PROCESSING AND GENETIC EFFECTS ON RESISTANT STARCH IN CORN FLAKES

BY

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THESIS

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ABSTRACT

A laboratory scale corn flaking procedure was developed at a batch size of 100 g grits to evaluate the effects of processing and genetics on resistant starch content in corn flakes. Cooking (15 psig/121°C, 50 min), drying (100°C, 30 min), tempering (room temperature, 30 min) and toasting (200°C, 60 s) resulted in flakes produced in laboratory that had similar color parameters (L, a and b values) but different RVA parameters (lower peak, trough and breakdown viscosities; higher final and setback viscosities) compared to commercial corn flakes. Seven corn hybrids were flaked with the developed procedure and resistant starch contents were determined at each processing stage for each hybrid. Cooking caused the largest decrease in resistant starch content and it remained at a similar level through subsequent processing stages of drying, flaking and toasting. Differences were observed in resistant starch content among hybrids at each processing stage. These results indicate that both genetic background and processing have an impact on the resistant starch content in corn. Hybrids with high intrinsic resistant starch content in raw flaking grits resulted in high levels of resistant starch in the final toasted flakes. Since resistant starch content is highly correlated with amylose content in corn, high amylose corn hybrids could be of potential use in the breakfast cereal industry for manufacture of corn flakes with higher nutritional quality, provided these hybrids have high grain yield and flaking grit yield for economic feasibility to corn producers and dry millers as well.

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TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

In the US, corn is a major source of carbohydrates and calories in the human diet through breakfast cereals, snacks and sweeteners. During dry milling from whole kernels to grits, grain components with high concentrations of nutrients, such as seed embryo and aleurone layer, are removed. With further use of high temperature, shear and moisture gradients during processing from flaking grits to flakes, changes in nutritional value might occur. US consumers are exposed to corn based foods and food ingredients daily, with concerns about their impact on health related issues, such as obesity and cardiovascular disease. Thus, it is important to monitor nutrient composition changes along the processing pipeline and in the final product.

Genetic background influences grain yield, corn dry milling efficiency and large grit yields, corn ethanol yields and wet milling fraction yields (Wang et al., 2011; Brekke et al., 1971; Rausch et al., 2009; Sharma et al., 2006; Zehr and Eckhoff, 1996). Improving grain yield and grain processing quality are important breeding objectives and of key importance to corn growers, dry millers and processors. However, nutritional needs and health of consumers often are neglected. We aim to provide insight into changes in nutrient value during processing and

link these effects to the genetic makeup of corn grain. This work focuses on resistant starch, which is a component of total dietary fiber, while nutrients such as bound and soluble phenolics, which have antioxidant properties, and tocopherols, which have vitamin E activity, are being investigated by collaborating groups. We hypothesize that:

1. Cooking would decrease RS content.
2. Toasting would increase RS content.
3. Corn hybrids would differ in their RS content in flaking grits.
4. There would be interaction between processing and hybrid effects.

Specific research objectives were to:

1. Develop a laboratory scale corn flaking procedure, evaluate its reproducibility and characterize the product.
2. Analyze processing and genetic effects on resistant starch in corn flakes prepared with the developed corn flaking procedure.

CHAPTER 2

LITERATURE REVIEW

2.1. Industrial Processing of Corn Flakes

Ready-to-eat (RTE) breakfast cereals originated in the United States in the late 19th century. Initially developed and used as healthy vegetarian foods in a clinical context, they soon caught on with the general population and an entire industry was thereby spawned (Fast, 1999). Fast and Caldwell (2000) is the only resource that gives an exhaustive description of the various existing manufacturing technologies of RTE breakfast cereals. The traditional corn flaking process, that uses corn flaking grits as raw material, is briefly described below. Flaking grits are the large endosperm fraction of the dry milling process. Dry milling of regular dent corn uses tempering and degermination processes to separate endosperm, pericarp and germ fractions (Rausch et al., 2009). The first step in converting raw flaking grits into corn flakes is to mix them with a flavor solution containing sugar, salt, malt syrup and other ingredients in water. A typical formula consists of corn grits (45.4 kg), granulated sugar (2.7 kg), malt syrup (0.9 kg), salt (0.9 kg) and sufficient water to yield cooked grits with a moisture content of not more than 32% after allowing for steam condensate (Fast and Caldwell, 2000).

2.1.1. Mixing and cooking

Raw flaking corn grits and flavor solution are placed in rotary type batch cookers that are built to withstand direct steam injection under pressure. The grits and flavor solution may be loaded simultaneously, or the grits may be added first and presteamed, followed by flavor addition and mixing. The mass of grits and flavor is cooked at 103.5 to 138 kPa for 2 hr. The rotational speed of these batch cookers is 1 to 4 rpm, with a higher rate during initial mixing, for thorough flavor distribution, and a lower rate during presteaming and cooking. Speeds too high can lead to attrition of grits, causing mushiness in the cooked product. Speeds too low can lead to uneven cooking within a batch (Fast and Caldwell, 2000).

Cooking is complete when each grit particle has been changed from hard, chalky white to light, golden brown and is soft and translucent. A batch is undercooked if large numbers of grits have chalky white centers, which can be verified by cutting the cooked grit in two with a knife. It is overcooked if the grits are excessively soft, mushy and sticky. Properly cooked grits are rubbery but firm and resilient under finger pressure; they contain no ungelatinized starch. Uncooked starch will appear as white spots in the finished flakes (Fast and Caldwell, 2000).

2.1.2. Dumping and delumping

When cooking is complete, steam supply is turned off, steam in the cooker is vented and

cooker contents are deposited on wide perforated plates with air blowing upwards. When the grits are cool to the touch, they are passed through delumping equipment for deagglomeration of lumps to obtain single grit pieces. Delumping is essential to obtain grits small enough for uniform circulation of heated air and hence uniform drying (Fast and Caldwell, 2000).

2.1.3. Drying and tempering

Drying of corn grits is best done at temperatures below 121°C using air with controlled humidity. Tempering is holding the grits in large bins under ambient conditions. This allows moisture to equilibrate in the grits, which is important for uniform blister formation on finished flakes during toasting. The desired moisture content at the end of drying is 10 to 14% (Fast and Caldwell, 2000).

2.1.4. Flaking

After tempering, grits are rolled or flattened into thin flakes by passing between pairs of metal rolls running at differential speeds. The gap between rolls can be adjusted to change flake thickness. For flaking of corn grits, roll surface temperature is 45°C. Temperatures over 50°C cause excessive roll wear and product sticking to the roll surface. Colder roll surfaces lack the grabbing ability needed to draw grits into the rolls. Roll knives are used to scrape flakes from the rolls (Fast and Caldwell, 2000).

2.1.5. Toasting

Flakes are toasted by suspending in a hot air stream rather than laying on a flat baking surface. The classic toasting oven is a rotating perforated drum. Drum rotation speed and perforation size must be appropriate to keep flakes suspended, maintain uniform airflow and avoid burning of flakes. Oven temperatures of 275 to 330°C with a residence time of 90 s are employed to obtain properly toasted flakes, which have the desired golden brown color and moisture content of 1.5 to 3% (Fast and Caldwell, 2000).

2.2. Laboratory Processing of Corn and Oat Flakes

Small scale procedures have been developed to produce corn and oat flakes in laboratory for evaluation of their physical properties. These are described below.

2.2.1. Small scale corn flaking

Using the formulation given by Fast and Caldwell (1990), corn flakes were produced at laboratory scale by Chaunier et al (2007) from 100 g samples of endosperm particles larger than 4 mm. The sample was cooked for 50 min and cooked particles were flattened into 0.5 mm thick petals between two parallel metal cylinders. The flaking setup was similar to that used by Levine et al., 2004. Cooking and flaking were followed by toasting at 215°C in three stages of 90 s each. Finally, 90 g corn flakes (moisture content of 8% to 9% w.b.) were obtained. Transformation or

degree of cook of starch in final toasted flakes was determined by differential scanning colorimetry (DSC) and polarized light microscopy. No gelatinization endotherms were detected and no polarization crosses were observed, indicating complete melting and loss of semicrystalline structure of starch granules during processing. The reason the same procedure was not used was because of unavailability of similar equipment, but parameters such as cook time (50 min) and toasting temperature (~200°C) were kept the same.

2.2.2. Importance of optimizing toasting temperature/time profile

Researchers have observed that the degree of cereal expansion is related to raw material compositional characteristics (Chen and Yeh, 2001; Jones et al., 2000) and processing conditions (Chinnaswamy and Bhattacharya, 1983; Chandrasekhar and Chattopadhyay, 1990; Owusu et al., 1984). Uniform soft crisp texture and bright yellow color are desired attributes of toasted flakes. Though the toasting operation is conducted at low moisture content (<10%), the use of high temperature for a short duration releases steam resulting in an expanded structure. Bhattacharya and Sumithra (2008) investigated development of crisp texture and characteristic flavor during toasting of raw corn flakes, at various moisture contents. Various toasting temperature/time profiles were tested for important product quality attributes such as puffed thickness, bulk density, color and overall sensory acceptability, employing response surface methodology.

