IDENTIFICATION OF SPECIFIC STARCH PROFILES IN NORTH DAKOTA STATE UNIVERSITY POTATO GERMPLASM FOR NUTRITIONAL AND INDUSTRIAL UTILIZATION

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ABSTRACT

Amylose and amylopectin, the mostly resistant and soluble forms of starch, respectively, are two forms of starch present in the granule. In this study, we examined the effect of a new cooking method, microwave steaming, on soluble starch and resistant starch in order to determine and determined that this method may be used as a more efficient means to cook tuber material for starch analysis. Using the steaming method, we found clones present in the North Dakota State University potato breeding program with unique levels of soluble or resistant starch. Clones with high or low levels of soluble or resistant starch displayed diverse granule sizes, pasting characteristics, gelatinization temperatures, and amylose and amylopectin molecular weight and abundance. Greenhouse-grown tubers were found to contain more soluble and resistant starch content than field-grown tubers, implying that greenhouse-grown tubers cannot be used to screen genotypes for starch content.

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ΔH	Enthalpy of gelatinization
ANOVA	Analysis of variance
BKD	Breakdown
CPV	Cold paste viscosity
DSC	Differential scanning calorimetry
GI	Glycemic index
GLM ANOVA	Generalized linear model ANOVA
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
HPV	Hot paste viscosity
HSD	Honest significant difference
LSD	Least significant difference
MALS	Multi-angle light scattering
NDSU	North Dakota State University
PC	Personal computer
PV	Peak viscosity
RDS	Rapidly digestible starch
RI	Refractive index
RS	Resistant starch
RVA	Rapid visco analyzer
SDS	Slowly digestible starch
SEM	Scanning electron microscopy
SS	Soluble starch
STB	Setback

T_c.....End

T_o .....Onset

T_p .....Peak

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CHAPTER 1. A REVIEW OF POTATO STARCH VARIABILITY AND ITS INFLUENCE IN PRODUCT DEVELOPMENT

Abstract

Potato starch is an important source of energy for many societies in the world and is also used in a variety of industrial applications. From a nutritional standpoint, soluble and resistant forms of starch, and their ratios, contribute to the glycemic index of food products. While soluble starch (SS) is known for its positive correlation with glycemic response, resistant starch (RS) has received attention for its health benefits, due to its similarity to dietary fiber. RS serves a beneficial role in gut microbial flora and assists in blood glucose control. A variety of modification processes are available for starch, in order to develop products tailored to consumer needs. This review summarizes potato starch, its uses for consumer applications, and highlights the need to produce improved potato cultivars that provide specific starch profiles for innovative applications.

Introduction

As an economically important staple crop across the world, the potato (*Solanum tuberosum* L.) has large scale production, consumption, and affordability. Potato is the most important non-cereal crop consumed in more countries than any other crop produced for consumption (CIP International Potato Center 2016). In 2015, the total value of production for potatoes was over $230 million, ranked fourth in value behind canola, barley, and hay (USDA 2015). About 30% of starch utilized within Europe and the US is used for consumption, where around 70% is utilized for industrial applications (Lillford and Morrison 1997). According to a recent review by Zaheer and Akhtar (2014), potatoes range in size, color, shape, starch content,

and flavor. There are over 4,000 varieties of potato worldwide (CIP International Potato Center 2016).

Many types of potato are grown for their unique starch attributes for specific end uses. Starch is composed of a mostly linear chain of α (1→4) linked D-glucose called amylose and a highly branched chain of α (1→4) D-glucose with α (1→6) branch points called amylopectin (Smith 2001). The total starch present among potato genotypes has been shown to be about 9 to 23% of the fresh weight (Burlingame et al. 2009) and between 66 and 80% of the dry matter (Liu et al. 2003 and Liu et al. 2007). Levels of amylose and amylopectin contribute to the glycemic index. The potential for carbohydrates to raise blood-glucose levels is referred to as the glycemic index (GI) (Jenkins et al. 1981). Foods with a GI above 70 are considered as high GI, whereas foods with a value of 56-69 are considered medium GI, and foods with a GI under 55 are considered low GI (ISO Standard 26642:2010). Studies have shown potatoes to range in GI from 56 to 104, indicating that consuming different genotypes elicit different glycemic responses (Fernandes et al. 2005, Henry et al. 2005, Leeman et al. 2005, Atkinson et al. 2008).

Since one single potato variety will not provide the appropriate attributes needed for every product, it is important to screen various cultivars and potato selections in order to find the most appropriate clone for specific end uses. Techniques have been developed in order to identify the most applicable potato genotype for a distinct industrial purpose (Singh et al. 2007). Potatoes, made primarily out of starch, may undergo modifications in order to meet consumer needs (Kraak 1992). Although potato producers are interested in increasing their domestic and global market shares, the media often associates potatoes with obesity, diabetes, and other nutritional issues. Contrary to the popular assumption that the potato provides negative attributes to human health, potatoes have been shown to be beneficial to the human diet (Stelljes 2001).

The purpose of this review is to introduce a variety of relatively recent studies that are related to factors that affect potato starch. The likelihood for a cultivar to possess strong desirability for a specific market depends on a wide variety of factors including specific gravity, dry matter content, and characterization of starch attributes (Haase 2003).

Starch Content and Digestibility

Raw potato starch consists of large amounts of RS that is converted to digestible starch after cooking. Foods high in rapidly digested starch have a high GI and elicit high insulin demand (Augustin et al. 2002). Jenkins et al. (1981) describes GI as the response of test foods compared to reference foods, such as glucose and white bread. Amylopectin typically makes up 70-80% of the available starch in the potato tuber, with the rest consisting of amylose (Zeeman et al. 2010). Amylopectin and amylose production are under enzymatic control. Granule-bound starch synthase (GBSS) is responsible for the production of amylose (Fulton et al. 2002), while many enzymes are responsible for amylopectin production (Smith 2001). GBSS also is capable of elongating amylopectin chains (Denyer et al. 1996). These enzymes are responsible for the production of starch granules in potato (Zeeman et al. 2010). The ratio of amylose to amylopectin, as well as their molecular structure influence, determine the end use of the potato's application (Blazek and Copeland 2008). Genotype and environment were shown to be the most significant factors contributing to variations in starch profiles among genotypes (Bach et al. 2013).

Amylose content and gelatinization temperature have both been shown to have a positive correlation with granule size (Geddes et al. 1965). Immature tubers contain a higher ratio of amylose and sucrose, compared to mature tubers (Jansky and Fajardo 2016). An increase in tuber growth from 1.0 to 2.5 cm in diameter has been shown to increase the starch content from

11.4 to 16.0% in white-skin potatoes, and from 6.6 to 11.5% in red-skin varieties (Qudrat-I-Khuda et al. 1964). This study also concluded that white-skinned potato stores more starch at all stages of growth, compared to red-skinned potato.

Three enzymes are responsible for the breakdown of straight-chain starches: α-amylase (α- 1,4-glucan glucanohydrolase; EC: 3.2.1.1), β-amylase (α-1,4-glucan malto- hydrolase; EC: 3.2.1.2), and α-glucosidase (α-1,4-glucosidase; EC 3.2.1.20) (Zobel et al. 2009). Amylopectin is a larger molecule than amylose, resulting in a larger surface area for amylolytic attack (Singh et al. 2010). The tight coiling of amylose also provides resistance to breakdown (Taiz and Zeiger 2010). The degradation of these starch chains is important in digestion, additionaly, the rate at which these starch chains are broken down is correlated with the glycemic index of the potato (Ek et al. 2012).

Retrogradation of starch occurs when starch recrystallizes during storage of starch paste or other products that contain starch. Retrogradation was first observed in 1852 (Boussingault 1852). This outcome influences food quality and other applications. The definition of retrogradation is the linkage of starch chains into organized crystalline structures (Eerlingen and Delcour 1995). Amylose, the linear branched starch, is the most susceptible to retrogradation due to its few branching sites (Tharanathan 2002). Amylose that has undergone crystallization results in resistance to amylase activity. Retrogradation of amylopectin is slow due to its highly branched nature (Miles et al. 1985). The process of retrogradation must be taken into account when extracting potato starch for food and industrial purposes in order to ensure the best quality of the product.

Repeated retrogradation has been studied on waxy potato starch, in order to determine structural differences (Xie et al. 2014). The maximum level of slowly digestible starch reached

in this study after repeated retrogradation was 40.41% (Xie et al. 2014). Other characteristics of the starch, including melting temperature range, onset temperature, and melting enthalpy also were studied. These analyses are important when preparing starch material for industrial products.

Gelatinization of starch is a process of breaking various intermolecular bonds within molecules of starch in the presence of water, ultimately causing the starch granules to swell (Parker and Ring 2001). The water that is absorbed by the starch granule is irreversible. As a result, the starch mixture turns viscous and transparent. Gelatinization is a common practice in the food industry that alters viscosity properties of the material that the starch is contained within (Parker and Ring 2001). The process of gelatinization also makes starch more susceptible to enzyme activity (Noda et al. 2008).

Structural and gelatinization characteristics were recently studied in five wild type potato starches, five amylose-free potato starches, and four high-amylose potato starches (Gomand et al. 2009). The molecular size of amylose and amylopectin were studied, along with amylopectin chain length distribution, crystallinity, and granular structure. Researchers found that wild-type potato starch granules were larger than amylose-free potato starch and high-amylose potato starch (Gomand et al. 2009).

A recent study examined starch fractions within a variety of potato genotypes after cooking and after cooking and storing cold (Monro et al. 2008). RS, rapidly digestible, and slowly digestible starch were measured within nine commercial potatoes from New Zealand, and also in 37 lines from a potato breeding program. Starch fractions were examined right after cooking, or after the cooked tuber material was stored at 4°C for 44 hours. In the immediately cooked potatoes, the potatoes consisted of 68% rapidly digestible starch, 3% slowly digestible

starch, and 3.9% RS based on mean and across-cultivar range on a dry matter basis (Monro et al. 2008). Cooling of the cooked tuber material resulted in altered starch fractions consisting of 44% rapidly digestible starch, 23% slowly digestible starch, and 7% RS. The study showed that the 37 potato lines within the breeding program contained 7-37% slowly digestible starch and 12-27% RS in the cooked-cooled potatoes. Clearly, cooling of cooked potato material has an impact on the starch profile of potato. The results indicated that the glycemic index of selected potatoes may be decreased after cooling of cooked potato. Differences in starch levels among potato lines should be taken into consideration when utilizing plant breeding as a means of altering the starch profile.

Salts have a significant effect on the properties of starch. Salting-out ions have been shown to increase gelatinization temperature and enthalpy, whereas salting-in ions have been shown to have the opposite effect (Zhou et al. 2014). Since the phosphate monoester groups in potato starch are negatively charged, the result is an ionic repulsion, weakening the organization of starch molecules and increasing the water-binding capacity (Zhou et al. 2014). This explains why salts have the ability to alter physicochemical properties within potato starch.

Environmental growing conditions also have been shown to have a significant effect on starch. Three potato cultivars (Shepody, Innovator, and Russet Burbank) were grown at two distinct locations in Canada (New Brunswick and Manitoba) (Chung et al. 2014). As a result of the study, the amount of total starch from dried potato was shown to be higher in cultivars grown in New Brunswick, than cultivars grown in Manitoba. The cultivar Innovator had the highest total starch, compared to the other two. This study concluded that growing conditions influence starch crystalline and molecular structures (Chung et al. 2014). Cultivar by location has a

significant effect and should be taken into account when growing cultivars for a desired starch profile.

With high-amylopectin starches on the rise for industrial purposes, like pastes and films, waxy potato cultivars have been developed for the purpose of improved material characteristics. Šimková et al. (2013) examined 16 potato cultivars, grown at five locations over four years, for changes in total starch, amylose, phosphorus content, and starch grain size. Cultivar was shown to have an affect on all parameters (starch, amylose, phosphorus, and starch granule size). Location and year were also shown to have a significant, but lower, effect. There was no significant effect of year on amylose levels. Similar to the study by Chung et al. (2014), Šimková et al. (2013) found growing location to influence starch properties.

Industrial Applications and Genetic Improvement

Potatoes are produced into a variety of forms including French fries, chips, baked, and mashed. As no single potato cultivar has been shown to be appropriate for all food applications, screening of cultivars is needed for specific end use, for their ability to provide optimum processing performance, and maximum product quality (Singh et al. 2005). Growing conditions, genotype, and tuber physiological age affect potato quality for processing (Freitas et al. 2012). Cultivar differences are mainly responsible for the variation in processed potato products (Arvanitoyannis et al. 2008). Approximately 30% of starch used in Europe and the US is used as native starch for consumption; whereas about 70% is used for industrial purposes (Lillford and Morrison 1997).

Potato starch is used in industrial applications such as, but not limited to, adhesives, paper, textiles, and biodegradables. Starch has traditionally been used in functions of thickening and adhesion, but demand for plant-based biodegradables has increased (Rosentrater and Otieno

2006). Researches have focused attention on modified starches for properties including stability, shelf life, expansion, and texture (Kraak 1992). Various alterations are applicable to starch in order to produce specific applications, such as water resistant material (Peltonen and Harju 1996), biodegradable films (Jobling 2004), and microcapsules for small molecules (Korus et al. 2003). Although starch from potato provides good texture stabilization and regulation in food applications, the low shear resistance, thermal decomposition and resistance, and high level of retrogradation have limited native starch use in industrial applications (Cousidine 1982). However, modification of starch can improve its functional characteristics (Hermansson and Svegmark 1996). Modifications are generally performed by etherification, esterification, cross-linking, and grafting (Singh et al. 2007). The structure and composition of starch granules differs across plants and ultimately affects the functions and specific properties of starches from different crops. A unique characteristic found within potato starch is the high level of phosphate groups linked covalently to the C3 and C6 positions of the glucose units (Hizukuri et al. 1970). The phosphate groups associated with the glucose monomers give potato starch a high power of swelling, which is related to pasting behavior and to rheological properties (Sitohy et al. 2000).

Potato starch also has been known for its applicability in films and other synthetic polymers. A recent study examined the material properties of genetically modified potatoes with varying amylopectin structure and amylose content. As a result of genetic modification, potato starch with high levels of amylose and an increase in amylopectin chain length was produced (Menzel et al. 2015). The modified starch content also resulted in different granule morphology due to the compositional change. The increase in amylose and change in amylopectin structure improved the starch's ability to form a film with enhanced tensile properties. The long chains of amylopectin were shown to be involved in the intertwined molecular network. The high amylose

content and long-chain amylopectin produced a cohesive film with a coarse surface and enhanced physical properties that have potential to be used as barrier coatings (Menzel et al. 2015).

Freeze-thaw ability is important to consider for many applications, especially those with cold storage of starchy products. Due to lack of amylose, waxy starch has relatively improved freeze-thaw stability when compared to normal starches (Zheng and Sosulski 1998). Potatoes may be advantageous in cold storage applications due to their high amylopectin content.

Health Concerns and Nutrient Content

The potato requires continued genetic improvement, to accommodate the needs of a changing world, due to increased demands for a healthier potato (Douches et al. 1996). The modern potato breeder has an opportunity to incorporate genomes that produce potatoes with unique starch characteristics desired for specific markets. Potato breeding is a difficult task due to inherent biological factors including cytoplasmic and nuclear sterility, tetrasomic inheritance, and inbreeding depression (Douches et al. 1996). Male sterility in potatoes results from the absence of pollen (Salaman and Lesley 1922). Unfavorable genes can remain "hidden" due to the tetraploid nature of the potato (Lindhout et al. 2011), resulting in limited genetic gain through traditional breeding. Inbreeding reduces the fitness of a species and has been studied for centuries. Darwin (1876) first demonstrated inbreeding depression.

In a review by Willet et al. (2002), it was noted that potatoes are rich in a variety of essential nutrients including carbohydrates, proteins, vitamin C, vitamin B6, magnesium, potassium, and fiber. Potatoes play an influential role in the production of the antioxidant defense system by contributing essential nutrients including vitamins, β-carotene, polyphenols, and minerals. Incorporating potatoes into the diet provides a good source of energy and

nutrients; however, potatoes have been associated with undesirable health effects. Due to their purported high glycemic index, potatoes are perceived as bad for health. Diets with high glycemic indices have been associated with increased risk of diabetes and cardiovascular disease (Willet et al. 2002). This supports the need to produce and examine improved cultivars of potato that have increased health benefits and a reduced glycemic index.

A recent study (Tahvonen et al. 2006) compared cooking method, peeling method, and processing method on the glycemic response of 22 healthy volunteers. Volunteers were chosen based on normal health and normal glucose resistance. As a result of this study, it was determined that cooking method, peeling method, and processing method (slicing or mashing) did not influence the glycemic index. However, cooling and cold storage were found to lower the glycemic index of the potato product by nearly 25%. Reheating of the cooled and cold-stored potato product did not influence the glycemic index compared to the cooled potato. After cooling, amylose goes through retrogradation, resulting in more crystalline starch and an increase in resistance to digestive enzymes (Miles et al. 1985), explaining why the glycemic index decreases for cold cooked potatoes compared to hot, cooked potatoes.

Potato Storage Influence on Starch

Storage is an important requirement in order to prolong the shelf life of potatoes past the growing season. Good storage conditions should be met in order to prevent a large amount of weight loss, invasion of pathogens, and the growth of sprouts (Schippers 1976). These ideal conditions include protection from light, and temperature and humidity control (Schippers 1976). Although weight loss is considered an economic factor comparable to yield reduction, it also is a factor that affects tuber appearance (Schippers 1976). Tuber weight loss occurs via transpiration and respiration (Schippers 1976). Weight loss between five and 10 percent will cause tubers to

feel progressively soft and they will begin to wrinkle at 8 or 9 percent (Schippers 1976). Deteriorated tubers cannot be used for table stock or for other uses, with the exception of livestock feed or starch extraction, implying that storage conditions for potatoes are extremely important for maintenance.

Formation of reducing sugars is of importance during potato storage. Starch is the source of sugars that are produced during low temperature induced sweetening (Isherwood 1973). Greater amylolytic activity was found in tubers stored at low, compared to high, temperatures (Bielinska-Czarnecka and Bialek 1977; Nowak 1977). The hydrolysis rate of α-amylase, β-amylase, and debranching enzyme have been shown to increase sharply during the first weeks of storage at 4°C; however, the activity of these enzymes increased slightly or remained constant when stored at 10°C (Cottrell et al. 1993). Although variety plays a role in the development of reducing sugars, storage conditions also contribute to this undesirable attribute. Once sugar formation begins, storage continuation influences the sugar level (Schippers 1976). Immature tubers contain a higher amount of sucrose, compared to mature tubers (Schippers 1976) and a higher ratio of amylose in starch, compared to mature tubers (Jansky and Fajardo 2016). Degree of brownness of chips (and similarly French fries) is a result of the level of reducing sugars (glucose and fructose) in the tuber (Schippers 1976). Upon frying, the sugars react with amino acids in the Maillard reaction, resulting in colored compounds that affect chip color and flavor (Shallenberger et al. 1959). Enzymatic conversion occurs simultaneously with starch synthesis from sugar and potato respiration (Singh et al. 1976). Respiration converts sugars into carbon dioxide and water and is highly temperature dependent; an increase in temperature results in an increase in respiration (Singh et al. 1976). A reduced rate in respiration is desired in order to achieve longer storage periods and reduced weight loss (Singh et al. 1976).

The end use of potatoes determines the storage conditions utilized. Generally, potatoes used for chips are stored at 10 to 12.8°C, those for French fries are kept at 7.2 to 8.8°C, and fresh and seed potatoes are kept near 4.4°C (The Potato Association of America 2010). Reconditioning is known as increasing the storage temperature for tubers to 15 or 20°C. The result of reconditioning on chipper varieties may decrease the level of reducing sugars via respiration, converting the sugars into starch (Schippers 1976). Varieties may be more or less sensitive to changes in storage conditions (Schippers 1976). Potatoes purchased fresh are usually advised to be stored unrefrigerated in a cool dark place (5.6-12.8°C); higher temperatures promote sprouting and cooler temperatures induce sugar accumulation (Woodell et al. 2009).

Kaur et al. (2009) compared starch properties of 11 potato cultivars at storage temperatures of 4, 8, 12, 16, and 20°C for 120 days. The researchers reported that the amount of amylose, as well as the swelling power, increased as the storage temperature increased. The amount of small-sized granules was more pronounced in potatoes that were stored at 4°C. The potatoes that were stored at 20°C exhibited a higher peak viscosity, set back, along with gel hardness, gumminess, and chewiness, than potatoes that were stored at the lower temperatures. The amylose content indicated a positive correlation with setback and gumminess. Swelling power was significantly positively correlated with hot-paste viscosity and peak viscosity. The results of this study may provide insight into proper storage techniques to maintain potato qualities for specific starch attributes and end uses.

Conclusions and Future Improvements

Although it is known that crystalline and amorphous regions are present within the granule, and that amylopectin forms the basis of the semi-crystalline starch granule, the precise location of amylose within the starch granule is often debated (Jane 2006). However, amylose is

proposed to be interspersed among amylopectin within the granule (Zobel 1988). Further advances are necessary in order to determine the specific pathway in which the granule is produced and also degraded after gelatinization. These improvements, along with further understanding of variables that influence starch content, can aid in proper handling of starch material in order to provide optimum quality in products.

Screening of cultivars is necessary to determine their specific end use, as well as their capacity to contribute optimum processing performance and quality, since no single potato cultivar has been shown to be applicable for all food and industrial applications. Potato starch profiles vary due to genotype, environmental factors, and storage conditions. Further research regarding environmental and storage influence on starch profiles is necessary to maintain desired starch characteristics for product developments and industrial applications.

