percentage of RS 2 is greater in high amylose starches, a portion of which is retained upon processing. There is one RS 2 flour that is commercially available, Hi-maize sold by National Starch Company.

Incorporation of RS 3 into food products is also possible. Retrogradation can be hastened by repeated heating and cooling cycles in an autoclave. Besides the addition of a pre-made RS 3

flour, RS 3 can be created upon storage. Most processed foods go through some amount of storage between the processing facilities and home use; the exact storage period can be hard to estimate. Storage studies to determine amount of retrogradation and RS formation over time should be done on high carbohydrate foods to see if RS is being made. Commercial sources of RS 3 include NOVELOSE 330 (National Starch Company) and CrystaLean (Opta Food Ingredients, Inc.).

The only type of RS not naturally present in foods is RS 4, which is made in the laboratory by various methods. It is possible to attach lipids to the starch. These large lipids groups block digestive enzymes. Other methods of RS 4 creation include starch that has been etherized, esterified, or cross-bonded (Czuchajowski et al., 1991, and Xie et al., 2006).

Resistant starch measurement

The measurement of RS is difficult in many ways. Only RS 2 or RS 3 is measured by any one method (Englyst et al., 2007). The current methods for RS are the Englyst method, the Prosky method (total dietary fiber method, AOAC 991.43), and the McCleary method/Megazyme RS Kit (AOAC 2002.02, AACC method 32-40). All methods give different RS values because they measure different DF components. The Englyst method measures plant cell wall non-starch polysaccharide, whereas the Prosky method measures the non-starch polysaccharide and lignin (Kontraszti et al., 1999). The Prosky method is best used for traditional DF components. The McCleary method is an extension of the Englyst method that attempts to mimic the human digestive tract.

The McCleary method involves incubating starch with pancreatic α-amylase and amylosglucosidase, at 37° C for 16 hrs, in a shaking water bath. The two enzymes hydrolyze the RDS and SDS to free glucose. The reaction ends when 100% ethanol is added, and then is washed with 50% ethanol twice. The RS is retained by centrifugation, and the supernatant, containing the free glucose, is discarded. The RS is then dissolved in 2 M potassium hydroxide (KOH) with stirring in an ice water bath. Once the solution is neutralized amyloglucosidase is added, and the RS is converted to free glucose. This glucose is measured by the glucose oxidase/peroxidase reagent (GOPOD), which turns pink in the presence of glucose. The pink color is then measured on a spectrophotometer at 510 nm.

No method accurately measures RS1 because the methods do not measure the starch as eaten. All methods require an extraction step which alters the surrounding matrix. The McCleary method accurately measures RS 2, but not RS 3. The majority of RS 3 is destroyed by gelatinization, and is underreported by the Prosky method. When 17 foods were measured by the Englyst and Prosky methods, the Prosky method was 98% higher in 16 of the foods (Kontraszti et al., 1999).

The McCleary/Megazyme method was accepted as an AACC/AOAC method (McCleary and Monaghan, 2002). This method is best suited for large numbers of samples, and requires a smaller sample size than the other methods. The packaging of the enzymes into a kit is also helpful to industry. Many other methods have been proposed to measure RS, including a method including chewing (Akerberg et al., 1998, and Saura-Calixto, 1993). Some papers have cited difficulties in accounting for differences between *in vitro* and *in vivo* assays (Danjo et al., 2003, Bauer et al., 2003, and Muir et al., 1993).

Differential scanning calorimetry

Gelatinization and retrogradation characteristics of starch provide valuable information regarding the characteristics of starches from new mutants. A starch-water slurry is heated by a Differential Scanning Calorimeter (DSC), and various parameters can be measured. Starch must first be extracted because other dietary components, such as proteins and lipids, can alter the output (Yamin, et al., 1997). Heating to 180° C ensures even high-amylose starches are fully gelatinized. Gelatinization creates a curve that reflects the change in enthalpy (ΔH) of the starch. The point of inflection is the onset temperature, the highest point is the peak temperature, and the return to baseline is the end point temperature (Figure 1). Measuring the area under the curve provides the ΔH, or the change in enthalpy. Retrogradation can be measured by reheating the starch-water slurry that has been stored for 7 days at 4° C. The amylose and amylopectin molecules that have recrystallized will melt again. The peak for retrogradation will be smaller, and occur at a lower temperature than the original analysis.

Stevens and Elton first used the DSC for starch gelatinization in 1971. The amylose-amylopectin ratio affects DSC characteristics, with a higher amylopectin content narrowing the gelatinization peak (Krueger et al., 1987). Heating starches on the DSC has shown that tropical conditions can narrow the gelatinization temperature range when compared to the same corn grown under temperate conditions, (White et al., 1991).

Rapid Visco-Analyser

The Rapid Visco-Analyser (RVA), a cooking and stirring viscometer, measures the pasting properties of starch (Tziotis et al., 2005). Pasting occurs after gelatinization, when starch swells and becomes thick. Pasting is the basis of the preparation of many food products, such as pudding. The RVA is capable of measuring parameters such as pasting temperature, peak viscosity, hot paste viscosity, final viscosity, breakdown, and setback (Seetharaman et al., 2001). Breakdown is the difference between peak viscosity and hot paste viscosity. Setback is the difference between the hot past viscosity and the final viscosity.

Texture Analyzer (TA.XT2)

A texture analyzer provides an instrumental way to analyze textural properties. One of the most commonly used instruments is a TA.XT2, made by Texture Technologies (Scarsdale, New York). The advantage of a TA.XT2 is that it comes with many different attachments that can be used for different food products. The TA.XT2 is often used to measure gel strength. It is possible to measure many different attributes with a TA.XT2 including hardness, chewiness, and adhesiveness (Sahai et al., 2001). Hardness is defined as the peak force of the first peak.

Corn kernel composition

Many different grains and legumes contain RS. *Zea Mays* L., commonly known in the United States as corn, or maize, is one of the main agricultural commodities grown around the world, with many human and animal feed uses, and industrial applications, including recent use in biofuels. Corn is a staple food product in many cultures. Corn is a good source

of calories, and forms the bulk of the diet for many people in Central and South America. The starch, protein, and lipid fractions of the kernel have been characterized.

The edible portion of the corn plant is called the kernel. The average hybrid ear has about 800 kernels (Watson, 2003). The corn kernel is a caryopsis, a single seeded fruit, containing an embryo, and all components required for growth and development (Watson, 2003). From the outside inwards, it is made up of the pericarp, aleurone, hilar layer, endosperm, and germ. Nixtamalized products are made from whole corn, and the texture is dependent upon all components being present, either in whole or part. But many corn products are milled to remove the germ, making the resulting flour more oxidatively stable.

The outermost kernel layer, the pericarp, is actually five layers thick. The pericarp is about five percent of the dry weight of the kernel, but makes up 51% of the dietary fiber content for the kernel (Table 1). The epidermis is the outermost, waxy cuticle layer, which helps retain moisture inside the kernel. All layers of the pericarp, with the exception of the seed coat, are dead cells. The seed coat, the inner most pericarp layer, adheres to the aleurone layer, and may have semipermeable properties.

The aleurone layer is just a single cell layer thick. While no starch is present, protein and oil bodies are. It is thought that this layer must be ground in order for digestive enzymes to attack the encased endosperm.

The hilar layer is underneath the tip cap, or pedicel, the portion of the kernel that is attached to the corn cob. The tip cap is made mostly of fibrous material. The hilar layer is used as an indicator of physiological maturity when it turns black. The black hilar layer has little effect

on end products except in white corn. The hilar layer can show up as specks in white tortillas, and is carefully removed by sifting.

The endosperm contains the bulk of the starch and protein of the kernel. The endosperm fraction is the majority of the kernel, making up 82-84% of the kernel dry weight, and is 86-89% starch by weight (Watson, 2003). The endosperm contains little to no traditional dietary fiber (hemicelluloses, cellulose, or lignin) (Table 1). The endosperm is made up of starch embedded in a continuous protein matrix, in the ratio of approximately 87% starch and 8% protein (Table 2). Moving from the outside of the endosperm towards the center of the kernel, more starch and less protein material are found. The starch is synthesized by amyloplasts, and deposited in granules because of this the granules will have some associated lipids on the surface, including those of the amyloplast membrane. Granules also contain internal free fatty acids and lysophospholipids (Watson, 2003).

The protein components in the protein bodies are mainly the four zeins (α, β, γ, and δ). The α- zein, comprising 70% of all zein proteins, is low in lysine but high in alanine and leucine (Lawton and Wilson, 2003). The β-zein contributes 5% of the total zein content, and lacks lysine and tryptophan. The γ-zein contributes 20% of the total zein, has no lysine or tryptophan, but is rich in proline and cysteine. The γ-zein is located on the outside of the protein bodies. Finally, the δ-zeins, making up less than 5% of the total zeins, are not present in all varieties, and are rich in methionine. Albumins and globulins exist in the aleurone, pericarp, and germ. Prolamins and glutelins make up the storage proteins, and are found in the endosperm (Lawton and Wilson, 2003). Protein concentration is dependent upon soil nitrogen, which is variable from location to location, and year to year (Watson, 2003).

Overall, corn protein is considered an inferior protein because it is deficient in the essential amino acids lysine and tryptophan. To make a complete protein corn should be combined with other protein sources high in lysine and tryphtophan.

The germ contains the immature embryo, and the bulk of the oil and the minerals. The germ provides 10-12% of the kernel dry weight, and 81-85% of total kernel oil, mostly in the triglyceride form (Watson, 2003). Within the germ is the scutellum, which is the food-storage organ of the germ. The oil is deposited in oil bodies, or sphereosomes, that are membrane bound. The oil is encased in a protein with polar ends facing out, and the hydrophobic ends pointing in. These bodies are very stable because of the presence of phosphotidylcholine and oleosins in the membrane. The germ also contains a small amount of starch and protein (Table 2). The proteins are largely enzymes that digest the starch and protein of the kernel in the event of germination (Watson, 2003).

Corn provides several essential vitamins and minerals. Most corn varieties are yellow because of the carotenoid pigment, 95-97% of which is contained in the endosperm protein (Watson, 2003). β-carotene has a 60% vitamin A conversion rate in the human body. It is susceptible to degradation when exposed to light and oxygen, thus, the concentration decreases during storage. Xanthophylls, such as lutein and zeaxanthin, are also present in the germ, and are more stable upon storage. Vitamin E (α-tocophorol), along with 78% of the minerals in corn, is found in the germ. The most abundant mineral is phosphorus, with 78% in the phytin form which is important for plant storage, but unavailable for human metabolism. Potassium is also present at an average of 0.37% (Watson, 2003).

Dent corn

Dent corn makes up the majority of all corn grown in the United States, with 98% being the yellow endosperm variety (no. 2 yellow) (Darrah et al., 2003). The endosperm of dent corn is composed of two types: the horny endosperm around the outside, and a soft, floury endosperm toward the middle of the kernel. When the kernels are dried a dent is formed on the top of the kernel giving dent corn its name. The more intense the yellow color the more vitamin A content.

Floury corn

Floury corn is common to Latin American cultures. The endosperm is soft, and there is almost no horny endosperm. It is easily ground, and is used for traditional foods like tortillas, humitas, and quimbolitas. When dried no denting occurs. Floury corn is not commonly grown in the US, but is grown in South America (Darrah et al., 2003). Floury corn has a low gelatinization onset temperature of 60.8° C and a wide range of 13.5° C (Seetharaman et al., 2001). When the pasting properties of a floury endosperm type were compared to dent, flint, semident, and semiflint the floury was significantly different from the other endosperm types. The floury endosperm had the highest pasting onset temperature and the lowest peak viscosity, breakdown, and setback (Seetharaman et al., 2001). The specialized corn type *opaque-2* is of the floury type. The *opaque-2* corn variety has double the normal lysine and tryptophan content (Mertz et al., 1964).

Corn starch

For food use, corn is often milled into corn starch, a product consisting of the endosperm without the germ and pericarp. The starch fraction of *normal* corn starch is made up of approximately 75% amylopectin and 25% amylose. Raw cornstarch is generally very low in RS, ranging from 0.7% (Themeier et al., 2005) to 6.9% (Zhang et al., 2006b). Corn starch gelatinization and gelation properties can be altered and enhanced through traditional corn breeding by focusing on particular gene expression. Typical starches hydrate between 40 and 120° C (Haralampu, 2000), and corn starch gels are thermally reversible at 100° C (Klucinec and Thompson, 1999). The amylose to amylopectin ratio affects the degree to which a starch gelatinizes (Zhang et al., 2008).

High-amylose starch

The amylose extender (*ae*) gene, first studied by R.P. Bear in 1950, provides increased amylose, with typical values ranging from 50 to 70%. Because it is a recessive gene, it must be grown in isolation so that dominant genes do not contaminate the plot by accidental cross-pollination. The amylose content is correlated to the RS present (Themeier et al., 2005, and Shu et al., 2007). When cereal starches (including corn starch) have less than 34% amylose the RS levels are very low, less than 1% according to the McCleary method (Themeier et al., 2005). These low RS starches came from A-type starches, such as *du* and *su2* (Tziotis et al., 2005). B-type starches, including *ae*, exhibit more RS (Tziotis et al., 2005). Corn starch with 70% apparent amylose resulted in 54.4% RS (Themeier et al., 2005).

High-amylose starches provide high amounts of RS, because when the amount of amylose is increased the individual chains of amylose lengthen to DP's greater than 30 (Klucinec and Thompson, 1999). Inside the starch granule these chains pack very tightly together. This

packing can cause incomplete hydration, and therefore reduced swelling and gelatinization. Incomplete gelatinization creates areas that digestive enzymes are unable to reach, thereby imparting enzymatic resistance and slowing/reducing digestion.

These long molecular chains of amylose also create ideal interactions for retrogradation and crystallization. Retrogradation is the thermodynamically reversible process by which a gelatinized starch cools, and amylose and amylopectin chains reform some internal structure held together with hydrogen bonds. The branching of amylopectin complicates the complete recrystallization of amylose. Amylose recrystallizes quickly, while branched chains force amylopectin to recrystallize more slowly (Zhang et al., 2008).

In a laboratory setting retrogradation is measured after 7 days at 4° C, but retrogradation begins immediately upon cooling. Over the 7 days RS increases until reaching a maximum at 7 days, whereas SDS reaches a maximum around 4 days and disappears after 7 days of storage as it is converted to RS, creating a bell shaped curve: RDS decreases as RS increases (Zhang et al., 2008). Retrogradation affects the digestibility of starch in several ways. Digestibility is decreased because retrogradation forms B-type crystalline structures, which are resistant to enzyme actions (Zhang et al., 2006b). Retrogradation of individual corn starch mutants will vary according to their amylose/amylopectin ratios (Zhang et al., 2008).

Other recessive mutants

The double mutants involved in the current study include: *du su2*, *ae su2*, *ae du*. The single mutant Guat *ae* is also used. Starch properties can be altered by genetic background and growing environment (Ji et al, 2005). Different genotypes may have different responses to

the same environmental conditions (Ji et al., 2005). While double mutants have not been extensively studied, single mutants have been more completely characterized. Single mutant characteristics can influence double mutant characteristics. Mutant genes can have substantial effects on kernel and starch granule development and morphology, and polysaccharide composition (Creech, 1968).

The *su2* mutant was first discovered in 1935 by Eyster. It is more easily digestible by pancreatic α-amylase (Sandstedt et al, 1962), making it have low RS values, and low potential for RS formation. While this makes it less desirable for low calorie foods, it is desirable for animal feed.

The *su2* gene lowers starch, and increases amylose by 10-15%. The starch from the *su2* mutant also has altered gelatinization characteristics. The starch onset temperature is lower than in *normal* corn (Pfahler et al, 1957, and Kramer et al., 1958). Onset temperatures can be as much as 10° lower than *normal*, with a ΔH value of 7.7 J g^{-1}, compared to *normal* having a ΔH of 14 J g^{-1}. The *su2* starches retrograde less than do *normal* starches (Campbell et al., 1994).

Pasting and viscosity temperatures are lower in *su2* starches than in typical starch. Pasting onset is delayed, no initial viscosity peak is exhibited, and there is a low final viscosity after cooling (Campbell et al, 1994). The gels formed by *su2* are much weaker gels than those formed by normal starch (Campbell et al., 1994). The granules have lowered birefringence (Pfahler et al, 1957 and Kramer et al, 1958, Inouchi et al, 1984).

The single mutants *ae*, *du* and *su2*, as well as the double mutants *ae du*, and *ae su2* endosperms have an apparent amylose content higher than that of normal maize starch (Shannon and Garwood, 1984). The *du* and *ae* are similar in that they have higher pasting temperatures, lower peak viscosity, and a lower breakdown than normal starch. The *du1* gene affects soluble starch synthase and branching enzyme IIa activity, while the *ae* mutation results in loss of starch synthase II (Gao et al., 1998). The *ae* starches contain branched molecules that have a higher proportion of longer chains (DP>30) than the amylopectin of common corn starch (Takdea at al., 1993).

The starch from corn having the *du 1* gene exhibits higher pasting onset temperature, lower viscosity, and relatively high amylose, which may cause a higher pasting temperature resulting from the increased bonding of amylose within the granule (Wang et al., 1992). The increase in amylose will vary depending on the genetic background (Shannon and Garwood, 1984). The *du1* gelatinization characteristics include a relatively low retrogradation ΔH value and low amount of retrogradation (percent retrogradation, Tziotis et al, 2005).

Health benefits

The Western diet is high in refined and processed grains, such as white bread, pasta, and white rice, all of which are low in RS. Low-fiber diets have been associated with increased risk of obesity and obesity related illnesses: diabetes, anthrosclerosis, hypertension, and colon cancer (Brennan, 2005). The health benefits of RS affect many areas of the body.