Toasting resulted in an expanded porous microstructure, leading to lower bulk density and increased product crispness. Corn flakes toasted with the optimized profile of 250°C for 40 s resulted in a product with puffed thickness of 2.4 mm, moisture content of 8.8% to 11.2% and the desired color attributes that lead to an overall acceptability of 8.9 on a scale of 1 to 10.

2.2.3. Small scale oat flaking

A benchtop motorized oat flaking machine, along with a small scale steaming and flaking procedure, was developed to make small quantities (50 g) of oat flakes representative of those made by commercial processes. Laboratory flakes made from whole groats had similar quality parameters, such as oat flake thickness, peak viscosity, gelatinization enthalpy and texture properties such as adhesiveness and stringiness, but different water absorption capacity compared to industry processed flakes. This small scale flaking method was developed by Ames and Rhymer (2003) to evaluate the impact of processing on flake functional properties such as rolled oat thickness, pasting and thermal properties, water binding capacity and oatmeal texture properties. These properties are also influenced by cultivar and growing environment (Lapveteläinen et al., 2001; Rhymer, 2002) thus making it important to develop oat cultivars with specific properties that would help millers and food manufacturers create end products to meet consumer preferences.

2.3. Starch and Resistant Starch

Starch is a major source of carbohydrates in the human diet and is used for many food and nonfood/industrial applications. Both native and chemically and/or physically modified starches are used as bulking agents, thickeners or stabilizers due to their texturizing ability (Ratnayake and Jackson, 2009). Starch granule size, shape and structure depend on botanical source, species, cultivar and genotype environment interactions (Parker and Ring, 2001; Ratnayake and Jackson, 2003; Trubell 1944). Starch is made of two major polymers, amylose (linear polymer) and amylopectin (branched polymer), which differ in the arrangement of glucose molecules. The polymer composition of starch (i.e. ratio of amylose and amylopectin) affects its physical and biological properties (Parker and Ring, 2001) and is controlled genetically. Plant breeding techniques can be used to obtain high amylose or high amylopectin (waxy) starches. Starch digestibility and hence its nutritional quality depends on factors such as source of starch, composition and processing conditions (Lehmann and Robin, 2007; Behall et al., 1988; Htoon et al., 2010). For nutritional purposes, starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS), depending on the rate of digestion *in vivo*. RDS and SDS are measured after incubation with pancreatic amylase and amyloglucosidase at 37°C for 20 min and 100 min, respectively. RS is the starch not hydrolyzed after 120 min of incubation (Englyst et al., 1992).

2.3.1. Health benefits of Resistant Starch

The term "resistant starch" was used initially to designate mainly enzyme resistant retrograded amylose but later was expanded to all fractions of starch that escape digestion in the human small intestine but may be fermented in the colon (Englyst and Cummings, 1986; Englyst and Macfarlane, 1986; Mathers, 1992; Schulze, 1992; Biliaderis, 1991; Björck et al., 1986; Brown, 1996). Due to its similar physiological properties, it is considered as a constituent of dietary fiber. Due to its low caloric content and low glycemic index, a higher intake of RS in food is recommended due to its preventative and therapeutic effects (Fuentes-Zaragoza et al., 2010; Ludwig, 2000; Nugent, 2005). RS is a fermentation substrate for colonic microflora and has potential for prebiotic applications. Fermentation of RS produces short chain fatty acids (SCFA) such as acetic, propionic and butyric acids. These fatty acids stimulate colonic blood flow, lower colon pH, hinder growth of harmful colonic bacteria, induce chemoprotective enzyme activity and thus play a role in protecting against colon cancer (Dhital et al., 2010; Garcia-Alonso et al., 1999; Haralampu, 2000; Nugent, 2005; Sajilata et al., 2006; Thompson, 2000; Topping and Clifton, 2001; Topping et al., 2003). Other benefits of RS include lowering of plasma cholesterol and blood lipids as well as improving glucose tolerance that ultimately help in controlling diabetes and obesity and reducing the risk of cardiovascular disease (Englyst and

Macfarlane, 1986; Jenkins et al., 1998; Voragen, 1998; Champ, 2004; Tomasik, 2004; Brand-Miller, 2005; Brennan and Tudorica, 2003; Morita et al., 2005). It also helps in increasing absorption of minerals such as Ca, Mg, Zn, Fe and Cu (Sajilata et al., 2006; Yue and Wang, 1998).

2.3.2. Types of Resistant Starch

Depending on the various reasons for enzyme resistance (Alsaffar, 2011), there are four types of resistant starches (table 1). RS1: physically inaccessible starch locked within cell walls found in partly milled grains, seeds and legumes; RS2: native (ungelatinized) granular starch resistant to enzyme action, e.g., in raw potato, pea, banana and high amylose corn; RS3: retrograded or crystalline starch and RS4: chemically modified starches (Garcia-Alonso et al., 1999). A fifth category, RS5: the amylose-lipid complexed starch has been identified and the reason behind its resistance to digestion is attributed to the complex being a native alpha-amylase inhibitor (Shainkin and Birk, 1970; Fuentes-Zaragoza et al., 2011). In human diets, a few starches, for example banana starch, are consumed in the native form (Zhou et al., 2013). Two types of RS granules can be defined within the RS2 class: RS2a – raw starch granules with non-elevated amylose contents (i.e. within the range 0-30% amylose). These typically lose their RS content on cooking and RS2b – high-amylose starches that retain some granular integrity on

processing (Morrell et al., 2004). Because of the physiological benefits of RS, substantial efforts have been made to enhance the production of RS3 and RS4, for commercial ingredient applications as dietary fiber and improvement in functional properties of foods, respectively (Haralumpu, 2000). As native starches contain less than 30% amylose, commercial RS3 is prepared with the starch containing high (70%) amylose (Zhou et al., 2013).

Table 1: Types, characteristics and cereal sources of resistant starch (adapted from Alsaffar, 2011).

Type	**Characteristics**	**Resistance reduced by**	**Cereal sources**
RS1	Granules that are physically inaccessible to enzymes due to entrapment in a non-digestible matrix	Milling, chewing	Whole or partly milled grains
RS2	Crystalline structure that is undigestible unless gelatinized	Food processing and cooking	High amylose starches
RS3	Retrograded starch	Processing conditions	Corn flakes, food products with prolonged and/or repeated moist heat treatment
RS4	Chemically modified starch, starch esters/ethers, cross-bonded starches	Less susceptible to digestibility *in vitro*	Synthetic

2.3.3. Resistant Starch Measurement

There is a standard method for the analysis of total RS (AACC method 32–40 and AOAC International official method 2002.02). It is a robust and reliable method that reflects *in vivo* conditions and yields values that are physiologically important. This method measures RS, solubilized or non-resistant starch and total starch content of samples. The method is applicable to samples containing more than 2% w/w RS and standard errors of ±5% can be achieved. Many studies available in literature have employed different analysis methods for the determination of RS and this complicates the comparison of results. The deployment of this accepted method in future studies may lead to a more straightforward evaluation and comparison of results (Alsaffar, 2011).

2.3.4. Effects of processing on Resistant Starch

Most researchers suggest RS formed during processing is associated mainly with amylose retrogradation. Commercial RS3 is produced by a two step process consisting of gelatinization, which is a disruption of the granular structure by heating starch with excess water (cooking), followed by retrogradation, which is slow recrystallization or reassociation of starch components (amylose and amylopectin) upon cooling and their dehydration to form tightly packed structures stabilized by hydrogen bonding, resulting in increased digestion resistance (Haralampu, 2000;

Chung at al., 2006; Annison and Topping, 1994; Shamai et al., 2003).

Food RS3 contents are low; 3 to 5% in baked foods, pasta, bread and processed cereals (Englyst and Cummings, 1985; Björck and Asp, 1994). Food applications of RS for fiber fortification are of interest to product developers, nutritionists and food technologists due to potential physiological benefits as well as unique technological properties (Björck and Asp, 1994; Annison and Topping, 1994; Englyst and Englyst, 2005). RS, as an ingredient, has the potential to result in high quality cereal products, especially in terms of texture (improved crispness and expansion), mouth feel, flavor and color, which are not attainable with traditional insoluble fibers (Fuentes-Zaragoza et al, 2010).