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CHAPTER 2. COOKING METHOD ASSESSMENT: IMPACT ON STARCH CONTENT

Abstract

The potato is a valued crop providing carbohydrate calories to the human diet. Complex carbohydrates in potato consist of amylose and amylopectin, making up the starch granule. The granule undergoes gelatinization after cooking, resulting in accessibility for digestive enzymes. Although baking and boiling potatoes for starch analysis is common, these techniques are time consuming when a large number of samples are to be analyzed. Steaming in microwave-safe bags is a relatively new technique that has not previously been studied for starch assessment purposes and could provide for a more efficient means to analyze a large number of samples. The research objective was to determine whether cooking method (baked, boiled, or steamed) influences soluble starch (SS) and/or resistant starch (RS) levels of potato genotypes. Three varieties (Red Norland, Russet Burbank, and Yukon Gold) were evaluated. Additionally, samples were examined from two temperatures (hot (60°C) and chilled overnight (4°C)). Results indicated that for both SS and RS, variety, cooking method, temperature, cooking method x temperature, and variety x cooking method x temperature were significant factors. The insignificance of variety x cooking method indicates that the three cooking methods did not influence SS or RS levels among varieties, implying steam bags may serve as a more efficient means for cooking and preparing a large number of potato genotypes for starch quantification.

Introduction

Potato (*Solanum tuberosum* L.) is a carbohydrate-rich staple crop, contributing a variety of vitamins and nutrients, including vitamins B1, B2, and B6, and C, as well as minerals such as potassium, magnesium, and phosphorus (Burlingame et al. 2009). Dietary energy intake is

composed of 40-75% available carbohydrate, in which starch is the most abundant (Nantel 1998). Although the potato has been negatively viewed in the media, due to its high carbohydrate nature and relatively high glycemic index, the potato yields more nutrient-dense food more quickly, on less land area, and in harsher climates than any other major food crop (FAO, 2005). Worldwide, potato has the ability to contribute to improved diets, resulting in reduced mortality rates caused by malnutrition.

Starch is packaged into granules that consist of varying amounts of amylose, a straight chain polyglucan, and amylopectin, a branched glucan (Zobel 1988). Cooking disturbs the starch granule, providing access to digestive enzymes. Soluble starch (SS) consists of starch that undergoes degradation by enzymes, such as α-amylase, β-amylase, and amyloglucosidase, and is utilized for energy in the body (Zobel et al. 2009). Resistant starch (RS) consists of starch degradation products that are unable to be absorbed by the small intestine and pass to the large intestine (Berry 1986). Amylopectin typically makes up 70-80% of the available starch in cooked potato, with the rest consisting of amylose (Zeeman et al. 2010).

Amylose is a straight chain polysaccharide composed of approximately 1000 α-D-(1-4) linked glucose units, while amylopectin is a highly branched polysaccharide compromised of approximately 4,000 glucose units with branches formed from α-D-(1-6) linkages (Haralampu 2000 and Sharma et al. 2008). Resistant starch (RS) is referred as a type of starch that resists digestion and passes through the gastrointestinal tract, where it functions as dietary fiber and prebiotic. A prebiotic is described as a non-digestible food component that stimulates the growth and/or activity of bacteria in the colon (Gibson and Roberfroid 1995). Indigestible starch is referred to as starch that has not been hydrolyzed within 120 min after being consumed (Fuentes-Zaragoza et al. 2011). Carbohydrates are broken down into glucose after consumption. The

potential for carbohydrates to raise blood-glucose levels, compared to either pure glucose or white bread, is referred to as the glycemic index (GI) (Jenkins et al. 1981). Foods with a GI above 70 are considered high, whereas foods with a value of 56-69 are considered medium, and foods with a GI under 55 are considered low (ISO Standard 26642:2010). The GI of potatoes ranges from 56, to as high as 104, depending on the genotype and service method (Soh and Brand-Miller 1999, Fernandes et al. 2005, Henry et al. 2005, Leeman et al. 2005a, Atkinson et al. 2008, Leeman et al. 2008, and Kinnear et al. 2011).

The relationship between carbohydrate digestion and glycemic index requires a different classification system than that of quantification of amylose and amylopectin content. This classification consists of rapidly digestible (RDS), slowly digestible (SDS), and RS. RDS and SDS compromise the starch that is hydrolyzed within the first 20 and 21-120 minutes of consumption, respectively (Fuentes-Zaragoza et al. 2011). RS is undigested until it enters the large intestine and is utilized by gut microflora (Brown et al. 1997). RS is fermented by anaerobic microflora in the colon, releasing short chain fatty acids that can be used as metabolic substrates (Johnson and Gee 1996). As an ingredient in food products, RS has a lower calorie value (8 kJ/g), than fully digestible starch (15 kJ/g); however, it can be introduced into food products, such as baked goods, without influencing processing properties, or the overall appearance and taste of the end product (Rochfort and Panozzo 2007). RS may not be digested in the body due to various reasons. The molecular structure is compact, limiting the accessibility of digestive enzymes and providing resistance of digestion of raw starch granules (Haralampu 2000). Starch granules are structured in a manner that prevents digestive enzymes from hydrolyzing them (Nugent 2005). Starch granules undergo gelatinization under heat stress, restricting the molecules' accessibility to digestive enzymes. Gelatinization occurs after starch

granules are heated during the cooking process of potatoes; intra- and inter- chain hydrogen bonds present between amylose and amylopectin are hydrolyzed, causing water molecules to bind with the hydroxyl groups (Parker and Ring 2001). However, starch gels that are cooled following heat stress can form starch crystals that are resistant to enzymatic hydrolysis. This process is known as retrogradation (Nugent 2005). Chemically modified starches that have altered etherisation or cross-bonding are unable to be hydrolyzed by digestive enzymes (Lunn and Buttriss 2007).

Potatoes can be prepared by several different cooking methods prior to consumption, including baking, boiling, microwaving, and frying. Studies have been performed on different potato varieties and indicate that cooked potatoes consist mostly of rapidly digested starch (Leeman et al. 2005b), which elicits a high glycemic index (Atkinson et al. 2008). Although potatoes provide many vitamins and minerals, some nutritionists advise that potatoes should be substituted with a carbohydrate evoking a lower glycemic index, in order to reduce the risk of chronic diseases (Brand-Miller et al. 2009). Most potato varieties generate a medium to high glycemic index; however, there are some varieties that elicit a low glycemic response (Ek et al. 2012). The method of preparation of cooked potato also influences GI (Kinnear et al. 2011).

The purpose of this study was to determine whether microwave steaming, which has not been investigated previously as a preparation method for starch analysis, affects SS or RS levels of potato genotypes. Although studies have been performed comparing baking and boiling (Raatz et al. 2016), the microwave steam bag method has not been previously employed.

Materials and Methods

Potatoes

Three commercial varieties (Red Norland, Russet Burbank, and Yukon Gold) grown by the NDSU Potato Research team and area growers during the 2014 growing season were used in this study. Russet Burbank and Yukon Gold were produced with irrigation, while Red Norland was from a non-irrigated site. The potatoes were stored at 3.3°C for approximately four months prior to analysis. Each variety was subjected to three cooking methods (baked, boiled or steamed using Ziploc® Zip'n Steam bags) and evaluated at two service temperatures: hot (60°C) and chilled overnight (4°C).

Starch Analysis

Two tubers of each variety were used for starch analysis. Evenly sized tubers were processed in one of three ways: 1) washed, pierced, wrapped in tinfoil, and baked in an oven at 177°C (350°F) for 1 hour (baked); 2) washed, peeled, cut into identically sized pieces (2.5 cm^2) and boiled in water at 100°C for 15 minutes until tender (boiled); and 3) washed, peeled, cut into identically sized pieces (2.5 cm^2) and placed into a Ziploc® Zip'n Steam bag, and microwaved (1200W) for 4 minutes on high (steamed). The cooked tubers were then riced and mixed for each variety.

SS and RS were determined using the Megazyme Resistant Starch Assay (K-RSTAR, Megazyme International Ireland, Ltd, Co. Wicklow, Ireland) kit. A modified miniaturization of the assay, as described by Raatz et al. (2016) was utilized, with the incorporation of sodium azide, in order to prevent alteration of the starch profile by microbes. Samples of potato were prepared and analyzed in triplicate. Riced samples of potato (0.50 g) were weighed into 15 ml centrifuge tubes and 4 mL of pancreatic amylase solution (10 mg/mL) (3U/mL

amyloglucosidase)/sodium azide (0.03%) was added into each tube. The tubes were capped and placed into a continuous shaking water bath at 37°C at 100 rpm for precisely 16 hrs.

Termination of the hydrolysis reaction was performed by adding 4 mL of 95% ethanol to each sample. Recovery of RS was performed by centrifugation (2000 x g, 10 min at RT). The supernatant, containing SS, was decanted into 100 ml volumetric flasks. The RS pellet was washed an additional two times with 8 mL of 50% ethanol, centrifuged (2000 x g, 10 min at RT), and decanted into 100 ml volumetric flasks.

The pellet containing RS was dissolved by adding 2 mL of 2 M KOH along with vigorous stirring, within an ice-water bath over a magnetic stirrer. The RS solution was neutralized by adding 8 mL of 1.2 M sodium acetate buffer (pH 3.8) and immediately adding 0.1 mL amyloglucosidase. The samples were incubated in a water bath at 50°C for 60 min. The contents in the tube were diluted 1:10 using a 100 mL volumetric flask. Aliquots of each solution were centrifuged (1500 x g, 10 min), 40 µL of the supernatant was transferred to 2.0 mL microtubes, and was mixed with 1.2 mL glucose oxidase-peroxidase-4-aminoantipyrine reagent (Megazyme Resistant Starch Assay, Megazyme International Ireland Ltd, Co. Wicklow, Ireland). The microtubes were placed in a water bath at 50°C for 20 min. The mixtures were transferred to a 96-well plate, where the absorbance was read against a reagent blank at 510 nm, utilizing a microplate reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA, USA).

The SS supernatant in the 100 mL volumetric flasks was filled to 100 mL with 100 mM sodium acetate buffer and mixed. A 1:2 dilution of the SS solution, composed of 20 µL SS solution and 20 µL deionized water, was added to 2.0 mL microtubes, along with 4 µL of dilute amyloglucosidase (300 U/mL) and 1.2 mL glucose oxidase-peroxidase-4-aminoantipyrine reagent (Megazyme Resistant Starch Assay, Megazyme International Ireland Ltd, Co. Wicklow,

Ireland). Samples were placed in a 50°C water bath for 20 minutes. The samples were then transferred to a 96-well plate, where the absorbance was read against a reagent blank at 510 nm utilizing a microplate reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA, USA). Two replicates of each sample were analyzed for moisture content. SS and RS content was calculated using the dry weight of each variety. Approximately 0.5 g of cooked tuber material for each variety was placed into glass tubes, placed in a freezer overnight, and freeze-dried for 2 days. The resulting dry weight was calculated using the following formula: 1-(weight following freeze drying/weight following cooking).

Statistical Analysis

The effects of cooking method (baked, boiled, or steamed) and service temperature (hot or chilled), and their influences on the SS and RS content in three potato varieties (Red Norland, Russet Burbank, and Yukon Gold) was evaluated using a 3-way analysis of variance (ANOVA). A factorial model was used with Factor A as variety, Factor B as cooking method, and Factor C as temperature. A generalized linear model (GLM ANOVA) was conducted using SAS 9.3 (SAS Institute, Inc., Cary, NC).

Results and Discussion

Soluble Starch

SS refers to the fraction of starch that is hydrolyzed by digestive enzymes. Multiple studies have shown that RS is composed of a linear molecule of α-1,4-D-glucan, which typically consists of retrograded amylose, and has a molecular weight that is relatively low (1.2×10^5 Da) (Tharanathan 2002). Although baking and boiling potatoes for starch analysis is common, these techniques are time consuming when a large number of samples are to be analyzed. Steaming in

microwave-safe bags is a relatively new technique that has not previously been explored for its influence on SS or RS levels. We hypothesized that this cooking method would not differ from baking or boiling. Each variety was hypothesized to vary in SS and RS, since genotype was shown to be a significant factor contributing to variations in starch profiles (Bach et al. 2013). The ANOVA output for SS is shown in Table 2.1. The significance of the three-way interaction (variety x cooking method x temperature) indicates that there is a two-way interaction that varies across levels of a third variable. To further analyze the three-way interaction, the two-way interactions are discussed. Although variety x cooking method lacked significance, the 2-way interaction plotted against different levels of temperature (hot and cold) expressed significant differences for SS. The significance of cooking method x temperature indicates that there was an interaction between cooking method and temperature on SS. Although the baked and steamed cooking methods varied by magnitude, with SS found to be more present in samples that were analyzed hot compared to cold, the steaming method resulted in a lower level of SS when the

Table 2.1. Analysis of variance for soluble starch for Red Norland, Yukon Gold, and Russet Burbank using three cooking methods (baked, boiled, or steamed in steam bags), and two service temperatures (hot (60°C) and chilled overnight (4°C)).

Source	DF	Type III SS	MS	F	Pr > F
Replicate	2	57965.89	28982.95	2.05	0.14^{ns}
Variety	2	264603.21	132301.61	9.36	0.01*
Cooking Method	2	240754.91	120377.45	8.51	0.01*
Variety x Cooking Method	4	85169.73	21292.43	1.51	0.22^{ns}
Temperature	1	126107.22	126107.22	8.92	0.01*
Variety x Temperature	2	23821.52	11910.76	0.84	0.44^{ns}
Cooking Method x Temperature	2	132551.61	66275.81	4.69	0.02*
Variety x Cooking Method x Temperature	4	182863.69	45715.92	3.23	0.02*

*significant at $\alpha \leq 0.05$, ns = not significant at $\alpha \geq 0.05$.

samples were analyzed hot compared to samples that were analyzed after refrigeration. Significance was shown for temperature x cooking method, suggesting that temperature impacted SS levels when factored by cooking method.

Analysis of variance indicated a lack of significance for variety x temperature and variety x cooking method, the source of variation most meaningful to this study. The lack of significance of variety x cooking method shows that SS did not have an interactive affect between variety and cooking method. This result indicates that the steam bag cooking method did not differ from the baking and boiling cooking method and could be used as a more efficient means of cooking tuber material for SS analysis. Although previous studies have used baking and boiling as a means to cook potatoes for starch analysis, these methods are not sufficient for examining a large number of potato varieties. Baking potatoes takes about an hour and boiling takes up four burners and takes 15-20 minutes at a time, whereas microwave steaming potatoes takes four minutes and can allow two clones to cook at once. The lack of significance of variety x temperature shows that variety and temperature did not have an interactive effect on SS. This insignificance indicates that cooked potato samples could be used at either service temperature without impacting the SS levels, however, the temperature was shown to vary across levels of cooking method when factored by variety, as discussed in the three-way interaction.

Significance was observed for the single effects of variety, cooking method, and temperature. Replicates were not significantly different. As expected, the three varieties used had varying levels of SS. Yukon Gold had a significantly higher average SS value (535.1 mg/g) than Red Norland (406.3 mg/g) and Russet Burbank (372.7 mg/g) (LSD = 80.6). Red Norland and Russet Burbank were not significantly different from one other. Cooking method also was

found to be a significant source of variation, with the baked potatoes containing a significantly higher average level of SS (527.6 mg/g) than boiled (419.0 mg/g) or steamed potatoes (367.4 mg/g), which were not significantly different (LSD = 80.6). Temperature was also a significant source of variation, with the hot service temperature containing a significantly higher average SS level (486.4 mg/g) than a cold service temperature (389.7 mg/g), which was expected. Retrogradation of starch occurs when starch recrystallizes during cooling of starch paste or other products that contain starch (Sharma et al. 2008). Therefore, it is not surprising that the hot service temperature displayed a higher SS level than the cold service temperature.

When comparing SS levels across variety, cooking method, and service temperature (Figure 2.1), SS levels were significantly higher when a hot service temperature was used, compared to a cold service temperature with baked samples of Yukon Gold and Red Norland, boiled samples of Yukon Gold and Russet Burbank, and steamed samples of Yukon Gold. There was no significant difference between hot and cold service temperatures on SS for Russet Burbank baked, and Red Norland boiled and steamed. SS was significantly higher when a cold service temperature was used for steamed Russet Burbank. These results could provide insight into producing an edible potato product with a relatively lower glycemic index.

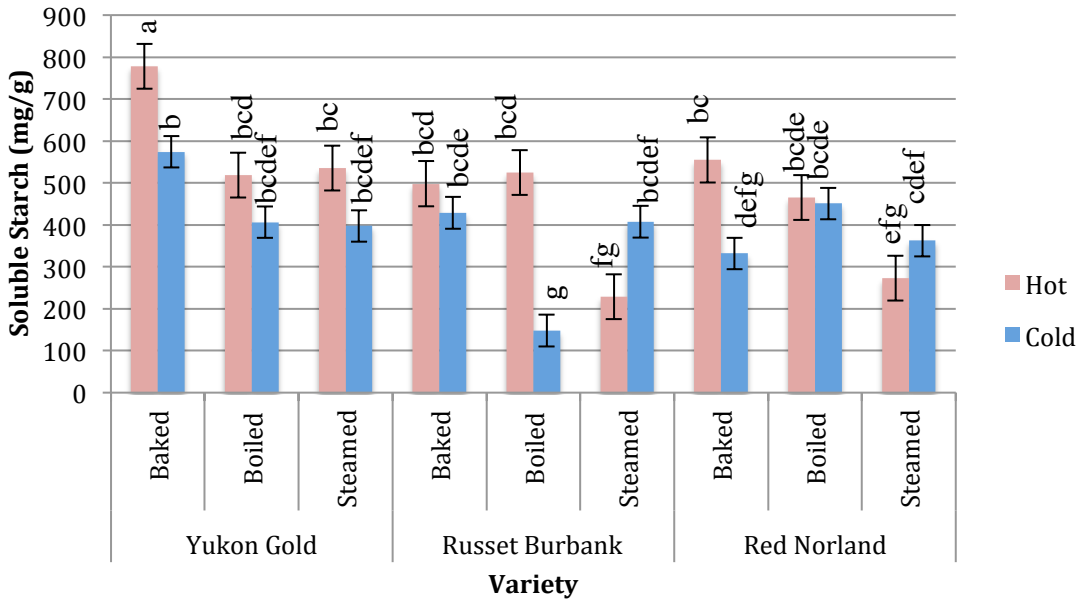

Figure 2.1. Soluble starch levels (mg/g dry weight) for three varieties, three cooking methods (baked, boiled, and steamed), and two service temperatures (hot and cold). SE for hot = 35.8; SE for cold = 28.4. LSD = 202.55.

Resistant Starch

The ANOVA output for RS is presented in Table 2.2. The significance of variety x cooking method x temperature indicates that there is a two-way interaction that varies across levels of a third variable. To further analyze the three-way interaction for RS, the two-way interactions are discussed. Although variety x cooking method lacked significance, the 2-way interaction plotted against different levels of temperature (hot and cold) were significantly different for RS. The significance of cooking method x temperature indicates that the samples analyzed hot differed from the samples analyzed after refrigeration when factored by cooking method. Cold samples from steamed potatoes (158.1 mg/g), and cold and hot samples of baked potatoes (157.8 mg/g and 140.1 mg/g, respectively) had the highest levels of RS and were not significantly different. Hot samples of boiled and steamed potatoes had significantly lower levels of RS (91.8 mg/g and 81.5 mg/g). The significance of the single effects are further

discussed. Temperature x cooking method was significant, indicating that RS levels were impacted by temperature when factored by cooking method.

Table 2.2. Analysis of variance for resistant starch for Red Norland, Yukon Gold, and Russet Burbank using three cooking methods (baked, boiled, or steamed in steam bags), and two service temperatures (hot (60°C) and chilled overnight (4°C)).

Source	DF	SS	MS	F	Pr > F
Replicate	2	876.63	438.31	0.44	0.65^{ns}
Variety	2	16592.16	8296.08	8.30	0.01*
Cooking Method	2	15521.93	7760.96	7.76	0.01*
Variety x Cooking Method	4	5699.11	1424.78	1.42	0.25^{ns}
Temperature	1	24667.07	24667.07	24.67	0.01*
Variety x Temperature	2	5212.62	2606.31	2.61	0.09^{ns}
Cooking Method x Temperature	2	8333.14	4166.57	4.17	0.02*
Variety x Cooking Method x Temperature	4	11255.56	2813.89	2.81	0.04*

*significant at $\alpha \leq 0.05$, ns = not significant at $\alpha \geq 0.05$.

ANOVA indicated a lack of significance for variety x temperature and variety x cooking method, the source of variation most meaningful to this study. The lack of significance of variety x cooking method shows that variety and cooking method did not have an interactive affect on RS levels. This indicates that the steam bag cooking method did not differ from the baking and boiling methods, and may be used to analyze a large number of potato clones for RS more efficiently. The lack of significance for variety x temperature shows that variety and temperature did not have an interactive affect on RS. This indicates that cooked potato samples can be used at either service temperature without impacting RS levels. However, temperature was shown to vary across levels of cooking method when factored by variety, as discussed in the three-way interaction. The significance of cooking method x temperature suggests that there was an interaction between cooking method and temperature on RS, but may have contributed mostly due to temperature since service temperature was highly significant.