The colon environment is positively affected by RS because RS functions as a prebiotic, a substance characterized by its ability to aid the proliferation of beneficial gut flora, such as

bifidobacteria (Nugent, 2005, and Brouns et al., 2002). Probiotics colonize the host but vary in attachment strength and preventive effects on other toxic/pathogenic bacteria (Macfarlane and Cummings, 1999). Probiotics aided by prebiotics have been beneficial in treating *Campylobacter jejuni* enteritis and *C. difficile* diarrhea. Probiotics may have beneficial properties even when the bacteria are dead when eaten. For example, dead and live bacteria can bind mutagenic pyrolsates making them antimutegenic. Live cells can carry antigens to the coloncytes improving immune response. Live cells also reduce the risk of irritable bowel syndrome (IBS), ulcerative colitis, and inflammatory bowel disease through the production of short-chain fatty acids (SCFA) (Macfarlane and Cummings, 1999).

As RS is digested it passes through the human digestive tract undigested by the enzymes, and passes to the large intestine where it is fermented by the gut flora, producing SCFA, including acetate, propionate, and butyrate. The production of SCFA is important to digestive health, but their production is hard to measure accurately because approximately 95% of SCFA are absorbed into the body (Topping and Clifton, 2001). The best way of measuring SCFA production in healthy humans is by measuring the excretion in feces, but this measure does not provide an accurate measure of production or absorption. Fecal studies are best for measuring changes in SCFA based on different diets. Another way of measuring SCFA is to measure the effluent of ileostomy patients, i.e., people who have had the large intestine removed either due to illness or injury. Not surprisingly, these conditions may not approximate the true conditions of healthy individuals.

Animal models have been used to better approximate human internal conditions but the perfect double of the human digestive track has not been found. Pigs and dogs seem to be the

best human substitutes, but rats are easier to work with (Topping and Clifton, 2001). *In vitro* models are also used, but do not always correlate to the activity in *in vivo* human feeding studies.

Mineral absorption is affected by SCFA depending on the model used and method of delivery. Na^+ and K^+ are cotransported with uptake of SCFA in the colonocyte of the rat (Fleming et al., 1991). Several studies have shown that SCFA can increase absorption of Ca^{2+} and Mg^{2+}, including after dietary supplementation (Courdray et al., 1997), after rectal infusion (Trinidad et al., 1996), and in pigs (Bird et al., 2000). However, phytate, sometimes associated with dietary fiber, can bind and complex with minerals (Persson et al., 1991).

Some work has been done examining SCFA's effect on blood vessels. There is a positive effect on colonic blood flow after addition of SCFA; the SCFA caused arterioles to dilate (Mortensen et al., 1991), which can be beneficial to post operative surgical patients. The increased blood flow is thought to increase tissue oxygenation and nutrient uptake. Local neurons, or chemoreceptors, are also affected by SCFA, causing smooth muscle to relax (Mortensen et al., 1991). Feeding RS after surgery could help patients heal quicker.

Being weak acids, SCFA can lower the digestive mucosa pH (Topping and Clifton, 2001). Lowering the pH of the lumin can prevent growth of pathogenic bacteria, including *E. coli* and *Salmonella* (Cherrington et al., 1991), and decrease active cholera disease and antibiotic-induced diarrhea by inducing water uptake (Ramakrishna et al., 2000). While pH changes have been observed in various models, the buffering of gut contents, or the mineral content present may dominate *in vivo* (Topping and Clifton, 2001). The three areas of the small intestine (duodenum, jejunum, and ileum) all have different optimal pHs, but it is not

possible to isolate and measure the changes in these areas *in vitro*. Many feeding studies have shown a lowered pH in the feces after feeding of fermentable carbohydrates (Noakes et al., 1996, and Kashtan et al., 1992), but there also are contradictory results showing no significant change in pH (Tomlin and Read, 1990, and Van Dokkum et al., 1999).

The effects of RS and DF on cholesterol are not well understood. The literature shows conflicting overall effects. Cholesterol absorption is thought to be altered by consumption of DF, but the underlying mechanism is not well understood (Scheppach et al., 2001). Possibly, the digestion of DF, and the resulting SCFA produced, suppress cholesterol synthesis in the liver (Hara et al.,1999). Hypotheses for the mode of action of DF on cholesterol include a lowering of total cholesterol due to excretion into the feces (Reddy et al., 1980), decreased cholesterol absorption (Vahouny et al., 1988), increased cholesterol oxidation (Carroll et la., 1978), or decreased cholesterol synthesis (Wright et al., 1990). A decrease in total lipids, total cholesterol, low density lipoproteins, high density lipoproteins, very low density lipoproteins, triglycerides, and triglyceride-rich lipoproteins after RS feeding (de Deckere et al., 1993, and Nugent, 2005). However, some studies contradict these results showing no change to the same parameters (Kim et al, 2003, and Noakes et al., 1996). More studies are needed to determine the impact of RS on lipid metabolism.

Butyrate is the preferred energy source for the colonocyte; however, it is not the most abundant product of RS fermentation. Acetate is present in a greater concentration than propionate, which is in a greater concentration than butyrate. Butyrate causes the most dramatic effects on the health of colonocytes, but may only affect abnormal cells by causing more cell turnover (Young et al., 2005). Butyrate decreases the inflammatory response,

modulates intestinal motility, and promotes normal cell types by increased apoptosis which inhibits proliferation of cancer cells, in the activity termed tumergensis (Nugent, 2005).

While many studies have found positive changes in the intestinal environment by feeding RS alone, Muir et al. (2004) suggest that only RS in combination with a traditional fiber source, such as wheat bran, will cause SCFA concentration to increase. Muir et al. (2004) evaluated fecal samples from humans and measured the excreted SCFA, which may not accurately reflect the SCFA absorbed. These researchers suggest that the optimal fiber source is a combination of RS and a non-starch polysaccharide, because the RS is a better substrate for fermentation and butyrate production, whereas the non-starch polysaccharide is a bulking agent (Topping and Clifton, 2001, and Noakes et al., 1996). The increased bulk can push the location of fermentation to a more distal location, where the most colon cancer is found (Muir et al., 2004). Reducing transit time and providing bulk also causes more cell turn over and constant removal of dead cells.

Fermentation of RS can reduce protein fermentation, or dilute the toxic end products. Products of protein fermentation, including ammonia and phenols, are toxic, and are thought to lead to cancer (Birkett et al., 1996). Ammonia promotes cell proliferation, which can cause cancer cells to multiply (Lin and Visek, 1991). Phenols, the degradation product of the aromatic amino acids, have been known to promote skin cancer since 1959, and are thought to promote bladder and bowel cancers (Boutwell and Bocsh, 1959). Conditions affecting the bowel, such as ulcerative colitis, reduce bowl function, making it difficult to clear the toxic components (Scheppach et al., 2001). The microbiota will only ferment protein when there is not enough starch in the diet. Probiotics can use the toxic end products as fuel, thereby

eliminating them from the lumin, and protecting the coloncytes from uptake (Birkett et al., 1996). The byproducts can be diluted by increasing the fiber content of the diet (Cummings et al., 1979, and Birkett et al., 1996). However, it is also proposed that combining RS with dietary protein could increase production of SCFA (Le Leu et al., 2006).

Glycemic index and glycemic load

RS has a low glycemic index (GI) which makes it a good food for diabetics looking to maintain low blood sugar. GI gives a measure of the impact a starch will have on blood glucose by ranking the glycemic potential of a food (McMillan-Price and Brand-Miller, 2006). The GI methodology was created in 1981, and involved comparing the example food to a standard (white bread) for the same amount of carbohydrate (Jenkins et al., 1981). This system made it difficult to work with complex food systems, and in 1991 the Food and Agriculture Organization (FAO) suggested its use be restricted to diabetics (McMillan-Price and Brand-Miller, 2006). A food that has a GI of 70-100+ is high, 55-70 medium GI, and below 55 is a low GI food (Brennan, 2005). Corn tortillas have a 87% predicted glycemic index, while the white bread standard received a 94 (Tovar et al., 2003). However, the high variability of tortilla manufacturing and preparation makes GI generalization difficult.

Low GI foods promote satiety by greater release of cholecystokinin (Holt et al., 1992), keep blood glucose stable, and promote higher rates of fat oxidation (McMillian-Price and Brand-Miller, 2006). High GI meals stimulate glucose and fatty acid uptake, reducing their

circulation, and raising levels of hunger-stimulating hormones (McMillian-Price and Brand-Miller, 2006). While the GI system is still used extensively in other countries, it is not widely used in the United States, United Kingdom, Canada, and Australia. The GI system is unproven to be helpful outside of diabetics.

Glucose load (GL) has been suggested as a better measure of the effect of an entire food system on blood glucose because it evaluates the glycemic effects of the whole diet, quality and quantity of carbohydrate (Brand-Miller et al., 2003). GL is the GI/100 x g of carbohydrate, which gives a better estimation of the total blood glucose. One unit of GL can be thought of as one g of white bread carbohydrate. GL can be reduced by either 1) lowering the GI of the carbohydrate, or 2) reducing total carbohydrate (Brand-Miller et al., 2003). Anything over 20 is considered to be a high GL, 11-19 is medium GL, and below 10 is a low GL (Brennan, 2005).

The appearance of high blood glucose soon after eating means the starch consumed is rapidly digestible. Refined grains and processed foods are high GI/GL foods. High GI foods eaten over many years put a strain on the insulinaemic response system, and can lead to insulin insensitivity and hyperinsuliema (too much circulating insulin). If these conditions continue, diabetes can develop, and lead to obesity and other obesity-related illnesses.

Tortillas and nutrition

Mexico, Central, and South America have large tortilla-making industries. Indeed, this industry represents one-fifth of the entire food industry in Mexico (Martinez-Bustos et al., 1996). The Latin American diet of all socioeconomic classes is largely based on corn

products, most of them nixtamalized (Bello-Perez et al., 2006), with the amount of nixtamalized corn increasing as income and social standing decreases (Burton et al., 2008). Estimates of tortilla consumption vary, but some estimates place total caloric intake from tortillas as high as 70% to 90% in rural Mexico (Flores-Farias et al., 2000, and Burton et al., 2008).

The two nutritional benefits of tortillas are that they are high in calcium as a result of the corn being soaked in calcium hydroxide solution during nixtimalization, and are a good source of carbohydrates. Calcium content can to increase by 400% when cooked at 92° C for 40 minutes without any steeping (Fernandez-Munoz et al., 2004). However, they offer few other health benefits. Many studies have focused on nutritional fortification of tortillas in an effort to make them more healthful. The Mexican government is considering fortifying dried masa flour in hopes of correcting iron, zinc, and folic acid deficiencies, but only approximately 40% of tortillas in Mexico are made from dried masa flour (Burton et al., 2008). Many fortification studies focused on increasing the protein content to address the concern that maize is deficient in the essential amino acids lysine and tryptophan (Obatolu et al., 2007). Other studies have focused on fortification of micronutrients, such as iron (Burton et al., 2008).

While tortillas are mostly made from white or yellow corn, heavily pigmented varieties, such as blue or red corn, can also be used. These varieties are often used for corn tortilla chips, and are sold for a price premium. The pigments, such as anthyocyanins, flavonoids, and other phenolic acids, function as antioxidants. Antioxidants scavenge free radicals, and may delay the effects of aging and disease.

Traditional tortilla preparation

Among Hispanic populations, homemade, or table tortillas, are commonly eaten with most meals. Making a table tortilla can be more of an art than a science, and many variations are possible. Tortillas were first made by the Aztecs (Cuevas-Rodriguez et al., 2009).

To prepare a high-quality tortilla, high-quality corn is needed. Kernels should be sound, free of cracks, of uniform size, and have intermediate-to-hard endosperms, with any broken pieces removed before milling (Rooney and Serna-Saldivar, 1990). Traditionally, the whole corn is heated in a calcium oxide, or lime, solution, and allowed to steep overnight in open vats. The original lime source is thought to be wood ashes; later, lime was mined from limestone, or cremated shells (Serna-Saldivar et al., 1990).

The corn is washed, the kernels rubbed between the hands to remove the pericarp, and then hand ground between stones. This grinding distributes the gelatinized and ungelatinized granules evenly among the masa (Rooney and Serna-Saldivar, 2003). The resulting masa dough is baked on a flat surface over a fire. There are still many places of the world where these same steps are used to produce tortillas.

Commercially sold tortillas are gaining popularity in both Hispanic countries and the United States because of ease of use, and low cost. Commercial tortillas often have a different taste and texture than those made traditionally. In the United States, wheat tortillas are more popular than corn tortillas because of the softer texture and extended shelf life compared to corn tortillas, but corn tortillas are still dominant in Latin American countries.

Commercially sold tortillas are made by an alternate procedure utilizing dry masa flour. Dry masa flours are made from traditional masa, but after grinding, the masa is dried, and the flour is separated by particle size. These fractions are combined in different ratios to result in flours for special functions; these flours can then be reconstituted at the processing facility. As a result, these dry masa tortillas differ in texture and appearance from traditional tortillas, but are accepted for ease of use. Dry masa flour is also sold in grocery stores for home use, and is gaining in popularity.

Nixtimalization and tortilla production

Nixtimalization, or alkaline cooking, is the process by which whole corn is prepared for tortilla and hominy production. As described in the literature, nixtimalization is variable, and must be tailored to local conditions and corn type. An average procedure involves cooking the whole corn for 20 min, (Del Pozo-Insfran et al., 2007), 30 min (Mendez-Montealvo et al., 2007), or 60 min (Martinez-Bustos et al., 2001), in a dilute lime solution of approximately 1%, but Rooney and Serna-Saldivar (1990) found variations of 0.8-5%. After cooking, the pot is taken off the heat, and steeped between 9.5 hr (Ratnayake et al., 2007) and 15 hr (Martinez-Bustos et al., 2001), at temperatures ranging from 80° C (Martinez-Bustos et al., 2001) to boiling or higher (Mendez-Montealvo et al., 2007, and Del Pozo-Insfran et al., 2007), until the kernels are swollen. Martinez-Bustos et al. (2001) insist that the solution should not be allowed to boil because high temperatures result in overcooked masa, but Del Pozo-Insfran (2007) noted that some varieties, including blue corn, must be cooked at boiling temperature to ensure nixtimalization.

After steeping the cooking liquor is discarded. The corn is washed with vigorous agitation to remove the pericarp. The resulting corn, named nixtamal, should have a high moisture content. The exact moisture content reported by others ranges from 48-50% (Del Pozo-Insfran et al., 2007, and Gomez et al., 1991), 46-51% (Sahai et al., 2001), and 55-58% (Rooney and Serna-Saldivar, 1990). The correct moisture content for the given conditions ensures machinability.

Grinding is usually done with stone plates. Many variations in grinding are possible. The gap size determination is often made empirically by operators (Ramierz-Wong et al., 1994). The best method to determine the correct gap size is to test different gap sizes, and select for coarse and medium masa based on rubbing the masa between the thumb and forefinger (Ramierz-Wong et al., 1994). The grinding stones are cut with grooves radiating outward. The nixtamal is fed into the center of the rotating plates, and as it is pushed outward it is cut, kneaded, and mashed (Rooney and Serna-Saldivar, 2003). Alternate masa production methods have been proposed including nixtimalization of corn meal (Cuevas-Rodriguez et al., 2009), and by extrusion (Arambula-Villa et al., 2001).

The tortilla is formed when the masa is pressed into a flat disc, and cooked on a heated press, or stove top. Desirable masa can be pressed between two metallic plates covered in plastic film and not stick to the plastic wrap (Martinez-Bustos et al., 2001). Excess water absorption results in a soft and sticky masa that cannot be machined, while dry masa is too hard, and can also be difficult to handle (Martinez-Bustos et al., 2001). Ideally, the tortilla will be cooked throughout, and the surface will puff, but retain a high moisture content.

Tortilla processing effects

Since the tortilla making process is so variable many studies have been done to try and perfect it for industrial use. The cooking and steeping steps have been pinpointed as greatly affecting the quality of the end product. While quite a few studies have been done to achieve an ideal product a consensus has not yet been achieved.

Both cooking and steeping involve lime treatment. The solubility of calcium hydroxide in water at 0° C is 0.185 g/100 mL, and becomes less soluble with increasing temperature (Bryant and Hamaker, 1997). Heating enhances the effects of lime and allow the kernels to take up more water than kernels soaked in lime without heat. For the lime to soften the endosperm the pericarp must become permeable. Once the pericarp is softened lime penetrates via the germ, and then into the endosperm; the germ will have the highest calcium content after cooking and steeping. In non-nixtamalized products the germ absorbs 3-5 times more steeping solution than the endosperm (Ratkovic et al., 1982). Very little lime is retained in the final tortillas, and lime concentration is very high in the discarded steeping liquor.

The first change that takes place during nixtimalization is alteration of the pericarp layer. Analysis of kernels at each hour of nixtimalization show that in the first 3 hr of steeping the pericarp absorbs the most calcium; this calcium is lost when the nixtamal is washed. The calcium oxide hydrolyzes the gums from the pericarp. The breakdown of the gums causes the pericarp to soften and allows for greater diffusion of calcium ions. Arabinoxylan and xylose are the main components of the corn pericarp, and are the main components of the cooking liquor when the nixtamal is excessively washed (Martinez-Bustos et al., 2001). The pericarp may contain pigments in the form of anthocyanins, flavonoids, and phenolic acids, in colored varieties. Excessive washing decreases the phenolics in the resulting masa

(Martinez-Bustos et al., 2001). Nixtimalization affects these antioxidant pigments by breaking ester linkages, and releasing free phenolic forms into the cooking solution (Del Pozo-Insfran et al., 2007). The result is tortillas that are lower in vitamin content that the original corn: acidifying the masa may help with vitamin retention (Del Pozo-Insfran et al., 2007).

Even though the pericarp is partly removed, its remaining components are still important to tortilla texture. Masa with partial pericarp will help tortillas to bend without breaking. In traditional nixtimalization 64% (w/w) of the pericarp material is lost to the cooking liquor (Pflugfelder et al., 1988). Total solid losses after washing were 1.26-2.23% (Fernandez-Munoz et al., 2004). Industrially much of the pericarp is lost, and to mimic the properties imparted by the pericarp, gums or carboxymethyl cellulose are added (Martinez-Bustos et al., 2001).