Cooking increases the rate of starch hydrolysis by gelatinizing the starch and making it more easily available for enzymatic attack (Bornet et al., 1989; Svihus et al., 2005; Roder et al., 2009; Singh et al., 2010). It is not possible to make such a generalization for RS contents of processed food, due to differences in processing conditions and raw materials (Alsaffar, 2011). Parchure and Kulkarni (1997) found that pressure cooking caused an increase in RS contents of rice and amaranth starches. However, Sagum and Arcot (2000) reported boiling and pressure cooking caused a decrease in rice RS content (from 10% to 1.6%, d.b.). Roopa and Premavalli (2008) reported a decrease in finger millet (ragi) RS content after cooking and puffing (from

1.0% to 0.6%, d.b.). Walter et al (2005) analysed the RS content of white and parboiled rice (autoclaved at 100°C for 10 min) by two methods and obtained different RS values for unprocessed white rice (1.5% vs 4.2%, d.b.). After parboiling, RS values were 3.8% vs 4.0%, d.b., respectively. It was observed that many studies have shown the effect of processing on RS content in grains such as rice, amaranth and finger millet but there is no data available for corn.

During the tempering (holding) process, cooked grains undergo time dependent changes that involve the restructuring of starch (i.e., retrogradation). Tempering allows for equilibration of moisture and may result in improvement of textural properties of grains (Whalen et al., 2000) and a decrease in starch digestibility (Tas, 2004). When starch gels are cooled, molecules comprising gelatinized starch begin to reassociate (retrograde); this leads to an increase in crystallinity. As a result, starch becomes less susceptible to hydrolysis with amylolytic enzymes (Gallant et al., 1992; Oates, 1997; Buleon et al., 1998). Retrogradation of starch can involve either amylose (Tharanathan and Tharanathan, 2001; Leeman et al., 2006; Wasserman et al., 2007) or amylopectin (Eerlingen et al., 1994; Cui and Oates, 1997; Mangala et al., 1999; Kohyama et al., 2004; Srichuwong et al., 2005; Cai et al., 2010). Amylose retrogrades at a more rapid rate than amylopectin (Miles et al., 1985; Fredriksson et al., 1998; Parker and Ring, 2001; Matalanis et al., 2009). Retrogradation of starch is affected by storage temperature (Farhat et al.,

2000). The storage of starch gels at lower temperatures increases retrogradation (Eerlingen et al., 1993; Gudmundsson, 1994; Blazek and Copeland, 2010). Storage of gelatinized starch at the cycled temperatures of 4 and 30°C resulted in the formation of amylopectin crystals with reduced digestibility when compared to conventional storage at a constant low temperature (Park et al., 2009). It is possible to modify the resistant starch content of foods during storage by applying temperature cycles (Slade and Levine, 1991).

The amount of total RS in processed foods is dependent on the degree of food processing, which can result in an increase or decrease in RS values from those found in the raw product (Englyst et al., 2007). Food processing, which involves heat, moisture and shear, can destroy RS1 and RS2 but it may form RS3 (Thompson, 2000). The highest RS value in cereal products, is found in corn flakes, 3.6 g/100 g food. These RS levels are not nutritionally significant (Englyst and Cummings, 1987). The challenge is to generate breakfast cereals with much higher RS contents (10 to 20%) to make a difference in physiological properties of foods (Alsaffar, 2011). The effects of cooking on RS content in rice and some other cereals have been studied but little is known about corn. Also, there is a vast amount of information about the RS contents in starches, flours and gel systems, but very few studies on RS contents in whole grains and their products.

CHAPTER 3

OBJECTIVE 1: DEVELOP A LABORATORY SCALE CORN FLAKING PROCEDURE

3.1. Introduction

The overall objective of this study is to identify corn hybrids that have good grain yield and perform well both in dry milling grit yield and nutritional stability during processing. Twelve inbreds (representing US commercial germplasm) and 72 hybrids (66 test hybrids produced by crossing the inbreds and six commercial hybrids), making a total of 84 varieties, grown over a period of 3 years (2009, 2010 and 2011) with 3 replications each year for each variety (84x3x3 = 756 samples), were evaluated for their test weight (lb/bu), grain yield (bu/acre) and dry milling grit yield (g grits/100 g corn d.b.) by Macke (2012). It was found that dry milling grit yield (mean 29.4 g grits/100 g corn d.b.; ranging from 24.0 to 36.0 g grits/100 g corn d.b.) was correlated phenotypically with grain yield (r = -0.50) and test weight (r = 0.52), correlated genetically with grain yield (r = -0.43), but not correlated with agronomic, ear and kernel traits. From the set of 66 test hybrids, a subset of 35 female x male crosses was chosen and from that subset, 5 best and 5 worst hybrids (in terms of dry milling grit yield) were selected in addition to 3 commercial PH207 (high grain yield) hybrids. Therefore, 13 hybrids and 12 inbreds, making a total of 25 varieties, with 3 replications each and spanning 3 years (25x3x3 = 225 samples), are

currently being analyzed for antioxidants such as soluble and bound phenolics (e.g., ferulic acid and p-coumaric acid), vitamins such as tocopherols and dietary fibers such as resistant starch, in whole kernels and flaking grits. To continue this study through finished corn flakes, it was imperative to first develop a laboratory scale corn flaking procedure by standardizing the three most important stages, i.e., cooking, drying and toasting. Therefore, specific objectives were to:

1. Develop a laboratory scale corn flaking procedure.
2. Measure the reproducibility of the developed procedure and characterize the product.

3.2. Materials and Methods

3.2.1. Commercial flaking grits

Commercial flaking grits obtained from Cargill, Paris, IL were stored in cold storage room at 4°C. Moisture content of grits was 12%. Other specifications, as provided by the manufacturer, are given in table 2.

Table 2: Analysis of commercial flaking grits.

Analysis	Result (%)
Fat	0.5
Granulation: U.S. 3.5	1.2
U.S. 4.0	35.9
U.S. 5.0	59.2

Grits were hand cleaned and sieved to remove any pieces of foreign material. A hammer mill (Retsch GmbH and Co.KG, Haan, Germany), equipped with a Syntron vibratory feeder

(FMC Corporation, Philadelphia, PA) and a 3 mm screen, was used for size reduction of flaking grits.

3.2.2. Moisture Content

Moisture content (MC) was determined using Approved Method 44-19 (AACC 2000). This method consisted of placing triplicate samples (2 g each) in an oven at 135°C for 2 hr and taking the average of three measurements.

3.2.3. Particle size distribution of large grit samples of 10 selected hybrids and sample preparation from commercial grits

Particle size distributions (PSD) of large grit samples, from the 1 kg dry milling procedure (Rausch et al., 2009), of 10 randomly selected hybrids were determined using the sieving procedure described in ASABE Standards (2006). A sieve shaker (Great Western Manufacturing Co., Inc, Leavenworth, KS), equipped with U.S. Standard mesh screens 16, 25, 40 (table 3) and pan, was used. Duplicate samples (100 g) were vibrated for 10 min and weight retained on each screen was recorded.

Table 3: Aperture sizes of test sieves, ASAE S319.3.

U.S. standard sieve No. (mesh)	Nominal sieve opening (mm)
16	1.19
25	0.71
40	0.42

3.2.4. Cooking

The total time needed to cook cereal products is a function of both moisture and temperature (Caldwell et al., 2000). Chaunier et al (2006) batch cooked 100 g samples (MC 12% w.b.) with 72 mL water at 100°C for 50 min. However, a detailed procedure pertaining to the kind of equipment used for the cooking step was not mentioned. This made it necessary to determine the amount of water needed to cook a 100 g sample of corn grits (MC 12% w.b.) as well as temperature and pressure conditions required during cooking.

The ingredient formula used for cooking was 6 g granulated sugar, 2 g liquid malt extract and 2 g salt per 100 g grits (Fast and Caldwell, 2000). Tap water (2.5 L) was poured in a 15 L canning pressure cooker (Model 921, Wisconsin Aluminium Foundry Co. Inc., Manitowoc, WI); the cooker was heated on an electric hot plate (Waring professional single burner model SB30, 1300W, East Windsor, NJ). A thermocouple was placed in the water and the cooker lid was closed. Once the temperature reached 98°C to 99°C (15 to 20 min), the sample(s) (one or four 1000 mL beaker(s), each containing the mixture of 100 g grits and flavor solution) were placed in the cooker. The pressure regulator weight was set at 103.5 kPa (15 psig). It took 10 to 12 min for the pressure in the cooker to reach 15 psig; it was cooked for an additional 50 min (total 1 hr). Once cooked, the burner was turned off and beakers containing the cooked sample(s) were removed after all the steam had vented out or once the pressure gauge read 0 psig.

A Carl Zeiss Apotome microscope Axio Observer.Z1, with built in cross polarizers, an attached imaging software Axiovision 4.8 and digital CCD camera AxioCam MRm (resolution 1388x1040 - 1.4 megapixels) was used to view and take images of raw and cooked grit samples to observe loss in birefringence at 20X magnification. The light source (halogen lamp) was set at 3.1 V and the bright field condenser was set at NA 0.5 to obtain a good compromise between resolution and contrast. The image size was 447.63 x 335.40 μm^2.