Results indicated significance for the single effects of variety, cooking method, and temperature on RS levels. There was no significance between replicates. As expected, the three varieties used had varying levels of RS. Bach et al. (2013) found genotype to be a contributing factor to varying starch levels. Yukon Gold had a significantly higher average RS value (147.6 mg/g) than Red Norland (125.2 mg/g) and Russet Burbank (104.7 mg/g) (LSD = 21.4). The significance of variety on RS is contradictory to a study performed by Raatz et al. (2016); they did not find a difference in RS levels between the varieties Yukon Gold, Red Norland, and Russet Burbank. However, the steam bag method was not incorporated into their study. The varieties used by Raatz et al. (2016) were grown at different locations in North Dakota and Minnesota in the 2013 growing season, which also could explain the variation in results. It was not clear whether the clones used by Raatz et al. (2016) were grown at the same location or how and for how long the potatoes were stored prior to analysis. Although genotype is a contributing factor, the effects of temperature, precipitation, and other climatic factors has been shown to impact starch production in potato genotypes grown at the same location in different years (Bach et al. 2013).

Significantly higher average RS levels were found in baked (149.0 mg/g) compared to steamed (119.8 mg/g) and boiled (108.8 mg/g) (LSD = 21.4); the latter two were not significantly different from one another. This result agreed with that of Raatz et al. (2016), who also found that baked potatoes had a higher level of RS than boiled. Potatoes analyzed with a cold service temperature had a significantly higher average RS level (147.2 mg/g) than potatoes analyzed at a hot service temperature (104.5 mg/g) (LSD = 17.5), which was expected. A previous study performed by Kinnear et al. (2011) indicated that cooling of potatoes resulted in a reduction in GI between 0-50%, depending on the variety. Starch from potatoes that are cooled

after cooking undergo retrogradation and become resistant to enzymatic digestion (Sharma et al. 2008).

Yukon Gold was significantly higher in SS and RS than either Red Norland or Russet Burbank (Figure 2.1 and Figure 2.2). Red Norland and Russet Burbank were not significantly different for SS and RS. Higher levels of SS and RS were found for baking, compared to boiling and steaming methods. Raatz et al. (2016) also observed baked potatoes to have higher RS content than boiled potatoes; however, Raatz et al. (2016) did not measure the SS content and only compared baking and boiling. The boiling and steaming methods were not significantly different from one another for SS and RS. The hot service temperature had significantly higher SS and RS levels than the cold. Gelatinized starch recrystallizes after cooling into a more solid state, which is less susceptible to pancreatic amylase; however, a small fraction of mainly retrograded amylose was found to remain even after reheating of potato (Englyst et al. 1992). Kingman and Englyst (1994) indicated that cooked potatoes have mostly rapidly digestible starch and only a small fraction of unhydrolysed starch after 20 minutes of incubation with pancreatin and amyloglucosidase; cooled potatoes had more RS compared to cooked potatoes due to recrystallization. Although precautionary measures were performed in order to prevent retrogradation during analysis, perhaps retrogradation took place, resulting in starch that was not physically accessible for hydrolysis enzymes.

RS levels were not impacted by variety for the three cooking methods, thus steaming may be utilized in studies determining RS. Steaming, using microwave-safe steam bags, is an efficient means to cook a large numbers of tuber samples for starch analysis compared to baking and boiling. Baking potatoes takes about an hour and boiling requires minimally 15-20 minutes

at a time. However, the steam bags take four minutes in the microwave and it is possible to cook two samples at once.

When comparing RS content across variety, cooking method, and service temperature (Figure 2.2), RS was significantly higher when a cold service temperature was used with boiled samples of Yukon Gold and Red Norland, baked samples of Red Norland, and steamed samples of Yukon Gold, Russet Burbank, and Red Norland. Interestingly, all cooking methods for Red Norland contained higher levels of RS when analyzed at a cold service temperature compared to a hot service temperature, which was not necessarily the case for the other clones. Raatz et al. (2016) found chilled potatoes to contain more RS than hot potatoes. The mean RS level across all clones for cold samples (147.2 mg/g) was significantly higher than the hot samples (104.5 mg/g) (LSD = 17.5), agreeing with the results from Raatz et al. (2016).

The glycemic index of starch-based foods may depend on a variety of factors, including the amylose: amylopectin ratio, starch gelatinization, water content, and the temperature at which the starch was cooked (Fuentes-Zaragoza et al. 2010). Variability in amylose content may demonstrate most of the variation in glycemic index values of rice, as well as other foods, due to the slower digestibility of amylose compared to amylopectin (Brand-Miller et al. 1992).

A study performed by Kinnear et al. (2011) determined that the effects of cooling on the glycemic index of potatoes varied for genotype; however, the glycemic response could not be explained by the consumption of amylose, rapidly digestible starch, slowly digestible starch, or RS examined *in vitro*. Due to the resistant nature of amylose, RS is expected to consist more of amylose than amylopectin (Englyst et al. 1992). On the contrary, a study performed by Soh and

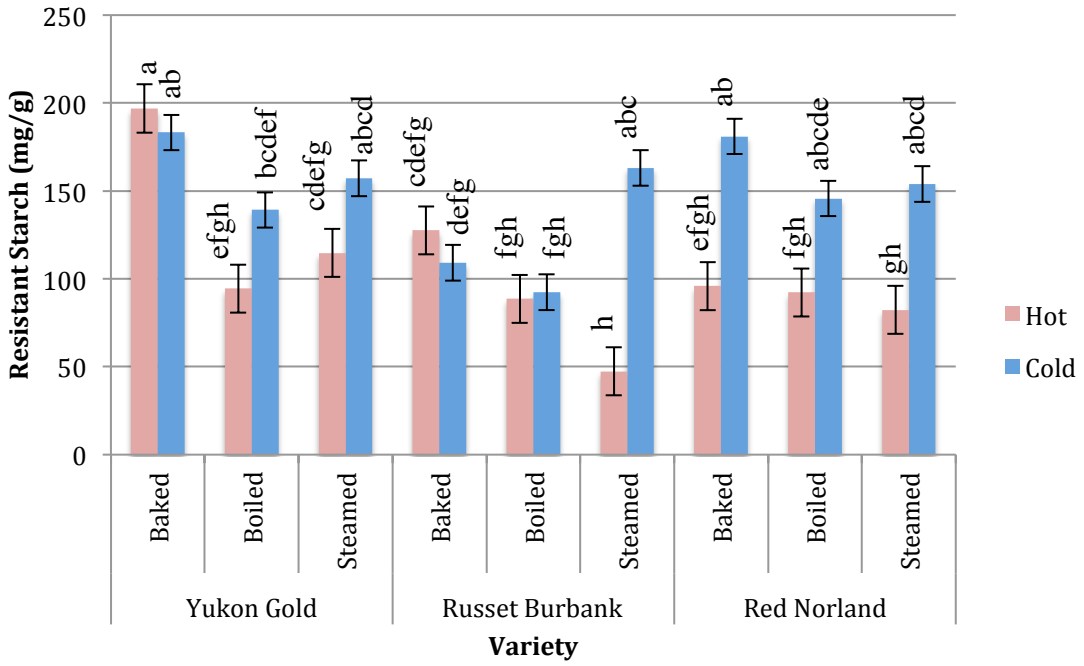

Figure 2.2. Resistant starch levels (mg/g dry weight) for three varieties, three cooking methods (baked, boiled, and steamed), and two service temperatures (hot and cold). SE for hot = 9.4; SE for cold = 7.2. LSD = 51.5.

Brand-Miller (1999) indicated that potatoes have an exceptionally high glycemic index, regardless of variety, cooking method, and tuber maturity. Immature tubers contain a higher ratio of amylose and sucrose compared to mature tubers (Jansky and Fajardo 2016).

Tahvonen et al. (2006) determined that cooking method (boiled or baked), peeling method (carbo- or steam- peeled), and processing method (slicing or mashing) did not influence the glycemic index; however, cooling and cold storage were found to lower the glycemic index of the potato product by nearly 25%. After cooling, amylose goes through retrogradation, resulting in more crystalline starch and an increase in resistance to digestive enzymes (Morris 1990). GI is primarily determined by the level of carbohydrate consumed; however, other dietary factors also influence GI, such as lipids, proteins, and fiber (Welch et al. 1987, Bjorck et al. 1994, and Foster-Powell and Miller 1995).

Yukon Gold had a significantly higher SS value than Russet Burbank and Red Norland, which did not significantly differ. In previous work, Bach et al. (2013) found genotype, as well as environment, to be the most significant factors contributing to variations in starch profiles among different genotypes. It is likely that Yukon Gold has unique genetic features that contribute to a higher level of SS. Research has not previously been performed on the variability in SS among potato genotypes.

Unlike results obtained from Jackson et al. (2013), which concluded that the varieties Yukon Gold, Russet Burbank, and Red Norland did not differ in their RS levels, the variety Yukon Gold was found to have a significantly higher level of RS than Russet Burbank and Red Norland in our study. Russet Burbank and Red Norland were not found to differ in their RS levels. This difference in significance may be due to a variety of factors, such as growing conditions and post harvest storage. Variation of starch profiles has been shown to differ due to environmental effects, especially due to location by year interactions, suggesting that a complex effect of temperature and moisture influences the production of starch profiles (Bach et al. 2013). Although Jackson et al. (2013) used the same varieties and they were also grown in North Dakota, it is possible that the varieties were grown under different environmental conditions or impacted by storage temperature and duration, resulting in differing starch profiles from those we had discovered. It is not clear whether Jackson et al. (2013) examined varieties from the same growing environment or whether they were examined fresh or after storage.

Baked potatoes were shown to have a significantly higher level of SS compared to boiled and steamed, which were not different. Although the average dry weight for steamed potatoes (28.7%) was higher than baked (24.1%) and boiled (21.9%), this alone does not explain the differences in SS among cooking methods. Perhaps the temperatures reached for each cooking

method had an impact on the enzymatic activity or starch content. Further research is needed to confirm this speculation. Baked potatoes in our study were also found to contain higher amounts of RS. This result agrees with that of Raatz et al. (2016), which concluded that baked preparations of the varieties Red Norland, Yukon Gold, and Russet Burbank contained higher RS content than boiled potatoes. Although differences were detected for each cooking method, it is important to note that variety x cooking method was not significant, indicating that for each variety, none of the three cooking methods impacted the amount of SS or RS. Thus, we can conclude that the Ziploc® Zip'n Steam bags may provide an efficient means to cook potato material in order to rapidly screen a large number of clones.

Conclusion

Although previous studies have examined the affect of baking and boiling potatoes for means of RS analysis, limited research has been performed on SS levels in potato. In order to screen a large number of clones for SS and RS, the baking and boiling methods are not ideal due to the labor and time demands required. The purpose of this study was to determine whether steaming in microwavable steam bags, which takes four minutes for two clones at a time, affected the SS and RS levels of tubers, compared to baking and boiling. Results indicated that variety x cooking method was not significantly different, indicating that for each variety, the cooking methods did not vary from one another. Thus, the microwavable steam bags can be implemented in research for SS and RS analysis.

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CHAPTER 3. EVALUATION OF PARENTAL GENOTYPES AND ADVANCING POTATO SELECTIONS FROM THE NDSU POTATO BREEDING PROGRAM FOR STARCH ATTRIBUTES

Abstract

Potato starch is unique compared to other sources of starch due to the large granule size and texture. Potato starch is used in various applications, including binding and thickening agents, anti-caking mixtures, pastes, pharmaceuticals, and biodegradables. The North Dakota State University (NDSU) potato improvement team has developed clones with high levels of starch and associated quality characteristics for French fry and chip processing. However, specific starch profiles of this germplasm have not been explored previously. A total of 219 clones were examined from the NDSU potato breeding program from potatoes grown at Baker, MN, and Absaraka, ND. Unique clones were found with varying levels of soluble and resistant starch. These may be further examined for their applicability in utilization and manufacture of products.

Introduction

Almost 70% of starch used in the US and Europe is used for industrial applications, whereas approximately 30% is used for consumption (Lillford and Morrison 1997). Although the potato contributes mostly carbohydrates to the human diet, other vitamins and nutrients are often over looked. For example, potatoes are high in potassium, with baked (with skin) and French-fries containing more potassium per 100g than banana (USDA 2013). Regardless of the additional vitamins and nutrients in the potato, potatoes have been perceived as bad for health, due to their relatively high glycemic index (Miller 1994). The potential for carbohydrates to raise blood-glucose levels compared to a reference food, such as pure glucose or white bread, is

referred to as the glycemic index (GI) (Jenkins et al. 1981). Foods with a GI above 70 are considered as high GI, whereas foods with a value of 56-69 are considered medium, and foods with a GI under 55 are considered low (ISO Standard 26642:2010). The GI of potatoes ranges from 56 to as high as 104, depending upon the genotype (Fernandes et al. 2005, Henry et al. 2005, Leeman et al. 2005a, Atkinson et al. 2008).

Amylose and amylopectin are linear and branched molecules, respectively, that make up the starch granule. Amylopectin typically makes up 70-80% of the available starch in the potato tuber (Zeeman et al. 2010), with the rest consisting of amylose; however, studies of different potato cultivars have indicated that cooked potatoes contain mostly rapidly digestible starch (Leeman et al. 2005b), as well as a high GI response (Atkinson et al. 2008). Starch, which has not been hydrolyzed within 120 min after being consumed, is considered indigestible (Fuentes-Zaragoza et al. 2011). Three enzymes are responsible for the breakdown of straight-chain starches: α-amylase (α- 1,4-glucan glucanohydrolase; EC: 3.2.1.1), β-amylase (α-1,4-glucan malto-hydrolase; EC: 3.2.1.2), and α-glucosidase (α-1,4-glucosidase; EC 3.2.1.20) (Zobel et al. 2009). Amylopectin is a larger molecule than amylose, resulting in a larger surface area for amylolytic attack (Singh et al. 2010). The tight coiling nature of amylose also provides resistance to breakdown (Taiz and Zeiger 2010). Degradation of these starch chains is important in digestion and the rate at which these starch chains are broken down is correlated to the GI of the potato (Ek et al. 2012). A lower rate of digestion of starch will reduce post-prandial blood glucose and insulin responses following consumption (Ek et al. 2012). A feature common among low GI foods is that they generally contain a fraction of starch that resists degradation by amylases (Fredriksson et al. 2000).

When starch granules are heated during the cooking process of potatoes, intra- and inter-chain hydrogen bonds present between amylose and amylopectin are hydrolyzed, causing water molecules to bind with the hydroxyl groups (Parker and Ring 2001). This process is called gelatinization and is known to disrupt the crystalline structure in the granule. Gelatinization results in an increase in solubility and swelling of the granule, a reflection of the interactive strength between starch chains (Sitohy et al. 2000). The starch can undergo an irreversible phase transition after increased heating, where the structural organization and native crystallinity are lost (Jenkins and Donald 1998).

Cooling of starch results in reassociation of starch polymers that retrograde gradually (Nugent 2005). Amylose molecules have been shown to retrograde more quickly than amylopectin molecules, which may take minutes to hours (Ring et al. 1987; Sievert and Wursch 1993). Linear amylose chain reassociation is inhibited by the presence of amylopectin (Sievert and Wursch 1993). The long chain properties of amylose also may contribute to the restriction of chain reassociation (Chung and Liu 2009). Soluble starch (SS) refers to starch that undergoes enzymatic degradation, and is used for energy in the body (Zobel et al. 2009). Resistant starch (RS) is composed of starch degradation products that cannot physically be absorbed by the small intestine and pass to the large intestine (Berry 1986). Starch that is retrograded has been shown to be more resistant to digestion (Englyst et al. 1992).

Textural properties of industrial products that incorporate starch are associated with physicochemical and functional properties (Singh et al. 2005). Starches with low levels of amylose have a waxy texture and contribute to improved freeze-thaw stability (Zheng and Sosulski 1998), which is desired in the food industry. The undesirable brown color in potato chips and fries is a result of the Maillard reaction, which occurs between reducing sugars and

free amino acids (Schallenberger et al. 1959). Cold-sweetening resistant potatoes contain higher levels of amylose and lower amylopectin levels than varieties susceptible to cold sweetening (Barichello et al. 1990).

The purpose of this study was to determine if clones present within the North Dakota State University potato breeding program possessed unique starch properties. Cultivars have been shown to differ in total starch and amylose content (Šimková et al. 2013). Clones in this study were expected to vary in SS and RS levels because genotype was shown to be a significant factor contributing to variations in starch profiles (Bach et al. 2013).

Materials and Methods

Genotypes

Two hundred nineteen clones were grown at two locations (Absaraka, ND, and Baker, MN) in 2014 on non-irrigated land. From Baker, 199 clones were investigated, and 43 genotypes from Absaraka were examined. Of the genotypes analyzed from Absaraka, 23 of the clones were also studied from Baker. Three check cultivars (Red Norland, Russet Burbank, and Yukon Gold) were used for each set of clones analyzed. Red Norland and Russet Burbank came from potato grower fields and Yukon Gold from research plots at Inkster in 2014. Russet Burbank and Yukon Gold were produced with irrigation. Clones were stored at 3.3° C for 12-16 months, depending on when the clones were analyzed. Three replicates were used for each clone, including the check varieties.

Starch Analysis

Based on the results obtained from Chapter 2, Ziploc® Zip'n Steam bags were used as a cooking method for our study. Two tubers of each clone were washed, peeled, cut into

identically sized pieces (2.5 cm^2), placed into a Ziploc® Zip'n Steam bag, and microwaved (1200W) for 4 minutes on high (steamed). Following cooking, cooked tuber tissue was riced and mixed. SS and RS was determined using the Megazyme Resistant Starch Assay (K-RSTAR, Megazyme International Ireland, Ltd, Co. Wicklow, Ireland) kit. A modified miniaturization of the assay was utilized (Raatz et al. 2016). Samples of potato were analyzed in triplicate. Riced samples of potato (0.50 g) were weighed into Corning® 15 ml centrifuge tubes and 4 mL of pancreatic amylase solution (10 mg/mL) (3U/mL amyloglucosidase)/sodium azide (0.03%)) was added into each tube. Tubes were capped and placed into a continuous shaking water bath at 37°C at 100 rpm for precisely 16 hrs.

Termination of the reaction was performed by adding 4 mL of 95% ethanol to each sample tube. Recovery of RS was performed by centrifugation (2000 x g, 10 min at RT). The supernatant, containing SS, was decanted into 100 ml volumetric flasks. The RS pellet was washed an additional two times with 8 mL of 50% ethanol, centrifuged (2000 x g, 10 min at RT), and decanted into 100 ml volumetric flasks.

The pellet containing RS was dissolved by adding 2 mL of 2 M KOH, along with vigorous stirring, within an ice-water bath, over a magnetic stirrer. The RS solution was neutralized by adding 8 mL of 1.2 M sodium acetate buffer (pH 3.8) and immediately adding 0.1 mL amyloglucosidase. The samples were incubated in a water bath at 50°C for 60 min. The contents in the tube were diluted 1:10 using a 100 mL volumetric flask. Aliquots of each solution were centrifuged (1500 x g, 10 min), 40 µL of the supernatant was transferred to 2.0 mL microtubes, and was mixed with 1.2 mL glucose oxidase-peroxidase-4-aminoantipyrine reagent (Megazyme Resistant Starch Assay, Megazyme International Ireland Ltd, Co. Wicklow, Ireland). The microtubes were placed in a water bath at 50°C for 20 min. The mixtures were transferred

to a 96-well plate where the absorbance was read against a reagent blank at 510 nm, utilizing a microplate reader (Multiskan FC, Thermo Scientific, Finland).

The SS supernatant in the 100 mL volumetric flasks was filled to 100 mL with 100 mM sodium acetate buffer and mixed. A 1:2 dilution of the SS solution, composed of 20 µL of the SS solution and 20 µL deionized water, was added to 2.0 mL microtubes, with 4 µL of dilute amyloglucosidase (300 U/mL) and 1.2 mL glucose oxidase-peroxidase-4-aminoantipyrine reagent (Megazyme Resistant Starch Assay, Megazyme International Ireland Ltd, Co. Wicklow, Ireland); samples were placed in a 50°C water bath for 20 minutes. Samples were then transferred to a 96-well plate, where the absorbance was read against a reagent blank at 510 nm, utilizing a microplate reader (Multiskan FC, Thermo Scientific, Finland). Two replicates of each sample were tested for moisture content.

Statistical Analysis

Variation among genotypes was determined by analysis of variance (ANOVA) using PROC MIXED (SAS Institute 2012). Genotypes were compared ($\alpha \leq 0.05$) using SAS 9.3. A protected mean separation test was conducted using PROC MIXED (SAS Institute 2012). Variation among market types was analyzed by analysis of variance (ANOVA) using Tukey's Range Test (SAS Institute 2012). Market types were compared ($\alpha \leq 0.05$) using SAS 9.3.

Results and Discussion

Differences in Soluble and Resistant Starch by Location

Starch profile analysis was performed for 219 clones, grown at Baker, MN, and Absaraka, ND, within the NDSU Potato Breeding Program, in order to assess differences in SS and RS levels. Three control varieties were used (Yukon Gold, Russet Burbank, and Red

Norland) for the comparative analysis with the 219 genotypes. A total of 12 genotypes could be analyzed at a time with the resistant starch assay kit (K-RSTAR, Megazyme International Ireland, Ltd, Co. Wicklow, Ireland), including the three control varieties, over a period of two days.