After the pericarp is softened the calcium affects the germ where the lipids are saponified and released to the cooking liquor (Martinez-Bustos et al., 2001 and Del Pozo-Insfran et al., 2007). The germ contributes to the nutritional quality of the tortilla. Fatty acid composition of the masa decreased after nixtimalization because myristic, palmitic, palmitoleic, stearic, oleic, and arachidic are released into the cooking liquor (Martinez-Bustos et al., 2001). When the corn is milled the germ contributes to machinability, and masa with germ has a higher breakdown tolerance than masa without germ (Martinez-Bustos et al., 2001).

After 3 hr the calcium continues into the endosperm, with more calcium being absorbed if the solution is oversaturated with calcium hydroxide (Fernandez-Munoz et al., 2002). Softer endosperm varieties take up water more rapidly (Serna-Saldivar et al., 1993). During heating

partial gelatinization of starch takes place (Martinez-Bustos et al., 2001), which facilitates milling and allows for further gelatinization upon final cooking. Nixtimalization increases amylose content, but decreases overall starch content compared to native starch (Mendez-Montealvo et al., 2007).

Lime affects the protein of the endosperm as well. The lime alters protein solubility, increasing the lysine and gluten availability (Fernandez-Munoz et al., 2004). Lime releases bound niacin, and improves the isoleucine-leucine ratio (Serna-Saldivar et al., 1990). The niacin naturally present is bound and unavailable to animals. The protein swells but does not disrupt starch granules (Gomez et al., 1989).

Cooking time affects all DSC parameters, while steeping time has an effect on peak temperature (Mondragon et al., 2004). Nixtimalization increased gelatinization temperature which was hypothesized to be because of annealing, or calcium ions stabilizing the structure during steeping (Mendez-Montealvo et al., 2007). Increasing lime concentrations (0.1-1.0%) can also increase the gelatinization peak temperature (Bryant and Hamaker, 1997). It is unclear whether annealing of starch takes place. Annealing is accomplished by incubating starch granules in greater than 40% water, held usually longer than 12 hr, at a temperature above the glass transition temperature, but below the gelatinization temperature. Ratnayake et al. (2007) suggested that an effect similar to but different than annealing, something more complex than annealing alone, takes place and is evidenced by the DSC data of nixtamalized corn versus raw corn starch. They found that the peak temperature was increased, but the enthalpy was unchanged.

Starch digestibility is altered by lime concentration. Concentrations of less than 0.4% lime made starch more digestible because the kernels hold more water, swell more, and solubilize more than greater lime concentrations (Bryant and Hamaker, 1997).

Longer cooking times allow for greater starch gelatinization, which results in a lower change in enthalaphy for gelatinization on DSC because some of the starch has already been gelatinized. It is expected that the weak granules will gelatinize during cooking and steeping, and therefore the stronger granules will be left for DSC gelatinization, and result in higher onset and peak temperatures (Mondragon et al., 2004).

It is not yet clear what the best length of time is for steeping. Based on crystallinity and rheological properties 7-9 hr of nixtimalization is best for high-quality tortillas (Fernandez-Munoz et al., 2002). In the first 7 hr the pericarp loses crystallinity, from 7-9 hr the pericarp begins to be affected but crystallinity is retained, 9-15 hr the crystallinity of the endosperm decreases, and when corn is steeped for more than 15 hr some crystallinity is regained, possibly due to retrogradation (Fernandez-Munoz et al., 2002). Nine hr is the peak for masa viscosity during pasting, which is also the peak crystallinity time period. During this time the calcium ions could interact with amylose and amylopectin creating cross linking, and strengthening the masa (Frenandez-Munoz et al., 2002).

Viscosity is affected by amount of washing. With minimal washing (two times) being beneficial to masa viscosity because more gums and saponified lipids are retained (Martinez-Bustos et al., 2001). Viscosity is also altered by lime concentration. The highest hot paste peak viscosity occurred at a lime concentration of 0.1% and further lime addition decreased

the hot paste peak viscosity (Bryant and Hamaker, 1997). However, the highest ending viscosity occurs at a lime concentration of 1.0% (Bryant and Hamaker, 1997).

Increasing pH and calcium concentration of masa are positively correlated. Addition of only 0.1% lime increased the pH of the solution from 7.44 to 11.59, but addition of lime up to 1% only caused an additional increase of the pH to 11.8 (Bryant and Hamaker, 1997). A concentration of 0.4% will saturate the solution at 60° C in the presence of corn starch (Bryant and Hamaker, 1997). Seven hours provided the peak for pH and calcium content. The alkaline conditions induced swelling and exposed reactive sites for calcium bonding. Nine hours provided the minimum for pH, and as was previously mentioned, the highest crystallinity. At this time calcium may be bound to form alkoxide which will decrease alkalinity (Bryant and Hamaker, 1997).

Resistant starch and retrogradation in tortillas

Tortillas can become stale within hours of removal from the oven, due to rapid retrogradation (Bello-Perez et al., 2006). Tortillas have a large surface area which aids in quick moisture loss. The RS content of tortillas has been shown to increase upon storage as RS 4 is formed (Islas-Hernandez 2006); however the sensory characteristics would make these tortillas very unpalatable as they would be very dry and prone to cracking (Rendon-Villalobos et al., 2006a). In fact, firmness and development of resistant starch followed similar trends (Campas-Baypoli et al., 2002). Rendon-Villalobos et al. (2006b) found RS increased by 50% over 7 days of refrigerated storage, starting at 2.74% to 5.04%, but an additional 7 days of storage did little, only increasing the RS by 0.19%. The peak temperature of retrogradation increased over storage from 51.6° C after 1 day to 55.9° C after 3 days (Bello-Perez et al.,

2003). This is thought to be a result of increasing crystal perfection of the reassociating amylose and amylopectin. The more time a starch retrogrades the more perfect the crystals are.

The low fat content of tortillas, which is dependent on corn type but near 4.4 % for yellow corn (Table 2), also favors retrogradation (Campas-Baypoli et al., 2002). Maximum retrogradation for corn tortillas occurs at 13° C (Limanond et al., 2001). Storage below the glass transition temperature of -5° C will inhibit retrogradation, but is not a standard storage condition (Gudmunsson, 1994). Tortillas have been shown to stale at a slower rate at room temperature, 25° C, than at 4° C (Bueso et al., 2006). Since retrogradation is thermally reversible texture can be improved some with heating. Minimal washing helps tortillas reheated after 24 hr to retain the best texture (Martinez-Bustos et al., 2001).

Extending shelf life with anti-staling agents

A problem with commercially sold tortillas is loss of flexibility during storage. A traditional tortilla has a shelf life of 3 days, 12 days if refrigerated (Serna-Saldivar et al., 1990). Water loss begins almost immediately after cooking, and too much water loss results in undesirable tortillas. Antistaling agents, like hydrocolloids and gums, bind water and retain texture, but also inhibit RS formation (Bello-Perez et al., 2006, and Rendon-Villalobos et al., 2006a). These processing aids enhance flexibility and strength, and reduce stickiness during processing and packing (Rendon-Villalobos et al., 2006b). Gums also help eliminate tortillas sticking together in packaging, and improve freeze-thaw tolerance (Serna-Saldivar et al., 1990). Over storage RDS decreased and RS increased in tortillas, but this trend was lower in tortillas with added hydrocolloids (Rendon-Villalobos et al., 2006a).

Tortillas also have a short shelf life because of microbial spoilage. Tortillas are a high-moisture food (38-46%) with a high water activity (Aw = 0.96), which increases the chances of microbial growth and spoilage (Serna-Saldivar et al., 1990). Preservatives and acidulants can inhibit microbial spoilage, but will also cause flavor changes. If preservatives are used, the masa must first be acidified to pH 5.5 for best results.

Tortillas and sensory evaluation

Not many researchers have looked at human evaluation of sensory aspects of tortillas. Herrera-Corredor et al. (2007) identified sensory characteristics that increase the likelihood that a consumer will purchase tortillas in a supermarket; they included overall appearance, rollability (resistance to cracking when rolled), chewiness, taste, and overall liking. The flavor of tortillas is enhanced by Maillard browning, the reaction of reducing sugars and peptides under heat (Serna-Saldivar et al., 1990).

The color of the tortilla is a result of many interactions: corn color, amount of lime, extent of washing, extent of pericarp removal, and pH (Rooney and Serna-Saldivar, 2003). Most tortillas on the market are made from white corn, and are expected to be white, but tortillas made from colored corn varieties do exist. Color is also affected by nixtimalization. Alkaline cooking makes the masa lighter, while excessive washing makes masa less yellow (Martinez-Bustos et al., 2001). Maize genotype, lime concentration, and processing may also affect color. Color intensity was related to pigments present and pH (Martinez-Bustos et al., 2001).

Texture is important to the quality of tortillas, thus, attempts at standardizing a method for measuring tortilla texture have been made. Excessive washing (defined as washing more than twice) makes tortillas more prone to cracking and tearing, less likely to have enough puffiness, and less able to roll without cracking (Martinez-Bustos et al., 2001). A subjective rollability method was originally used to measure the impact of staling on texture. For this test, panelists were asked to roll a tortilla around a dowel and rate the amount of cracking on a scale of 1 to 5, with 1 being unrollable, and 5 being very rollable with no cracking (Flores-Farias et al., 2000). Rollability is important because tortillas are generally folded or rolled to combine with various fillings. Aged tortillas are firmer, more rigid, and therefore less rollable. The rollability when measured by a panel has inherent variability between people, and is not sensitive to changes over the first several hours after baking (Suhendro et al., 1998b).

Suhendro et al. (1998a) attempted to create a method that was more reliable and sensitive to the beginning changes in texture caused by the onset of retrogradation by using the texture analyzer. A special attachment was made to mechanically roll the tortilla around a dowel, with the force required to pull the tortilla around the dowel being recorded. This method was able to detect changes in the first 24 hr after baking. This method is hard for other labs to replicate because of the required special TA.XT2 attachment.

The same group also developed an extensibility method. Extensibility is when something is pulled apart using tensile forces. Extensibility is a component of rollability. Again the TA.XT2 was used to pull a tortilla strip apart. Fresh tortillas are soft and extensible, which results in longer distances of extension (Suhendro et al., 1999).

The same group also recognized that bending is an important characteristic of tortillas. Another method using the TA.XT2 was developed that evaluate strips of tortillas bent at 40° angles, measuring the force versus distance curve (Suhendro et al., 1998b). All of these methods were attempts to make the measurement of texture attributes of tortillas more objective. Unfortunately without the necessary attachments, labs cannot perform these measures and the subjective measure of rollability is often used.

Conclusions

While RS has a similar chemical structure to starch, it is classified as a type of DF because it resists digestion by human digestive enzymes. The four types of RS are resistant to digestion for different reasons, but all can be used as a fermentable substrate for beneficial gut bacterial. Fermentation produces SCFAs that lower intestinal pH, and provides protection again colon cancer by regulating apoptosis. Because RS is a low GI food it increases satiety, has a low blood glucose effect, and can help with weight regulation. Ingestion of RS also has effects on cholesterol metabolism.

Measurement of all four types of RS is difficult because there is no way currently to measure RS as eaten. Another way to characterize a starch is to examine the gelatinization characteristics with a DSC. Different mutants produce different gelatinization patterns. Retrogradation affects RS percentage, and can also be measured by DSC. Combining the information gained from DSC analysis with the RS percentage could provide a clearer understanding of how a high-RS starch will behave in a food product.

Dent corn is low in RS, and has a harder endosperm than *floury* corn. Tortillas are made from floury corn and are a staple food product in Latin American countries. They are also gaining popularity in the United States. Tortillas are traditionally produced by nixtimalization of *floury* corn, the process of soaking corn in lime to soften it, and aid in grinding. The process of nixtimalization is variable, but because the process conditions affect the end product, finding ideal processing conditions is the key to producing a high-quality product.

Tortillas are high in calories and calcium, but otherwise have little nutritional benefit. Making tortillas from corn mutants, including high-amylose mutants, could alter the RS content, and consequently the DF content. Tortillas are a high GI food, but this could be altered by increasing the DF content.

The texture of tortillas is very important to tortilla quality. Tortillas stale rapidly, and become unpalatable not long after they are produced. Commercial tortillas make use of anti-staling agents which retard staling. Slowing staling also slows formation of RS 3 in tortillas. Instrumental methods have been created to quantify textural changes in tortillas. Corn varieties high in amylose provide increased RS, but could adversely affect the texture of products.

LITERATURE CITED

Arambula-Villa, G., S.R.A. Mauricio, J.D. Figueroa-Cardenas, J. Gonzales-Hernandez, and C.A. Ordorica-Falomir. 2001. Corn masa and tortillas from extruded instant corn flour containing hydrocolloids and lime. *J. Food Sci*. 64: 120-124.

Akerberg, A.K.E., H.G.M. Liljeberg, Y.E. Granfeldt, A.W. Drews, and I.M.E. Bjorck. 1998. An in vitro method, based on chewing, to predict resistant starch content in foods allows parallel determination of potentially available starch and dietary fiber. *J. Nutr.* 128: 651-660.

American Association of Cereal Chemists. 2001. The definition of dietary fiber. *Cereal Foods World*. 46: 112-126.

AACC Board of Directors. 1999. http://www.aaccnet.org/definitions/wholegrain.asp. Visited April 1, 2008.

American Dietetic Association. 2008. Position of the American Dietetic Association: Health Implications of Dietary Fiber. *J. Am. Diet. Assoc.* 108: 1716-1731.

Bauer, L.L., M.R. Murphy, B.W. Wolf, and G.C. Fahey Jr. 2003. Estimates of starch digestion in the rat small intestine differ from those obtained using in vitro time-sensitive starch functional assays. *J. Nutr*. 133: 2256-2261.

Bear, R.P., M.L. Vineyard, M.M. MacMasters, and W.L. Deatherage. 1958. Resistant starch: formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *Cereal Sci*. 4: 301-314.

Bello-Perez, L.A., J.R. Rendon-Villalobos, E. Agama-Acevedo, and J.J. Islas-Hernandez. 2006. In vitro digestibility of tortillas elaborated by different masa preparation procedures. *Cereal Chem.* 83 (2): 188-193.

Bello-Perez, L.A., P. Osorio-Diaz, E. Agama-acevedo, J. Solorza-Feria, J. F. Toro-Vazquez, and O. Paredes-Lopez. 2003. Chemical and physiochemical properties of dried wet masa and dry masa flour. *J. Sci. Food Agri*. 83: 408-412.

Bird, A.R., T. Hayakawa, Y. Marsono, J.M. Gooden, R.L. Correll, and D.L. Topping. 2000. Coarse brown rice increases fecal and large bowel short-chain fatty acids and starch but lowers calcium in the large bowel of pigs. *J. Nutr*. 130: 1780-1787.

Birkett, A., J.G. Muir, J. Phillips, G.P. Jones, and K. O'Dea. 1996. Resistant starch lowers fecal concentrations of ammonia and phenols in humans. *Am. J. Clin. Nutr*. 63: 766-72.

Boutwell, R.K., and D.K. Bosch. 1959. The tumor-promoting action of phenol and related compounds for mouse skin. *Cancer Res*. 19: 413-27.

Brand-Miller, J., M. Thomas, V. Swan, Z. Ahmad, P. Petcock, and S. Claggier. 2003. Physiological validation of the concept of glycemic load in lean young adults. *J. Nutr*. 133: 2728-2732.

Brennan, C.S. 2005. Dietary fibre, glycaemic response, and diabetes. *Food Res*. 49: 560-570.

Brouns, F., B. Kettlitz, and E. Arrigoni. 2002. Resistant starch and "the butyrate revolution." *Trends in Food Sci. and Tech.* 13: 251-261.

Bryant, C.M., and B.R. Hamaker. 1997. Effect of lime on gelatinization of corn flour and starch. *Cereal Chem.* 74 (2): 171-175.

Bueso, F.J., R.D. Waniska, R. Moreira, K. Seetharaman, and L.W. Rooney. 2006. Effect of temperature on texture of corn tortilla with and without antistalling agents. *Cereal Chem*. 83 (4): 348-353.

Burton, K.E., F.M. Steele, L. Jefferies, O.A. Pike, and M.L. Dunn. 2008. Effect of microfortification of nutritional and other properties of nixtamal tortillas. *Cereal Chem*. 85 (1): 70-75.

Cagampang, G.B., and A.W. Kirleis. 1985. Properties of starches isolated from sorghum floury and corneus endosperm. *Starch*. 37: 253-257.

Carrol, K.K., R.M.G. Hamilton, M.W. Huff, and A.D. Falconer. 1978. Dietary fiber and cholesterol metabolism in rabbits and rats. *Am. J. Clin. Nutr*. 31: S203-S207.

Campas-Baypoli, O.N., E.C. Rosas-Burgos, P.I. Torres-Chavez, B. Ramirez-Wong, and S.O. Serna-Saldivar. 2002. Physicochemical changes of starch in maize tortillas during storage at room and refrigeration temperatures. *Starch*. 54: 358-363.

Campbell, M.R., P.J. White, and L.M. Pollak. 1994. Dosage effect at the *sugary-2* locus on maize starch structure and function. *Cereal Chem*. 71:464-468.

Cherrington, C.A., M. Hinton, G.R. Pearson, and I. Chopra. 1995. Short-chain organic acids at pH 5.0 kill *Esherichia coli* and *Salmonella spp* without causing membrane perturbation. *J. Appl. Bacteriol.* 70: 161-165.

Coudray; C., J. Bellanger, C. Castigila-Delavaud, C. Remesy, M. Vermo-Rel, J. Bellanger, and Y. Rayssiguier. 1997. Effects of soluble or partly soluble dietary fibres supplementation

on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *Eur. J. Clin. Nutr.* 51: 275-280.

Creech, R.G. 1968. Carbohydrate synthesis in maize. *Adv. Agron.* 20:275.

Cuevas-Rodriguez, E.O., C. Reyes-Moreno, S.R. Eckhoff, and J. Milan-Carrilo. 2009. Nixtimalized instant flour from corn (*Zea mays* L.) meal: Optimization of nixtamilzation conditions. *Cereal Chem.* 86 (1): 7-11.

Cummings, J.H., M.J. Hill, E.S. Bones, W.J. Branch, and D.J.A. Jenkins. 1979. The effect of meat protein and dietary fiber on colonic function and metabolism. II. Bacterial metabolites in feces and urine. *Am. J. Clin. Nutr.* 32: 2094-101.