Samples of raw corn grits, used to obtain the most representative and clear images of starch showing the maltese cross, consisted of the fraction that passed U.S. mesh size 200. 0.2 mL double distilled water was placed at the center of the microscopic cover glass. Using a spatula, the sample was dispersed into the droplet and mixed gently until it formed a uniform slurry or paste. This suspension was covered with a cover slip without allowing formation of air bubbles.

3.2.5. Drying and tempering followed by flaking

Drying, for moisture removal, was carried out in a laboratory scale oven (Lindberg/Blue M, Asheville, NC). This was followed by tempering, for moisture equilibration, in a closed vessel at room temperature for 30 min. Four drying treatments (50°C/30 min, 50°C/60 min, 100°C/30 min and 100°C/60min) were evaluated in triplicates for ease of flaking of dried and tempered grits using a hand cranked tortilla press (Ra Chand Tortilla Maker CTM-2000, E & A

Hotel & Restaurant Equipment and Supplies, NJ). The dried and tempered grits were compressed into dough by hand; the dough was flaked into sheets with the tortilla press; flaked sheets were cut into individual flake pieces with a cookie cutter. Using a feeler gauge, flake thickness was monitored and controlled by adjusting the gap between the rolls of the press. Flakes of thickness 0.8 to 1 mm were obtained. Flaked samples were kept in an open container overnight at room temperature (20°C to 25°C) overnight; untoasted flakes were toasted the next day.

3.2.6. Toasting

Toasting imparts color, texture and sensory attributes to the final product, which are essential quality parameters to consumers. Therefore, it was important to standardize the temperature/time profile for toasting corn flakes. Color of the finished product, an important quality parameter, is a direct result of the toasting time/temperature profile. Measurement of color properties requires small sample size (5 g) and hence was the preferred method for selecting the toasting treatment that resulted in laboratory flakes with color similar to that of commercial flakes.

Toasting was carried out in a laboratory scale convection pizza oven (Model 1302-12, 6 kW, Lincoln Foodservice Products Inc., Fort Wayne, IN). Three toasting treatments (50 s, 60 s and 70 s, each at 200°C) were evaluated for color parameters (*L*, *a* and *b* values) of toasted flakes

using a colorimeter (Model LabScan XE, HunterLab, Reston, VA) by placing the corn flake samples in a 9 cm petri dish. Ten readings per toasting treatment were recorded.

Color is a physical perception by which the human eye detects reflection of light at certain wavelengths. Color is influenced in corn by the presence of carotenoids and other pigments. Changes in color are also a result of cooking or caramelization and Maillard reaction. A change in particle size of the same material could result in a lighter color (Harper, 1989). Color changes also have been found to be reflective of nutritional value (Berset, 1989). The values of *L* denote lightness or brightness, *a* indicate redness or greenness with positive and negative values, and *b* mean yellowness or blueness with positive and negative values. The colorimeter permits color measurements of products based on assignment of numerical values for hue, lightness and intensity in a three dimensional system. A beam of light from a tungsten lamp hits the product and is reflected to a sensor. Three values define each point in this three dimensional color space: "L" value (for lightness) ranges from 100 (white) to 0 (black), "a" value ranges from red (+) to green (-) and "b" value ranges from yellow (+) to blue (-) (Hutchings, 1999).

3.2.7. Procedure reproducibility for commercial grits and hybrid grits

Five flaking runs were carried out with the final developed procedure for commercial grits, each run on a different day and reproducibility was evaluated in terms of the following:

1. Moisture content at every processing stage, except after cooking since that was stable at 60%.

2. Viscous properties of final toasted flakes.

Five flaking runs were carried out for hybrid grits (pooled sample of 5 randomly selected hybrids K330, 11026, 12059, 11082 and 12002), each run on a different day and reproducibility was evaluated in terms of moisture content at every processing stage, except after cooking since that was stable at 63.5%.

3.2.8. Characterization of laboratory flakes

Cooking or pasting characteristics of flours and starches can be studied by heating samples with standard time temperature profiles in excess water using a Rapid Visco Analyzer (RVA). It is a rotational viscometer which uses small sample sizes (3 to 5 g) and continuously records the viscosity of the sample under conditions of controlled temperature and shear, is easy to operate and gives quick and reproducible results (Deffenbaugh and Walker, 1989). Therefore, laboratory corn flake samples were characterized in terms of RVA parameters and these were compared to those of commercial corn flake samples.

A Rapid Visco Analyzer (Model RVA-4, Newport Scientifc, Warriewood, Australia), or RVA, with Thermocline for Windows version 2.0 software was used to create pasting profiles of commercial corn flakes and lab produced flakes. This was done in five replicates with the RVA profile given in table 4, used for breakfast cereals (Chaunier et al., 2006; Carvalho and Mitchell,

2000). The formula given below (AACC Method 76-21) was used to calculate the amount of ground sample (S) and water (W) required for 14% moisture basis, based on the actual moisture content M of the sample.

$S = \frac{86*3.5}{100-M}$ & $W = 25 + (3.5 - S)$

Samples were ground in a kitchen blender (Model 6694-B, 450 W, Osterizer 14 speed all metal blender, Mexico) for 1 min and then sieved through US mesh size 60 using an ultrasonic sifter (Model L3P, ATM Corporation, Milwaukee, WI) for 1 min. The fraction that passed through 60 mesh (<250 μm) (Chaunier et al., 2006) was used for RVA analysis.

Table 4: Temperature time profile for RVA analysis.

Elapsed Time (min:sec)	Temperature (°C)	Speed (rpm)
00:00	50	960
00:10	50	160
02:00	50	160
09:30	95	160
14:30	95	160
22:00	50	160
23:00	50	160

3.2.9. Statistical analysis

Color parameters and pasting characteristics of laboratory prepared corn flakes were compared with those of commercial corn flakes using a simple linear CRD model in proc glm. Analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) were used to

detect differences at a certainty of $p \leq 0.05$ using Statistical Analysis System (Version 9.4, SAS Institute, Cary, NC, USA).

3.3. Results and Discussion

3.3.1. Particle size distribution of large grit samples of 10 selected hybrids and sample preparation from commercial grits

The fraction retained over 16 mesh and the fraction obtained in pan had the highest coefficients of variance (11% and 14%, respectively). The fractions retained on 25 mesh and 40 mesh had lower (≤5%) coefficient of variance. Most of the material (40.2%) was retained on the 25 mesh screen and least (14.7%) was retained on the 16 mesh screen (>1.2 mm (table 5).

Table 5: Grits retained on each screen per 100±0.5 g sample (as is) for 10 hybrids.

Hybrid	16 mesh	25 mesh	40 mesh	Pan	Total
1	13.2	42.5	27.3	16.4	99.4
2	16.6	41.2	26.2	15.6	99.4
3	15.4	39.4	26.4	18.5	99.2
4	15.2	40.4	26.3	17.7	99.4
5	12.2	35.4	27.6	24.0	99.2
6	16.7	41.8	24.4	16.3	99.7
7	13.0	39.7	27.5	19.0	99.2
8	15.4	39.4	25.7	18.9	99.6
9	16.5	42.0	25.5	15.7	99.6
10	12.9	39.8	27.1	19.6	99.7
Mean±S.D.	14.7±1.7	40.2±2.0	26.4±1.0	18.2±2.5	99.4±0.2
CV (%)	11.7	5.0	3.9	13.8	0.2

Commercial grits were milled using the hammer mill equipped with a 3 mm sieve and the ground grits passed over the sieve shaker to collect fractions retained on 16, 25, 40 mesh and pan. These fractions were stored in sealed plastic bags in a refrigerator (Glenco Refrigeration Co., Philadelphia, PA) at 4°C. For preparing 100 g grit samples to simulate PSD of hybrid grits, 15 g from the 16 mesh fraction, 40.2 g from the 25 mesh fraction, 26.4 g from the 40 mesh fraction and 18.4 g from the pan fraction were mixed, following means of fractions observed for hybrid large grit samples.

3.3.2. Cooking

Final moisture content of samples, when cooked for 50 min at 121°C, was 60%. Raw or ungelatinized starch granules, when observed under polarized light, exhibit an optical birefringence pattern known as a ‘‘Maltese cross’’ (fig. 1), which implies a high degree of molecular order within the granule (Greenwood, 1979). Birefringence (or double refraction) is the decomposition of a light ray into two rays when it passes through certain types of crystalline materials. This occurs only when the material is anisotropic, that is, the material has different characteristics in different directions. Amylose and amylopectin polymers are organized into radially anisotropic, semicrystalline units in the starch granule. This radial anisotropy is responsible for the distinctive Maltese cross (Blanshard, 1979). The crystallinity of starch is caused by amylopectin polymer interactions (Banks and Greenwood, 1975; Biliaderis, 1998;

Donald, 2004; Hizukuri, 1996). When raw starch is heated in excess water, granules swell and lose their birefringence (Alsberg, 1928). Complete loss in birefringence was observed in cooked grits obtained from the 50 min cooking period, when seen under polarized light (fig. 2).