Differences in SS and RS among locations are presented in Table 3.1. Potatoes grown at Baker, MN, had significant differences for SS among clones. However, no significant differences were detected between genotypes grown at Baker, MN, for RS. Potatoes grown at Absaraka, ND, showed no significant differences for SS or RS among genotypes. The difference in significance for SS between locations may be explained by environmental factors. Although genotype is a contributing factor, the influence of temperature, precipitation, and other climatic factors have been shown to impact rapidly digested, slowly digested, and RS in potato genotypes grown at the same location in different years (Bach et al. 2013). Yearly interactions were not measured in our study. However, Baker, MN, and Absaraka, ND, had similar growing conditions. The clones grown in Baker, MN, contributed to a higher degree of freedom than Absaraka, ND, which also may have contributed to the difference in significance for SS between locations.

Table 3.1. Results of the type 3 test of fixed effects from PROC MIXED for soluble and resistant starch from potato clones grown at Baker, MN, and Absaraka, ND, in 2014.

Location	Starch Type	Source	Num[a] DF	Den[b] DF	F	Pr > F
Baker, MN	Soluble	Genotype	201	44	1.60	0.03*
	Resistant	Genotype	201	44	1.27	0.18[ns]
Absaraka, ND	Soluble	Genotype	45	10	1.77	0.17[ns]
	Resistant	Genotype	45	10	1.61	0.21[ns]

[a]Num = numerator
[b]Den = denominator
*significant at $\alpha \leq 0.05$, ns = not significant at $\alpha \geq 0.05$.

Unique clones within the NDSU Potato Breeding program were discovered with significantly higher and lower levels of SS (Table 3.2). ND102687AB-1Russ, ND113256C-2R, and Lenape contained the highest amount of SS, at 358.9 mg/g, 350.0 mg/g, and 347.3 mg/g, respectively (dry weight basis). ND113438CB-8R and ND113419CB-1R had the lowest levels of SS at 117.9 mg/g, and 134.3 mg/g, respectively, based on dry weight. ND113517ABC-9 had the highest level of RS (152.1 mg/g) and ND102921C-3 had the lowest (40.9 mg/g), although they were not found to be significantly different in our model. Our statistical model indicated that clones grown at Absaraka, ND, did not differ in their SS or RS levels. SS and RS levels, as well as the percentage of SS and RS discovered, are listed in Tables A.1 and A.2.

Table 3.2. Unique potato clones found within NDSU germplasm resources compared to control varieties, for soluble and resistant starch, grown at Baker, MN, in 2014.

	Clone	Soluble Starch (mg/g)		Clone	Resistant Starch (mg/g)
Highest 5	ND102687AB-1Russ	358.9	a*	ND113517ABC-9	152.1
	ND113256C-2R	350.0	ab	ND102822CAB-1	143.1
	Lenape	347.3	ab	ND113163-1	137.5
	ND113043B-6RY	340.2	b	ND102549TB-2Russ	129.4
	ND102719B-1Russ	338.5	b	ND113517ABC-6	127.9
Lowest 5	ND113487C-1	171.6	d	ND113438CB-8R	54.0
	ND113418CB-2RY	159.4	de	ND102990B-3R	49.1
	Inka Dawn	158.9	de	ND113060-1	47.4
	ND113419CB-1R	134.3	ef	ND102903-1R	46.0
	ND113438CB-8R	117.9	f	ND102921C-3	40.9
Controls	Red Norland	230.8	c	Red Norland	75.0
	Russet Burbank	224.6	c	Russet Burbank	81.4
	Yukon Gold	235.6	c	Yukon Gold	91.3
	Mean	246.6		Mean	85.8
	LSD ($\alpha = 0.05$)	18.6		LSD[a] ($\alpha = 0.05$)	8.9

*Different letters signify means are significantly different using PROC MIXED ($\alpha \leq 0.05$).
[a]differences between clones for resistant starch were not detected, so a protected mean square was used.

The control varieties, Red Norland, Russet Burbank, and Yukon Gold, were not shown to differ significantly in their SS levels. This result contradicts the study from Chapter 2, in which Yukon Gold was found to produce significantly higher amounts of SS, compared to Russet Burbank and Red Norland. This contradiction could be due to the number of clones analyzed in this study compared to Chapter 2, since clones were found to contain significantly higher and lower levels of starch compared to Red Norland, Russet Burbank, and Yukon Gold. The control varieties showed intermediate SS levels compared to the unique clones that had relatively high or low levels of SS (Figure 3.1). Our results for SS levels in Russet Burbank and Red Norland were similar to that seen from Chapter 2; however, Yukon Gold had a higher level of SS in Chapter 2 (535.3 mg/g) compared to this study (230.8 mg/g). Tubers used in Chapter 2 were examined approximately four months after harvest, where tubers utilized in this study were

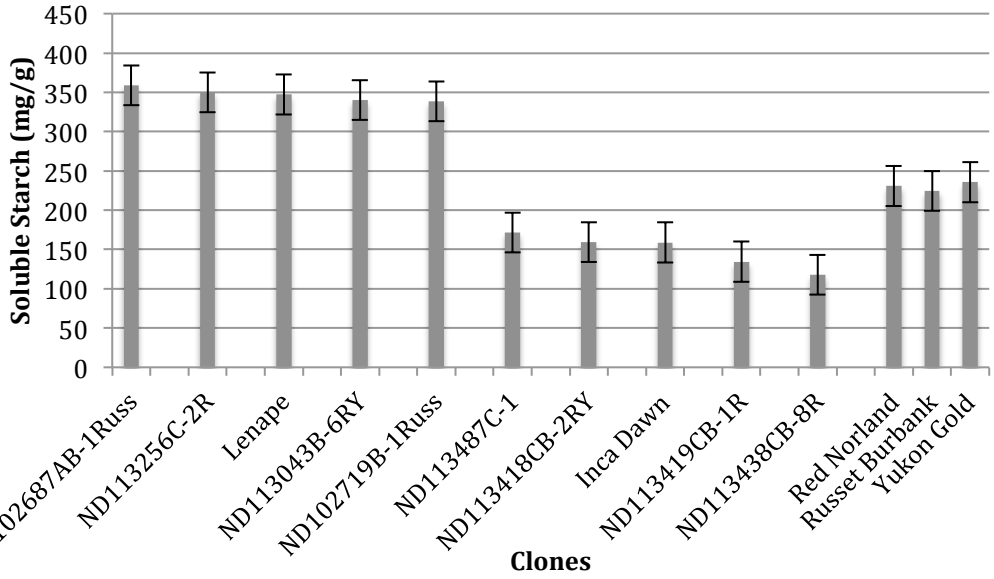

Figure 3.1. Unique clones grown at Baker, MN with highest or lowest levels of soluble starch (mg/g) based on dry weight. Error bars represent ±SE. LSD for soluble starch (mg/g) = 18.6.1

stored 12-16 months, although the same storage conditions were used. Once sugar formation begins during storage of tubers, storage continuation influences the sugar level (Schippers 1976), suggesting that the tubers stored for a longer period of time should produce more SS. It is unclear why Yukon Gold displayed lower SS content after longer storage duration.

Stored tubers of Russet Burbank and Yukon Gold have been found to contain 28.8% and 27.5% amylose, respectively (Fajardo et al. 2013). Little information is available from other studies regarding SS content in potato. Instead, potato starch has been analyzed in terms of rapidly and slowly digested starch (Bach et al. 2013), amylose and amylopectin content (Noda et al. 2004; Fajardo et al. 2013), and glycemic elicitation (Ek et al. 2012). Amylose and amylopectin are not considered completely soluble or insoluble, respectively. However, amylose is more difficult to hydrolyze than amylopectin and more enzymes are able to degrade amylopectin than amylose, contributing to the variability in digestion rates of these two forms of starch (Taiz and Zeiger 2010). This suggests that there is more amylopectin degraded into SS and more amylose present in the RS fraction, although research is needed in order to verify this. Our study found similar results to Fajardo et al. (2013) in terms of RS for Yukon Gold (27.9%); however, Russet Burbank had slightly lower results, at 24.5% (Table A.1). Further research is required to correctly compare our RS levels to amylose content as reported by Fajardo et al. (2013). Amylose levels of at least 50% are considered nutritionally desirable, due to the high fiber content and low glycemic index (Behall and Hallfrisch 2002). The control varieties showed intermediate RS levels compared to the unique clones that had relatively high or low levels of RS (Figure 3.2). Our RS levels were similar to the results reported in Chapter 2 for varieties examined while hot.

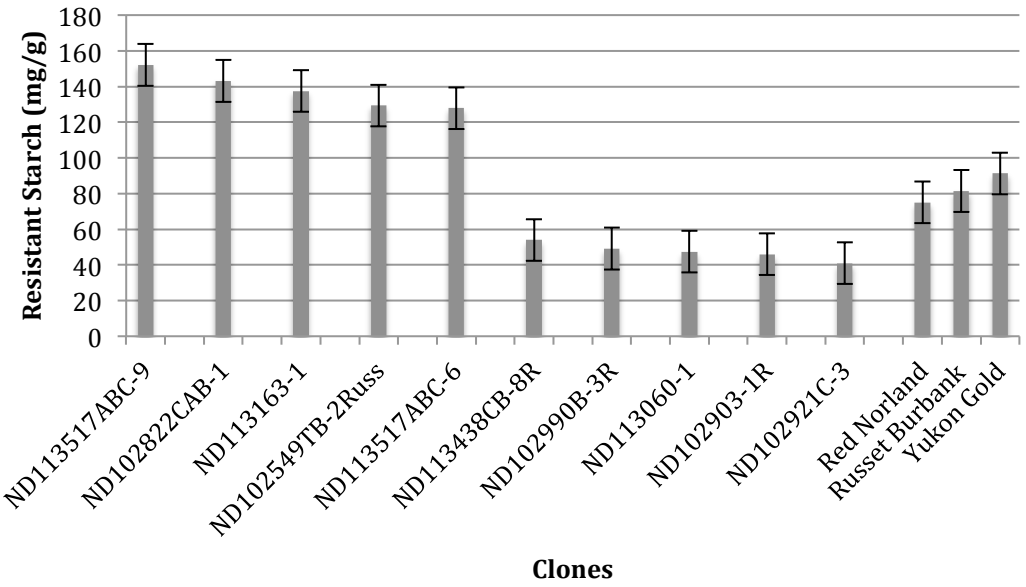

Figure 3.2. Unique clones grown at Baker, MN, with highest or lowest levels of resistant starch (mg/g) based on dry weight. Error bars represent ±SE. The statistical model used did not find differences in resistant starch between clones.

Differences in Soluble and Resistant Starch by Market Class

Various market classes of potato clones were used in this study, including dual-purpose, frozen processing, chip processing, specialty, germplasm, fresh, and flake. The analysis of variance for market types analyzed at Baker, MN, and Absaraka, ND, is presented in Table 3.3. Significant differences were found for SS and RS among market types grown at both locations. Although studies have been performed that examine the starch profiles of various clones, no known research has been performed on the starch profiles between specific market types. Mean starch profiles for market classes grown at Baker, MN, and Absaraka, ND, are presented in Table 3.4. Frozen and flake market types were not grown at Absaraka, ND. From Baker, MN, there were 14 dual-purpose, 19 frozen processing, 34 specialty, 41 germplasm, 45 fresh, 45 chip processing, and one flake market class analyzed. From Absaraka, ND, eight dual-purpose, 11 specialty, one germplasm, 11 fresh, and 12 chip processing market genotypes were analyzed.

Table 3.3. Analysis of variance for potato market classes grown at Baker, MN, and Absaraka, ND, in 2014.

Location	Starch	Source	DF	SS	MS	F	Pr > F
Baker, MN	Soluble	Market	6	46954.15	7825.69	2.66	0.02*
		Error	586	1720962.77	2936.80		
		Corrected	592	1767916.92			
	Resistant	Market	6	69894.34	11649.06	9.57	0.01*
		Error	590	718364.01	1217.57		
		Corrected	596	788258.35			
Absaraka, ND	Soluble	Market	4	50722.59	12680.65	3.95	0.05*
		Error	124	397832.70	3208.33		
		Corrected	128	448555.29			
	Resistant	Market	4	12099.53	3024.88	5.72	0.01*
		Error	124	65602.70	529.05		
		Corrected	128	77702.23			

*significant at $\alpha \leq 0.05$, ns = not significant at $\alpha \geq 0.05$.

The flake market type was found to have the highest SS level at Baker, MN (323.6 mg/g). The chip processing market type had the highest level of RS (104.6 mg/g) and the flake market type had the lowest level of RS (53.3 mg/g), among all classes from Baker, MN. The high and low levels of SS and RS, respectively, of the flake market type from Baker, MN, may be explained by the low number of clones analyzed for this market class.

The levels of starch in potato cells, as well as the size and shape of the granules, influence the final texture (Linehan and Hughes 1969; McComber et al. 1994). Textural properties have been associated with physicochemical and functional properties (Singh et al. 2005). Research on specific potato market classes and their desired starch profiles is limited. Amylose, the mostly resistant form of starch, is undesirable in some applications in the food industry (Potze 1976). Waxy starches, which contain low levels of amylose, have improved freeze-thaw stability (Zheng and Sosulski 1998), which is desired in the food industry. Native potato starch is not usually optimal for specific applications. In order to obtain the properties needed for specific end uses, modifications of starch are performed (Zobel 2009).

Table 3.4. Average soluble and resistant starch levels between market classes and growing locations.

	Location			
	Baker, MN		Absaraka, ND	
Market Class	Soluble (mg/g)	Resistant (mg/g)	Soluble (mg/g)	Resistant (mg/g)
Dual purpose	266.8[b*]	74.6[bc]	212.0[a]	92.4[b]
Frozen	260.4[b]	93.8[ab]	na[**]	na
Specialty	255.5[b]	79.9[ab]	177.5[a]	99.7[ab]
Germplasm	245.1[b]	84.1[ab]	200.6[a]	95.9[b]
Fresh	244.6[b]	78.6[ab]	195.6[a]	103.4[ab]
Chip	257.8[b]	104.6[a]	229.6[a]	119.0[a]
Flake	323.6[a]	53.3[c]	na	na

[*] Different letters signify means are significantly different using Tukey's Range Test ($\alpha \leq 0.05$).

[**]The frozen and flake market classes were not analyzed from genotypes grown at Absaraka.

Sugar levels in the tuber are an important component affecting the quality of processed products, such as chips, French fries, and other fried products. Sugar levels in potato are affected by several factors, including environmental, cultural practices during tuber maturation, storage (Kumar et al. 2004), and genotype (Stevenson et al.1964). The Maillard reaction, which occurs between reducing sugars and free amino acids, is responsible for the undesirable brown color in fried potato products (Schallenberger et al. 1959). Fructose, glucose, and sucrose concentrations present in the native tuber contribute to chip color variation (McCann et al. 2010). Potatoes that are resistant to cold sweetening contained higher levels of amylose and lower amylopectin levels and had more intact granules when introduced to α-amylase, than varieties susceptible to cold sweetening (Barichello et al. 1990). According to our results, clones within the chip processing market class have a significantly higher level of RS, suggesting that higher levels of amylose may contribute to improved chip color and quality. Potato clones used for frozen products posses similar qualities as chip processing market clones, although higher sugar content can be tolerated (Smith and Davis 1977).

Starch from potato is preferred by the food industry due to the low level of lipids and protein, and thus good paste clarity (Glicksman 1969). Potato starch dextrins are advantageous over other sources of starch for adhesives due to their remoistenability and rheology, resulting in a desirable direct tack (Zobel 2009). The textile industry also produces better products with potato starch, due to its film properties (Kerr 1950). Potato, tapioca, and waxy maize starch have desirable traits in oil drilling technology, because of their excellent fluid loss properties (Kraak 1992). Potato starch also is preferred as a precoat on filters due to large granule size (Zobel 2009).

Conclusion

The North Dakota State University (NDSU) potato improvement team has developed clones with high levels of starch and associated quality characteristics for French fry and chip processing. However, specific starch profiles of this germplasm have not previously been explored. The objectives of this research were to evaluate parental genotypes and advancing potato selections from the NDSU potato breeding program for starch attributes, focusing on the genetic diversity, contained within this germplasm collection and to aid in the development of novel products. Results indicated that the germplasm has unique starch profiles that may aid in the development of diverse products, such as pharmaceuticals, coatings, textiles, and biodegradables. Clones grown at Baker, MN, displayed significant differences for SS; however, no differences were found for RS at this location. Clones grown at Absaraka, ND, did not vary in SS or RS content, possibly due to the lower number of clones analyzed from this location, compared to Baker, MN. Market types analyzed from Baker, MN, and Absaraka, ND, indicated significant differences in SS and RS. Further examination is required for a better understanding of the factors impacting starch profiles, such as storage temperature, storage duration, and

environment. Additional research is required to determine the amount of amylose and amylopectin that make up the SS and RS fractions.

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CHAPTER 4. PHYSICOCHEMICAL ASSESSMENTS OF UNIQUE POTATO GENOTYPES

Abstract

Starch samples from 12 diverse potato clones, compared to three commercial varieties (Red Norland, Russet Burbank, and Yukon Gold), were isolated and their physicochemical properties investigated. These clones were selected based on their unique soluble starch (SS) and/or resistant starch (RS) levels. Six clones were chosen based on their relatively high or low levels of SS, and six clones were chosen due to their relatively high or low levels of RS. The research objective was to establish a foundation of fine chemistry research to further explain the unique SS and/or RS content that these clones elicit. Starch granules from the pith (center) and near the cortex (outer flesh) of the tubers were examined by Scanning Electron Microscopy. Granules ranged in length from 3 μm to 92 μm. Starch granule length is important for specific industrial applications, such as filters, and aids in explaining the digestion of starch. Pasting profiles were examined by rapid visco analyzer (RVA), which showed different pasting profiles for clones. Gelatinization characteristics, studied using differential scanning calorimetry (DSC), exhibited different gelatinization parameters. Isolated starch samples were examined by high performance size exclusion chromatography (HPSEC), in order to determine amylopectin and amylose molecular weights and abundance. Amylopectin and amylose percentages ranged from 77.5% to 83.7% and 16.3% to 22.5%, respectively. Differences in amylopectin and amylose molecular weight also were found. The results of this study indicated that diverse potato clones within the NDSU breeding program possess starch with unique physicochemical characteristics. This may provide insight into the development of various products, including bioplastics, coatings, textiles, and pharmaceuticals from these or similar genotypes.

Introduction

Potato starch is unique, compared to starch from cereal grains, due to its large granule size, relatively long amylose and amylopectin chain lengths, the phosphate linkages on amylopectin, and its ability to form viscous gels upon heating and subsequent cooling (Vasanthan et al. 1999). Although considerable differences have been found between potato varieties for their physicochemical properties (Yusuph et al. 2003), environmental factors have been shown to contribute to the variation in granule size, amylose content, pasting properties, and thermal properties (Kaur et al. 2007a). Analysis of physicochemical properties in potato starch is influential on the development of products such as biodegradables, water binding agents, adhesives, and food items.

Starch occurs naturally as granules of different sizes, shapes, size variation, and forms (Tester et al. 2004). Degradation of these granules depends on a variety of influences, including molecular structure, reaction conditions, and enzyme specificity (Buleon et al. 1998; Yook and Robyt 2002). Examination of the enzyme hydrolysis on starch granule size has been performed (Kasemwong et al. 2008; Sushil et al. 2010), and the general conclusion is that smaller granules undergo enzymatic hydrolysis more quickly than larger granules due to their higher available surface area. Physicochemical properties have been examined for potato starch previously (Hoover 2001). However, physicochemical properties have not been examined for genotypes from the NDSU potato breeding program. The objective of this research was to examine the granule size, pasting profile, gelatinization characteristics, and composition of dried starch from potato genotypes that were found to have unique starch profiles based on soluble (SS) and resistant starch (RS) levels. The outcome of this study may provide insight for applications of potato starch in various industries, including bioplastics, pharmaceuticals, coatings, and textiles.

Materials and Methods

Genotypes

Fifteen clones, including three commercially acceptable control cultivars (Red Norland, Russet Burbank, and Yukon Gold), were chosen for physicochemical evaluation (Table 4.1). These clones were selected based on unique SS and RS levels found in Chapter 3, in addition to selection based on availability of tuber material. The clones were grown at Absaraka, ND, without irrigation in 2014. Red Norland and Russet Burbank came from potato grower fields, and Yukon Gold, from research plots at Inkster, ND, in 2014. Russet Burbank and Yukon Gold were produced with irrigation, while all other genotypes were produced under non-irrigated conditions.

Table 4.1. Clones selected for physicochemical assessment based on soluble and resistant starch levels.

	Clone	Soluble Starch (mg/g)		Clone	Resistant Starch (mg/g)
Highest 3	ND102687AB-1Russ	358.9	a*	ND113517ABC-9	152.1
	Lenape	347.3	ab	ND102549TB-2Russ	129.4
	ND113508C-4	337.4	b	ND113517ABC-6	127.9
Lowest 3	ND113487C-1	171.6	d	ND113060-1	47.4
	Inka Dawn	158.9	de	ND102903-1R	46.0
	ND113438CB-8R	117.9	f	ND102921C-3	40.9
Controls	Red Norland	230.8	c	Red Norland	75.0
	Russet Burbank	224.6	c	Russet Burbank	81.4
	Yukon Gold	235.6	c	Yukon Gold	91.3
	Mean	246.6		Mean	85.8
	LSD ($\alpha = 0.05$)	18.6		LSD[a] ($\alpha = 0.05$)	8.9

*Different letters signify means are significantly different using PROC MIXED ($\alpha \leq 0.05$).

[a]differences between clones for resistant starch were not detected, so a protected mean square was used.