Czuchajowska, Z., D. Sievert, and Y. Pomeranz. 1991. Enzyme-resistant starch. IV. Effects of complexing lipids. *Cereal Chem.* 68: 537-542.

Danjo, K., S. Nakaji, S. Fukuda, T. Shimoyama, J. Sakamoto, and K. Sugawara. 2003. The resistant starch level of heat moisture-treated high amylose corn starch is much lower when measured in the human terminal ileum than when estimated in vitro. *J. of Nutr.* 133: 2218-2221.

Darrah, L.L., M.D. McMullen, and M.S. Zuber. 2003. *Breeding, genetics, and seed corn production.* In: Advances in Cereal Science and Technology. American Assoc. of Cereal Chemists, Inc. St. Paul, MN.

de Dekere, E.A.M, W.J. Kloots, and J.M.M. van Amelsvoort. 1993. Resistant starch decreases serum total cholesterol and triacylglycerol concentrations in rats. *J. Nutr*. 123: 2142-2151.

Del Pozo-Insfran, D., S.O. Serna Saldivar, C.H. Brenes, and S.T. Talcott. 2007. Polyphenolics and antioxidant capacity of white and blue corns processed into tortillas and chips. *Cereal Chem*. 84 (2): 162-168.

DeVries, J.W., L. Prosky, B. Li, and S. Cho. 1999. A historical perspective on defining dietary fiber. *Cereal Foods World*. 44: 367-369.

Earle, F.R., J.J. Curtis, and J.E. Hubbard. 1946. Composition of the component parts of the corn kernel. *Cereal Chem*. 23: 504-411.

Englyst, K.N., S. Liu, and H.N. Englyst. 2007. Nutritional characterization and measurement of dietary carbohydrates. *Euro J. Clin Nutr*. 61 S1: S19-S39.

Englyst, K.N., S. Vinoy, H.N. Englsyt, and V. Lang. 2003. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *Br. J. Nutr*. 89: 329-339.

Englyst, H.N., and G.J. Hudson. The classification and measurement of dietary carbohydrates. 1996. *Food Chem*. 57: 15-21.

Eyster, W.H. 1934. Genetics of *Zea mays*. *Bibliogr. Genet*. 11: 87.

Fernandez-Munoz, J.L., M.E. Rodriguez, R.C. Pless, H.E. Martinez-Flores, M. Leal, J.L Martinez, and L. Banos. 2002. Changes in the nixtamalized corn flour dependent on post cooking steeping time. *Cereal Chem*. 79: 162-166.

Fernandez-Munoz, J.L., I. Rojas-Molina, M.L. Gonzalez-Davalos, M. Leal, M.E. Valtierra, E. San Martin-Martinez, and M.E. Rodriguez. 2004. Study of calcium ion diffusion in components of maize kernels during traditional nixtamalization process. *Cereal Chem*. 81 (1): 65-69.

Fleming, S.E., Y.S. Choi, and D.M. Fitch. 1991. Absorption of short-chain fatty acids from the rat cecum in vivo. *J Nutr*. 121: 1787-1797.

Flores-Farias, R., F. Martinez-Bustos, Y. Salinas-Moreno, Y. Kil Chang, J.G. Hernandez, and E. Rios. 2000. Physicochemical and rheological characteristics of commercial nixtamalised Mexican maize flours for tortillas. *J. Sci. Food Agric*. 80: 657-664.

Gao, M., J. Wanat, P.S. Stinard, M.G. James, and A.M. Myers. 1998. Characterization of *dull1*, a maize gene coding for a novel starch synthase. *Plant Cell* 10:399-412.

Gomez, M.H., R.D. Waniska, and L.W. Rooney. 1991. Starch characterization of nixtamalized corn flour. *Cereal Chem*. 68 (6): 578-582.

Gomez, M.H., C.M. McDonough, L.W. Rooney, and R.D. Waniska. 1989. Changes in corn and sorghum during nixtamalizaiton and tortilla baking. *J. Food Sci*. 54: 330-336.

Gregory, J.F. 2004. Dietary folate in a changing environment: bioavailability, fortification, and requirements. *J Food Sci*. 69: 59-60.

Gudmundsson, M. 1994. Retrogradation of starch and the role of its components. *Thermochim. Acta.* 246: 329-341.

Hara, H., S. Haga, Y. Aoyama, and S. Kiriyama. 1999. Short-Chain Fatty Acids suppress cholesterol synthesis in rat liver and intestine. *J Nutr*. 129: 942-948.

Haralampu, S.G. 2000. Resistant starch—a review of the physical properties and biological impact of RS3. *Carb. Polymers*. 41: 285-292.

Harris, P.J., and B.G. Smith. 2006. Plant cell walls and cell-wall polysaccharides: structures, properties and uses in food products. *Intl. J. Food Sci. and Tech.* 41 S2: 129-143.

Herrera-Corredor, J.A., J.E.P. Saidu, A. Khachatryan, W. Prinyawiwatkul, A. Carballo-Carballo, and R. Zepeda-Bautista. 2007. Identifying drivers for consumer acceptance and purchase intent of corn tortilla. *J. Food Sci*. 72: S727-S731.

Holt, S.H., J. Brand, C. Soveny, and J. Hansky. 1992. Relationship of satiety to postprandial glycemic, insulin and cholecystokinin responses. *Appetite*. 18: 129-141.

Inouchi, N., Glover, D.V., Sugimoto, Y., and H., Fuwa. 1984. Developmental changes in starch properties of several endosperm mutants of maize. *Starch*. 36:8.

Islas-Hernandez, J.J., R. Rendon Villalobos, E. Agama-Acevedo, F. Gutierrez-Meraz, J. Tovar, G. Arambula-Villa, and L.A. Bello-Perez. 2006. In vitro digestion rate and resistant starch content of tortillas stored at two different temperatures. *LWT*. 39: 947-951.

Jenkins, D., T. Wolever, R. Taylor, H. Barker, H. Fielden, and J.M. Baldwin. 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am. J. Clin. Nutr*. 34: 362-366.

Ji, Y., L.M. Pollak, S. Duvick, K. Seetharaman, P.M. Dixon, and P.J. White. 2005. Gelatinization properties of starches from three successive generations of six exotic corn lines grown in two locations. *Cereal Chem*. 81(1): 59-64.

Kashtan, H., H.S. Stern, D.J. Jenkins, A.L. Jenkins, L.U. Thompson, K. Hay, N. Marcon, S. Minkin, and W.R. Bruce. 1992. Colonic fermentation and markers of colo-rectal cancer risk. *Am. J. Clin. Nutr*. 55: 723-238.

Kim, W.K., M.K. Chung, and N.E. Kang. 2003. Effect of resistant starch from corn or rice on glucose control, colonic events, and blood lipid concentrations in streptozotocin-induced diabetic rats. *J. Nutr. Bio*. 14: 166-72.

Klucinec, J.D., and D.B. Thompson. 1999. Amylose and amylopectin interact in retrogradation of dispersed high-amylose starches. *Cereal Chem*. 76: 282-291.

Kontraszti, M., G.J. Hudson, and H.N. Englyst. 1999. Dietary fibre in Hungarian foods measured by the Englyst NSP procedure and the AOAC Prosky procedure: a comparison study. *Food Chem*. 64: 445-450.

Kramer, H.H., P.L. Pfahler, and R.L. Whistler. 1958. Gene interaction in maize affecting endosperm properties. *Agron. J.* 50: 207.

Krueger, B.R., C.E. Walker, C.A. Knuston, and G.E. Inglett . 1987. Differential scanning calorimeter of raw and annealed starch isolated from normal and mutant maize genotypes. *Cereal Chem.* 64: 187-190.

Lawton, J.W., and C.M. Wilson. 2003. *Proteins of the kernel*. In: Advances in Cereal Sceince and Technology. American Assoc. of Cereal Chemists, Inc. St. Paul, MN.

Limanond, B., M.E. Castell-Perez, and R. Moreira. 2001. Modeling the kinetics of corn tortilla staling using stress relaxation data. *J. Food Eng.* 53: 237-247.

Lin, H.C., and W.J. Visek. 1991. Large intestinal pH and ammonia in rats: Dietary fat and protein interactions. *J Nutr*. 121: 832-43.

Le Leu, R.K., I.L. Brown, Y. Hu, T. Morita, A. Esterman, and G.P. Young. 2006. Effect of dietary resistant starch and protein on colonic fermentation and intestinal tumourigenesis in rats. *Carcinogenesis*. 28: 240-245.

Macfarlane, G.T., and J.H. Cummings. 1999. Probiotics and prebiotics: Can regulating the activities of intestinal bacteria benefit health? *British Medical J.* 318: 999-1003.

Martinez-Bustos, F., H.E. Martinez-Flores, E. Sanmartin-Martinez, F. Sanchez-Sinencio, Y.K. Chang, D. Barrera-Arellano, and E. Rios. 2001. Effect of the components of maize on the quality of masa and tortillas during the traditional nixtamalisation process. *J. Sci. Food Agric.* 81: 1455-1462.

Mertz, E.T., L.S. Bates, and O.E. Nelson. 1964. Mutant gene that changes protein composition and increases lysine content of maize endosperm. *Science*. 145: 279-280.

McCleary, B.V. and D.A. Monaghan. 2002. AOAC official method 2002.02 resistant starch in starch and plant materials. *J. AOAC Int*. 85: 1103-1111.

McMillian-Price J., and J. Brand-Miller. 2006. Low-glycaemic index diets and body weight regulation. *Intl. J. Obesity*. 30: S40-S46.

Mendez-Montealvo, G., J.L. Trejo-Espino, O. Paredes-Lopez, and L.A. Bello-Perez. 2007. Physicochemical and Morphological Characteristics of Nixtamalized Maize Starch. *Starch*. 59: 277-283.

Mondragon, M., L.A. Bello-Perez, E. Agama-Acevdo, D. Betancur-Ancona, and J.L. Pena. 2004. Effect of cooking time, steeping and lime concentration on starch gelatinization on corn during nixtamalization. *Starch*. 56: 248-253.

Mortenson, F.V., I. Hessov, H. Birke, N. Korsgaad, and H. Nielsen. 1991. Microcirculartory and trophic effects of short chain fatty acids in the human rectum after Hartmann's procedure. *Br. J. Surg*. 78: 1208-1211.

Muir, J.G., and K. O'Dea. 1993. Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. *Am. J. Clin. Nutr*. 57: 540-56.

Muir, J.G., E.G.W. Yoew, J. Keogh, C. Pizzey, A.R. Bird, K. Sharpe, K. O'Dea, and F.A. Macrae. 2004. Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. *Am. J. Clin. Nutr*. 79: 1020-8.

Noakes, M., P.M. Clifton, P.J. Nestel, R. Leu, and G. McIntosh. 1996. Effect of high amylose starch and oat bran on metabolic variables and bowel functions in subjects with hypertriglyceridemia. *Am. J. Clin. Nutr*. 64: 944-951.

Nugent, A.P. 2005. Health properties of resistant starch. *Nutr. Bull*. 30: 27-54.

Obatolu, V.A., O. Augustine, and J.E. Iken. 2007. Improvement of home-made maize tortilla with soybean. *Intl. J. of Food Sci. and Tech.* 42: 420-426.

Ostlund R.E., S.B. Racette, and W.F. Stenson. (2003). Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. *Am. J. Clin. Nutr.* 77 (6): 1385–1589.

Persson, H., M. Nyman, H. Liljeberg, G Onning, and W. Frolich. 1991. Binding of mineral elements by dietary fibres components in cereals. In vitro (III). *Food Chem*. 40: 169-178.

Pfahler, P.L., H.H. Kramer, and R.L. Whistler. 1957. Effect of genes on birefringence and end-point temperature of starch grains in maize. *Science*. 125: 441.

Pflugfelder, R.L., R.D. Waniska, and L.W. Rooney. 1988. Fractionation and composition of masa. *Cereal Chem*. 65: 262-266.

Ramakrishna, B.S., S. Venkataraman, S. Srinivasan, P. Dash, G.P. Young, and H.J. Bidner. 2000. Amylase-resistant starch plus oral rehydration solution for cholera. *N. Engl. J. Med.* 342: 308-313.

Ramirez-Wong, B., V.E. Sweat, P.I. Torres, and L.W. Rooney. 1994. Cooking time, grinding and moisture content effect on fresh corn masa texture. *Cereal Chem*. 71: 337-343.

Ratkovic, S., M. Denic, and G. Lahajnar. 1982. Kinetics of water imbibtion by seed: why normal and *opaque-2* maize kernels differ in their hydration properties. *Period. Biol*. 84: 180-182.

Ratnayake, W.S., A.B. Wassinger, and D.S. Jackson. 2007. Extraction and characterization of starch from alkaline cooked corn masa. *Cereal Chem*. 84(4): 415-422.

Reddy, B.S., K. Watanabe, and A. Sheinfil. 1980. Effect of dietary wheat bran, alfalfa, pectin and carrageenan on plasma cholesterol and fecal bile acid and neutral sterol excretion in rats. *J. Nutr*. 110: 1247-1254.

Rendon-Villalobes, R., E. Agama-Acevedo, J.J. Islas-Hernandez, O. Paredes-Lopez, and L.A. Bello-Perez. 2006a. Effect of hydrocolloid type and concentration on in vitro starch digestibility of stored tortillas using response surface methodology. *J. of Food Engr*. 74: 153-159.

Rendon-Villalobes, R., E. Agama-Acevedo, J.J. Islas-Hernandez, J. Sanchez-Munoz, and L.A. Bello-Perez. 2006b. In vitro starch bioavailability of corn tortillas with hydrocolloids. *Food Chem*. 97: 631-636.

Robertson, J.B., and P.J. Horvath. Detergent analysis of foods. *CRC Handbook of Dietary Fiber in Human Nutrition.* 1993. Spiller GA, Boca Raton, FL.

Rooney, L.W., and S.O. Serna-Saldivar. 2003. *Food use of whole corn and dry-milled fractions.* In: Advances in Cereal Science and Technology. American Assoc. of Cereal Chemists, Inc. St. Paul, MN.

Sahai, D., J.P. Mua, I. Surjewan, M.O. Buendia, M. Rowe, and D.S. Jackson. 2001. Alkaline processing (nixtamalization) of white Mexican corn hybrids for tortilla production: Significance of corn physicochemical characteristics and process conditions. *Cereal Chem*. 78: 116-120.

Salovaara, H., F. Gates, and M. Tenkanen. 2007. *Dietary fibre—components and functions*. 1st ed. Wageningen Academic Publishers, The Netherlands.

Sandstedt, R.M., D. Strahan, S. Ueda, and R.C. Abbot. 1962. The digestibility of high-amylose corn starches compared to that of other starches: the apparent effect of the *ae* gene on the susceptibility of amylase action. *Cereal Chem*. 39: 123.

Saura-Calixto, F., I. Goni, L. Bravo, and E. Manas. 1993. Resistant starch in foods: modified method for dietary fiber residues. *J Food Sci*. 58: 642-645.

Scheppach, W., H. Luchrs, and T. Menzel. 2001. Beneficial health effects of low-digestible carbohydrate consumption. *British J. Nutr*. 85: S23-S30.

Seetharaman, K., A. Tziotis, F. Borras, P.J. White, M. Ferrer, and J. Robutti. 2001. Thermal and functional characterization of starch from Argentinean corn. *Cereal Chem*. 78: 379-386.

Seifter, J., D. Sloane, and A. Ratner. 2005. *Nutrition, digestion and absorption*. In: Concepts in medical physiology. Lippincott Williams & Wilkins.

Serna-Saldivar, S.O., M.H. Gomez, H.D. Almeida-Dominguez, A. Islas-Rubio, and L.W. Rooney. 1993. A method to evaluate the lime-cooking properties of corn (*Zea mays*). *Cereal Chem*. 70: 762-764.

Serna-Saldivar, S.O., M.H. Gomez, and L.W. Rooney. 1990. *Technology, chemistry, and nutritional value of alkaline-cooked corn products*. In: Advances in Cereal Science and Technology. American Assoc. of Cereal Chemists, Inc. St. Paul, MN.

Shannon, J.C., and D.L. Garwood. 1984. *Genetics and physiology of starch development*. In Starch: Chemistry and technology (2nd edition). Orlando, FL: Academic Press.

Shu, X., L. Jia, J. Gao, Y. Song, H. Zhao, Y. Nakamura, and D. Wu. 2007. The influence of chain length of amylopectin on resistant starch in rice (*Oryza sativa* L.). *Starch*. 59: 504-509.

Stevens, D.J., and G.A.H. Elton. 1971. Thermal properties of the starch/water system. Part I. Measurement of heat of gelatinization by differential scanning calorimeter. *Starch*. 23: 8.

Suhendro, E.L., H.D. Almeida-Dominguez, L.W. Rooney, and R.D. Waniska. 1998a. Objective rollability method for corn tortilla texture measurement. *Cereal Chem*. 75(3): 320-324.

Suhendro, E.L., H.D. Almeida-Dominguez, L.W. Rooney, R.D. Waniska, and R.G. Moreira. 1998b. Tortilla bending technique: An objective method for corn tortilla texture measurement. *Cereal Chem*. 75 (6): 854-858.

Suhendro, E.L., H.D. Almeida-Dominguez, L.W. Rooney, R.D. Waniska, and R.G. Moreira. 1999. Use of extensibility to measure corn tortilla texture. *Cereal Chem*. 76 (4): 536-540.

Takeda, C., Takeda, Y., and Hizukuri, S. 1993. Structure of the amylopectin fraction of amylomaize. *Carb. Res*. 246:273-281.

Themeier, H., J. Hollmann, U. Neese, and M.G. Lindhauer. 2005. Structural and morphological factors influencing the quantification of resistant starch II in starches of different botanical origin. *Carb Polymers*. 61: 72-79.

Tomlin J., and N.W. Read. 1990. The effect of resistant starch on colon function in humans. *Br. J. Nutr* 64: 589-595.

Topping, D.L., and P.M. Clifton. 2001. Short-chain fatty acids and human colonic function: Roles of resistant starch and non-starch polysaccharides. *Physio. Reviews*. 81: 1031-1064.