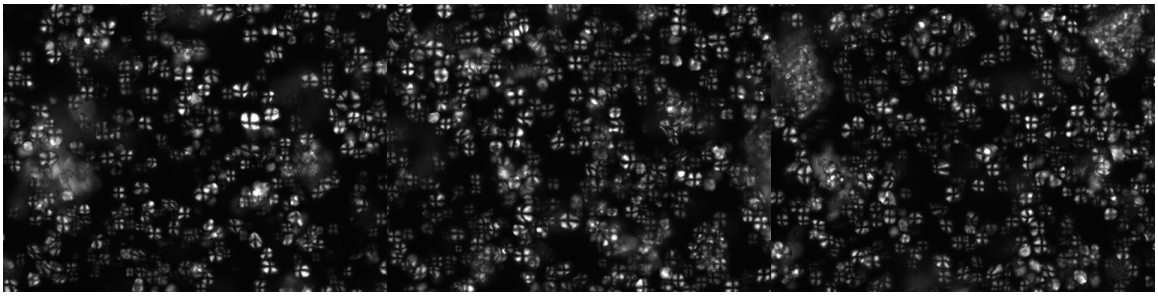

Figure 1: Raw corn grits showing Maltese crosses.

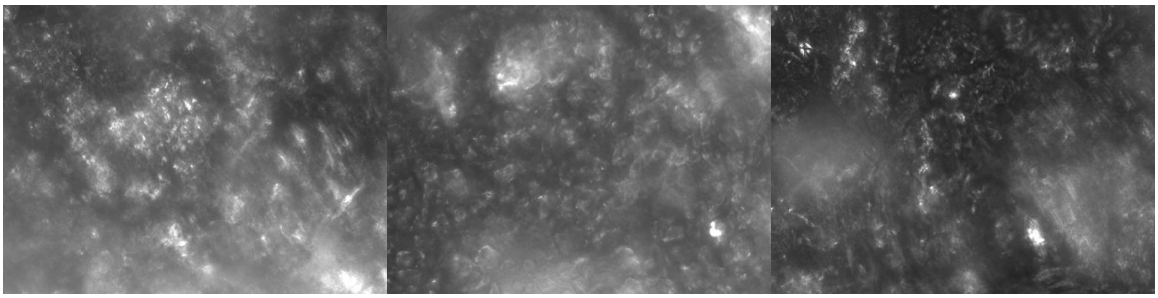

Figure 2: Cooked grits with complete loss in birefringence.

3.3.3. Drying and tempering followed by flaking

The 50°C drying treatment did not change the moisture content of the cooked sample (MC 60%), irrespective of the duration it was kept in the oven i.e., 30 min or 60 min. Drying at 100°C for 30 min reduced the moisture content of the cooked sample by 4% and drying at 100°C for 60 min reduced the moisture content of the cooked sample by 8% (table 6). Both 50°C drying treatments resulted in dough that was too moist to flake and 100°C, 60 min drying treatment

resulted in dough that was too dry to flake. Therefore, 100°C, 30 min treatment was selected as the final time temperature profile for drying.

Table 6: Mean MC of dried and tempered grits after each drying treatment.

Drying profile	Moisture content (%)
50°C, 30 min	60
50°C, 60 min	60
100°C, 30 min	56
100°C, 60 min	52

3.3.4. Toasting

The 70 s toasting treatment had values of *L*, *a*, and *b* different from those of commercial corn flakes. For 50 and 60 s treatments, only *a* values were different; *L* and *b* values were similar to those of commercial corn flakes (table 7). Therefore, both these treatments were found to be appropriate for toasting the flakes. However, 60 s toasting treatment was used in the final procedure as these flakes also had more visual similarity with commercial corn flakes. The 70 s treatment gave brownish or over toasted appearance; 50 s treatment gave yellowish or under toasted appearance.

Table 7: Color parameters of commercial and laboratory prepared corn flakes.

Toasting treatment	L	a	b
Commercial	$51.0 \pm 0.9^{a,b}$	10.6 ± 0.9^{c}	37.2 ± 1.8^{b}
50 s	51.4 ± 1.4^{a}	13.1 ± 0.6^{b}	41.9 ± 0.9^{a}
60 s	49.7 ± 2.5^{b}	$13.2 \pm 0.5^{a,b}$	38.0 ± 1.5^{b}
70 s	47.4 ± 1.5^{c}	13.7 ± 0.4^{a}	35.7 ± 2.1^{c}

Different letters in the same column indicate significant ($p \leq 0.05$) differences
LSD (L=1.5, a=0.6, b=1.5)

3.3.5. Procedure reproducibility for commercial grits and hybrid grits

During procedure development using commercial grits, it was identified that consistency in moisture content was very important at every step for the procedure to work at all times. In addition to that, it was important to make sure the procedure worked for hybrid grits as well since it was ultimately being developed to flake a huge sample set of hybrid grits. Moisture content of dried and tempered grits had low CV (2%), ranging from 53.5% to 56.0%. However, MC of untoasted flakes had high CV (10.1%) ranging from 9% to 11.5%. MC of toasted flakes remained constant at 5% (table 8). CV of RVA parameters of toasted flakes produced from commercial grits ranged from a minimum of 6.8% for setback viscosity to a maximum of 12.9% for breakdown viscosity (table 9). Therefore, the corn flaking procedure was reproducible for commercial grits.

Table 8: Moisture content (%) at each step of the corn flaking procedure for commercial grits.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean±S.D.	CV (%)
Dried and tempered grits	53.5	55.0	56.0	53.5	55.0	54.6±1.1	2.0
Untoasted flakes	9.5	11.5	9.5	9.0	10.0	9.9±1.0	10.1
Toasted flakes	5.0	5.0	5.0	5.0	5.0	5.0	-

Table 9: RVA parameters of toasted flakes produced in laboratory from commercial grits.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean±S.D.	CV (%)
Peak	113	101	96	96	109	103±7.7	7.5
Trough	90	80	78	78	92	84±6.8	8.2
Breakdown	23	21	18	18	17	19±2.5	12.9
Final	289	267	248	255	276	267±16.4	6.9
Setback	199	187	170	177	184	183±10.9	6.8

Initial runs with 100 g hybrid grit samples (MC 13%) did not yield good flaking results. Cooked hybrid grits had higher MC, 63.5% compared to 60.0% for cooked commercial grits. These cooked grits, when dried at 100°C for 30 min and tempered at room temperature for 30 min, resulted in dough too moist and stuck to the rolls of the flaking machine. This was due to the fact that hybrid grits were produced with the laboratory dry milling procedure and therefore contained parts of germ and pericarp, which absorb moisture at a higher rate than pure endosperm fraction (Pietruszewski et al., 2001). So difference in the inherent composition of commercial and hybrid grit samples was the reason behind this consistent difference. Therefore,

the corn flaking procedure had to be tweaked at the drying step for hybrid grits. The 120°C/30 min drying treatment (against 100°C/30 min for commercial grits), followed by 30 min tempering time resulted in dough with the right consistency for easy flaking and the remaining procedure was the same. Similar to the previous results, MC of dried and tempered grits had low CV (2.5%); MC of untoasted flakes had high CV (5.4%), but this was half the CV of untoasted flakes for commercial grits (10.1%). MC of toasted flakes remained constant at 6% (table 10).

Table 10: Moisture content (%) at each step of the corn flaking procedure for hybrid grits.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean±S.D.	CV (%)
Dried and tempered grits	53.0	56.5	55.5	56.0	54.5	55.1±1.4	2.5
Untoasted flakes	9.0	10.0	9.0	9.5	9.0	9.3±0.5	5.4
Toasted flakes	6.0	6.0	6.0	6.0	6.0	6.0	-

3.3.6. Characterization of laboratory flakes

Mean pasting profiles of commercial and laboratory corn flakes were different; peak, trough and breakdown viscosities were lower and final and setback viscosities were higher for laboratory flakes (table 11 and fig. 3). However, these results were similar to results of previous studies that found RVA characteristics are dependent on the process (Chaunier et al., 2006; Ames and Rhymer, 2000).

Table 11: Mean viscosity parameters of commercial and laboratory corn flakes.