Starch Granule Morphology

Raw potatoes were stored at 3.3°C until ready for SEM (Scanning Electron Microscopy) evaluation. Potatoes were cut crosswise with a knife and a cork borer was used to remove plugs from two areas near the cortex and near the pith of the potatoes. Plugs were immediately placed into a hole drilled into a brass sample-holder cryostub (JEOL USA, Peabody, Massachusetts, USA), which was supplied with Teflon feet in order to isolate it thermally from the SEM stage, and allowed to warm at a slower rate. Plugs of potato were secured in the hole utilizing Tissue-Tek O.C.T. Compound (Sakura Finetek USA, Inc., Torrance, California). The cryostub containing the potato tissue was submerged in liquid nitrogen and after complete freezing of the sample the tissue that extended out of the hole above the surface of the cryostub was fractured using a razor blade that was previously cooled in liquid nitrogen. Any excess fractured potato tissue was removed and discarded. The brass holder was then inserted into the SEM holder, where it was positioned onto a stage of a variable-pressure scanning electron microscope (SEM; JEOL JSM-6490LV, JEOL USA, Peabody, Massachusetts). Samples were held in the SEM vacuum for 5-10 minutes, in order for the surface moisture/frost to sublimate prior to examining the fractured surface. Images were acquired within a 10 minute window. Backscattered electron images were acquired in a low-vacuum mode at a pressure of 30 Pascals. The images taken were used for starch granule size distribution analysis by comparing the reference bar to the granules present in the images.

A Tukey-Kramer test was performed on starch granule length and widths. However, due to the variation in the number of granules analyzed between each scanning electron micrograph, multiple honest significant difference (HSD) values were calculated. Thus, HSD values are not presented in the granule size distribution tables.

Dried Potato Flour

Potatoes were washed, peeled, cut into thin slices, and placed in the freezer overnight. Frozen potatoes were then freeze-dried for three days, ground to a fine texture, and used for determination of pasting properties, gelatinization characteristics, and molecular weight and chain length distribution.

Pasting Properties

Dried potato flour was used for the determination of pasting profile, gelatinization properties, and amylose and amylopectin content and molecular weight. Two replicates were performed. The pasting profile of the dried potato samples were analyzed according to the AACC-I approved method 76-21.01 (AAC-I, 2009) with a rapid visco analyzer (RVA). The samples (3.5g on a 14% moisture basis) were weighed into a can containing 25% water. The samples and water were mixed vigorously and the can was loaded into the RVA. Peak viscosity, hot paste viscosity, breakdown, cold paste viscosity, setback, and peak time were determined.

Gelatinization Characteristics

Starch gelatinization properties were measured with a Perkin-Elmer Differential Scanning Calorimeter, DSC-7. Dried potato flour samples of 3.5 mg were weighed into aluminum pans and 8µl of deionized water was added. Sealing of the pans was performed hermetically, and pans were stored at room temperature overnight prior to analysis. The samples were heated from 10 to 100°C at 10°C/min. The reference was an empty aluminum pan. Onset (T_o), peak (T_p), enthalpy of gelatinization (ΔH), and end (T_c) were obtained from the curve using the data processing software equipped with the DSC instrument (Kim et al. 1997).

Starch Molecular Weight

Amylose and amylopectin content and molecular weight were examined. Potato starch was isolated from dried potato flour by defatting and precipitating the starch. Defatting was initiated by adding 2.5 mL methanol to 30-40 mg of each sample and heating at 100°C for 30 minutes, followed by centrifugation for 5 minutes at 2,000 RPM. Samples were decanted and dried in an oven at 55°C. Starch extraction was initiated by adding 2 mL potassium hydroxide/urea solution (4.5 mL 1.0 *M* KOH with 0.5 mL 6.0 *M* urea), followed by heating for 15 minutes at 100°C. Starch was precipitated by adding 6 mL 95% ethanol (3 mL at a time) prior to centrifugation for 5 minutes at 2,000 RPM and then dried. Defatted potato samples were prepared for high performance size exclusion chromatography (HPSEC) by treating with KOH and urea, as described by Grant et al. (2002) with modifications (Simsek et al. 2012). Approximately 30 mg of starch was solubilized by adding 4.5 mL KOH (1.0 *M*) and 0.5 mL urea (6.0 *M)* and heated for 90 minutes at 100°C. After solubilizing, 1.0 mL aliquots were neutralized with 1.0 *M* HCl. The samples were filtered through a hydrophilic 0.45 μm nylon syringe filter, prior to running HPSEC. Samples were examined using an Agilent 1200 series high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA), which was supplied with an auto sampler, a refractive index (RI) detector, and a Wyatt Dawn Helios-II multi-angle light scattering (MALS) detector. Starch separation was performed with an Ultrahydrogel guard column and Ultrahydrogel 1000 and Ultrahydrogel linear size exclusion columns (Waters, Milford, MA, USA). The temperature of the column and detector was fixed at 50°C. HPLC-grade water was used for the mobile phase and was pumped at a flow rate of 0.4 mL/min with an injection volume of 60 μL. Control and integration were conducted with a personal computer (PC) equipped with a ChemStation (HP ChemStation for LC Rev.

A.04.01), in order to determine the percent amylose and amylopectin present. Calculation of molecular weights (Mw) for amylose and amylopectin were performed on Astra 6.0.5. data processing software (Wyatt Technology Corporation, Santa Barbara, CA, USA). The RI value was defined according to You et al. (1999). Data was normalized from Pullulan standards prior to baseline corrections and peak alignments. Molar mass calculations were performed using the Berry model, with a fit degree of two and a second-order polynomial fit. The Berry model utilizes mathematical equations to examine the light scattering intensity and scattering angle given off by molecules in light scattering systems (Berry 1966).

Statistical Analysis

Significance of granule size morphology was performed using Tukey-Kramer's test for significance using SAS 9.3 (SAS Institute, 2012). All other analyses were replicated two times (n=2). Analyses of variance (ANOVA p=0.05) mean values and least significant differences were determined by Fischer's least significant difference (LSD) using SAS 9.3 (SAS Institute, 2012).

Results and Discussion

Starch Granule Morphology

Starch granule morphology was determined using scanning electron microscopy (SEM), and is presented in Table 4.2. A Tukey-Kramer test was performed on starch granule length and widths. Granules were examined from tuber tissue near the pith and cortex of tubers to determine whether granules were evenly distributed. Although the clones used for this study expressed unique SS and/or RS levels based on cooked tuber tissue, SEM images were analyzed from raw potato. Heating starch granules in the presence of water breaks down the granule and

causes gelatinization (McGee 1984). Excessive swelling after cooking results in ruptured cells and extrusion of gelled starch (Reeve 1967). For these reasons, raw potato was utilized for SEM images.

The length of granules near the cortex ranged from 3.0 μm to 92.0 μm (Table 4.2). ND102549TB-2Russ displayed the least variation (13.0-45.0 μm), and ND113508C-4 displayed the largest variation (12.0-92.0 μm). The width of granules near the cortex ranged from 3.0 μm to 52 μm (Table 4.2). ND113438CB-8R had the least variation (16.0-33.0 μm) and ND113508C-4 had the largest variation (3.0-52.0 μm). The length of granules near the pith ranged from 4.0 μm to 78.0 μm. ND113438CB-8R and ND102549TB-2Russ had the least variation (30.0-54.0 μm and 12.0-36.0 μm, respectively); Yukon Gold had the largest variation (6.0-78.0 μm). The width of granules near the pith ranged from 3.0 μm to 112.0 μm. ND113438CB-8R had the least variation (21.0-34.0 μm) and ND102549TB-2Russ had the most variation (7.0-112.0 μm). Genetic variation has previously been demonstrated to influence granule morphology (Fajardo et al. 2013).

Although it has been reported that granule size distribution and length vary between the pith and the cortex (Reeve 1967), the mean lengths and widths for all of the clones used in this study are similar near the both the cortex and pith (average granule length equaled 24.2 μm and 24.4 μm for granules near the cortex and pith, respectively; average granule width equaled 17.6 μm and 17.9 μm for granules near the cortex and pith, respectively). However, when granule length distribution is presented (Table 4.3 and 4.4), we see variation in granule size between the pith and cortex. Reeve (1967) examined the distribution of starch granule lengths in Russet Burbank, and found that larger granules, although prevalent near the pith, are less abundant compared to the storage parenchyma (between the pith and the cortex). Our results indicated that

Russet Burbank remained consistent in its larger starch granule distribution (or lack thereof) (Table 4.3 and 4.4). Most of the granule sizes for Russet Burbank near the cortex were between 30 μm and 39 μm, whereas most of the granules near the pith ranged between 20 μm and 29 μm.

Table 4.2. Starch granule size distribution for raw potato tissue near the cortex and pith of the tuber.

	Starch granule sizes (μm)							
	Length of cells cortex (μm)		Width of cells cortex (μm)		Length of cells pith (μm)		Width of cells pith (μm)	
Clone	Mean	Range	Mean	Range	Mean	Range	Mean	Range
ND102687AB-1Russ	28.8	12.0-56.0	22.6	7.0-48.0	21.0	11.0-55.0	16.0	10.0-30.0
Lenape	23.5	4.0-52.0	19.0	4.0-34.0	34.8	7.0-50.0	26.2	7.0-68.0
ND113508C-4	36.7	12.0-92.0	25.2	3.0-52.0	34.6	12.0-62.0	24.9	11.0-35.0
ND113487c-1	24.9	12.0-50.0	18.6	9.0-27.0	24.6	9.0-58.0	17.5	7.0-30.0
Inka Dawn	13.2	3.0-40.0	11.7	3.0-25.0	13.9	4.0-44.0	13.5	5.0-28.0
ND113438CB-8R	30.9	15.0-53.0	24.5	16.0-33.0	37.9	30.0-54.0	26.4	21.0-34.0
ND113517ABC-9	20.7	5.0-53.0	13.9	5.0-33.0	22.1	6.0-49.0	15.3	4.0-29.0
ND102549TB-2Russ	26.5	13.0-45.0	17.1	9.0-33.0	20.7	12.0-36.0	20.4	7.0-112.0
ND113517ABC-6	34.0	10.0-52.0	22.4	12.0-33.0	22.0	4.0-42.0	17.3	3.0-32.0
ND113060-1	23.8	5.0-46.0	17.4	6.0-35.0	25.7	7.0-62.0	16.7	7.0-45.0
ND102903-1R	21.5	6.0-41.0	15.1	4.0-26.0	23.1	8.0-51.0	15.6	5.0-27.0
ND102921C-3	28.5	8.0-53.0	19.5	9.0-33.0	25.2	12.0-54.0	17.1	8.0-32.0
Red Norland	23.1	8.0-48.0	17.0	7.0-39.0	25.0	10.0-45.0	16.0	7.0-25.0
Russet Burbank	26.1	6.0-40.0	17.8	6.0-29.0	20.8	4.0-41.0	15.5	4.0-38.0
Yukon Gold	22.6	4.0-46.0	17.1	6.0-36.0	25.9	6.0-78.0	20.9	5.0-50.0
Mean	17.6		17.6		24.4		17.9	

Table 4.3. Size distribution of starch granules near the cortex for raw potato tissue of unique clones compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

Clone	Total number granules measured	% of Total granule sizes						
		Length near cortex (μm)						
		<10	10-19	20-29	30-39	40-49	50-59	60-69
ND102687AB-1Russ	17	0	35	35	6	6	18	0
Lenape	34	26	18	15	26	9	6	0
ND113508C-4	23	0	22	22	22	13	9	13
ND113487c-1	17	0	29	47	12	6	6	0
Inka Dawn	53	30	51	15	2	2	0	0
ND113438CB-8R	13	0	31	15	38	0	15	0
ND113517ABC-9	35	11	43	23	23	0	0	0
ND102549TB-2Russ	34	0	16	63	11	11	0	0
ND113517ABC-6	25	0	16	20	28	32	4	0
ND113060-1	52	10	33	29	13	15	0	0
ND102903-1R	30	7	43	23	23	3	0	0
ND102921C-3	25	4	12	44	20	16	4	0
Red Norland	24	4	29	50	4	13	0	0
Russet Burbank	21	10	24	14	48	5	0	0
Yukon Gold	33	14	45	24	10	21	0	0

Table 4.4. Size distribution of starch granules near the pith for raw potato tissue of unique clones compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

Clone	Total number granules measured	% of Total granule sizes						
		Length near pith (μm)						
		<10	10-19	20-29	30-39	40-49	50-59	60-69
ND102687AB-1Russ	31	0	65	19	6	6	3	0
Lenape	25	4	20	20	20	16	12	8
ND113508C-4	14	0	29	14	0	50	0	7
ND113487c-1	18	6	28	44	17	0	6	0
Inka Dawn	38	18	45	29	5	3	0	0
ND113438CB-8R	12	0	0	8	67	17	8	0
ND113517ABC-9	31	16	23	35	16	10	0	0
ND102549TB-2Russ	14	0	43	43	14	0	0	0
ND113517ABC-6	18	6	28	44	17	6	0	0
ND113060-1	23	4	39	26	13	9	4	4
ND102903-1R	25	17	21	42	13	0	8	0
ND102921C-3	21	0	19	52	19	5	5	0
Red Norland	17	0	35	35	6	24	0	0
Russet Burbank	20	20	20	30	25	5	0	0
Yukon Gold	19	10	25	40	10	10	0	5

however, this is not consistent with all of our results. For example, only 5% of Lenape's granules near the cortex were at least 50 μm, whereas 20% of granules near the pith were at least 50 μm. No current research is available that suggests genotype influences the size distribution of starch granules near the pith compared to the cortex. Granule size distributions have been reported to vary by storage temperature (Reeve 1967); however, the unique clones used in this study were all stored at the same temperature.

Scanning micrographs of the commercial cultivars Red Norland, Russet Burbank, and Yukon Gold are presented in Figure 4.1. The lengths, widths, and variation of the granules in these cultivars were intermediate compared to the other clones used in this study. However, Yukon Gold expressed the largest variation in granule length near the pith (6.0-78.0 μm), although the average granule size near the cortex and the pith was similar to Red Norland and Russet Burbank. Interestingly, most of the granule lengths for Red Norland, Russet Burbank, and Yukon Gold were between 20-29 μm, 30-39 μm, and 10-19 μm near the cortex, respectively (Table 4.3). A recent study found starch granules of Russet Burbank to have an average length of 36.0 μm and width of 25.1 μm (Fajardo et al. 2013). Our results indicated a smaller average length for Russet Burbank (26.1 μm and 20.8 μm near the cortex and the pith, respectively) and width (17.8 μm and 15.5 μm near the cortex and the pith, respectively.

Granules from Russet Burbank have remained consistent during prolonged storage (Johnston et al. 1968). Previous research on Yukon Gold starch granules reported the average length to be 34.5 μm and width to be 24.4 μm (Fajardo et al. 2013). Our results indicated smaller average lengths (22.6 μm and 25.9 μm near the cortex and the pith, respectively) and widths (17.2 and 20.9 near the cortex and pith, respectively). The differences in the average granule lengths could be explained by environmental factors, which have been demonstrated to influence

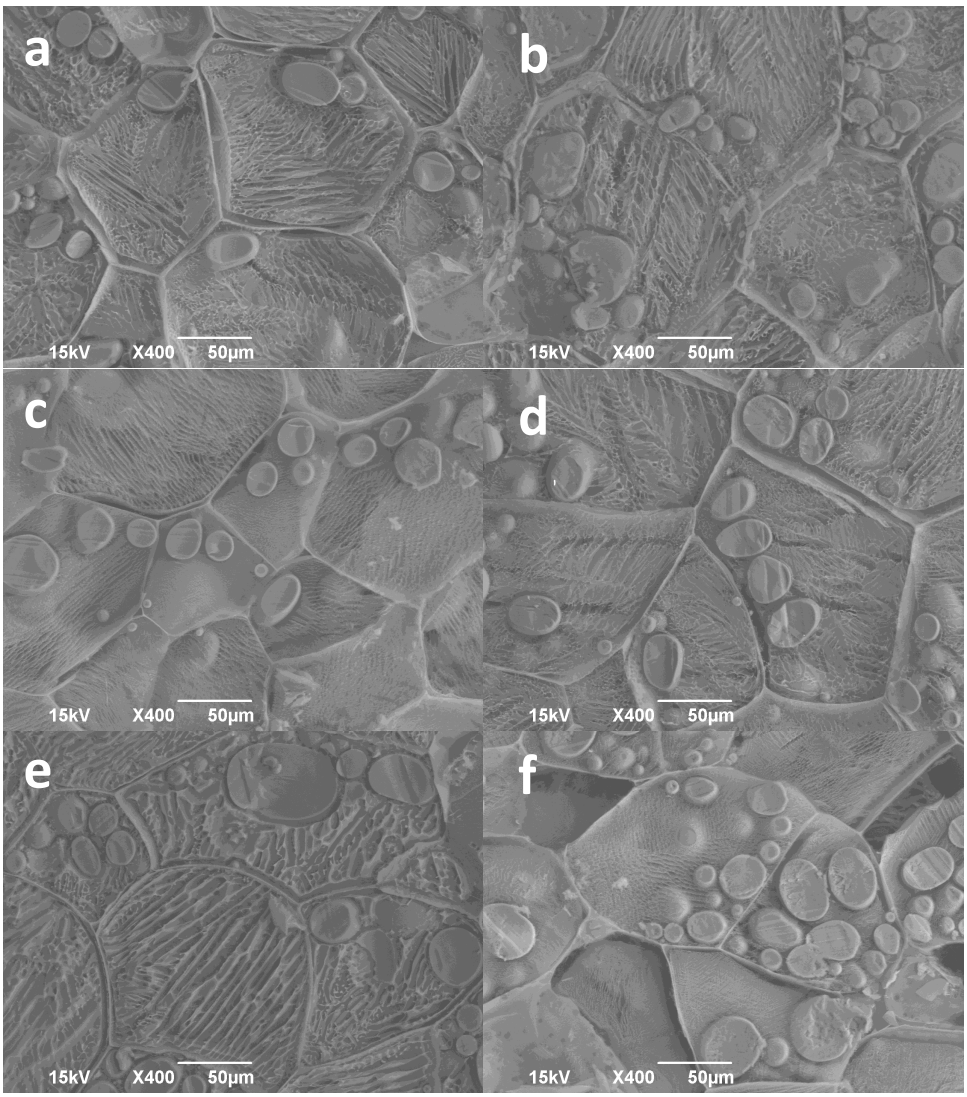

Figure 4.1. Scanning electron micrographs of tuber flesh of Red Norland: pith (panel a) and cortex (panel b); Russet Burbank: pith (panel c) and cortex (panel d); and Yukon Gold: pith (panel e) and cortex (f). All panels have a magnification of 400X, the bars represent 50 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

starch granule sizes (Kaur et al. 2007a). Kaur et al. (2007a) grew different genotypes in Gwalior, Jalandhar, Modipuram, and Patna, India, and found larger granule sizes from genotypes grown in Jalandhar, likely due to lower temperature. Granule sizes have been shown to decrease with an increase in growing season temperature (Cottrell et al. 1995). Perhaps the lower temperature range in ND compared to locations in India resulted in smaller granule sizes. Results from

Fajardo et al. (2013) were analyzed from cultivars grown in WI, whereas our clones were grown in ND. WI generally has warmer day and night time temperatures in the primary potato production areas than North Dakota production areas.

Scanning electron micrographs of clones with unique SS levels are presented in Figure 4.2. Clones ND102687AB-1Russ, Lenape, and ND113508C-4 had significantly higher levels of SS, whereas ND113487c-1, Inka Dawn, and ND113438CB-8R had significantly low levels of SS. Keeping in mind the magnification differences between images (Figure 4.2), the starch granules from the highest SS level clones appear to have a greater distribution of larger size starch granules (Figure 4.2a, 4.2b, 4.2c, 4.2d, 4.2e, and 4.2f) compared to starch granules from clones with low levels of SS (Figure 4.2g, 4.2h, 4.2i, 4.2j, 4.2k, and 4.2l). Our size distribution data (Table 4.3 and 4.4) agrees that larger sized granules are more prevalent in ND102687AB-1Russ, Lenape, and ND113508C-4.