Tovar, J., S.G. Sayago-Ayerdi, C. Penalver, O. Paredes-Lopez, and L.A. Bello-Perez. 2003. In vitro starch hydrolysis index and predicted glycemic index of corn tortilla, black beans (*Phaseolus vulgaris* L.), and Mexican "taco". *Cereal Chem*. 80: 533-535.

Trinidad, T.P., T.M.S. Wolever, and L.U. Thompson. 1996. Effect of acetate and propionate on calcium absorption from the rectum and distal colon of humans. *Am. J. Clin. Nutr*. 63: 574-578.

Tziotis, A., K. Seetharaman, J.D. Klucinec, P. Keeling, P.J. White. 2005. Functional properties of starch from normal and mutant corn genotypes. Carbohydrate polymers. 61: 238-247.

US Department of Agriculture, 2005. http://www.health.gov/dietaryguidelines/dga2005/document/default.htm. Visited November 2008.

Vahouny, G.V., S. Satchithanandam, I. Chen, S.A. Tepper, D. Kritchevsky, F.G. Lightfoot and M.M. Cassidy. 1988. Dietary fiber and intestinal adaptation: effects on lipid adsorption and lymphatic transport in the rat. *Am. J. Clin. Nutr.* 47: 201-206.

Von Dokkum, W., B. Wezendonk, T.S. Srikumar, and E.G. Van Den Heuvel. 1999. Effect of non-digestible oligosaccharide on large bowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. *Eur. J. Clin. Nutr*. 53: 1-7.

Wang, Y.-J., P. White, and L. Pollak. 1992. Thermal and gelling properties of maize mutants form Oh43 inbred line. *Cereal Chem*. 69: 328-334.

Watson, S.A. 2003. *Description, development, structure, and composition of the corn kernel*. In: Advances in Cereal Science and Technology. American Assoc. of Cereal Chemists, Inc. St. Paul, MN.

White, P.J., L.M. Pollak, and S. Burkhart. 1991. Thermal properties of starches from corn grown in temperate and tropical environments. (Abstr.) Cereal Foods World. 36: 704.

Wright, R.S., J.A. Anderson, S.R. Bridges. 1990. Propionate inhibits hepatocyte lipid synthesis. *Proc. Soc. Exp. Biol. Med*. 195: 26-29.

Xie, S., Q. Liu, and S.W. Cui. 2006. Studies on the granular structure of resistant starches (type 4) from normal, high amylose and waxy corn starch citrates. *Food Res. Intl*. 39: 332-341.

Yamin, E.F., L. Svendsen, and P.J. White. 1997. Thermal properties of corn starch extraction intermediates by different scanning calorimetry. *Cereal Chem.* 74: 407-411.

Young, G.P., Y. Hu, R.K. Le Leu, and L. Nyskohus. 2005. Dietary fibre and colorectal cancer: a model for environment—gene interactions. *Mol. Nutr. Food Res*. 49: 571-584.

Zhang, G., Ao, Z., and Hamaker, B.R. 2006a. Slow Digestion Property of Native Cereal Starches. *Biomacromolecules*. 7: 3252-3258.

Zhang, G. M. Venkatachalam, and B.R. Hamaker. 2006b. Structural basis for the slow digestion property of native cereal starches. *Biomacromolecules*. 7: 3259-3266

Zhang, G., Sofyan, M., and Hamaker, B.R. 2008. Slowly Digestible State of Starch: Mechanism of Slow Digestion Property of Gelatinized Maize Starch. *J. Agric. Food Chem*. 56: 4695-4702.

Table 1. Dietary fiber components of corn kernel, starch endosperm, and pericarp.

Fraction	Percent of Kernel Dry Substance	Hemi-cellulose	Cellulose	Lignin	Soluble Fiber	Total Fiber	Percentage of Kernel Fiber
Whole Kernel	100	6.7	3.0	0.2	0.1	9.5	100
Starchy Endosperm	81	--	--	--	0.5	1.5	12
Pericarp	5.3	67	23	0.1	0.2	90.7	51

Based on Watson, 2003

Table 2. Average composition of seven yellow dent corn hybrids (% dwb)

Fraction	Percent Dry Weight of Whole Kernel	Starch	Fat	Protein	Ash	Sugar
Whole Kernel	100	73.4	4.4	9.1	1.4	1.9
Endosperm	82.9	87.6	0.8	8.0	0.3	0.62
Germ	11.1	8.3	33.2	18.4	10.5	10.8
Pericarp	5.3	7.3	1.0	3.7	0.8	0.34

Based on Watson, 2003, Earle et al. 1946

Fig. 1. Example of a Differential Scanning Calorimeter (DSC) curve indicating gelatinization of a starch-water slurry. The vertical dashes indicate where onset, peak, and end point temperatures would be measured. The ΔH value is calculated by measuring the area under the curve.

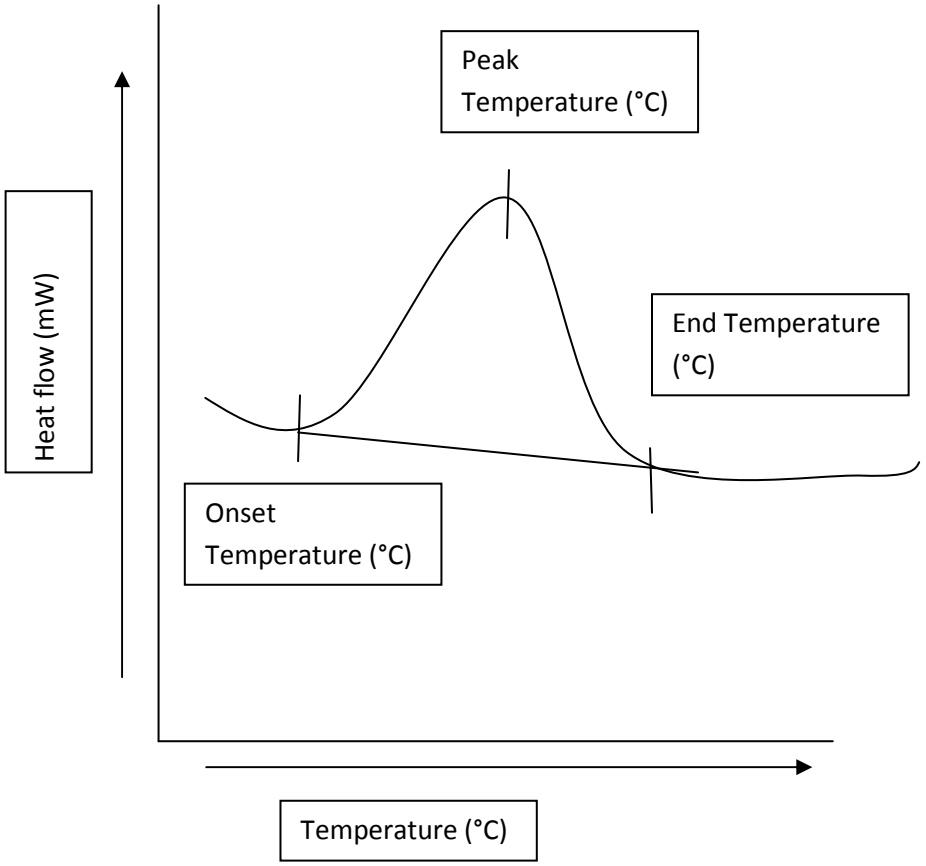

Fig. 1

THERMAL CHARACTERISTICS OF STARCH FROM CORN MUTANTS AND EXOTICS WITH DIFFERENT AMOUNTS OF RESISTANT STARCH

A paper to be submitted to *Cereal Chemistry*

Kim A. Rohlfing, Linda M. Pollak, and Pamela J. White

Abstract

Ten parent corn lines, comprised of four mutants (*dull sugary2, amylose-extender sugary2, amylose-extender dull,* and an *amylose-extender* with introgressed Guatemalen germplasm (GUAT *ae*)) and six lines with introgressed exotic germplasm backgrounds, were crossed with each other to create 20 progeny crosses. The parents and progeny crosses varied in resistant starch (RS) percentage. The lines and crosses with increased RS might be used in breeding corn to use as a means to increase dietary fiber in cornstarch-based foods. The RS was measured from the extracted starch, targeting the measurement of RS 2, which is present in ungelatinized starch, by using the Megazyme Resistant Starch kit. The RS values from the 10 parent lines varied from 18.3 % to 52.2 %, and the values from the 20 progeny crosses ranged from 16.6 to 34.0 %. The % RS of parents was not additive in the offspring, but greater RS in parents was correlated to greater RS in the progeny crosses ($r = 0.63$). The Differential Scanning Calorimeter (DSC) was used to measure the gelatinization and retrogradation characteristics of the starches. Peak gelatinization temperature and change in enthalpy were positively correlated to % RS ($r = 0.65$ and $r = 0.67$, $P \leq 0.05$); however, the retrogradation parameters, a measure of RS 3, did not correlate with % RS (RS 2 type). All parents and progeny crosses, with the exception of the Guat *ae* parent (52.5 %), had % RS

greater than that of commercial corn starch (8.9%), but lower than that of a high-amylose (*ae*) standard (50 % apparent amylose, 40.2 % RS). The % RS and onset temperature increased with the addition of the *ae* gene. Understanding the impact of RS on the gelatinization characteristics of starches will help the food industry understand its impact on food processing, especially processing involving heating.

Introduction

Four types of resistant starch (RS) have been defined. RS 1 is resistant due to the surrounding food matrix, RS 2 is present in ungelatinized, raw starches, RS 3 is created by retrogradation, and RS 4 is produced through chemical alteration (Englyst et al., 1996). Incorporation of RS into the diet provides many health benefits: it serves as a prebiotic, or fermentable substrate, for the growth of probiotics, lowers the pH of the colon (Cherrington et al., 1991), increases mineral absorption (Courdray et al., 1997), and increases cell turnover (Young et al., 2005). Cholesterol metabolism may be down regulated by RS, by production of short-chain fatty acids that may either suppress cholesterol synthesis in the liver (Hara et al., 1999), or decrease cholesterol absorption (Vahouny et al., 1988).

Corn endosperm mutants can affect the appearance of the kernel and/or underlying component quality, while double mutants can have synergistic effects on endosperm appearance and quality. Exotic germplasm used for food may have been selected for unusual endosperm quality. Corn-starch properties can be modified via traditional plant breeding methods by using major (e.g. naturally occurring mutant genes), or minor (modifying genes) genetic factors (Ji et al., 2004). Exotic corn lines may provide unusual traits of interest, including increased % RS, through the presence of modifying genes. High-amylose

(*amylose-extender*, *ae*) corn lines provide greater amounts of RS 2 than *normal* corn through a major (mutant) gene; thus, crossing *ae* and exotic corn types could increase the RS, provide unique materials for food use, and possibly provide cooking properties better than *ae* corn lines used alone.

Whereas four types of RS have been defined, only RS 2, 3, and 4 are routinely measured. The process of extraction may alter RS 1 because it destroys the surrounding food matrix. There are several options available for measuring RS 2, 3, and 4. The Megazyme RS kit measures RS 2 effectively, and is designed to screen large numbers of samples (McCleary and Monaghan, 2002). However, most starch is not eaten in an ungelatinized form: the starch is generally cooked. Thus, the Megazyme kit, which includes no gelatinization step, may not be an accurate measure of RS as eaten.

A Differential Scanning Calorimeter (DSC) can measure starch gelatinization characteristics, including retrogradation. Retrogradation is thought to create RS 3, thus, starches with a high percentage of retrogradation might be predicted to have high RS percentages (Haralampu, 2000). The starch properties from many corn mutants, including *sugary2 (su2)*, *amylose extenter (ae)*, and *amylose dull* (*ae du)* have been examined on a DSC (Tziotis et al., 2005). Less work has been done on double mutants, and especially on other mutants that vary in RS. An *ae* starch might be predicted to provide a large amount of RS 2 and 3. The *ae* starches will not gelatinize completely under boiling temperatures (Champ, 1992), leaving some of the RS 2 intact. The starch that did gelatinize is available for retrogradation and can be converted to RS 3.

Thermal characterization of starches from corn lines with elevated levels of RS has not been done, especially in relation to both RS 2, measured with the Megazyme kit, and RS3, measured with a DSC. These evaluations would be useful in predicting behavior of the starches, and thus the corn, in food products.

The objectives of this study were to:

1) Identify new corn breeding crosses containing both high % RS and potential for use in high quality foods, by crossing four mutants and six lines with introgressed exotic backgrounds with each other;
2) Evaluate the thermal characteristics of the starch from these parents and their crosses on the DSC;
3) Compare the percentage of RS 2 in the starches measured by using the Megazyme Resistant Starch kit (RS 2), with the % RS 3 and other thermal characteristics measured on the DSC.

Materials and Methods

Corn Materials

Ten corn lines (*Zea mays* L.), four mutants and six lines with introgressed exotic backgrounds (Table 1), termed 'parents,' were crossed with each other to create 20 progeny crosses (Table 2). The GUAT *ae* parent is the first public 70% amylose line (Campbell et al., 2007). For easier discussion and comparison in this paper, the progeny crosses are separated into four groups and identified by their first parent, creating the mutant groupings of *dull*

sugary2 (*du su2),amylose-extender sugary2(ae su2), amylose-extender dull (ae du),* and an *amylose-extender* with introgressed Guatemalan germplasm (GUAT *ae).*

All progeny crosses were grown at Juana Diaz, Puerto Rico in 2006 and 2007 and were of the second selfed generation in the process of developing inbred lines. Ears were harvested at full maturity, and dried at 37.5° C to approximately 12% moisture. Seeds were stored at 4° C and 10 % relative humidity until needed for starch extraction. Seeds from individual ears were pooled and 15 kernels were randomly selected for each starch extraction. Commercial corn starch (Sigma Chemical Co., St. Louis, MO) was used as a typical corn-starch control, and a high-amylose control, (High Am-C, amylogel 03001, 50% apparent amylose, Cargill, Inc, Cedar Rapids, IA) was used as an *ae* control in the analyses.

Starch Extraction

From each parent and progeny cross, starch was extracted twice from two sets of 15 randomly selected kernels from an individual ear. The two extractions were treated as replicates. Starch was extracted from the 15 randomly selected kernels based on the procedures of Krieger et al. (1997) with the following modifications. A 100-µm filter (N100C Cellmicrosieves^{TM}, Biodesign Inc., New York, NY) was used as suggested in the Megazyme RS Kit (McCleary and Monaghan, 2002). The filtrate was allowed to settle at 4° C for 24 hr, after which the supernatant was discarded. The remaining slurry was centrifuged at 1000 x g revolutions per min, for 10 min, and the supernatant was again discarded. The pellet was dried at 45° C for 24 to 48 hr. After extraction, starches were stored in a desiccator until needed for RS and DSC analyses.

RS determination

The Megazyme RS Kit (K-RSTAR, Megazyme International, Bray, Ireland) was used to determine the RS content of ungelatinized starch. The following modification was used: 50-µl of amyloglucosidase was added to 50 µl of diluted RS solution to ensure all RS was converted to glucose before the addition of glucose oxidase/peroxidase reagent (GOPOD). All analyses for each replicate were conducted twice and the averages were computed.

DSC

The method of White et al. (1990), with modifications by Krieger et al. (1997) was followed. Briefly, starch (4 mg, dwb) was weighed in stainless steel pans, 8-µL of distilled water was added, and the pan was sealed. Pans were added to the DSC Diamond by using an autosampler, and Intercooler 2P (Perkin-Elmer, Norwalk, CT). The intercooler kept the pans in the autosampler at -100° C. Once the pans entered the DSC oven they were equilibrated at 25° C for 5 min, and then scanned from 25 to 180° C at 10° C per min. Data was then analyzed with Pyris Step Scan software (V3.7, Perkin-Elmer, Norwalk, CT). All analyses for each replicate were conducted twice, and the averages were computed. Onset temperature (T_{oG}), peak temperature (T_{pG}), and change in enthalpy (ΔH_G) were computed for the initial gelatinization. The gelatinized pans were stored for 7 days at 4° C and re-scanned to determine retrogradation by using the same program as for gelatinization, and measuring retrogradation onset (T_{or}), retrogradation peak temperature (T_{pr}), and change in enthalpy of retrogradation (ΔH_r) (White et al., 1989). Endpoint was recorded, but is not presented

because differences were not significant. The % retrogradation (% r) is the ΔH_r divided by the ΔH_G.

Statistical analyses

The proc ANOVA from Statistical Analysis Systems (SAS Institute, Cary, NC) was used to determine significant differences ($\alpha = 0.05$) between lines. Correlations were evaluated between RS and all DSC parameters by using Excel (Microsoft Office 2007, Seattle, WA). Regression analyses, using SAS, were done between the % RS averages of progeny crosses and the parents of those progeny crosses to determine if % RS was an additive trait.

Results and Discussion

RS

Of the parents studied, the ones with the *ae* gene in their backgrounds had the greatest % RS (Table 3). The GUAT *ae* parent was greatest overall in RS at 52.2%, and different from all other lines. The *ae du* parent also had a high % RS at 30.6, and was intermediate compared to the GUAT *ae* parent and the remaining parent with values ranging from 18.3 to 23.9 %.

The progeny crosses had more variability than the parents. Progeny crosses with GUAT *ae* in their background had the greatest % RS, ranging from 23.7 to 34.0%, but none retained the % RS of the GUAT *ae* parent (52.2 %, Table 3). In general, the % RS increased in the order: *du su2* < *ae su2* < *ae du*. The exotic parent did not affect % RS enough to produce differences between the lines of the first three mutant parent groups (*du su2*, *ae su2*, *ae du*).

For example, the % RS of the progeny crosses, (*du su2*/AR)-3-1 through (*du su2*/CU)-3-2, are not statistically different (Table 3). The AR and CU parents provided similar amounts of RS to their progeny. Mutant progeny crosses with *ae su*2 and *ae du* followed similar trends.