Viscosity parameter	Commercial	Lab
Peak	220±6.6[a]	103±7.7[b]
Trough	173±5.8[a]	84±6.8[b]
Breakdown	47±5.8[a]	19±2.5[b]
Final	214±4.9[a]	267±16.4[b]
Setback	40±5.8[a]	183±10.9[b]

Different letters in the same row indicate significant ($p \leq 0.05$) differences
LSD (peak=10.5, trough=9.3, breakdown=6.5, final=17.6, setback=12.8)

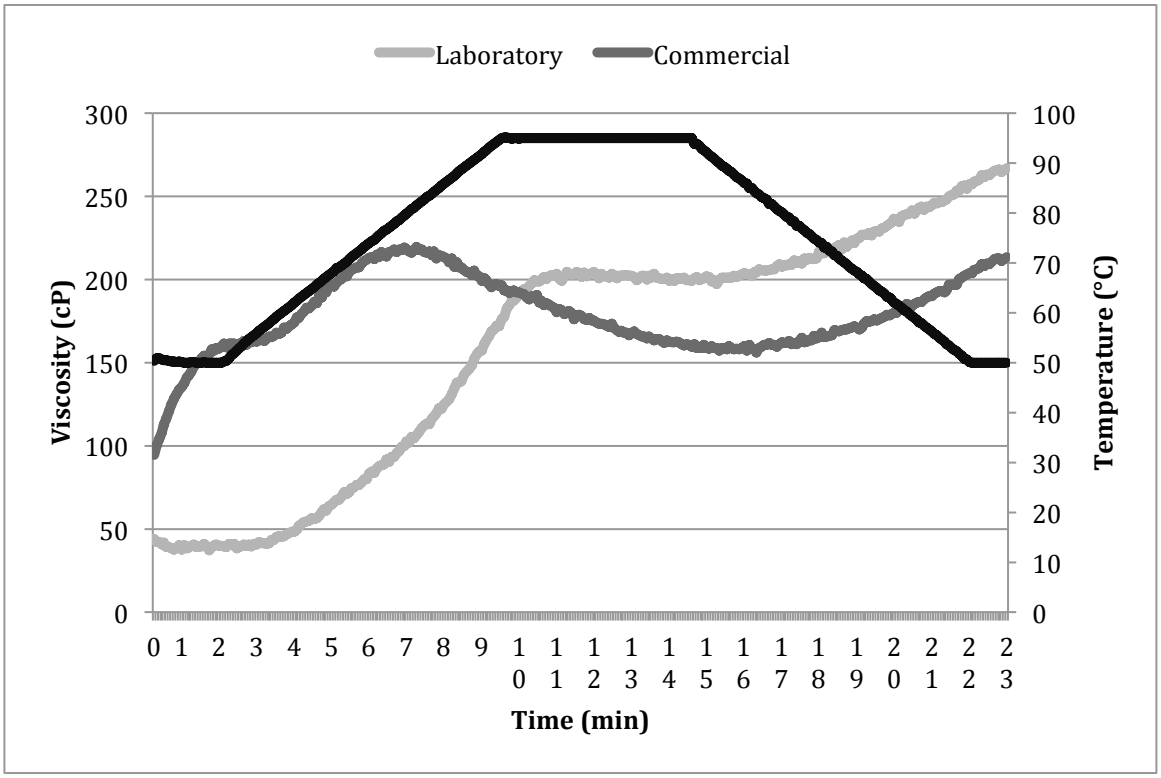

Figure 3: Mean pasting profiles of commercial and laboratory flakes.

CHAPTER 4

OBJECTIVE 2: ANALYZE THE EFFECT OF PROCESSING AND GENETICS ON RESISTANT STARCH IN CORN

4.1. Introduction

After developing the procedure and measuring its reproducibility, the next objective was to determine total RS content at each processing stage using commercial grits to identify stages of significant RS content change. Once these stages were identified, a set of corn hybrids was flaked with the developed procedure and total RS content was determined at those particular stages to evaluate the effects of genetics and processing on corn RS. Agronomic (grain yield, test weight and dry milling grit yield) and nutritional (tocopherol and soluble phenolic levels) data has been collected for 13 hybrids (Macke, 2012 and Butts et al., unpublished). Cluster analysis (performed using Ward's method) resulted in formation of two major clusters, the first being the cluster with good nutrient levels and the second being the cluster with poor nutrient levels but high dry milling grit yields. A diverse genetic pool of seven hybrids, representative balance of the two clusters, was selected for RS evaluation.

4.2. Materials and Methods

For commercial grits, RS content was measured in raw corn grits, cooked grits, tempered grits, fresh flakes, untoasted flakes and toasted flakes in five replicates at each processing stage. For the grits of seven hybrids, RS content was measured in raw grits, cooked grits and toasted flakes in three replicates at each processing stage, where each replicate value was a mean of two determinations. The seven hybrids used in this study (table 12) were grown in 2009 at the University of Illinois Crop Sciences Research and Education Center in Urbana, IL. Whole grains of these hybrids were processed into flaking grits with the laboratory dry milling procedure shown in fig 4. Details about the hybrids can be found in Macke, 2012.

Table 12: List of hybrids tested. Different codes represent three replications.

Genotype	Hybrid	Replicates (year 2009)
B73xMO17	H1	K172, K213, K322
B73xPHG47	H2	K148, K235, K329
LH1xMO17	H3	K155, K226, K305
LH123HTxPHJ40	H4	K160, K275, K351
MO17xPHJ40	H5	K122, K269, K345
PH207xPHG47	H6	K129, K263, K303
PHG39xPHZ51	H7	K143, K250, K348

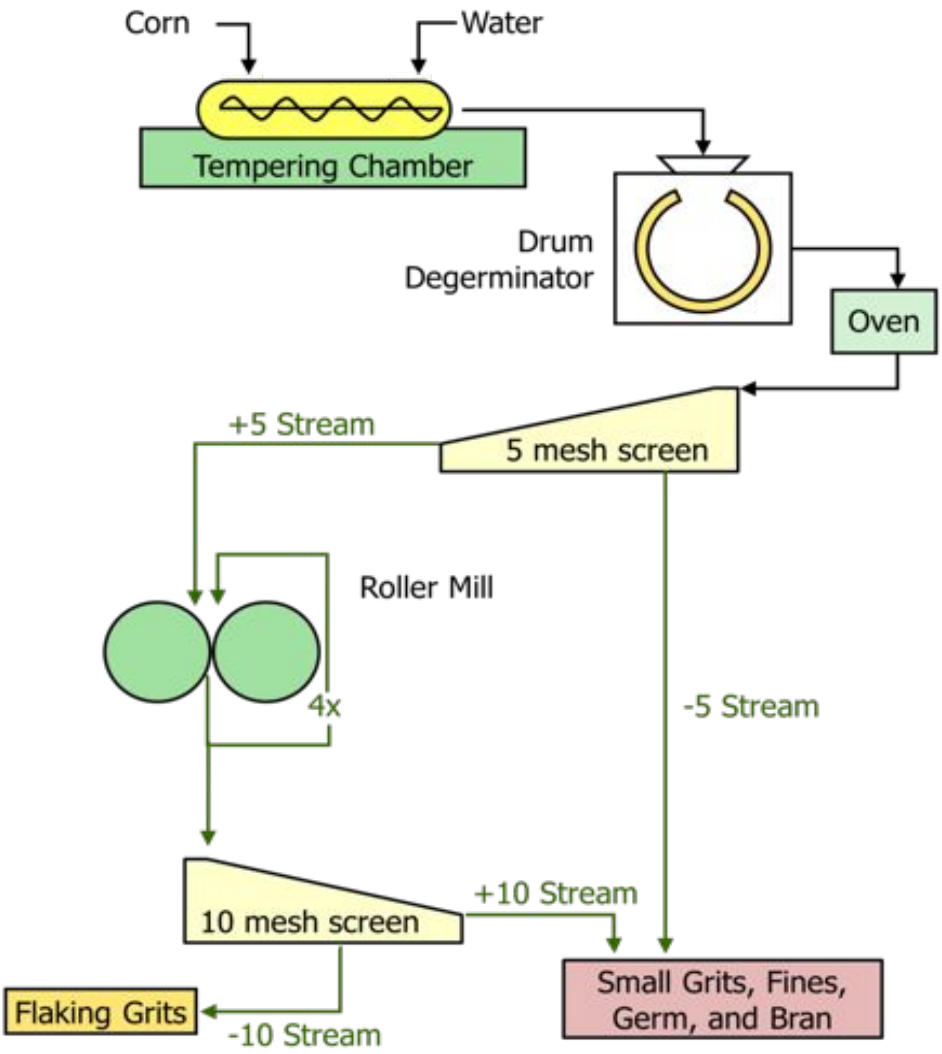

Figure 4: Laboratory dry milling procedure (Rausch et al., 2009).