A study performed by Noda et al. (2005) on potato starch indicated that as granule size decreased, the hydrolysis rate of raw starch by amylase increased. This indicates that smaller starch granules digest more quickly than larger granules. Larger starch granules have a smaller surface area than smaller granules. The substrate's smaller surface area in the larger granules decreases the ability for amylase absorption. The rate of amylase degradation on granules was not examined in our study, but may provide insight into the relationship between granule size and SS and RS levels found within our clones. Although larger granule sizes have been associated with higher amylose content (Geddes et al. 1965), contradictory studies have found little to no differences in amylose content among various sized potato starch granules (Fujita et al. 1983, Chen et al. 2003, and Noda et al. 2005). However, it is not clear how the potatoes used

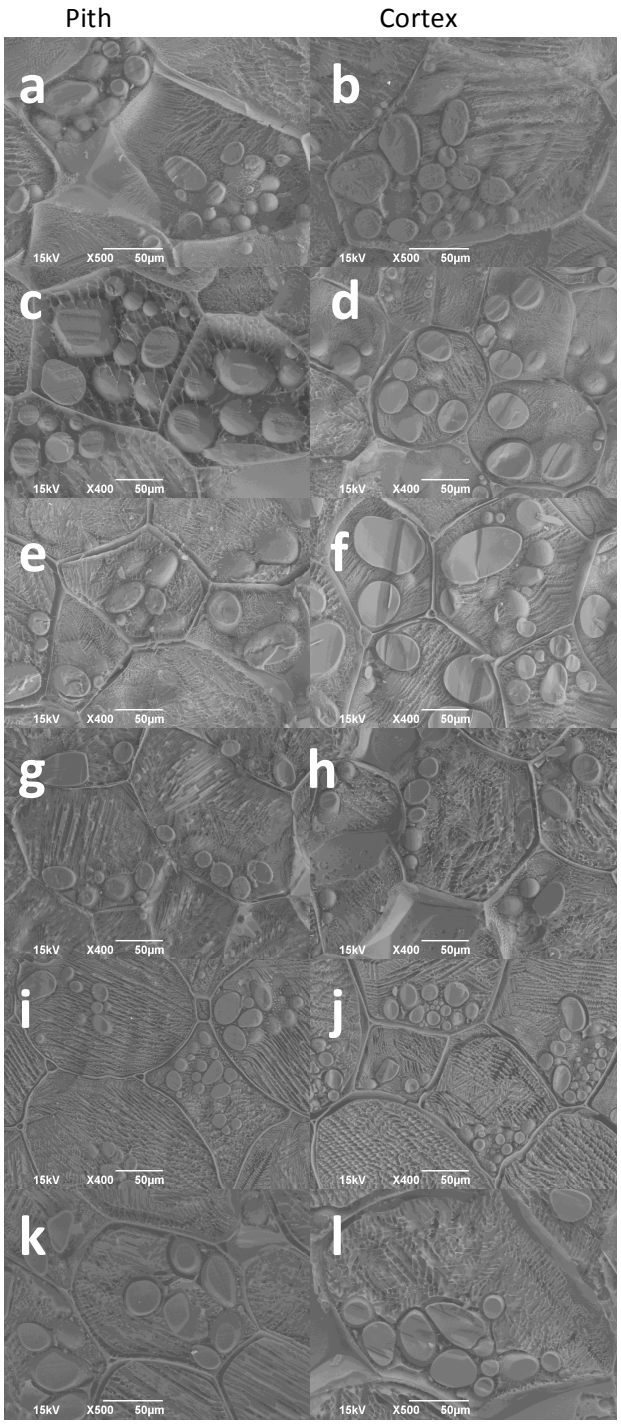

Figure 4.2. Scanning electron micrographs of tuber flesh of ND102687AB-1Russ (panels a and b); Lenape (panels c and d),); ND113508C-4 (panels e and f), ND113487c-1 (panels g and h), Inka Dawn (panels i and j), and ND113438CB-8R (panels k and l). Panels a, c, e, g, i, and k were analyzed near the pith, and panels (b, d, f, h, j, and l were analyzed near the cortex. Panels a, b, k, and l have magnification of 500X,. Panels c, d, e, f, g, h, i, and j have a magnification of 400X. The bars represent 50 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

for these studies were grown (irrigated vs. non-irrigated) or stored. Granules from potato are smooth-surfaced with oval and irregular shapes, whereas wheat granules are spherical and lenticular shaped (Singh et al. 2003). Potato granules (<110 μm) are larger than wheat (<30 μm), corn (<25 μm), and rice (<20 μm) granules (Singh et al. 2003).

Scanning electron micrographs for clones with unique RS levels are presented in Figure 4.3. Clones ND113517ABC-9, ND102549TB-2Russ, and ND113517ABC-6 displayed high levels of RS. Clones ND113060-1, ND102903-1R, and ND102921C-3 expressed low levels of RS. Our statistical design used for RS analysis in Chapter 3 indicated that differences were not significant for RS levels between clones. RS consists of starch degradation products that are unable to be absorbed by the small intestine and pass to the large intestine (Berry 1986). There is not an obvious granule size difference when comparing the clones with high RS levels (Figure 4.3a, 4.3b, 4.3c, 4.3d, 4.3e, and 4.3f) compared to clones with low RS (Figure 4.3g, 4.3h, 4.3i, 4.3j, 4.3k, and 4.3l). Although our size distribution data (Table 4.3 and 4.4) seems fairly consistent between the clones unique for RS, the clones with the lowest levels of RS all displayed large granule lengths (>50 μm near the pith), whereas the clones with the highest levels of RS did not display any granules larger than 49 μm near the pith. Reeve (1967) examined raw potato starch and discovered that although large granules occur in the pith, they are minimal in abundance compared to the cortex of the tuber. Interestingly, larger granules were more abundant near the pith of the tuber than the cortex for clones with unique RS levels. We do not believe that this difference in granule size distribution among pith and cortex of the potato contribute to the unique RS levels found within these clones because tuber samples used for RS analysis in Chapter 3 contained all tuber tissues mixed, and not a specific part of the tuber flesh.

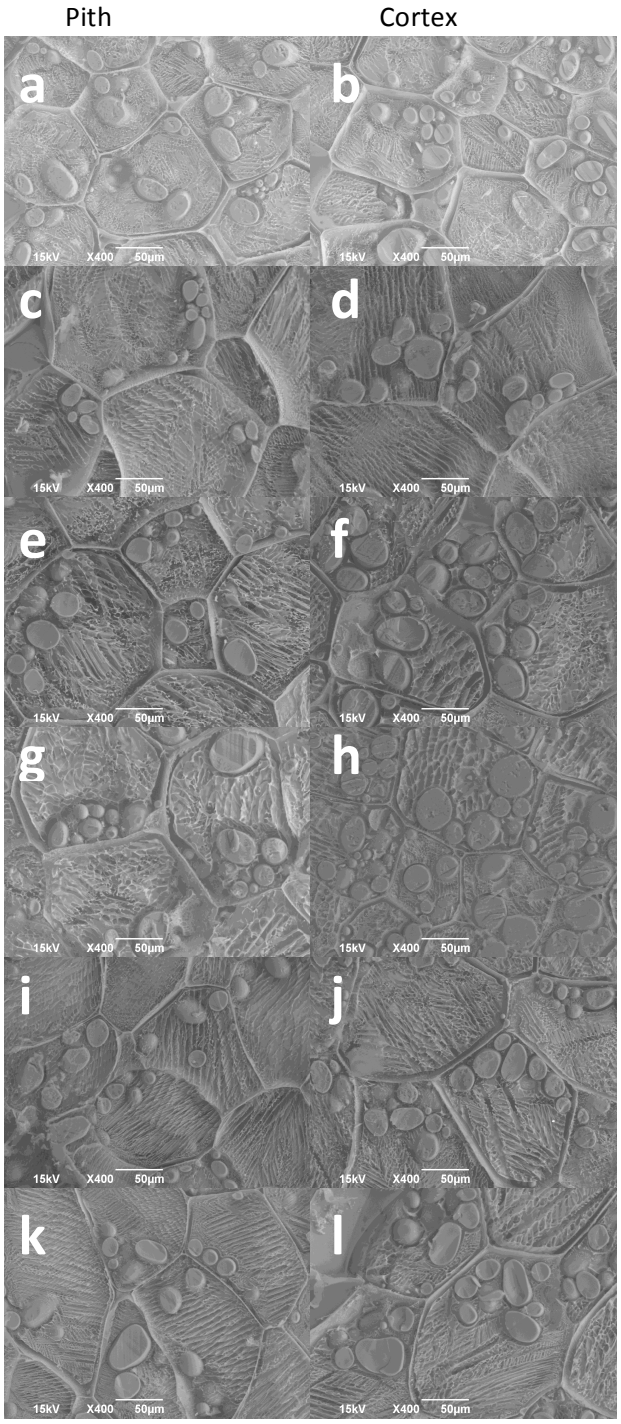

Figure 4.3 Scanning electron micrographs of tuber flesh of ND113517ABC-9: pith (panel a) and cortex (panel b); ND102549TB-2Russ: pith (panel c) and cortex (panel d); ND113517ABC-6: pith (panel e) and cortex (panel f); ND113060-1: pith (panel g) and cortex (panel h); ND102903-1R: pith (panel i) and cortex (panel j); and ND102921C-3: pith (panel k) and cortex (panel l). All panels have magnification of 400X, the bars represent 50 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

There is increasing interest in the use of plant-based material for non-food applications. Modified potato starches are produced in order to meet consumer needs and provide durable materials (Kraak 1992). Derivatization of starch is used to adjust viscosity, improve stability, clarity, and to adjust dissolving rates and hydrophobicity (Kraak 1992). Arun et al. (2012) produced biodegradable starch composites from potato with nanocellulose and ramie textile fabric, but the potato starch used was purchased through a chemical manufacturer and did not identify specific starch characteristics. However, Fonseca et al. (2015) evaluated the effect of oxidation on potato starch in biodegradable films for physiological, morphological, pasting, thermal, and gel parameters. Results of the study indicated that oxidation did not affect the morphology of the granules or gelatinization temperature, but influenced paste characteristics. Fonseca et al. (2015) concluded that although films produced with oxidized starch had decreased tensile strength compared to native starch films, the oxidized starch films had lower water solubility, enabling the use of oxidized starch films in products with higher water activity. Potato and casein complexes, in the ratio of 1:1, have been formed into biodegradable polymers (Grega et al. 2003); Superior, a fresh market variety, was used in this study. Starch granules from Superior average in length from 16.9 μm to 32.2 μm, depending on maturity (Liu et al. 2003). Most of the clones used in our study fall into this range of average granule length, depending on whether the granules are analyzed near the cortex or the pith. Thus, our clones may provide suitable characteristics for use in biodegradable polymers. Additionally, potato starch has been used in pharmaceutical tablets; attributes include its easy preparation, release rate controllability, and possibility to incorporate drugs of high percentages with diverse chemical properties (Te Wierik et al. 1997). Approximately 30% of starch used in Europe and the US is used as native

starch for consumption; whereas about 70% is used for industrial purposes (Lillford and Morrison 1997).

Potatoes are popularly produced into a variety of forms including French fries, chips, baked, and mashed. Cultivar differences are mainly responsible for the variation in processed potato products (Arvanitoyannis et al. 2008). Texture properties of cooked potato, such as mealiness, consistency, and sogginess, are highly correlated with starch content (Kirkpatrick et al. 1951; Unrau and Nylund 1957). Barrios et al. (1963) indicated that mealy potatoes had a higher proportion of large starch granules (>50 μm in diameter) than waxy cultivars. The clones ND102687AB-1Russ, ND113508C-4, ND113438CB-8R, and Lenape, had over 15% of starch granules over 50 μm, depending whether they were analyzed near the pith or the cortex. Further research is needed to correlate starch granule size in these clones with mealiness. Waxy potato starch has good paste clarity and stability and can be used in the food and paper industry. Waxy starches also have improved freeze-thaw stability, an important characteristic for frozen food products (Zheng and Sosulski 1998). Tuber starches have larger granules and lower levels of protein and lipids than cereals, providing a clearer paste (Jobling 2004).

Pasting Properties

The gelatinization behavior of starches from the 12 diverse clones identified in Chapter 3 was studied using RVA and is reported in Table 4.5. The clones with the highest levels of SS (ND102687AB-1Russ, Lenape, and ND113508C-4) had a significantly higher peak viscosity (PV) than the clones with the lowest levels of SS (ND113487C-1, Inka Dawn, and ND113438CB-8R). Clones with the greatest level of SS (ND102687AB-1Russ and Lenape) had a significantly higher hot paste viscosity (HPV) than the clones that had the lowest levels of SS; however, the third highest clone for SS, ND113508C-4, did not differ significantly from the

clone that had the third lowest level of SS, ND113487c-1. The clones with the highest levels of RS (ND113517ABC-9, ND102549TB-2Russ, and ND113517ABC-6) had significantly higher cold paste viscosity (CPV) and setback (STB) than the clones with the lowest levels of RS (ND102921C-3, ND102903-1R, and ND113060-1).

Table 4.5. Gelatinization behavior based on Rapid Visco Analyzer (RVA) of starches from 12 potato clones with unique soluble or resistant starch levels, compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

Clone	RVA* Pasting characteristics[b]					
	PV* (cP**)	HPV* (cP)	BKD* (cP)	CPV* (cP)	STB* (cP)	PT (min)
ND102687AB-1Russ	5505[g***]	3814[a]	1691[j]	5417[a]	1603[a]	3.8[cde]
Lenape	7227[b]	3840[a]	3387[c]	4763[c]	923.0[ef]	3.7[e]
ND113508C-4	7315[ab]	2674[g]	4642[a]	3486[g]	812.0[fgh]	3.3[h]
ND113487c-1	4885[i]	2668[g]	2217[h]	3662[f]	994.5[de]	3.9[bcd]
Inka Dawn	1151[k]	831.0[k]	320.0[k]	1272[k]	441[i]	4.7[a]
ND113438CB-8R	2028[j]	1637[j]	391.5[k]	2069[j]	432.5[i]	4.0[b]
ND113517ABC-9	6126[d]	3131[c]	2995[d]	4194[d]	1063[d]	3.4[gh]
ND102549TB-2Russ	4825[i]	2855[e]	1970[i]	4224[d]	1369[b]	3.8[de]
ND113517ABC-6	7370[a]	3694[b]	3677[b]	4911[b]	1218[c]	3.8[cde]
ND113060-1	5740[f]	3056[d]	2684[f]	3886[e]	830.0[fgh]	3.9[bc]
ND102903-1R	5328[h]	2582[h]	2746[f]	3318[h]	737.0[h]	4.0[b]
ND102921C-3	5955[e]	3029[d]	2926[de]	3914[e]	886.0[efg]	3.3[h]
Red Norland	5656[f]	2781[f]	2876[e]	3523[g]	742.5[h]	3.5[fg]
Russet Burbank	6360[c]	2763[f]	3598[b]	3555[fg]	792.5[gh]	3.5[f]
Yukon Gold	4796[i]	2261[i]	2535[g]	3089[i]	828.0[fgh]	3.3[h]
Mean	5351	2774	2577	3686	911.4	3.7
LSD	99.6	64.01	88.04	122	106.9	0.11

* RVA = rapid visco analyzer, PV = peak viscosity, HPV = hot paste viscosity, BKD = breakdown, CPV = cold paste viscosity, STB = setback, PT = peak time.
** cP = centipoise
***Values with different letters are significantly different ($\alpha \leq 0.05$) using Fischer's LSD.

Genotypic differences have been reported in the pasting profile (Leivas et al. 2013). Smaller starch granules are correlated with lower PV and BKD values (Noda et al. 2005). More than 80% of cortical starch granules for Inka Dawn were under 20 μm. Inka Dawn had the

lowest values for PV (1151 cP) and BKD (320.0 cP). Likewise, ND113508C-4 displayed the highest average length and width for granules near the cortex (36.7 μm and 25.2 μm, respectively), and also expressed one of the highest levels for PV (7315 cP) and BKD (4642 cP). Our results confirm that starch granule size is correlated with PV and BKD, as reported by Noda et al. (2005).

Kaur et al. (2007b) observed that PV and CPV were lower for potato starches with small granules (1-20 μm). The study also observed that BKD and STB were highest for large granule fractions and lowest for small granule fractions. This agrees with our study, which found Inka Dawn to have the lowest mean granule length (13.9 and 13.2 μm for pith and cortex, respectively) and also the lowest PV, CPV, BKD. Our results support the correlation between low PV, CPV, BKD, and STB values and small starch granule size reported by Kauer et al. (2007b). Although ND113508C-4, the clone with the highest mean granule length near the cortex (36.7 μm) and third highest mean granule length near the pith (34.6 μm), had the highest BKD (4642 cP), the clone had the sixth lowest STB value (812 cP). Perhaps the variability of starch granule lengths present in each clone accounted for these differences. Although ND113508C-4 contained large starch granule percentages near the pith (57% of the granules were at least 40 μm), 76% of the cortex granules were under 30 μm. The raw potato flour used for this study was mixed from whole dried potato, resulting in various shaped granules for each clone. This may explain the low value of STB for ND113508C-4.

Gelatinization Characteristics

Starch gelatinization is a process that describes the breakdown of intermolecular bonds of starch molecules and disruption of the starch granule structure (Zobel et al. 2009). Gelatinization of starch and the separation of the cell wall are considered two of the main changes that occur in

potato tissue during heating. Starch gelatinization occurs prior to retrogradation, which is a process in which gelatinized starch associates into a crystalline order (Atwell et al. 1988). DSC is widely used to study starch-water systems and potato tissue and was used in this study.

Gelatinization characteristics were analyzed for our 12 unique genotypes; gelatinization temperatures and enthalpies are presented in Table 4.6. Significant differences were found for all DSC parameters. All clones, except Red Norland, Russet Burbank, and Yukon Gold, were selected at the same growing location and harvest period. The clones were grown at Absaraka, ND, whereas Red Norland, Russet Burbank, and Yukon Gold were grown from various locations in 2014. Geddes et al. (1965) found that small starch granules have a higher gelatinization temperature than larger starch granules; however, only the variety Pentland Crown, grown at the Scottish Plant Breeding Station in Scotland in 1962, was used for this conclusion. Inka Dawn, which had the lowest mean granule length (13.2 μm and 13.9 μm near the cortex and pith, respectively), was the third highest clone for onset temperature for gelatinization, after Lenape and ND102687-1Russ. Although a similar granule size measuring system was used, our results contradict those from Geddes et al. (1965), but may be further explained by genotypic differences.

A previous study (Karlsson and Eliasson 2003) found that dry matter content and gelatinization temperature varied between the pith and other areas of the tuber. The dried potato flour used in our study was a mixture of ground whole, raw potato. The percentage of granule lengths over 20 μm in Inka Dawn was 19% for the cortex and 37% for the pith. Perhaps this variation in granule size among the pith and cortex influenced the gelatinization temperature.

Table 4.6. Gelatinization temperatures and enthalpies, based on starches from 12 potato clones with unique soluble or resistant starch levels, compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

Clone	Thermal properties				
	Onset (°C)	Peak (°C)	Conclusion (°C)	Temperature Range (°C)	Enthalpy (J/g)
ND102687AB-1Russ	68.5[a*]	73.3[a]	79.3[a]	10.9[b]	11.3[bcd]
Lenape	68.7[a]	72.3[b]	77.3[b]	8.6[cd]	13.6[a]
ND113508C-4	61.0[i]	66.9[f]	72.9[g]	11.9[a]	12.7[abc]
ND113487c-1	66.0[de]	69.6[ef]	75.6[cde]	9.6[c]	11.4[bcd]
Inka Dawn	67.9[b]	72.8[b]	78.6[a]	10.7[b]	5.39[e]
ND113438CB-8R	67.0[c]	71.4[c]	79.6[a]	12.6[a]	7.3[e]
ND113517ABC-9	66.4[d]	70.0[de]	74.7[ef]	8.3[d]	10.0[d]
ND102549TB-2Russ	63.9[h]	70.5[d]	76.7[bc]	12.9[a]	11.2[cd]
ND113517ABC-6	66.2[d]	70.2[d]	75.6[cde]	9.4[c]	14.9[a]
ND113060-1	65.5[ef]	70.2[d]	76.2[cd]	10.7[b]	13.4[ab]
ND102903-1R	64.4[g]	69.0[f]	75.3[de]	10.9[b]	12.7[abc]
ND102921C-3	69.0[ef]	73.5[f]	73.5[g]	8.0[d]	12.7[abc]
Red Norland	67.4[bc]	71.1[c]	76.1[cd]	8.8[cd]	11.1[cd]
Russet Burbank	65.3[f]	69.1[f]	73.9[fg]	8.6[cd]	10.6[cd]
Yukon Gold	67.5[b]	71.6[c]	76.2[cd]	8.7[cd]	12.7[abc]
Mean	66.1	70.5	76.1	10.0	11.4
LSD	0.5	0.6	1.1	0.9	2.0

[*] Values with different letters are significantly different ($\alpha \leq 0.05$) using Fischer's LSD.

Karlsson and Eliasson (2003) also found that gelatinization temperature varied by genotype. Our results indicate that there is variation between starch granule size between the pith and cortex of the tuber and for genotype. Further examination of the gelatinization temperatures between the pith and cortex may aid in understanding the relationship between granule size and gelatinization temperature.

Starch Molecular Weight

Isolated potato starches were analyzed by HPSEC after defatting and precipitating the starch. The percentage of amylopectin and amylose, as well as the molecular weights for both starch components, are presented in Table 4.7. Potato starch is generally composed of 20-30% amylose, with the rest consisting of amylopectin (Hoover 2001). Our data agrees with Hoover (2001); ND102687AB-1Russ had the highest percentage of amylopectin (83.70%) and Russet Burbank had the lowest (77.65%). Russet Burbank had the highest percentage of amylose (22.54%), while ND102687-1Russ had the lowest (16.30%). ND102903-1R had the highest M_w for amylopectin (2.45×10^7) and amylose (6.72×10^6). ND102921C-3 had the lowest amylopectin M_w (1.02×10^7), while ND113517ABC-9 had the lowest amylose M_w (1.17×10^6). Although larger granule sizes have been associated with higher amylose content (Geddes et al. 1965), contradictory studies have found little to no differences in amylose content among various sized potato starch granules (Fujita et al. 1983, Chen et al. 2003, and Noda et al. 2005). In our study, Russet Burbank displayed the highest percentage of amylose (22.54%) among clones. Interestingly, 53% of starch granules near the cortex of Russet Burbank were at least 30 μm. On the contrary, 57% of granules near the cortex from ND113508C-4 were at least 30 μm; however, ND113508C-4 ranked third lowest in percent amylose, which was significantly lower than Russet Burbank. Our data suggests that granule sizes are not associated with amylose content. Fajardo et al. (2013) examined amylose content in 20 cultivars grown at Hancock, WI, and indicated that genotype and environment contributed to variation for amylose content. All of our clones, except Red Norland, Russet Burbank, and Yukon Gold, were selected from the same growing location and harvest period. Thus, we believe that the differences in amylose and amylopectin percentage and molecular weights are due to genotypic differences. Although our

clones were selected and examined based on the amount of SS and RS present, amylopectin and amylose are generally not considered completely soluble or insoluble, respectively.