The progeny crosses with the mutant parent, GUAT *ae,* differed between exotic crosses. The exotic sources URS and BR contributed less RS than one of the AR crosses and the DK parents. All of the progeny crosses are of the S2 generation when homozygosity of a trait throughout an entire ear of corn is not ensured. Successive generations of the GUAT *ae*/AR cross should be grown and analyzed.

All parents with the exception of GUAT *ae* and progeny crosses had RS values greater than the commercial cornstarch (8.9%), but lower than the High Am-C (40.2%). Regression analysis showed that greater RS in the parents lead to greater RS in the progeny crosses ($r = 0.59$, $P \leq 0.05$, Figure 1), but expression of RS was not additive. Parents with greater % RS did not combine to create progeny crosses with RS greater than the parent with greater % RS. The recessive genes, *ae*, *su2*, and *du,* previously were shown to increase amylose expression (Shannon and Garwood, 1984). Presence of the *ae* gene increased the amounts of amylose, which lead to increased amounts of RS, as previously noted by Shu et al. (2007). The *ae du* gene also increased the amylose content of corn starch by 10-15% (Shannon and Garwood, 1984).

DSC

The parents had less differentiation in both gelatinization and retrogradation profiles than did the progeny crosses (Table 3). The GUAT *ae* parent showed a large ΔH_G of 34.8 J g^{-1}, but a very small ΔH_r of 3.2 J g^{-1}. The parent with the greatest % r was the BR parent, which also had the lowest % RS as measured by the Megazyme kit.

The progeny crosses showed a slightly greater T_{pG} range (68.3 to 76.4° C) than the parents (61.8 to 74.7° C, Table 3). The strongest correlations were between % RS and T_{pG} (r = 0.65), and % RS and ΔH_G (r = 0.67). The greatest ΔH_G was for the (GUAT *ae*/DK)-1-1 cross with a ΔH_G of 17.5 J g^{-1}. Starch from all progeny crosses had a ΔH_G near that of the commercial corn starch (13.1 J g^{-1}).

The % r was highly variable among the mutants, ranging between 6 and 52%, with no correlations noted between RS and any retrogradation parameter (Table 3). Differences did not depend on mutant groups.

The starch from the GUAT *ae* group had among the highest onset and peak temperatures, with the rest of the progeny crosses following the trend *du su2* < *ae su2* < *ae du* (Table 3). Previously, the presence of the *su2* gene was shown to increase the starch component, amylose, by approximately 10%, (Campbell et al., 1994). In other work, the *su2* gene decreased the T_{oG} and the ΔH_G values, especially compared to *normal* corn starch (Inouchi et al., 1984). Although increased amylose leads to increased RS, the *su2* gene causes increased digestibility, possibly because of the long B-chains and few branch points of the amylose in *su2* mutants (Takeda and Preiss, 1993). Branching slows digestion, thus fewer branch points could increase digestibility and reduce the % RS. These observations were reflected in the data with progeny crosses containing the *su2* gene, which had among the lowest onset

temperatures and among the lowest RS. This observation and explanation does not take into account the possibility of RS creation through tangled amylose chains forming enzyme-inaccessible areas. Starches with greater gelatinization temperatures might retain more of their RS 2 during heating. Temperatures of up to 180° C may be needed to fully gelatinize starches containing % amylose of 50% or higher.

Retrogradation of starch from individual mutants should vary according to their amylose to amylopectin ratios (Zhang et al., 2008). Amylose recrystallizes quickly upon cooling, whereas amylopectin recrystallizes more slowly. Starch with increased % r should have greater RS 3 content than starches with low % r. The % RS of the corn starch in the raw state is likely to be altered by gelatinization. Either a decrease or increase in RS is possible, although the mechanism is not perfectly understood (Yao et al., 2009). An increase in RS could be caused by annealing during gelatinization and a decrease could be caused by rupture of the starch granule, entry of water into the granule, followed by less ability of the starch chains to realign.

Both parents and progeny crosses in this study showed unusual DSC peak shapes with a large amount of tailing. The tailing at the beginning and end of the peak made the peak measurement difficult with the software available. The T_{oG} and T_{or} were most affected by tailing of the peak, creating a large variance for onset temperature, but also for the ΔH_G and ΔH_r. More precise ΔH measurements would provide less variation in % r.

Conclusions

There are several commercial options available for manufacturing RS, but traditional plant breeding methods have not been fully examined for developing varieties used for producing food ingredients with enhanced amounts of RS. This study examined 20 exotic crosses of maize from 10 parents with mutant or exotic backgrounds for their RS 2 and thermal characteristics as a measure of RS 3. The progeny crosses did not have greater % RS than their parents, showing no transgressive segregation. A moderate correlation occurred between % RS and T_{pG}, suggesting that RS 2 remains after processing involving heat treatment. The GUAT *ae* mutants had the greatest % RS and T_{pG}, and the starches from the parents and progeny crosses had more RS 2 than commercial corn starch. Little RS 3 was found when the starches were evaluated for retrogradation, the ΔH_r values were smaller than normal cornstarch. Using traditional plant breeding to develop corn lines for increased RS seems promising, particularly with the identification of parent lines high in amylose (greater than 50%). Although RS 2 is present in ungelatinized starches, starch is not usually eaten in this raw form, and much RS 2 may be lost upon cooking. Use of RS 3 as a food ingredient should provide more RS and dietary fiber retention after cooking. The development of high-amylose, high-RS corn types could provide new sources of high-fiber products useful to the food industry in its quest for healthful foods and food ingredients.

Literature Cited

Campbell, M.R., J. Jane, L. Pollak, M. Blanco, and A. O'Brien. 2007. Registration of Maize Germplasm Line GEMS-0067. *J. Plant Registrations*. 1: 60-61.

Campbell, M.R., P.J. White, and L.M. Pollak. 1994. Dosage effect at the *sugary-2* locus on maize starch structure and function. *Cereal Chem*. 71:464-468.

Champ, M. 1992. Determination of resistant starch in foods and food products: interlaboratory study. *Eur. J. Clin. Nutr*. 46: 51-62.

Cherrington, C.A., M. Hinton, G.R. Pearson, and I. Chopra. 1995. Short-chain organic acids at pH 5.0 kill *Esherichia coli* and *Salmonella spp* without causing membrane perturbation. *J. Appl. Bacteriol.* 70: 161-165.

Coudray; C., J. Bellanger, C. Castigila-Delavaud, C. Remesy, M. Vermo-Rel, J. Bellanger, and Y. Rayssiguier. 1997. Effects of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *Eur. J. Clin. Nutr.* 51: 275-280.

Englyst, H.N., and G.J. Hudson. The classification and measurement of dietary carbohydrates. 1996. *Food Chem*. 57: 15-21.

Hara, H., S. Haga, Y. Aoyama, and S. Kiriyama. 1999. Short-Chain Fatty Acids suppress cholesterol synthesis in rat liver and intestine. *J. Nutr*. 129: 942-948.

Inouchi, N., D.V. Glover, Y. Sugimoto, and H. Fuwa. 1984. Developmental changes in starch properties of several endosperm mutants of maize. *Starch*. 36:8.

Ji, Y., K. Seetharaman, K. Wong, L.M. Pollak, S. Duvick, J. Jane, and P.J. White. 2003. Thermal and structural properties of unusual starches from developmental corn lines. *Carbohydr. Polymers.* 51: 439-450.

Krieger, K.M., S.A. Duvick, L.M. Pollak, and P.J. White. 1997. Thermal properties of corn starch extracted with different blending methods: microblender and homogenizer. *Cereal Chem*. 74: 553-555.

McCleary, B.V., and Monaghan D.A. 2002. Measurement of alpha-amylase activity in white wheat flour, milled malt, and microbial enzyme preparations, using the cealpha assay: Collaborative study. *J. Amer. Org. Anal. Chem. Intl*. 85:665-675.

Shannon, J.C., and D.L. Garwood. 1984. *Genetics and physiology of starch development*. In Starch: Chemistry and Technology (2nd edition). Orlando, FL: Academic Press. p. 44-61.

Shu, X., L. Jia, J. Gao, Y. Song, H. Zhao, Y. Nakamura, and D. Wu. 2007. The influences of chain length of amylopectin on resistant starch in rice (*Oryza sativa* L.) *Starch*. 59: 504-509.

Takeda, Y., and J. Preiss. 1993. Structures of B90 (sugary) and W64A (normal) maize starches. *Carbohydr. Res*. 240: 265.

Tziotis, A., K. Seetharaman, J.D. Klucinec, P. Keeling, P.J. White. 2005. Functional properties of starch from normal and mutant corn genotypes. *Carohydr. Polymers* 61: 238-247.

Vahouny, G.V., S. Satchithanandam, I. Chen, S.A. Tepper, D. Kritchevsky, F.G. Lightfoot and M.M. Cassidy. 1988. Dietary fiber and intestinal adaptation: effects on lipid adsorption and lymphatic transport in the rat. *Am. J. Clin. Nutr*. 47: 201-206.

White, P.J., I. Abbas, L. Pollak, and L. Johnson. 1990. Intra- and interpopulation variability of thermal properties in maize starch. *Cereal Chem*. 67: 70.

White, P.J., I.R. Abbas, and L.A. Johnson. 1989. Freeze-thaw stability and refrigerated-storage retrogradation of starches. *Starch* 5: 176-180.

Yao, N., A.V. Paez, and P.J. White. 2009. Structure and function of starch and resistant starch from corn with different doses of mutant amylose-extender and floury-1 alleles. *J. Agri. Food Chem.* 57: 2040-2048.

Young, G.P., Y. Hu, R.K. Le Leu, and L. Nyskohus. 2005. Dietary fibre and colorectal cancer: a model for environment—gene interactions. *Mol. Nutr. Food Res*. 49: 571-584.

Zhang, G., Sofyan, M., and Hamaker, B.R. 2008. Slowly Digestible State of Starch: Mechanism of Slow Digestion Property of Gelatinized Maize Starch. *J. Agri. Food Chem.* 56: 4695-4702.

Table 1. Pedigree information and ID labels for 10 parent lines.

Pedigree	**ID Label**	**When/Where grown**	**Generation**
HSY99 *du su2*	*du su2*	Juana Diaz Puerto Rico, 2005-2006	Inbred line
HSY99 *ae su2*	*ae su2*	Juana Diaz Puerto Rico, 2005-2006	Inbred line
HSY99 *ae du*	*ae du*	Juana Diaz Puerto Rico, 2005-2006	Inbred line
GUAT209:S13//Oh43*ae*/H99*ae*-1-2-1	GUAT *ae*	Iowa Agronomy Farm 2006	S_3
AR01105:S01-1082	AR	Iowa Agronomy Farm, 2005	S_1
UR13061:S22-1092	URS	Iowa Agronomy Farm 2006	S_1
CU110:N17-1172	CUBA	Iowa Agronomy Farm, 2005	S_1
B/3/DK212T:S610-8-1-3-4-8-2	DK	Iowa Agronomy Farm, 2003	S_6
BR52051:S17-1112	BR	Iowa Agronomy Farm, 2005	S_1
UR13085:N214-14-1	URN	Iowa Agronomy Farm 2006	S_2

Table 2. Pedigree information and ID labels for 20 progeny crosses, grown in Juana Diaz Puerto Rico in 2006-2007, of the S2 generation.

Cross Pedigree[1]	**ID Label**
(HSY99 *du su2*/AR011050:S01-1082)-2-2	(*du su2*/AR)-2-2
(HSY99 *du su2*/CU110:N17-1122)-3-1	(*du su2*/CU)-3-1
(HSY99 *du su2*/CUBA110:N17-1122)-3-2	*(du su2*/CU)-3-2
(HSY99 *du su2*/AR011050:S01-1082)-2-1	*(du su2*/AR)-2-1
(HSY99 *du su2*/AR011050:S01-1082)-2-3	*(du su2*/AR)-2-3
(HSY99 *du su2*/CUBA110:N17-1122)-3-1	*(du su2*/AR)-3-1
(HSY99 *du su2*/CUBA110:N17-1122)-3-2	*(du su2*/AR)-3-2
(HSY99 *ae su2*/AR011050:S01-1082)-3-1	(*ae su2*/AR)-3-1
(HSY99 *ae su2*/UR13061:S22-1092)-2-1	(*ae su2*/URS)-2-1
(HSY99 *ae su2*/AR011050:S01-1082)-03)-02	(*ae su2*/AR-)3-2
(HSY99 *ae du*/AR011050:S01-1082)-1-1	(*ae du*/AR)-1-1
(HSY99 *ae du*/UR13085:N215-14-1)-2-2	(*ae du*/URN)-2-2
(HSY99 *ae du*/AR011050:S01-1082)-1-2	(*ae du*/AR)-1-2
(HSY99 *ae du*/UR13061:S22-1092)-3-2	(*ae du*/URS)-3-2
(GUAT209:S13/Oh43*ae*/H99*ae*/AR011050:S01-1082)-1-1	(GUAT *ae*/AR)-1-1
(GUAT209:S13/Oh43*ae*/H99*ae*/UR13061:S22-1092)-2-1	(GUAT *ae*/URS)-2-1
(GUAT209:S13//Oh43*ae*/H99*ae*/UR13061:S22-1092)-2-2	(GUAT *ae*/URS)-2-2
(GUAT209:S13//Oh43*ae*/H99*ae*/3/BR52051:S17-1112)-1-2	(GUAT *ae*/BR)-1-2
(GUAT209:S13//Oh43*ae*/H99*ae*/3/DK212T:S610-8-1-3-4-8-2)-1-1	(GUAT *ae*/DK)-1-1
(GUAT209:S13//Oh4 *ae*/H99*ae*/3/DK212T:S610-8-1-3-4-8-2)-1-2	(GUAT *ae*/DK)-1-2

[1]Numbers outside of pedigree parenthesis refer to ear number.

Table 3. The % RS as measured by the Megazyme Resistant Starch kit, a measurement of RS 2 for parents and 20 progeny crosses. Gelatinization and retrogradation characteristics as measured by a differential scanning calorimeter (DSC), scanning from 25 to 180° C. ID labels can be found in Table 1. Values are the average of three replications. Values having the same letter in the same column are not significantly different ($\alpha = 0.05$).

		Gelatinization			Retrogradation			
Parent ID	RS (%)	T_{oG} (°C)	T_{pG} (°C)	ΔH_G	T_{or} (°C)	T_{pr} (°C)	ΔH_r	% r
BR	18.3 c	70.6 a	74.6 a	15.3 ab	44.9 b	59.9 ab	9.0 a	.62 a
DK	18.8 c	67.6 ab	72.6 a	13.5 ab	47.7 ab	52.4 b	1.0 bc	.07 bc
CUBA	22.0 c	70.4 a	74.7 a	13.5 ab	49.7 ab	58.6 b	2.4 bc	.17 abc
URS	21.9 c	69.0 a	73.7 a	11.8 ab	53.2 ab	57.8 b	1.0 bc	.10 bc
du s2	23.9 c	63.8 bc	66.9 b	3.3 b	53.0 ab	73.5 a	1.9 bc	.56 a
ae su2	18.5 c	60.95 c	61.8 b	8.5 ab	54.2 ab	57.9 b	0.5 c	.05 c
ae du	30.6 b	69.0 a	73.3 a	6.4 ab	51.3 ab	57.2 b	5.9 ab	.53 ab
AR	18.7 c	69.6 a	72.7 a	10.1 ab	54.8 ab	60.0 ab	1.7 bc	.18 abc
GUAT *ae*	52.2 a	62.3 c	66.7 b	34.8 a	60.8 a	62.7 ab	3.2 bc	.30 abc
URN	19.1 c	71.3 a	73.7 a	11.1 ab	59.8 ab	63.2 ab	1.0 bc	.09 bc
Cross ID Label								

(*du su2*/AR)-3-1	19.5 defg	65.7 fgh	71.5 fgh	11.3 def	52.7 bcd	60.5 bcdefgh	2.1 bcd	.19 abcd
(*du su2*/AR)-3-2	20.6 cdefg	64.0 gh	68.3 ij	10.7 defg	53.0 bcd	58.9 defgh	1.7 cd	.15 bcd
(*du su2*/AR)-2-1	16.9 g	62.5 h	67.5 j	7.9 hij	54.2 bcd	59.4 cdefgh	3.2 bcd	.43 abcd
(*du su2*/AR)-2-2	16.6 g	63.9 gh	71.1 ghi	8.2 hij	55.9 abcd	57.7 efgh	1.7 cd	.21 abcd
(*du su2*/AR)-2-3	17.1 g	66.7 efg	71.8 efgh	7.2 ij	50.4 cd	56.4 gh	2.7 bcd	.28 abcd
(*du su2*/CU)-3-1	17.3 g	67.6 defg	72.6 defgh	9.4 efghi	48.8 d	57.5 fgh	3.0 bcd	.32 abcd
(*du su2*/CU)-3-2	16.9 g	64.6 gh	70.2 hij	12.3 cd	53.1 bcd	59.8 cdefgh	2.2 bcd	.19 abcd
(*ae su2*/AR)-3-1	17.9 fg	62.3 h	68.5 ij	8.8 ghij	51.3 bcd	58.3 efgh	1.4 d	.17 abc
(*ae su2*/AR)-3-2	18.2 fg	65.6 fgh	72.9 cdefgh	8.4 ghij	51.3 bcd	57.6 efgh	1.2 d	.14 cd
(*ae du*/AR)-1-1	20.9 cdefg	68.6 bcdef	73.2 bcdefg	9.7 efgh	59.3 abc	64.1 abcd	1.2 d	.13 cd
(*ae du*/AR)-1-2	20.6 cdefg	69.4 abcdef	73.8 abcdefg	6.5 j	56.9 abcd	61.0 bcdefg	2.8 bcd	.44 abcd
(*ae du*/URN)-2-2	22.6 bcde	71.9 ab	75.8 ab	8.0 hij	50.8 bcd	54.8 h	4.0 bcd	.50 abc
(*ae du*/URS)-3-2	22.4 bcdef	67.7 cdefg	71.9 efgh	9.3 fghi	50.8 bcd	56.5 gh	1.3 d	.06 d
(GUAT *ae*/AR)-1-1	34.0 a	66.8 efg	74.2 abcdef	12.4 cd	64.6 a	67.3 a	5.7 abc	.47 abc
(GUAT *ae*/AR)-1-2	25.6 b	71.1 abcd	74.3 abcde	8.3 hij	50.7 bcd	58.5 defgh	4.4 abcd	.47 abc
(GUAT *ae*/BR)-1-2	24.4 bc	70.5 abcde	75.0 abcd	13.7 bc	48.1 d	56.8 fgh	2.7 bcd	.20 abcd

(GUAT *ae*/URS)-2-1	23.7 bcd	73.2 a	76.4 a	12.7 cd	60.4 ab	63.3 abcde	4.3 abcd	.31 abcd
(GUAT *ae*/URS)-2-2	24.6 bc	71.6 abc	75.7 abc	16.0 ab	55.1 abcd	66.2 ab	8.4 a	.52 ab
(GUAT *ae*/DK)-1-1	33.2 a	70.2 abcde	73.0 cdefgh	17.5 a	59.5 abc	64.9 abc	6.1 ab	.34 abcd
(GUAT *ae*/DK)-1-2	31.4 a	69.0 bcedef	74.2 abcdef	11.7 cde	55.2 abcd	62.4 abcdef	5.9 ab	.40 abc

Figure 1. Relation of % RS in parents to % RS in progeny crosses ($r = 0.59$, $P \leq 0.05$). Each data point is the average of the % RS for the parents and the progeny crosses for each line.