Total RS content was measured using the Megazyme kit, which follows AOAC Method 2002.02 and AACC Method 32-40. Samples of raw corn grits, untoasted and toasted flakes were ground in a kitchen blender (Model 6694-B, 450 W, Osterizer 14 speed all metal blender, Mexico) for 1 min and then sieved using U.S. mesh 18 (1 mm opening) in a sieve shaker (Model

RX-86, W. S. Tyler, Incorporated, Mentor, OH) for 5 min. The fraction that passed through the sieve was used for RS assay. Samples of cooked grits, tempered grits and fresh flakes were kept overnight in a laboratory oven (Model 160DM, Thelco Laboratory Oven, Precision Scientific Instruments Inc., Chicago, IL or Model DKN-400 Yamato Constant Temperature Oven, Japan) at 60°C. Dried samples were ground and sieved as described above and used for RS analysis. Moisture contents were determined using AOAC Method 925.10.

Samples were incubated in a shaking water bath with pancreatic alpha-amylase and amyloglucosidase (AMG) for 16 hr at 37°C, during which time nonresistant starch was solubilized and hydrolyzed to D-glucose by the combined action of the two enzymes. The reaction was terminated with the addition of an equal volume of ethanol or industrial methylated spirits (IMS, denatured ethanol) and the RS was recovered as a pellet on centrifugation. Free liquid was removed by decantation. RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice water bath over a magnetic stirrer. This solution was neutralized with acetate buffer and the starch was hydrolyzed quantitatively to glucose with AMG. D-Glucose was measured with glucose oxidase/peroxidase reagent (GOPOD); this is a measure of sample RS content. Nonresistant (solubilized) starch was determined by pooling the original supernatant and the washings, adjusting the volume to 100 mL and measuring D-glucose content with GOPOD.

For commercial grits, analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) were tested in proc glm using a simple linear CRD model with five replications to detect differences at a certainty of $p \leq 0.05$ using Statistical Analysis System (Version 9.4, SAS Institute, Cary, NC, USA). For hybrid grits, a two factor factorial model was tested in CRD with three replications.

4.3. Results and Discussion

Raw grits contained 14.9% RS content, which reduced to 5.3% with cooking (table 13). Breakdown of starch granular structure, caused by cooking corn at high temperature with excess water, resulted in increased availability of starch for enzymatic action. Drying, tempering, flaking and toasting did not result in any change in RS content, as it remained in the range of 5% to 6%. Therefore, all processing stages after cooking, which included heating and cooling cycles, did not retrograde or recrystallize gelatinized starch enough to change the RS content. Starting from 14.9% in raw grits, the corn flaking procedure reduced the RS content in laboratory corn flakes to 5.9%.

Greater variability was observed in RS measurement of raw grits and toasted flakes (CV 12.1% and 10.2%, respectively) compared to that of cooked grits, tempered grits, fresh flakes and untoasted flakes (CV 7.6, 5.5, 4.1 and 5.8%, respectively). Commercial corn flakes had maximum variability (CV 20.7%). Since the largest change in RS content was caused by cooking

in commercial grits (fig. 4), subsequently the seven selected hybrids were tested for RS only at three processing stages, i.e., raw grits, cooked grits and toasted flakes. RS content of commercial (market) corn flake samples was at 2.9%, 3% lower than that of laboratory corn flake samples (5.9%) (table 13).

Table 13: Mean RS content at various stages of processing for commercial grits.

Stage of processing (MC)	Mean±SD (g/100 g sample)	CV (%)
Raw grits (14.0%)	14.9 ± 1.8^{a}	12.1
Cooked grits dry (4.0%)	5.3 ± 0.4^{b}	7.6
Tempered grits dry (4.5%)	5.5 ± 0.3^{b}	5.5
Fresh flakes dry (2.5%)	4.9 ± 0.2^{b}	4.1
Untoasted flakes (10.8%)	5.2 ± 0.3^{b}	5.8
Toasted flakes (5.0%)	5.9 ± 0.6^{b}	10.2
Commercial flakes (6.0%)	2.9 ± 0.6	20.7

Different letters indicate significant ($p \leq 0.05$) differences
(MC - moisture content, LSD=1.1)

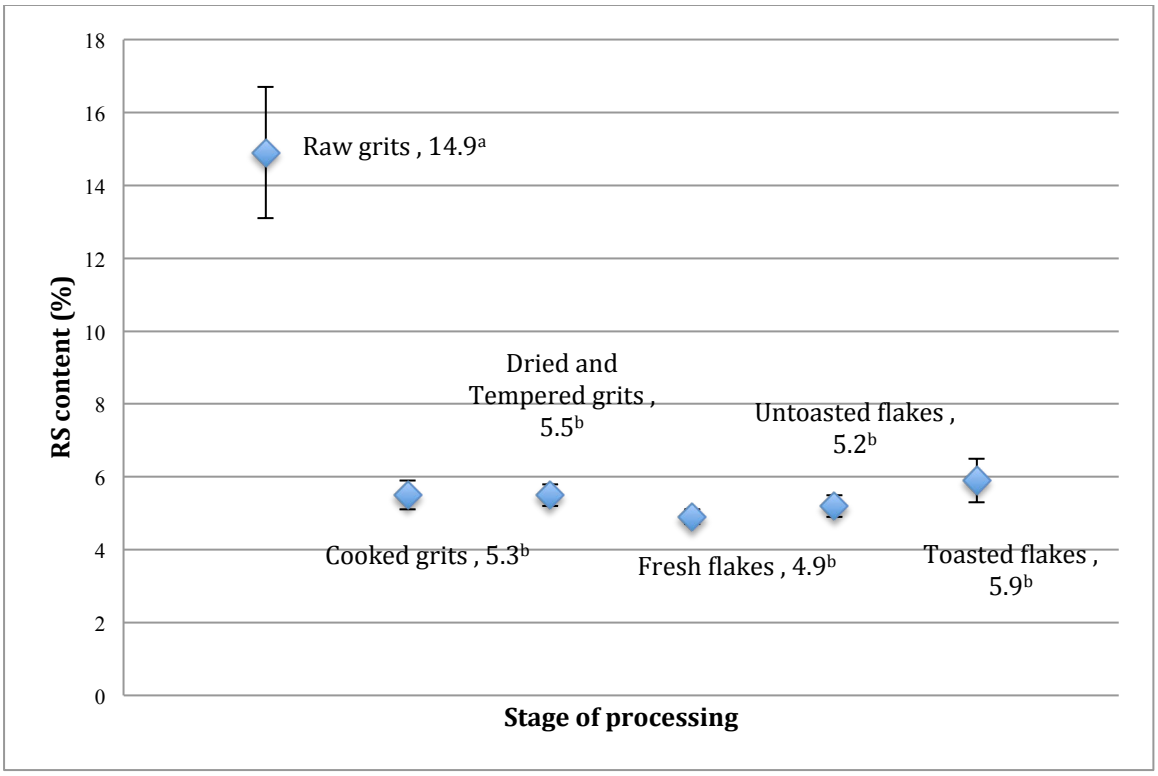

Figure 5: Change in RS content during corn flaking procedure for commercial grits.

Change in RS content in the seven hybrids followed the same pattern as it did in commercial grits (fig. 5). For each hybrid, cooking decreased total RS contents but subsequent processing stages did not change this level. The corn flaking procedure reduced RS content to almost half its original level (from an average of 6.7% in raw grits to 2.9% in cooked grits to 3.2% in toasted flakes). Differences were found among the hybrids at each processing stage (table 15). Enzymatic digestibility of raw starch granules varies among corn varieties due to the differences in amylose content (table 14). Resistant starch content in corn is correlated with amylose level and depends on processing conditions (Berry, 1986; Sharma et al., 2008). Genetic dependence of RS content could be attributed to differences in amylose contents in raw grits of

the seven hybrids tested, but this needs to be verified in future experimental work. In addition, no interactions were observed between genetic and processing effects.

Table 14: Amylose and RS content of various starches (Themeier et al., 2005).

Source	Amylose (% d.b.)	Resistant Starch* (% d.b.)
Maize	66.5	54.4
Maize	65.8	49.1
Maize	30.0	0.7
Maize	7.6	0.5
Wheat	30.2	0.3
Wheat	33.7	0.2
Rye	31.1	0.2

*Determined by AOAC method (AOAC 2002.02).