Table 4.7. High performance size exclusion chromatography (HPSEC) parameters of starches from 12 potato clones with unique soluble or resistant starch levels, compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

Clone	Amylopectin %	Amylose %	Amylopectin $M_w \times 10^7$	Amylose $M_w \times 10^6$
ND102687AB-1Russ	83.70[a*]	16.30[g]	1.76[e]	2.92[c]
Lenape	79.77[f]	20.23[b]	1.38[j]	1.80[g]
ND113508C-4	81.63[b]	18.37[f]	2.10[b]	2.33[d]
ND113487c-1	79.79[f]	20.21[b]	1.20[l]	1.30[k]
Inka Dawn	80.36[d]	19.64[d]	2.04[c]	1.54[i]
ND113438CB-8R	81.10[c]	18.90[e]	1.74[f]	1.40[j]
ND113517ABC-9	79.75[f]	20.25[b]	1.35[k]	1.17[n]
ND102549TB-2Russ	81.24[c]	18.76[e]	1.07[n]	1.27[l]
ND113517ABC-6	81.26[c]	18.74[e]	1.65[g]	1.09[o]
ND113060-1	81.76[b]	18.24[f]	1.64[h]	3.35[b]
ND102903-1R	80.40[d]	19.60[d]	2.45[a]	6.72[a]
ND102921C-3	80.48[d]	19.52[d]	1.02[o]	1.56[h]
Red Norland	80.01[e]	19.99[c]	1.17[m]	2.02[e]
Russet Burbank	77.46[g]	22.54[a]	1.49[i]	1.23[m]
Yukon Gold	79.65[f]	20.35[b]	1.78[d]	1.89[f]
Mean	80.56	19.44	1.59	2.11
LSD	0.18	0.18	0.02	0.01

[*] Values with different letters are significantly different ($\alpha \leq 0.05$) using Fischer's LSD.

Amylopectin generally digests more quickly than amylose due to the higher number of reducing ends available for enzymatic degradation; however, the branch points on amylopectin are resistant to enzymatic hydrolysis by amylase (Miles et al. 1985). Thus, we cannot determine the levels of amylopectin and amylose present in our SS and RS calculations.

Conclusion

Starch is an important component for diverse applications, such as food products, binding, textiles, films, biodegradables, and pharmaceuticals. Raw potato starch consists of large amounts of resistant starch (RS) that is converted to digestible starch after cooking. Foods high in rapidly digested starch have a high glycemic index (GI) and elicit high insulin demand (Augustin et al. 2002). Amylopectin typically makes up 70-80% of the available starch in the potato tuber, with the rest consisting of amylose (Zeeman et al. 2010). The ratio of amylose and amylopectin determines the end use of the potatoes application. Separation of the two starch molecules for processing is costly and may lead to a high level of water waste. For this reason, applications that require potato starch must be selected based on their starch profile.

In this study, 12 potato clones, considered unique for their SS and/or RS content presented in Chapter 3, were analyzed for their granule morphology, pasting profile, gelatinization characteristics, and starch composition. The commercial cultivars Red Norland, Russet Burbank, and Yukon Gold also were examined. All of the clones were selected from the same location and harvesting period, except for the commercial cultivars, which were grown from various locations in North Dakota in 2014. Thus, we believe that the variability in starch properties is due to genotypic factors.

Our results concluded that there are unique granule size distributions between the clones. Starch granule length is important for specific industrial applications, such as filters, and aids in explaining the digestion of starch. Significant differences were found between clones and commercial cultivars for their pasting characteristics for peak viscosity, hot paste viscosity, breakdown, cold paste viscosity, setback, and peak time. Pasting occurs after heat treatment of starch and water suspensions, resulting in a highly viscous solution. Pasting characteristics are

important in food and non-food applications, such as films and adhesives. Differences were examined between clones and commercial cultivars for gelatinization temperatures for onset, peak, conclusion, and temperature range. Enthalpy differences were also displayed. Starch components, such as amylose and amylopectin percentage and molecular weight, varied between clones and commercial cultivars. These results confirm that genotypic differences are present within the clones and commercial cultivars used in this study. Our findings will aid in the development of products for diverse industries, and help potato breeders develop clones with unique starch profiles for specific end uses.

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CHAPTER 5. STARCH PROFILE VARIABILITY BETWEEN GREENHOUSE AND FIELD GROWN TUBERS

Abstract

Diverse soluble (SS) and/or resistant starch (RS) levels have been discovered in the North Dakota State University (NDSU) potato breeding program germplasm. However, these clones only have been analyzed when grown in the field. The purpose of this study was to determine whether clones grown in the greenhouse vary in their SS or RS concentration compared to clones grown in the field. Screening tubers of clones grown in the greenhouse versus the field could possibly provide a more rapid and efficient assessment. A total of 48 clones were analyzed between the field locations of Baker, MN, Absaraka, ND, and from in the greenhouse. Results indicated that growing environment significantly impacts SS and RS content. Greenhouse-grown clones contained significantly reduced levels of SS and RS compared to field grown tubers, indicating that there are environmental factors that dramatically impact the starch profiles within the clones. The findings presented in this study suggest that greenhouse-grown tubers should not be used to analyze SS or RS content due to the large variation in the starch profile compared to field-grown tubers.

Introduction

Chemically, starches are composed of polysaccharides consisting of α-D-glucose networked by α-D-(1—4) or α-D-(1—6) linkages. Amylose, the mostly linear linked glucoses, and amylopectin, the highly branched glucoses, make up the starch granule. Amylopectin is a larger molecule than amylose, resulting in a larger surface area for amylolytic attack (Singh et al. 2010). The potato typically is compromised of 70-80% amylopectin, with the remainder consisting of amylose (Zeeman et al. 2010). Raw potato starch consists of large amounts of

resistant starch (RS) that is converted to digestible starch after cooking. Foods high in starch rapidly digested to glucose have a high glycemic index (GI) and elicit high insulin demand (Augustin et al. 2002). Starch is considered indigestible if it has not been hydrolyzed within 120 min after being consumed (Fuentes-Zaragoza et al. 2011).

Research has not been performed previously that compares soluble starch (SS) and RS levels of greenhouse-grown potatoes to field-grown potatoes. The greenhouse offers advantages, including more control over environmental factors, and the opportunity to use tubers during convenient times of a breeding programs calendar. The purpose of this research was to determine if starch profiles for clones grown in the greenhouse vary from those grown in the field.

Materials and Methods

Genotypes

Forty-eight potato clones from the North Dakota State University potato breeding program were grown at two locations (Baker, MN and Absaraka, ND) in 2014; 45 from Baker, and 11 from Absaraka, were analyzed. Eight clones were common to the two locations. Tubers were harvested from Baker on October 23 and Absaraka on October 10 in 2014. The 48 genotypes were also grown in the Agricultural Experiment Station greenhouse in 2016 at North Dakota State University (NDSU) in 10-12 inch clay pots (Table 5.1).

Table 5.1. Clones grown at Absaraka, ND, Baker, MN, and in the greenhouse.

Location		
Absaraka, ND	Baker, MN	Greenhouse
ND102642C-2	90245.1	90245.1
ND113207-1R	95043.11	95043.11
ND113278-3	463-4	463-4
Dakota Pearl	Dakota Jewel	Dakota Jewel
Dakota Ruby	Dakota Pearl	Dakota Pearl
Dakota Russet	Dakota Ruby	Dakota Ruby
ND102775C-5RR	Dakota Russet	Dakota Russet
ND113224C-	Dakota Trailblazer	Dakota Trailblazer
ND7743C-2RS	Etb-6-21-3	Etb-6-21-3
Romanze	Etb-6-21-4	Etb-6-21-4
Russet Norkotah	Etb-6-5-5	Etb-6-5-5
	Gala	Gala
	J103-K7	J103-K7
	J138-A12	J138-A12
	ND060735-4Russ	ND060735-4Russ
	ND081557c-5P	ND081557C-5P
	ND081571-3R	ND081571-3R
	ND081577-1R	ND081577-1R
	ND092019C-4Russ	ND092019C-4Russ
	ND102663B-3R	ND102642C-2
	ND102775C-5RR	ND102663B-3R
	ND102921C-3	ND102775C-5RR
	ND102990B-2R	ND102921C-3
	ND113060-1	ND102990B-2R
	ND113113B-1PSY	ND113060-1
	ND113224C-3Russ	ND113113B1PSY
	ND113230C-1	ND113207-1R
	ND113289C-1	ND113224C-3Russ
	ND113338C-3R	ND113230C-1
	ND113460c-3PS	ND113278-3
	ND113461-1RS	ND113289C-1
	ND113461-2P	ND113338C-3R
	ND113508C-4	ND113460C-3PS
	ND113526CB-1Russ	ND113461-1RS
	ND113541-1	ND113461-2P
	ND4100C-19	ND113508C-4
	ND7743C-2RS	ND113526CB-1Russ
	ND8068-5Russ	ND113541-1
	ND8331Cb-2	ND4100C-19
	ND8527B-94Y	ND7743C-2RS
	NDJL64BV-1R	ND8068-5Russ
	Romanze	ND8331Cb-2
	Russet Norkotah	ND8527B-94Y
	Shepody	NDJL64BV-1R
	WND8625-2Russ	Romanze
		Russet Norkotah
		Shepody
		WND8625-2Russ

Starch Analysis

Two tubers of each clone from each location were washed, peeled, cut into identically sized pieces (2.5 cm^2), placed into a Ziploc® Zip'n Steam bag, and microwaved (1200W) for 4 minutes on high (steamed). Following cooking, tuber tissue was riced and mixed. SS and RS was determined using the Megazyme Resistant Starch Assay (K-RSTAR, Megazyme International Ireland, Ltd, Co. Wicklow, Ireland) kit. A modified miniaturization of the assay was utilized (Raatz et al. 2016). Samples of potato were analyzed in triplicate. Riced samples of potato (0.50 g) were weighed into Corning® 15 ml centrifuge tubes and 4 mL of pancreatic amylase solution (10 mg/mL) (3U/mL amyloglucosidase)/sodium azide (0.03%)) was added into each tube. The tubes were capped and placed into a continuous shaking water bath at 37°C at 100 rpm for precisely 16 hrs.

Adding 4 mL of 95% ethanol to each sample resulted in termination of the reaction. Recovery of RS was performed by centrifugation (2000 x g, 10 min at RT). The supernatant, containing SS, was decanted into 100 ml volumetric flasks. The RS pellet was washed an additional two times with 8 mL of 50% ethanol, centrifuged (2000 x g, 10 min at RT), and decanted into 100 ml volumetric flasks.

Adding 2 mL of 2 M KOH, along with vigorous stirring, within an ice-water bath, over a magnetic stirrer, dissolved the pellet containing RS. The RS solution was neutralized by adding 8 mL of 1.2 M sodium acetate buffer (pH 3.8) and immediately adding 0.1 mL amyloglucosidase. The samples were incubated in a water bath at 50°C for 60 min. The contents in the tube were diluted 1:10 using a 100 mL volumetric flask. Aliquots of each solution were centrifuged (1500 x g, 10 min), 40 µL of the supernatant was transferred to 2.0 mL microtubes, and was mixed with 1.2 mL glucose oxidase-peroxidase-4-aminoantipyrine reagent

(Megazyme Resistant Starch Assay, Megazyme International Ireland Ltd, Co. Wicklow, Ireland). The microtubes were placed in a water bath at 50°C for 20 min. The mixtures were transferred to a 96-well plate where the absorbance was read against a reagent blank at 510 nm utilizing a microplate reader (Multiskan FC, Thermo Scientific, Finland).

The SS supernatant in the 100 mL volumetric flasks was filled to 100 mL with 100 mM sodium acetate buffer and mixed. A 1:2 dilution of the SS solution, compromised of 20 µL SS solution and 20 µL deionized water, was added to 2.0 mL microtubes, with 4 µL of dilute amyloglucosidase (300 U/mL) and 1.2 mL glucose oxidase-peroxidase-4-aminoantipyrine reagent (Megazyme Resistant Starch Assay, Megazyme International Ireland Ltd, Co. Wicklow, Ireland); samples were placed in a 50°C water bath for 20 minutes. Samples were then transferred to a 96-well plate, where the absorbance was read against a reagent blank at 510 nm utilizing a microplate reader (Multiskan FC, Thermo Scientific, Finland). Two replicates of each sample were analyzed for moisture content.

Statistical Analysis

Variation between the field locations and greenhouse clones was analyzed by analysis of variance (ANOVA) using GLM (SAS Institute 2012). Genotypes were compared ($\alpha \leq 0.05$) using SAS 9.3 (SAS Institute 2012). A mean separation test was performed for the eight clones grown at all locations, using Fischer's least significant difference (LSD) ($\alpha \leq 0.05$) using SAS 9.3 (SAS Institute 2012).

Results and Discussion

Soluble Starch

Analysis of variance for SS levels of clones grown in three environments is presented in Table 5.2. SS refers to the fraction of starch that is hydrolyzed by digestive enzymes. Location x clone was significant, indicating that the clones, when grown in another environment, differed in their SS levels. Bach et al. (2013) found genotype, as well as temperature, precipitation, and other environmental factors, to impact starch profiles for potato genotypes grown within the same location over different years, implying that there is a complex effect of moisture and temperature that results in varied starch profiles. Previously, genotype and growing conditions had been reported to influence the composition and physical properties of starch granules (Cottrell et al. 1995), suggesting that growing locations and genotypes used in our study impacted the starch granule configuration and may have influenced the SS and RS levels. Replication within location was not significant, indicating that the starch levels of replicates within each growing environment were not significantly different. Location was significant, indicating that significant differences were found for SS levels between the growing environments. Clone was a significant factor, as expected, based on previous research (Bach et al. 2013) and our findings in Chapter 3 and 4. Although our findings in Chapter 3 reported significant differences among clones grown at Baker, MN, for SS, our results indicated no significant differences among clones grown at Absaraka, ND. However, sample size for Absaraka was small, compared to the number of clones analyzed for Baker.

Table 5.2. Analysis of variance for soluble starch levels for clones grown at Baker, MN, Absaraka, ND, and in the greenhouse.

Source	DF	SS	MS	F	Pr>F
Location	2	778326.5	389163.	362.6	0.01*
Replication(Location)	6	10999.8	1833.3	1.7	0.12^{ns}
Clone	47	289870.8	6167.5	5.8	0.01*
Location x Clone	54	322989.7	5981.3	5.6	0.01*
Error	20	216807.0	1073.3		
Corrected Total	31	1598035.8			

* significant at $P \leq 0.05$, ^{ns}= not significant at $\alpha \geq 0.05$.
^{ns} = not significant

Little information is available from other studies regarding SS content in potato. Instead, potato starch has been analyzed in terms of rapidly and slowly digested starch (Bach et al. 2013), amylose and amylopectin content (Noda et al. 2004), and glycemic elicitation (Ek et al. 2012). Amylose and amylopectin are not considered completely soluble or insoluble, respectively. However, amylose is more difficult to hydrolyze than amylopectin, and more enzymes are able to degrade amylopectin than amylose, contributing to the variability in digestion rates of these two forms of starch (Taiz and Zeiger 2010). This suggests that there is more amylopectin degraded into SS and more amylose present in the RS fraction, although research is needed to verify this. Thus, we may not directly compare our findings to studies that compare SS levels via other definitions of starch. Raatz et al. (2016) found that baked potatoes contained higher levels of RS than boiled potatoes for three commercial cultivars, indicating that cooking method impacts RS levels. However, our results from Chapter 2 indicated no significant difference in SS or RS levels among the same cultivars used by Raatz et al. (2016) for baking, boiling, and steaming with microwavable steam bags.

A mean separation test was performed among the eight clones common to out three locations using Ficher's LSD (Table 5.3). SS and RS levels for all clones listed in Table 5.1 are presented in Tables A.3, A.4, and A.5. Among the clones grown across all three growing environments, ND113224C-3Russ had the highest SS level (367.7 mg/g) when grown at Absaraka, ND, and ND102775C-5RR had the lowest when grown in the greenhouse (91.6 mg/g) (Table 5.3). For all clones, except ND102775C-5RR, the SS content was highest from Baker, MN. For all clones, except Dakota Ruby and Romanze, SS content was lowest when grown in the greenhouse.

Our data suggests that the growing conditions at Baker, MN, during the 2014 growing season, positively impacted SS levels. However, ND774C-3Russ displayed a significantly higher SS level when grown at Absaraka, ND, than any of the clones that were grown at all three locations (Table 5.3). Clones grown in the greenhouse exhibited the lowest levels of SS compared to field grown clones. Research has not been performed previously on the variability of SS or RS levels between greenhouse and field grown tubers. However, environmental factors, such as temperature and rainfall, as well as soil attributes, may have contributed to these differences in RS. The tubers in the greenhouse were watered daily, whereas field-grown tubers were grown under non-irrigated conditions. The temperature in the greenhouse was kept consistent at 22.2°C (±4°C) during the day and 12.8°C (±4°C) at night, whereas temperatures at Baker, MN, ranged from 7.8°C to 24.4°C, and Absaraka, ND, ranged from 7.8°C to 26.7°C between June and September 2014.

Nitrogen fertilization was shown to negatively impact starch content in potatoes (Bártová et al. 2011), although only raw tuber tissue was examined, our starch was extracted from cooked tuber tissue. Cooking disrupts starch granules, making amylose and amylopectin susceptible to

Table 5.3. Comparison of soluble and resistant starch levels (based on dry weight) for clones grown at Baker, MN, Absaraka, ND, and in the greenhouse.

	Location[a]					
	Baker, MN		Absaraka, ND		Greenhouse	
Clone	Soluble Starch (mg/g)	Resistant Starch (mg/g)	Soluble Starch (mg/g)	Resistant Starch (mg/g)	Soluble Starch (mg/g)	Resistant Starch (mg/g)
Dakota Pearl	238.0^{abcd*}	77.4^{ab}	200.5^{b}	99.9^{ab}	171.5^{a}	30.5^{de}
Dakota Ruby	261.0^{abc}	74.0^{ab}	163.1^{b}	108.1^{ab}	177.5^{a}	24.3^{ef}
Dakota Russet	273.0^{ab}	90.9^{a}	202.8^{b}	87.2^{b}	171.5^{a}	40.9^{cd}
Russet Norkotah	231.9^{abcd}	56.7^{bc}	170.9^{b}	117.0^{ab}	132.0^{b}	18.7^{ef}
Romanze	224.5^{bcd}	41.0^{c}	164.0^{b}	91.1^{b}	183.2^{a}	26.7^{a}
ND102775C-5RR	220.7^{cd}	94.3^{a}	179.3^{b}	127.8^{a}	91.6^{c}	13.8^{f}
ND113224C-3Russ	203.6^{d}	61.5^{bc}	367.7^{a}	101.6^{ab}	132.0^{b}	56.0^{b}
ND7743C-2RS	279.9^{a}	77.2^{ab}	153.8^{b}	94.4^{b}	104.6^{c}	53.9^{bc}
Mean	241.6	71.6	200.3	103.4	143.1	41.1
LSD	51.5	29.1	60.4	32.3	21.0	13.7

*Values with different letters are significantly different (α=0.05) using Fischer's LSD.

enzymatic degradation. Perhaps the soil fertility differences between the field- and greenhouse-grown potatoes impacted the SS levels and contributed to these differences between growing locations. Bogucka (2014) found that an increase in soil-applied fertilizer resulted in a decrease in starch content and a higher proportion of smaller granules (<20μm). Similar results were obtained by Westermann et al. (1994), which found increased levels of nitrogen and potassium to negatively effect starch concentrations in field-grown Russet Burbank.

Eppendorfer and Eggum (1992) studied the effect of nitrogen, phosphorus, potassium, and sulfur, as well as, three different levels of water, on total starch content in greenhouse-grown potatoes. Results of this study indicated that a deficiency in phosphorus, potassium, or sulfur produced tubers with decreased starch content in boiled potatoes. Günel and Karadoğan (1997)

studied the effects of irrigation at three growth stages (planting, stolon initiation, and tuber bulking after available soil water dropped to 25%, 50%, and 75%). Results indicated frequent irrigation during planting and stolon initiation to positively affect potato starch; however, frequent irrigation at the final growth stage had negative effects. Research plots at Baker and Absaraka were supplied with about 90.7 kg of nitrogen prior to planting. Greenhouse grown pots were fertilized at planting using a mixture of slow release and rapid release fertilizer and were not fertilized again, despite being watered twice daily. It is possible that soil and watering differences resulted in a decrease in total available starch in greenhouse-grown tubers, explaining the significant decrease in SS and RS content compared to field-grown tubers.

Harvest dates have been shown to influence starch content and granule characteristics (Noda et al. 2004). Tubers harvested at later dates resulted in a significant increase in granule size and decrease in amylose content (Noda et al. 2004). In contrast, Christensen and Madsen (1996) observed amylose levels to stay consistent during later tuber development stages. Liu et al. (2003) reported dry matter to increase as the growth time increased. The tubers grown in the greenhouse and field for our study were grown to full maturity. Therefore, environmental conditions, such as soil fertility and watering, must have contributed to more variation in SS and RS content than tuber maturity.