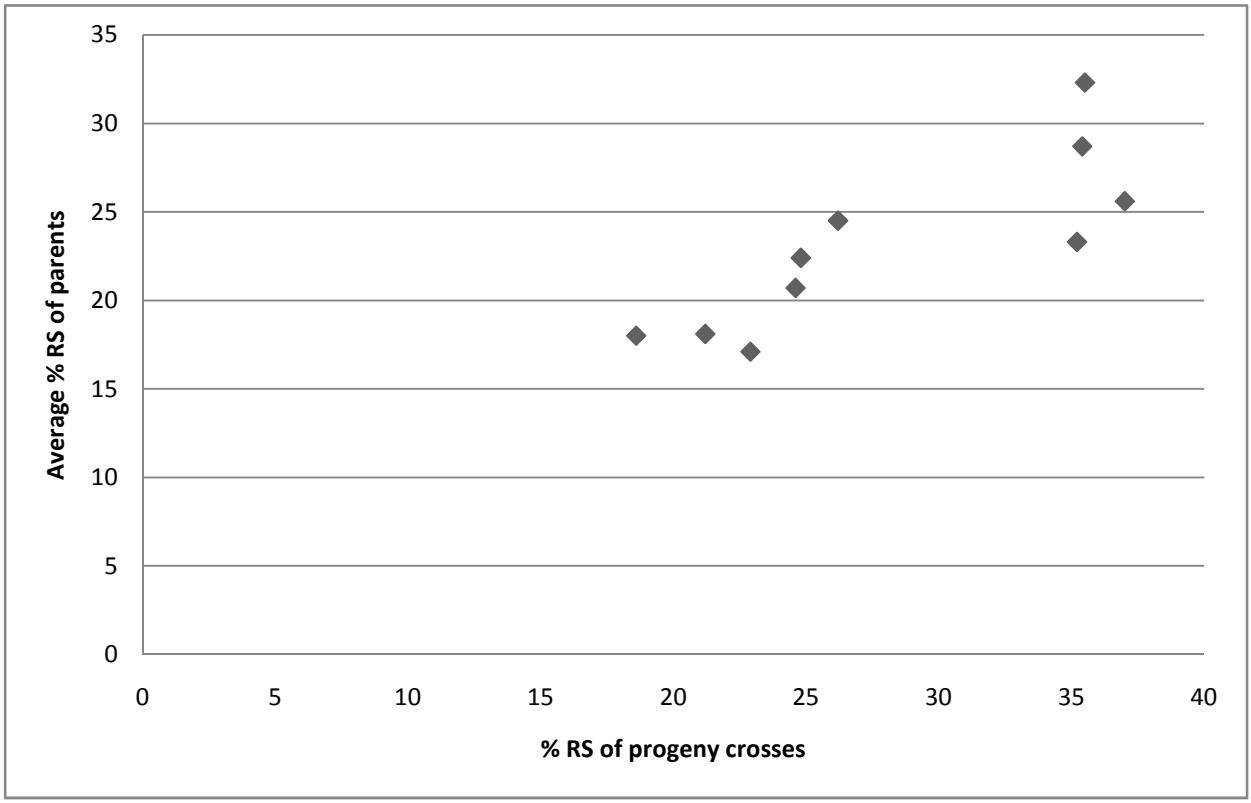

Fig. 1

RESISTANT STARCH EFFECTS ON TORTILLA TEXTURE

A paper to be submitted to *Cereal Chemistry*

Kim A. Rohlfing, Alix Paez, and Pamela J. White

Abstract

A high-amylose, non-*floury* corn type with 55.2% resistant starch (RS), a *floury* corn type with 1% RS, and a 1:1 blend with 28.2% RS were used to make traditional tortillas. Whole corn was nixtamalized and ground to make masa. The masa was evaluated for pasting properties on a Rapid-Visco Analyser. The high-amylose masa slurry gelatinized only slightly, as noted by a small change in peak viscosity during the 95° C heat treatment. The *floury* masa had the greatest peak viscosity, whereas the blend was intermediate in value. Tortillas were evaluated by an 11-member sensory panel who evaluated the textural attributes of grittiness, moistness, chewiness, rollability, and tearability. The *floury* tortillas were chewier, more rollable, and grittier than the high-amylose tortillas. The blend tortillas were intermediate in most parameters. The cutting force of the high-amylose tortillas, as measured by a texture analyzer, was very low, whereas the blend and *floury* tortillas required more force. Chewiness was correlated to rollability ($r = 0.99$, $P = 0.05$). The RS percentage was correlated to rollability ($r = 0.99$), and cutting force ($r = 0.99$). The *floury* and blend tortillas had a firm texture that would be expected when eating a tortilla with a filling. The high-amylose tortillas fell apart with very little force, and would not roll around a filling, making them unsuitable for this use. Although the high-amylose tortillas had increased dietary fiber

in the form of RS, it had very poor textural attributes. The blend tortillas retained enough of the textural properties of the *floury* tortilla to make it a suitable product.

Introduction

Corn tortillas, a staple food product in Latin America, are gaining popularity in the United States and Europe. Consumption of corn tortillas is very high in Mexico, Central and South America. Traditional tortillas are low in dietary fiber, with the actual amount varying depending on the corn variety and processing conditions. The fiber contribution results from the pericarp and tip cap materials remaining in the masa after processing. The diets of most individuals in the United States fall short of the recommendations for dietary fiber of 25 g/day for adult females, and 38g/day for adult males (American Dietetic Association, 2008). Development of a high-fiber tortilla would provide a more healthful food choice, not only because of the contribution of the fiber, but also because calories from available starch would be replaced with low-calorie resistant starch (RS, Behall and Howee, 1996). A high-fiber tortilla could be produced using masa from high-RS corn types, especially high-amylose varieties.

Four types of RS exist, categorized by the source of resistance. The first type of RS is made resistant by the surrounding food matrix; RS 2 is present in ungelatinized, raw starches; RS 3 is created by retrogradation; and RS 4 is chemically altered to be resistant (Englyst et al., 1996). High-amylose starches are high in RS 2 (Themeier et al., 2005). RS has many beneficial effects on digestion. Prebiotics, including RS, function as fermentable substrates for probiotics, which are beneficial gut bacteria. Fermentation of RS produces short-chain fatty acids (SCFA), which can increase calcium and magnesium absorption (Courdray et al.,

1997). SCFA lower the pH of the digestive mucosa which inhibits growth of pathogenic bacteria (Cherrington et al., 1991). Cholesterol absorption may be affected by RS, but the mechanism is not well understood: SCFA may suppress cholesterol synthesis in the liver (Hara et al, 1999), or decrease cholesterol absorption (Vahouny et al., 1988). Butyrate, a SCFA, helps the colonocyte regulate apoptosis, and reduces the risk of colon cancer (Young et al., 2005).

Inclusion of RS in the diet also helps lower the glycemic index (GI) of a given food product. Low GI foods promote satiety by release of cholecystokinin (Holt et al., 1992), keep blood glucose stable, and promote higher rates of fat oxidation (McMillian-Price and Brand-Miller, 2006). A typical corn tortilla made from *floury* corn has a GI of 87, compared to a white-bread standard of 94 (Tovar et al., 2003). High-RS tortillas would have a far lower GI. Eating foods high in RS and dietary fiber, along with a healthy, balanced diet, may decrease rates of obesity and obesity-related illness.

Tortillas are a simple food system of nixtamalized corn that is ground, and baked into a flat disc. Traditional methods of nixtamalization increase the digestibility of corn, and increase the calcium content by as much as 400% (Fernandez-Munoz et al., 2004). Traditional tortillas are made with *floury* corn, which has a soft endosperm that grinds easily, but the corn source can vary according to local preferences and availability. Starch from the *floury* endosperm, when evaluated as a slurry in a Rapid Visco Analyser, had a low gelatinization onset temperature of 60.8° C, a wide range of gelatinization 13.5° C, a high pasting onset temperature, and low values for peak viscosity, breakdown, and setback (Seetharaman et al., 2001). Flour from high-amylose corn types has increased amylose content (50% or higher),

and the corn starch has been successfully incorporated into many food systems, including breads, muffins, and spaghetti (Akerberg et al., 1998, Yamada et al., 2005, Sanz et al., 2008, and Sozer et al., 2006). The dietary fiber content of corn flour and masa, and thus a finished tortilla, might be increased by crossing a high-amylose corn mutant with a *floury*-type corn.

Texture is important to tortilla functionality. An ideal tortilla should withstand wrapping around a filling without cracking, a property known as rollability. Foods containing high-amylose starches can suffer from some of the textural problems associated with foods high in traditional dietary fiber: too dense, dry, and coarse (Sanz et al., 2007). Increasing the RS of tortillas could make them nutritionally beneficial for Americans, but also negatively affect the texture. The overall objective of this study was to examine the texture of tortillas made from corn containing different levels of RS, by both instrumental and human sensory analyses.

Materials and Methods

Corn Material

Two corn types from Genetic Enterprises International were used for this study, a *floury* variety with an Ecuadorian background (*floury*, *fl1fl1fl1*1) and a high-amylose variety (amylose-extender, *ae; aeaeae*). The high-amylose variety was obtained by selfing two *ae ae* hybrids of similar maturities. The *floury* variety was obtained by selfing two *fl fl* hybrids. All lines were grown in Sheldahl, Iowa in the summer of 2007. These lines were previously characterized by Yao et al. (2009).

Proximate Analysis

Each corn type was analyzed for protein, oil, and starch by using near infrared spectroscopy (NIR, Foss 1241 transmission scanner, Eden Prairie, MN), and the values estimated from predicted values (Fox et al., 1992). All values are reported on a dry-weight basis (dwb). The values for the 1:1 blend were mathematically calculated.

Resistant starch measurement

Masa was filtered through a 100-µL filter (N100C Cellmicrosieves^{TM}, Biodesign Inc., New York, NY). The starch was centrifuged at 1000 x g revolutions per min, for 10 min, the supernatant discarded, and the pellet dried overnight at 45° C. The extracted starch was then analyzed with the Megazyme RS Kit (K-RSTAR, Megazyme International, Bray, Ireland) to determine the RS content of the masa (after nixtamalization and grinding). The following modification was used: 50-µl of amyloglucosidase was added to 50-µl of diluted RS to ensure all RS was converted to glucose before addition of glucose oxidase/peroxidase reagent (GOPOD). All analyses were conducted twice, and the averages were computed.

Masa Preparation

The procedure for masa and tortilla preparation was modified from previous reported formulas, and tailored to the high-amylose corn type. Masa preparation was kept consistent among all three tortilla treatments, even though conditions may not have been optimum for all three, so the specific effects could be measured of the different corn types on the tortilla. One kilogram of each corn type was cooked at 100° C for 30 min in 1% lime solution (CaOH), and then allowed to steep at room temperature for 14 hr (adapted from Martinez-

Bustos et al., 2001 and Mendez-Montealvo et al., 2007). The steeping liquor was discarded, and the nixtamal was washed twice with agitation. The *floury* and high-amylose corns were then made into masa using the following procedure. The corn was ground in a Glenn Mill (model LV-15K, Glenn Mills, Ill.) with 400 mL water added to facilitate grinding. A third masa type, identified as 'blend', was made from a 1:1 blend of the *floury* and high-amylose masa types. Masa was prepared three times for each treatment, to provide replicate preparations for all additional tests.

RVA

Pasting properties of masa were measured by using a Rapid Visco-Analyser (RVA-4, Newport Scientific Pty. Ltd, of Warriewood, Australia). Slurries were prepared by combining 2.44 g of masa on a dry-weight basis (Flores-Farias et al., 2000). Data was collected and analyzed by using Thermocline software (V. 2.3, Newport Scientific). Using a standard pasting profile, STD1, an initial speed of 960 rpm was applied for the first 10 sec, followed by a test speed of 160 rpm for the duration of the program. The slurry was equilibrated at 50° C for 1 min, heated to 95° C for 4 min 42 sec, held at 95° C for 3 min 30 sec, then cooled to 50° C over 3 min (Yao et al., 2009). The RVA parameters measured included peak viscosity (cP), trough (cP), setback (cP), final viscosity (cP), and pasting temperature (°C).

Tortilla Preparation

Thirty-six grams of masa was pressed, and then cooked by using a Saachi tortilla press (SA-1650, amazon.com) for 30 sec, flipped, and cooked for an additional 30 sec. Tortillas were

then plated and allowed to cool for 30 min to room temperature. Results are the average of three replications.

Tortilla Cutting Force

Cutting force was measured by using a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY and Stable Micro Systems, Godalming, Surrey, UK). After cooking, tortillas were cooled to room temperature for 30 min. The cutting force was the peak force (g) obtained from a blade attachment which cut through the middle of a 10.5 cm tortilla approximately 2 mm in height. Each tortilla was measured once (Martinez-Bustos et al., 2001). The probe moved downward at a rate of 2 mms^{-1} to a penetration depth of 2 mm, until the tortilla was cut. Each treatment was reported as the average of three cut tortillas from each replicate, with three replicates being analyzed.

Sensory Evaluation

An 11-member sensory evaluation panel was trained in three, 30-min sessions during which they were presented with standards for textural tortilla attributes. The tortillas were evaluated on three separate days (replications), during which the panelists were presented with three treatments and a reference tortilla. Treatments consisted of tortillas made from *floury*, high-amylose, and blend masa. The reference was made by mixing equal parts of commercially sold, dry masa flour and water (Goya Masarica, Secaucus, NJ).

On a 15-cm line scale, panelists were asked to indicate grittiness, chewiness, moistness, rollability, and tearability: 0 was smooth not gritty, not chewy, not moist, and easily tearable, and 15 was gritty, chewy, moist, and difficult to tear. Tearability was measured by the

amount of force required to tear a 10.5 cm tortilla down the middle, with 0 being very little force, and 15 being a lot of force (Herrera-Corredor et al., 2007). Panelists also judged rollability. This attribute was a measure the amount of cracking observed when a tortilla was rolled around a 3/4" dowel and held for 30 sec (Flores-Farias et al., 2000). Rollability was rated on a scale of 1 to 5, with 1 as cracking over the entire surface, and 5 as no cracking.

Panelists were trained for each of these attributes as noted: grittiness: plain cornmeal and tortillas made with added cornmeal; chewy: purchased crisp-style chocolate-chip cookie and chewy chocolate-chip cookie. The standards for rollability and tearability were reference tortillas cooked for two different periods of time. An overcooked tortilla cooked for two minutes was used as the unrollable and very difficult to tear standard. A slightly undercooked tortilla (45 sec) was used for the easy to roll and easy to tear standard.

During actual testing, panelists were given two tortillas of each treatment during each session. The first tortilla was used to judge the oral attributes, whereas the second tortilla was used for rolling and tearing. Each testing session was considered a replicate; values from the three replicates were averaged and reported.

Statistical Analyses

Results were analyzed by using proc ANOVA with three replicates ($\alpha = 0.05$) from Statistical Analysis Systems (SAS Institute, Cary, NC, 2003). Correlations were done between RS, sensory data, and instrumental data by using Excel regression, $P \leq 0.05$ (Microsoft Office 2007, Seattle, WA).

Results and Discussion

Proximate Analysis and RS

The protein and starch concentrations of the high-amylose, whole corn were greater (13.2 and 68.4%, respectively) and the oil concentration less (4.7%) than the *floury*, whole corn (10.7, 66.5%, and 4.1%, respectively; Table 1). The values for the blend treatment were mathematically calculated and, thus, were intermediate in all values.

The extracted *floury* starch had the least percentage of RS among the three treatments (1%, Table 1), followed by the blend treatment (calculated at 28.2%), and the high-amylose starch (55.2%). Creation of masa involved an initial heating step, steeping, and grinding which increased digestibility in corn types low in RS (Table 1). When gelatinized at 95° C the extracted *floury* corn starch increased in RS to 2.2%, and the extracted high-amylose starch decreased to 28.2%. This decrease in high-amylose RS suggests that the RS is type-2 RS, which is known to be high in native high-amylose starches, and to decrease upon gelatinization (Berry, 1986).

RVA

The RVA parameters of breakdown, peak time, and peak temperature did not differ among the three masa slurry treatments (Table 2). The starch in the high-amylose masa slurry did not fully thicken and gelatinize, thus resulting in the lowest final viscosity (98.7 cP). The blend was intermediate in value, and the *floury* masa slurry had a much greater final viscosity of 439 cP. The three treatments showed similar trends in the order of values for the peak viscosity, trough, final viscosity, and setback *floury* < blend < high-amylose. The starch in

the high-amylose masa slurry showed minimal pasting when analyzed by the RVA, which previously was demonstrated in a high-amylose starch (Tziotis et al., 2005).