Table 15: Total RS content of seven hybrids at three processing stages [lsmeans±S.D. (CV)]

Hybrid	Raw	Cooked	Toasted
H1	6.3±1.5 (23.8%)^{a,b,A}	2.5±0.4 (16%)^{c,d,B}	2.7±0.7 (26.0%)^{b,B}
H2	6.6±0.7 (10.6%)^{a,b,A}	3.3±0.3 (9%)^{a,b,B}	3.3±0.5 (15.2%)^{a,b,B}
H3	6±1.2 (20%)^{b,A}	2.3±0.1 (4.4%)^{d,B}	2.7±0.6 (22.2%)^{b,B}
H4	7±1.4 (20%)^{a,b,A}	3.5±0.4 (11.4%)^{a,B}	3.9±0.5 (12.8%)^{a,B}
H5	8.5±1.9 (22.4%)^{a,A}	3.4±0.3 (8.8%)^{a,b,B}	4.1±0.6 (14.6%)^{a,B}
H6	7±1.7 (24.3%)^{a,b,A}	3±0.1 (3.3%)^{b,c,B}	3±0.2 (6.7%)^{b,B}
H7	5.3±0.9 (17.0%)^{b,A}	2.5±0.3 (12%)^{c,d,B}	2.8±0.2 (7.1%)^{b,B}

Different letters (upper case) in the same row indicate significant ($p \leq 0.05$) differences (effect of processing)

Different letters (lower case) in the same column indicate significant ($p \leq 0.05$) differences (effect of genotype)

LSD (H1=2.0, H2=1.1, H3=1.6, H4=1.8, H5=2.3, H6=2.0, H7=1.1, Raw=2.4, Cooked=0.5, Toasted=0.9)

Hybrid 5 (MO17xPHJ40) had maximum RS content in raw grits (8.5%) and hybrid 7 (PHG39xPHZ51) had minimum (5.3%). Only these two hybrids were different from each other. While commercial raw grits were at 14.9% RS content, hybrids averaged at only 6.7% RS content. For cooked grits and toasted flakes, differences were observed among the seven hybrids, but these were not practically relevant. For cooked grits, RS content ranged from a minimum of 2.3% to a maximum of 3.5% (mean 2.9%) and for toasted flakes, RS content ranged from a minimum of 2.7% to a maximum of 4.1% (mean 3.2%). The hybrid with maximum initial RS content (i.e., H5 - MO17xPHJ40 at 8.5%) had high RS content in the final toasted flakes (4.1%). Likewise, the hybrid with minimum initial RS content (i.e., H7 - PHG39xPHZ51 at 5.3%) had relatively low RS content in the final toasted flakes (2.8% against a minimum 2.7%). Change in RS content occurs almost proportionally (i.e., higher in the beginning, higher in the end and lower in the beginning, lower in the end). RS content did not increase with toasting for any of the hybrids; therefore for future RS analyses of hybrid samples, it would be necessary to measure RS only for raw grits and for toasted flakes (eliminating cooked grits), as it would capture the overall change in RS content with the corn flaking procedure.

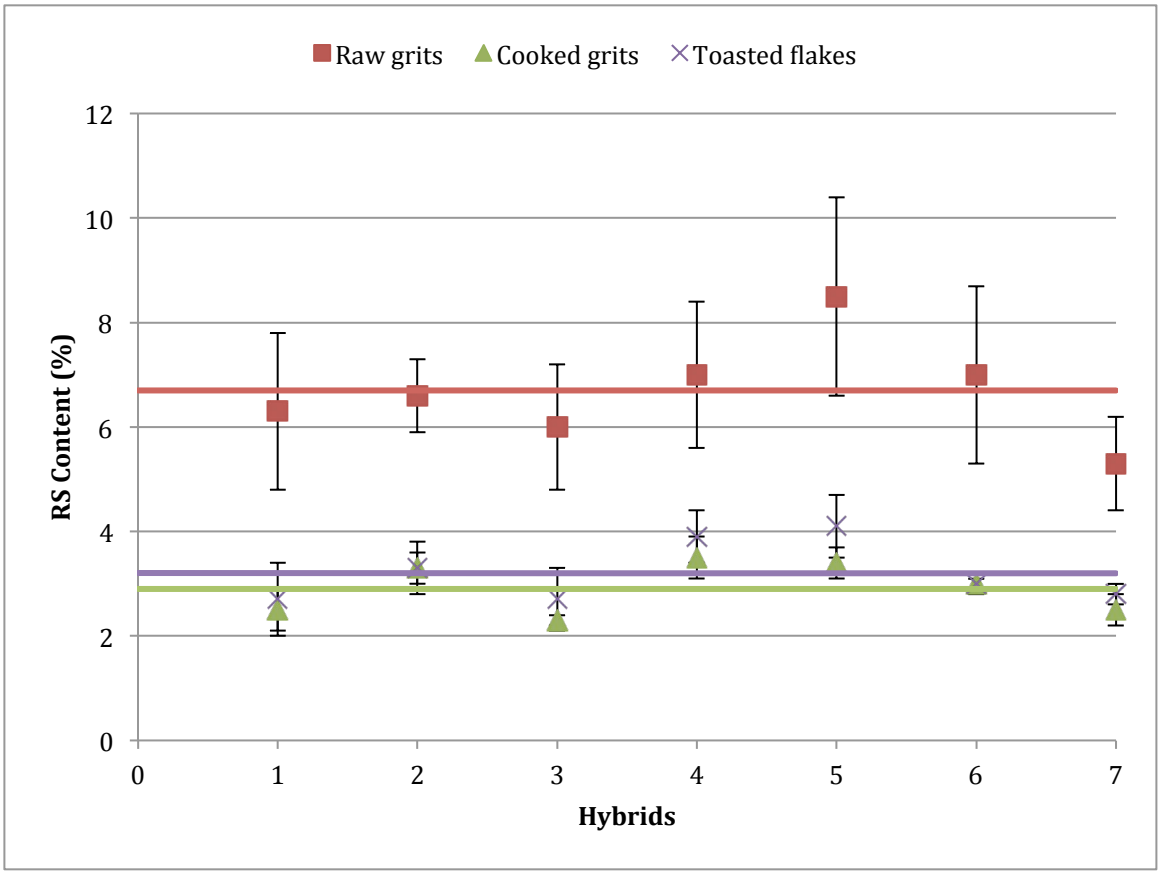

Figure 6: Change in RS content during corn flaking procedure for seven hybrids (error bars indicate ± S.D.).

CHAPTER 5

CONCLUSIONS

A laboratory scale corn flaking procedure was developed at a batch size of 100 g grits, with a throughput of four samples per day. The procedure was found to be reproducible for both commercial and hybrid grits. Flakes produced in laboratory had similar color properties but different pasting properties compared to commercial corn flakes. Processing conditions at laboratory scale simulated the commercial processing conditions (table 16).

Table 16: A summary and comparison of the commercial and laboratory corn flaking procedures.

	Commercial process (Fast and Caldwell, 2000)	**Laboratory process**
Cooking	Batch cooking of commercial corn grits in a rotary cooker at 15 to 20 psi for 2 hr, MC not mentioned. End point of cooking for batch process: details not mentioned.	Batch cooking of lab corn grits (100 g) in a pressure cooker at 15 to 16 psi for 50 min, MC 60%. End point of batch cooking: loss in birefringence.
Drying and Tempering	Drying at 120°C and tempering at room temp, time not mentioned, MC 10% to 14%.	Drying at 100°C and tempering at room temperature, 30 min each. MC 55%.
Flaking	Metal rolls running at high but differential speed, flake thickness not mentioned.	Metal rolls running at low but same speed (manual), flake thickness – 0.8 to 1 mm.
Toasting	275°C to 330°C for 90 s, MC 1.5% to 3%.	200°C for 60 s, MC 5%.

Resistant starch content decreased in corn as it was processed from raw grits to toasted flakes, the largest decrease caused by the single processing stage of cooking and no changes caused by subsequent steps. Differences were observed among the hybrids at each processing stage. Processing and genetic background both affected RS content in corn but no interaction was observed between the two effects. Hybrids with high RS content in flaking grits resulted in high final RS content in toasted flakes. Since RS content is highly correlated with amylose content, high amylose corn hybrids could be used in the breakfast cereal industry, as these would result in high RS levels (high fiber content) in corn flakes and hence improve their nutritional quality. But there is a need to evaluate if high amylose hybrids have good grain yield and flaking grit yield as well, to ensure economic feasibility to farmers and processors.

CHAPTER 6

RECOMMENDATIONS

1. For future hybrid work, RS content should only be measured for flaking grits and for toasted flakes, as this would capture the total change from beginning to end during the corn flaking procedure.
2. Since RS content is correlated positively with amylose content, the set of hybrids should be tested for amylose content.
3. Different storage temperature/time conditions for untoasted flakes could be evaluated to see if these result in increased RS content in toasted flakes.
4. It was observed that laboratory flakes had lower peak and trough viscosities compared to commercial flakes but higher RS content, therefore correlation should be established between RS content in toasted flakes and RVA properties.
5. It was observed that the hybrid with highest RS content in raw grits was low ranking in terms of grain yield but high ranking in terms of flaking grit yield, while the hybrid with lowest RS content in raw grits was high ranking in terms of grain yield but low ranking in terms of flaking grit yield. Once all the hybrids have been evaluated for RS content, correlation should be established between RS content, flaking grit yield and grain yield.

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