TA.XT2

The *floury* tortillas had the greatest cutting force (1633.7 g^{-1}), followed by the blend tortillas, and the high-amylose tortillas (Table 2). The high-amylose tortillas cut with very little force, and actually fell apart during handling. The *floury* tortillas did not cut cleanly through, perhaps because they seemed denser than the other tortillas.

Sensory Evaluation

The panel judged the *floury* and blend tortillas to be grittier, chewier and more moist than the high-amylose tortillas (Table 3). The three treatments were different from each other for the attribute of rollability. The high-amylose tortillas were judged to be unrollable, with a value of 1 (cracking over the entire surface), the blend was rated 4 (a small amount of cracking) and the *floury* was rated 5 (almost no cracking, Table 3). All three treatments were different from each other for the attribute of tearability. The *floury* tortillas were the most resistant to tearing, the blend was intermediate, and the high-amylose tortillas the least resistant to tearing (Table 3). Rollability and tearability also were positively correlated to cutting force ($r=0.99$, $P \leq 0.05$, Table 5). Previous sensory evaluation of tortillas reported that consumers' overall liking was based on the three textural attributes of rollability, chewiness, and tearability (Herrera-Corredor et al., 2007).

Very likely, the high-amylose corn had less water uptake during steeping, resulting in the low value for moistness. Less water uptake could mean there were fewer overall changes

resulting from nixtamalization in the high-amylose masa because water is the vehicle for the calcium ions that create the necessary changes in corn texture. Nixtamalization results in hydration of the endosperm which leads to easier gelatinization upon cooking. Less hydration of the high-amylose corn would result in less gelatinization and a less cohesive structure, which seemed to be the case in the current study. The authors observed that the high-amylose tortillas had a greater degree of spread because of this less cohesive dough and the tortillas fell apart very easily during normal handling. The nixtamalization did not affect the high-amylose endosperm to the degree that it affected the *floury* endosperm as evidenced by their very different texture and handling ability.

The blend tortilla was not different from the *floury* type for chewiness and moisture, indicating that making tortillas from corn with RS up to 28.2% can result in a functional tortilla. Previously, during traditional masa preparations, a low percentage of granules either gelatinized fully or in part during normal cooking; indeed, a full 76% of starch granules were not physically altered (Ratnayake et al., 2007). However, greater amounts of gelatinization occurred with increased lime concentrations during nixtamalization (Bryant and Hamaker, 1997).

Conclusions

Tortillas made from high-amylose corn were judged by a sensory panel to be less chewy, less moist, less rollable, and to require less tearing force than tortillas made from a *floury*-type corn and tortillas made from a 1:1 blend of high-amylose and *floury* corn types. The *floury* tortillas required the most tearing force when evaluated on a TA.XT2, followed by the blend

tortillas, and the high-amylose tortillas. The high-amylose tortillas would be less desirable to the consumer because they would not easily roll around a filling. The 1:1 blend tortillas were similar to the *floury* tortilla in rollability and tearability, suggesting it is possible to create high-fiber corn tortillas made from corn with RS of up to 28.2% while retaining the rollability of a *floury* tortilla. Sensory evaluation of tortillas with RS levels between 28.2 and 55.2% should be conducted to find the maximum level of RS incorporation that retains the desired textural properties.

Literature Cited

Akerberg, A., H. Liljeberg, and I. Bjorck. 1998. Effects of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. *J. Cereal Sci*. 28: 71-80.

American Dietetic Association. 2008. Position of the American Dietetic Association: Health Implications of Dietary Fiber. *J. Am. Diet. Assoc.* 108: 1716-1731.

Behall, K.M. and, J.C. Howee. 1996. Resistant starch as energy. *J. Amer. Co. Nutr.* 15(3):248-54.

Berry, C.S. 1986. Resistant starch: Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *J. Cereal Sci.* 4: 301-314.

Bryant, C.M., and B.R. Hamaker. 1997. Effect of lime on gelatinization of corn flour and starch. *Cereal Chem.* 74 (2): 171-175.

Cherrington, C.A., M. Hinton, G.R. Pearson, and I. Chopra. 1995. Short-chain organic acids at pH 5.0 kill *Esherichia coli* and *Salmonella spp* without causing membrane perturbation. *J. Appl. Bacteriol.* 70: 161-165.

Coudray, C., J. Bellanger, C. Castigila-Delavaud, C. Remesy, M. Vermo-Rel, J. Bellanger, and Y. Rayssiguier. 1997. Effects of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *Eur. J. Clin. Nutr.* 51: 275-280.

Englyst, H.N., and G.J. Hudson. The classification and measurement of dietary carbohydrates. 1996. *Food Chem*. 57: 15-21.

Fernandez-Munoz, J.L., I. Rojas-Molina, M.L. Gonzalez-Davalos, M. Leal, M.E. Valtierra, E. San Martin-Martinez, and M.E. Rodriguez. 2004. Study of calcium ion diffusion in components of maize kernels during traditional nixtamalization process. *Cereal Chem*. 81 (1): 65-69.

Flores-Farias, R., F. Martinez-Bustos, Y. Salinas-Moreno, Y.K. Chang, J.G. Hernandez, and E. Rois. 2000. Physicochemical and rheological characteristics of commercial nixtamalized Mexican maize flours for tortillas. *J. Sci. Food Agric*. 80: 657-664.

Fox, S.R., L.A. Johnson, C.R. Hurburgh, Jr., C. Dorsey-Redding, and T.B. Bailey. 1992. Relations of grain proximate composition and physical properties to wet-milling characteristics of maize. *Cereal Chem*. 69: 191-197.

Hara, H., S. Haga, Y. Aoyama, and S. Kiriyama. 1999. Short-Chain Fatty Acids suppress cholesterol synthesis in rat liver and intestine. *J. Nutr*. 129: 942-948.

Herrera-Corredor, J.A., J.E.P. Saidu, A. Khachatryan, W. Prinyawiwatkul, A. Carballo-Carballo, and R. Zepeda-Bautista. 2007. Identifying Drivers for consumer acceptance and purchase intent of corn tortilla. *J. Food Sci*. 9: S727-731.

Holt, S.H., J. Brand, C. Soveny, and J. Hansky. 1992. Relationship of satiety to postprandial glycemic, insulin and cholecystokinin responses. *Appetite*. 18: 129-141.

Martinez-Bustos, F., H.E. Martinez-Flores, E. Sanmartin-Martinez, F. Sanchez-Sinencio, Y.K. Chang, D. Barrera-Arellano, and E. Rios. 2001. Effect of the components of maize on the quality of masa and tortillas during the traditional nixtamalisation process. *J. Sci. Food Agric.* 81: 1455-1462.

McMillian-Price J., and J. Brand-Miller. 2006. Low-glycaemic index diets and body weight regulation. *Intl. J. Obesity*. 30: S40-S46.

Mendez-Montealvo, G., J.L. Trejo-Espino, O. Paredes-Lopez, and L.A. Bello-Perez. 2007. Physicochemical and morphological characteristics of nixtamalized maize starch. *Starch*. 59: 277-283.

Ratnayake, W.S., A.B. Wassinger, and D.S. Jackson. 2007. Extraction and characterization of starch from alkaline cooked corn masa. *Cereal Chem*. 84(4): 415-422.

Sanz, T. A. Salvador, and S.M. Fiszman. 2008. Evaluation of four types of resistant starch in muffin baking performance and relationship with batter rheology. *Eur. Food Res. Technol*. 227: 813-819.

Seetharaman, K., A. Tziotis, F. Borras, P.J. White, M. Ferrer, and J. Robutti. 2001. Thermal and functional characterization of starch from Argentinean corn. *Cereal Chem*. 78: 379-386.

Sozer, N., A.C. Dalgic, A. Kaya. 2006. Thermal, textural and cooking properties of spaghetti enriched with resistant starch. *J. Food Engr*. 81: 476-484.

Themeier, H., J. Hollmann, U. Neese, and M.G. Lindhauer. 2005. Structural and morphological factors influencing the quantification of resistant starch II in starches of different botanical origin. *Carbohydr. Polymers*. 61: 72-79.

Tovar, J., S.G. Sayago-Ayerdi, C. Penalver, O. Paredes-Lopez, and L.A. Bello-Perez. 2003. In vitro starch hydrolysis index and predicted glycemic index of corn tortilla, black beans (*Phaseolus vulgaris* L.), and Mexican "taco". *Cereal Chem*. 80: 533-535.

Tziotis, A., K. Seetharaman, J.D. Klucinec, P. Keeling, P.J. White. 2005. Functional properties of starch from normal and mutant corn genotypes. *Carbohydr. Polymers*. 61: 238-247.

Vahouny, G.V., S. Satchithanandam, I. Chen, S.A. Tepper, D. Kritchevsky, F.G. Lightfoot and M.M. Cassidy. 1988. Dietary fiber and intestinal adaptation: effects on lipid adsorption and lymphatic transport in the rat. *Am. J. Clin. Nutr*. 47: 201-206.

Yamada, Y., S. Hosoya, S. Nishimura, T. Tanaka, Y. Kajimoto, A. Nishimura, and O. Kajimoto. 2005. Effect of bread containing resistant starch on postprandial blood glucose levels in humans. *Biosci. Biotechnol. Biochem*. 69: 599-566.

Yao, N., A.V. Paez, and P.J. White. 2009. Structure and function of starch and resistant starch from corn with different doses of mutant amylose-extender and floury-1 alleles. *J. Agri. Food Chem.* 57: 2040-2048.

Young, G.P., Y. Hu, R.K. Le Leu, and L. Nyskohus. 2005. Dietary fibre and colorectal cancer: a model for environment—gene interactions. *Mol. Nutr. Food Res*. 49: 571-584.

Table 1. Proximate analysis of whole corn using a Foss 1241 transmission scanner and resistant starch (RS) percentage in whole corn, and in starch gelatinized at 95° C measured by the Megazyme Resistant Starch kit (K-RSTAR, Megazyme International, Bray, Ireland).

Corn Type	Protein (%)[1]	Oil (%)[2]	Starch (%)[3]	Extracted Starch RS (%)[4]	Gelatinized Extracted Starch RS (%)[5]
Floury	10.7 c	4.7 a	66.5 c	1.1	2.2
Blend[6]	11.9 b	4.4 b	67.4 b	28.2	12.0
High-amylose	13.2 a	4.1 c	68.4 a	55.2	21.7

[1, 2, 3,] Values reported on a dry weight basis (0% moisture).

[4]Values for *floury* and high-amylose previously reported by Yao et al. (2009).

[5]Values for *floury* and high-amylose previously reported by Yao et al. (2009), starch was gelatinized on a Rapid Visco-Analyser (RVA).

[6]Blend is a 1:1 blend of *floury* and high-amylose corn flours, all values are predicted.

Table 2. Tortilla cutting force measured on a TA.XT2 texture analyzer, and masa pasting properties measured on a Rapid Visco-Analyser (RVA).

Tortilla Type	**TA.XT2**	**RVA**						
	Cutting Force (g)[1]	Peak Viscosity (cP)	Trough (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)	Peak Temperature (°C)
Floury	1633.7 a	439 a	391.6 a	47.8	565 a	173.4 a	6.5	84.6
Blend	1211.3 ab	222.7 b	189.1 b	34.1	275.9 b	86.8 ab	5.8	82.8
High-amylose	804.6 b	98.7 b	70.2 b	69.8	100.7 b	34.6 b	6.2	78.6

[1]Results with the same letter in the same column are not significantly different, α=0.05. Cutting force measured as peak force. Values reported are the average of three replications, in columns with no letters are not significantly different, α=0.05.

Table 3. Sensory panel scores (n = 11) for textural attributes of tortillas made from three treatments varying in resistant starch (RS).

Mutant Type	Grittiness[2]	Chewiness[2]	Moistness[2]	Tearability[2]	Rollability[2]
Floury	11.0 a	11.7 b	10.3 b	5.4 a	4.8 a
Blend[3]	9.7 a	11.3 b	11.6 b	3.3 b	4.2 b
High-amylose	5.7 b	9.0 a	7.0 a	1.3 c	1.3 c

[1]Results with the same letter in the same column are not significantly different. Values reported are the averages of three replications, and analyzed by ANOVA, $\alpha = 0.05$.

[2]On a 15-cm line scale, panelists were asked to indicate grittiness, chewiness, moistness, and tearability: 0 was smooth not gritty, not chewy, not moist, and easily tearable, and 15 was gritty, chewy, moist, and difficult to tear. Tearability was measured by the amount of force required to tear a 10.5 cm tortilla down the middle, with 0 being very little force, and 15 being a lot of force (Herrera-Corredor et al., 2007). Rollability was an objective measure noting the amount of cracking observed when a tortilla was rolled around a 3/4" dowel and held for 30 sec (Flores-Farias et al., 2000). Rollability was rated on a scale of 1 to 5, with 1 as cracking over the entire surface, and 5 as no cracking.

[3]Blend is a 1:1 blend of *floury* and high-amylose masa.

GENERAL SUMMARY

The health benefits of resistant starch (RS) consumption, including production of short-chain fatty acids and a decrease in cholesterol absorption, make RS an attractive option for addition of dietary fiber by food companies. Increasing the RS of a starch also decreases its calories making it ideal for healthful, low-calorie foods. Creation of high-RS foods will be beneficial to overweight and obese individuals looking for foods that help with weight loss and maintenance. The health of the digestive tract can be assisted by a diet containing prebiotics and probiotics, which together help prevent colon cancer. Fermentation of RS leads to short-chain fatty acid production by the colonocyte which helps these cells regulate apoptosis.

Some corn mutants have been characterized for amylose content and thermal characteristics, but not RS content. Commercial corn starch is very low in RS, but corn mutants differ in % RS. It is possible to increase the RS content of corn starch through traditional plant breeding methods. Data from the current study suggests great potential for increased RS with the Guat *ae* corn type. Overall, the % RS values ranged from 16.6% to 34.0% for the four mutant groups (*du su2*, *ae su2*, *ae du*, and Guat *ae*). The possibility of a wider variety of RS is likely when more double mutants are examined.

Thermal characteristics of the starches were measured on a differential scanning calorimeter. The strongest correlations were found between % RS and T_{pG} ($r = 0.65$, $P \leq 0.05$) and ΔH_G ($r = 0.67$, $R \leq 0.05$). These mutants did not have high ΔH_r values compared to the original ΔH_G values, showing that RS 3, the type of resistant starch formed during retrogradation, was not formed in large amounts. The % r was highly variable, ranging from 6 to 52%, and differences did not depend on mutant groups.

Floury corn is ideal for nixtamalization and tortilla production. *Floury* corn is also low in RS (1%). While high-amylose corn is greater in % RS (55.2), it was unknown if it would make a functional tortilla. A mid-level RS blend (28.2%) was made from a combination of the *floury* and high-amylose corn types, to provide three treatments for evaluation in tortillas. The high-amylose tortillas were not functional as rated by a sensory panel. They fell apart too easily, and would not roll without cracking. The % RS was correlated to the rollability ($r = 0.99$, $P \leq 0.05$) and cutting force ($r = 0.99$, $P \leq 0.05$). The *floury* and blend tortilla treatments were able to roll around a filling and not fall apart when handled making them functional.

The high-amylose tortilla's poor handling could relate to the pasting properties as evaluated by an RVA. The high-amylose masa slurry did not thicken, and had a low final viscosity (98.7 cP) because it was not gelatinized at the RVA high temperature of 95^{o} C. Without complete swelling, pasting, and gelatinization the high-amylose tortilla had a poor structure and fell apart under normal handling. The high-amylose tortillas were very easy to cut by the texture analyzer, with 804.6 g of force, compared to the floury tortillas which had a cutting force of 1633.7 g. The blend tortilla was intermediate in value but not different from the other two treatments in cutting force. The blend masa was not significantly different from the high-amylose masa for all RVA properties.

The presence of RS can have positive effects on health, but negative effects on food properties, such as texture. The functionality of RS at different levels in different foods needs further examination to pinpoint substitution levels that can be used and have minimal effects on texture. Overall, the current study showed that the blend of *floury* and high-

amylose masas that provided a % RS of 28.2% in the tortilla did not negatively impact the textural attributes of rollability, tearability and cutting force.

Recommendations for Future Research

There are many more mutant and exotic corn lines yet to be characterized for % RS and thermal characteristics. Further plant breeding can be used to create corn lines that meet specific % RS targets. It is also helpful to examine specific high-RS starches in food systems. Ideal high-RS starches will not have adverse effects on texture such as dryness, coarseness, or denseness. Other corn mutants could also be evaluated under nixtamalization conditions to find which mutants produce the best tortillas.

APPENDIX A. SENSORY SCORE SHEET

Panelist Number_____ Date_____

Please evaluate each sample for the attributes listed below. Completely evaluate one food sample before moving to the next one. Please take a drink of water between samples. Place a mark perpendicular to the line indicating its intensity for the attribute being evaluated. Label each mark with the appropriate 3-digit code.

**for the first three attributes please tear the tortilla down the middle and eat from the center of the tortilla.

Texture—place sample in mouth and eat normally. Do you sense any grit or large particles?

Very gritty Smooth/not gritty

Chewiness

Very chewy Not chewy

Moisture

Very moist Not moist

**SWITCH TORTILLAS

Rollability—place dowel near edge of tortilla. Roll dowel and tortilla to opposite edge and hold for 30 sec. Rate amount of cracking, tearing, and tortilla destruction. 1=a lot of tearing and cracking, 5= no cracking and tearing. Circle your answer below.

1 2 3 4 5

Tearability—tear tortilla down the middle, rate ease of tearing

---

Very **easy** to tear Very **hard** to tear

ACKNOWLEDGMENTS

I would like to thank my major professor Dr. Pamela J. White, program of study committee (Dr. Linda Pollak and Dr. Cheryl Reitmeier), family, and friends for all their support and help during the last 3 years and especially during the writing of this thesis. My lab group, including Jose Gerde, Ni Yao, Krisha Miller, Valerie Rosberg, Yan Jun Liu, and Hyun Jung Kim, deserve a special mention for all of their help, suggestions, and knowledge of statistics! The members of my sensory panel provided great input, and their participation was greatly appreciated. Brenda Emery and Breanna Wetzler, along with the rest of the administrative staff of the FS HN department who were always there, even at the last minute! Thanks to all.