Resistant Starch

Analysis of variance between clones for RS levels grown in three different environments is presented in Table 5.4. Multiple studies have shown that RS is compromised of a linear molecule of α-1,4-D-glucan, which typically consists of retrograded amylose, and has a

Table 5.4. Analysis of variance for resistant starch (based on dry weight) levels between clones grown at Baker, MN, Absaraka, ND, and in the greenhouse.

Source	DF	SS	MS	F	Pr>F
Location	2	3748.4	1874.2	7.3	0.01*
Replication(Location)	6	8745.4	1457.6	5.7	0.01*
Clone	47	722930.4	15381.5	60.0	0.01*
Location x Clone	54	794375.5	14710.7	57.4	0.01*
Error	202	51812.7	256.5		
Corrected Total	311	1590189.9			

* significant at $\alpha \leq 0.05$, ^{ns}= not significant at $\alpha \geq 0.05$.

molecular weight that is relatively low (1.2×10^5 Da) (Tharanathan 2002). The significance of location x clone suggests that the clones, when grown in different environments, vary in their RS levels. Genotypes grown in heated glasshouse conditions were shown to have higher amylose content in raw potato, compared to nonheated glasshouse and field conditions (Cottrell et al. 1995). Although amylose content was not directly analyzed in our study and our samples were examined after cooking, the clones grown in the greenhouse displayed dramatically lower levels of RS, which would contradict the findings of Cottrell et al. (1995). Research has not been previously reported regarding the difference in amylose content in raw versus cooked potato. Amylose concentration was shown to decrease for cooked rice compared to raw rice (Jain et al. 2012). Perhaps soil conditions between the growing environments influenced RS content. Clones analyzed from the field were stored for a longer duration prior to analysis (approximately 14 months) compared to greenhouse-grown tubers (about 4 months). A study performed by Fajardo et al. (2013) reported that storage duration had little affect on amylose content compared to fresh tubers; however, tubers from Fajardo et al. (2013) were only stored for two months prior

to analysis. Further research is required to determine the affect of prolonged storage on RS content.

Replication(Location) was significant, indicating that the replicates within locations were significantly different. This result was unexpected, as replicates were not anticipated to vary. Small changes in temperature or time during enzymatic hydrolysis could have contributed to these differences. Small changes in temperature have been shown to influence the saccharide composition after amylase degradation (Marchal et al. 1998). However, temperatures and times during hydrolysis were kept consistent. Repeated hydrolysis of samples may help determine the amount of RS not detected after our initial hydrolysis. Pure amylose has been shown to be extremely resistant to enzymatic hydrolysis (Rendleman 2000), which is not surprising for starch that has undergone retrogradation. Perhaps the differences between replicates found in our study are due to varying levels of retrograded starch, or due to room temperature changes during enzymatic hydrolysis. Location was significant, suggesting that RS levels did varied between locations. Results reported in Chapter 3 indicated that clones grown at Baker, MN, and Absaraka, ND, did not vary in RS levels, although significant differences were not compared between the locations. Clone was a significant factor, implying that genotypes differed in their RS levels. Our results from Chapter 3 did not find significant differences between clones grown at Baker, MN, or Absaraka, ND, for RS. However, the sample size for Chapter 3 was much larger than this study, and the genotypes used in Chapter 3 were predominantly advanced or advancing selections, whereas this study used predominantly cultivars, perhaps intimating adaptability and more uniform performance for widely grown commercial cultivars

The clone ND102775C-5RR had the highest level of RS when grown at Absaraka, ND (127.8 mg/g), and surprisingly had the lowest level of RS among all three growing environments

when grown in the greenhouse (13.8 mg/g) (Table 5.3). Although genotypic differences were detected, there is evidence in our results indicating that RS content varies among growing environments. There are various influences on starch content, including precipitation and temperature (Bach et al. 2013).

Most clones grown at Absaraka, ND, have higher RS content, compared to clones grown at Baker, MN, and in the greenhouse (Figure 5.2). Amylose content of at least 50% is desired for nutritional benefits resulting from high fiber content and a reduced glycemic index (Behall and Hallfrisch 2002). Clones grown in the greenhouse have a significantly lower level of RS, indicating that these clones would likely elicit a higher glycemic index compared to clones grown at Baker, MN, and Absaraka, ND. According to Abe et al. (1982), the hydrolysis of potato is impacted by the concentration of phosphorus; the higher the phosphorus content, the lower the hydrolysis rate. Hydrolysis action by amylase is prevented by the esterified phosphate groups attached to residues of starch (Abe et al. 1982). It is possible that soil used in the greenhouse had an increased level of phosphorus compared to the field locations, or the clones had a higher inherent phosphorus level, thus impacting the hydrolysis rates by amylase, preventing degradation of the starch granule. Further examination is needed to determine which factors in the greenhouse cause such dramatic decreases in SS and RS content.

Digestible and RS in raw potato starch have reported to be affected by genotypic and environmental factors (Bach et al. 2013). Raw potato starch consists of large amounts of RS that is converted to digestible starch after cooking. Cooking disrupts starch granules, making amylose and amylopectin susceptible to enzymatic degradation. Little research is available regarding differing SS and RS profiles from cooked potatoes among different growing environments. Although studies have compared cooked potato tissue from genotypes, these

studies describe starch as amylose and amylopectin content (Noda et al. 2004), and glycemic elicitation (Ek et al. 2012), and only describe RS among few genotypes (Raatz et al. 2016). More research is required to determine the amount of amylose and amylopectin that was converted to SS and RS, in order to more accurately compare our findings with other studies. Additional research also is required to provide insight into environmental factors impacting the starch profile among potato genotypes, especially greenhouse versus field grown environments. These parameters include precipitation differences, temperature, soil components, and tuber growth periods.

Conclusion

The objective of this research was to determine whether SS and/or RS levels among clones within the North Dakota State University potato breeding program varied between greenhouse and field environments. The microwave steam bag method was employed to cook tuber tissue for SS and RS analysis. Clones grown at Baker, MN (45), and Absaraka, ND (11), were compared to 48 greenhouse-grown clones. Of the clones grown at Absaraka, ND, eight of them were also grown at Baker, MN. Clones that were grown in all three growing environments were compared for their SS and RS levels. Results indicated that there is variability in SS and RS levels among clones and locations, suggestion that environmental factors impact the levels of SS and RS. Greenhouse clones had significantly lower levels of SS and RS, most likely due to environmental conditions such as watering, temperature, and soil components that varied from the field grown clones. Although other studies have compared starch from baked tuber tissue, most studies define starch as a percentage of amylose and amylopectin, or the glycemic index that they elicit. Future research is required to determine the levels of amylose and amylopectin that contribute to SS and RS levels. Further analysis should be performed regarding the effect of

environmental factors, tuber maturity, and storage conditions on tubers grown in the field and in the greenhouse.

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CHAPTER 6. CONCLUSION

Our studies show the diverse soluble starch (SS) and resistant starch (RS) content and characterizations found in the North Dakota State University (NDSU) potato breeding program germplasm. Some genotypes may provide adequate starch characteristics that can be utilized in various industrial applications, such as biodegradables, pharmaceuticals, textiles, and filters. Still, the need for further assessment of the environmental and storage factors that influence starch profiles among the germplasm should be emphasized. The development of clones with high or low levels of amylose or amylopectin is desired for certain applications. The experimental approach consisted of examining the factors that influence starch content in potato (Chapter 1), determining the applicability of a more efficient cooking method, microwave steaming, to cook tuber material for starch analysis (Chapter 2), screening clones present in the NDSU potato breeding program for unique starch profiles (Chapter 3), examining the unique clones found in Chapter 3 for fine chemistry attributes using scanning electron microscopy (SEM), rapid visco analyzer (RVA), differential scanning calorimetry (RVA), and high performance size exclusion chromatography (HPSEC) (Chapter 4), and determining whether clones grown in the greenhouse differed from field-grown clones for SS and RS content (Chapter 5).

In order to provide a more efficient examination of starch content, microwave steaming was shown to have no influence on SS and RS. The microwave steam method was used in the remainder of our indicated specific clones present in the NDSU potato breeding program have uniquely high, or low, levels of SS or RS. Distinct starch profiles were also indicated for market types, suggesting that the end-use of a genotype depends on the starch profile.

Unique clones found within the NDSU germplasm were further analyzed for their starch granule morphology, pasting properties, gelatinization characteristics, and starch molecular weight and chain length distribution. These clones displayed varying granule size distributions, and exhibited significantly different parameters for fine chemistry characteristics. Clones with unique starch characteristics may provide approproate attributes for specific industrial or nutritive product applications.

In order to examine a large number of clones for starch properties, efficiency and rapid assessment are preferred. Greenhouse grown clones were compared to field grown clones for their SS and RS. Results indicated that clones grown in the greenhouse contained significantly lower levels of SS and RS than field-grown clones, suggesting that environmental conditions impact the starch profile. The duration of storage may have been a factor contributing to the altered starch profile, since greenhouse tubers were stored for approximately four months, whereas field-grown tubers were stored for 12-16 months prior to analysis. Research and development for starch-based applications should examine clones grown from the field, as most applications desire high starch content. However, for nutritive purposes, a low-starch potato is preferred.

Environmental conditions, such as soil fertility, rainfall, and temperature and storage conditions, such as duration, temperature, and humidity, have been shown to impact the starch profile. Further research is needed to determine the factors that impact the starch profile in clones that may provide applicability to food or industrial products. Unique clones in this study may provide insight into breeding and developing potato cultivars for specific starch profiles in order to obtain optimum starch characteristics for specific product applications.

APPENDIX

Table A.1. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Baker, MN, in 2014.

Clone	Market Class	Soluble Starch (mg/g)	Soluble Starch % of total	Resistant Starch (mg/g)	Resistant Starch % of total
ND102647-3Russ	Dual purpose	307.7	75.9	97.7	24.1
ND1026638-3R	Fresh	248.2	68.7	113.3	31.3
ND102687AB-1Russ	Frozen	358.9	76.0	113.2	24
ND102719B-1Russ	Frozen	338.5	77.4	98.7	22.6
ND102735CB-4R	Fresh	292.4	76.1	91.9	23.9
ND102775C-5RR	Specialty	228.0	65.5	120.0	34.5
ND102800ABC-1	Germplasm	251.0	70.4	105.6	29.6
ND102784B-3R	Fresh	285.8	70.8	118.1	29.2
ND102809AB-2	Germplasm	282.5	69.0	127.2	31.1
ND102814CAB-1	Germplasm	289.8	71.9	113.1	28.1
ND102822CAB-1	Germplasm	328.8	69.7	143.1	30.3
ND102857CB-1	Chip processing	237.2	68.9	107.2	31.1
ND102858CB-2	Chip processing	245.1	72.3	94.1	27.7
ND113338C-3R	Fresh	241.6	66.7	120.6	33.3
ND102903-1R	Fresh	273.2	85.6	46.0	14.4
ND102908-4R	Fresh	190.5	70.0	81.7	30.0
ND102921C-3	Chip processing	243.9	85.7	40.9	14.4
ND102990B-2R	Fresh	259.7	74.6	88.6	25.4
ND102990B-3R	Fresh	205.8	80.7	49.1	19.3
ND113060-1	Chip processing	196.3	80.5	47.4	19.5
ND113065CB-12Russ	Dual purpose	232.0	75.1	76.8	24.9
ND113091B-2RY	Specialty	207.4	75.5	67.4	24.5
ND113207-1R	Fresh	257.0	78.5	70.3	21.5
ND113200B-1RY	Specialty	230.0	71.4	92.0	28.6
ND113100-1Russ	Dual purpose	262.9	73.2	96.4	26.8
ND113174B-2Russ	Frozen	321.0	74.9	107.4	25.1
ND113163-1	Chip processing	256.2	65.1	137.5	34.9
ND113203-2R	Fresh	225.5	78.9	60.2	21.1
ND113070B-1R	Fresh	239.0	74.9	80.0	25.1
ND113089B-2RY	Specialty	225.8	76.5	69.5	23.5
ND113300C-3RSY	Specialty	282.9	77.5	82.4	22.6
ND113289C-1	Chip processing	253.8	69.1	113.4	30.9
ND113243ABC-2Russ	Germplasm	252.9	73.2	92.7	26.8
ND113174B-1Russ	Frozen	286.1	73.5	103.3	26.5

Table A.1. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Baker, MN, in 2014 (continued).

Clone	Market Class	Soluble Starch (mg/g)	Soluble Starch % of total	Resistant Starch (mg/g)	Resistant Starch % of total
ND113256C-2R	Fresh	350.0	76.4	108.0	23.6
ND113266C-3	Chip processing	298.1	77.6	86.2	22.4
ND113337-4RS	Specialty	276.4	73.8	98.1	26.2
ND113224C-3Russ	Frozen processing	242.7	74.3	83.8	25.7
ND113307C-3	Chip processing	273.9	72.4	104.4	27.6
793101.3	Germplasm	286.1	71.9	111.6	28.1
463-4	Germplasm	205.0	75.1	68.1	24.9
ND102733Cb-1R	Fresh	240.4	77.4	70.3	22.6
ND113335B-4R	Fresh	255.2	77.4	74.7	22.6
ND113338C-1R	Fresh	289.6	72.2	111.4	27.8
ND102721b-1Russ	Dual purpose	252.4	77.9	71.5	22.1
ND113230C-1	Chip processing	235.9	76.0	74.4	24.0
ND113356B-2PEY	Specialty	196.3	73.1	72.2	26.9
ND113298-2RS	Specialty	245.5	74.4	84.7	25.7
90245.1	Germplasm	303.0	73.6	109.0	26.5
93057.1	Germplasm	299.0	78.5	82.1	21.6
All Blue	Specialty	256.3	76.3	79.4	23.7
95043.11	Germplasm	210.2	67.1	102.9	32.9
Bison	Fresh	250.5	74.1	87.4	25.9
DakChip	Chip processing	254.5	76.7	77.4	23.3
Crystal	Chip processing	285.7	74.6	97.4	25.4
Dakota Crisp	Chip processing	216.7	73.1	79.7	26.9
Dakota Diamond	Chip processing	237.5	74.2	82.5	25.8
Lenape	Germplasm	347.3	75.3	114.3	24.8
Dakota Jewel	Fresh	271.6	79.0	72.1	21.0
Dakota Trailblazer	Dual purpose	268.0	80.1	66.6	19.9
Inka Dawn	Specialty	158.9	71.1	64.5	28.9
Gala	Specialty	198.8	70.9	81.7	29.1
Dakota Pearl	Chip processing	259.3	73.3	94.6	26.7
Dakota Russet	Dual purpose	294.4	73.2	108.1	26.9
Dakota Ruby	Fresh	282.3	75.6	91.2	24.4
Dakota Rose	Fresh	252.7	81.5	57.5	18.5
NorValley	Chip processing	279.3	75.3	91.8	24.7
Norland	Fresh	258.0	73.1	95.2	27.0
Norking Russet	Fresh	228.6	75.2	75.3	24.8
Stirling	Chip processing	330.3	73.3	120.4	26.7

Table A.1. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Baker, MN, in 2014 (continued).

Clone	Market Class	Soluble Starch (mg/g)	Soluble Starch % of total	Resistant Starch (mg/g)	Resistant Starch % of total
Snowflake	Flakes	315.5	82.4	67.4	17.6
Viking	Fresh	233.0	75.9	74.0	24.1
Shepody	Frozen processing	215.8	70.1	92.0	29.9
Russet Norkotah	Fresh	223.8	75.9	70.9	24.1
Romanze	Fresh	216.4	79.7	55.2	20.3
Ebt5-31-2	Germplasm	253.6	70.8	104.6	29.2
AND99362B-1Russ	Germplasm	194.5	63.8	110.2	36.2
Etb-6-21-4	Germplasm	187.1	67.5	90.3	32.6
Etb-5-31-3	Germplasm	178.3	65.7	93.1	34.3
Etb-5-31-7	Germplasm	207.6	69.3	92.0	30.7
Etb-6-5-3	Germplasm	234.3	71.9	91.7	28.1
Etb-6-21-3	Germplasm	294.2	74.4	101.3	25.6
Etb-6-5-5	Germplasm	255.8	74.6	87.2	25.4
Etb-6-21-5	Germplasm	233.7	72.3	89.4	27.7
J101-K6	Germplasm	235.4	73.9	83.3	26.1
J138-A12	Germplasm	233.1	72.6	88.0	27.4
J103-K7	Germplasm	283.0	77.2	83.5	22.8
N142-71	Germplasm	244.4	73.1	90.1	26.9
ND2861-1	Germplasm	202.2	76.0	63.7	24.0
ND4100C-19	Germplasm	291.5	74.5	99.9	25.5
ND4659-5R	Fresh	266.3	76.5	81.8	23.5
ND6956b-13	Germplasm	225.3	75.8	71.8	24.2
ND7132-1R	Fresh	204.7	74.8	68.8	25.2
ND7743C-2RS	Specialty	275.1	76.5	84.6	23.5
ND7982-1R	Fresh	229.6	71.2	92.7	28.8
ND8068-5Russ	Dual purpose	223.2	73.0	82.7	27.0
ND8291C-2Russ	Germplasm	276.9	77.0	82.7	23.0
ND8304-2	Chip processing	236.0	74.6	80.2	25.4
ND8331Cb-2	Chip processing	256.1	75.4	83.7	24.6
ND8527B-94Y	Specialty	212.5	70.6	88.5	29.4
ND039166CB-53R	Fresh	225.6	75.4	73.6	24.6
ND049251B-9Russ	Dual purpose	203.1	74.5	69.6	25.5
ND050060CB-4R	Fresh	199.4	72.1	77.3	27.9
ND059804C-13	Germplasm	299.5	80.8	71.2	19.2
ND060735-4Russ	Dual purpose	262.0	81.5	59.6	18.5
ND060761B-3Russ	Dual purpose	311.8	77.9	88.5	22.1

Table A.1. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Baker, MN, in 2014 (continued).

Clone	Market Class	Soluble Starch (mg/g)	Soluble Starch % of total	Resistant Starch (mg/g)	Resistant Starch % of total
ND081557c-5P	Specialty	238.8	78.8	64.4	21.3
ND081571-2R	Fresh	253.5	79.0	67.2	21.0
ND081571-3R	Fresh	270.5	74.0	94.9	26.0
ND081764B-4Russ	Dual purpose	334.4	76.8	100.8	23.2
ND081577-1R	Fresh	268.5	73.7	96.1	26.4
ND091890-1RR	Specialty	222.7	70.5	93.1	29.5
ND091896ABC-3	Germplasm	307.4	75.5	99.9	24.5
ND091905ABC-4	Germplasm	263.0	73.8	93.4	26.2
ND091997BT-3Russ	Frozen processing	278.6	77.3	82.0	22.8
ND092018C-1	Germplasm	255.0	75.5	82.7	24.5
ND092018C-3	Germplasm	249.9	72.3	95.7	27.7
ND092019C-4Russ	Germplasm	241.5	72.4	92.1	27.6
ND092150b-5pinto	Fresh	265.2	72.6	100.2	27.4
NDJL78B-1R	Fresh	236.6	71.6	94.1	28.5
NDJL64BV-1R	Fresh	285.4	77.6	82.4	22.4
NDJL21C-1	Germplasm	192.3	71.9	75.1	28.1
ND092049C-1	Germplasm	286.6	73.6	103.0	26.4
ND092417-2R	Fresh	251.0	72.5	95.1	27.5
NDJL23C-1	Germplasm	198.3	72.4	75.8	27.6
ND102573B-3R	Fresh	206.7	69.2	92.0	30.8
ND092355CR-2Russ	Frozen processing	180.3	66.1	92.4	33.9
ND102549TB-2Russ	Frozen processing	274.6	68.0	129.4	32.0
P2-4	Germplasm	291.5	75.8	92.9	24.2
P2-5	Germplasm	252.8	68.3	117.2	31.7
Q115-6	Germplasm	313.2	72.7	117.4	27.3
WND8624-2Russ	Dual purpose	239.9	73.2	87.8	26.8
WND8625-2Russ	Dual purpose	256.8	75.7	82.5	24.3
ND113027c-5	Chip processing	270.1	76.6	82.7	23.5
ND113030c-1	Chip processing	334.0	78.9	89.6	21.2
ND113032-1RY	Specialty	264.7	73.3	96.6	26.7
ND113032-5RSY	Specialty	330.7	73.7	118.0	26.3
ND113033b-1R	Fresh	243.5	74.0	85.6	26.0
ND113035b-1	Chip processing	287.9	75.7	92.3	24.3
ND113043B-6RY	Specialty	340.2	76.3	105.6	23.7
ND113043B-8RY	Specialty	209.8	71.6	83.1	28.4
ND113054b-3Y	Specialty	337.5	78.3	93.8	21.8
ND113085B-1Y	Specialty	253.0	75.6	81.8	24.4

Table A.1. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Baker, MN, in 2014 (continued).

Clone	Market Class	Soluble Starch (mg/g)	Soluble Starch % of total	Resistant Starch (mg/g)	Resistant Starch % of total
ND113113B-1PSY	Specialty	279.9	79.2	73.7	20.9
ND113355B-4Russ	Frozen processing	234.1	71.2	94.5	28.8
ND113361c-2	Chip processing	204.4	73.1	75.3	26.9
ND113361c-4	Chip processing	259.5	76.4	80.0	23.6
ND113364B-3	Chip processing	230.3	76.9	69.1	23.1
ND113370CAB-1	Chip processing	211.1	77.2	62.3	22.8
ND113372CAB-5	Chip processing	257.9	77.4	75.5	22.7
ND113374CAb-4	Chip processing	227.3	79.3	59.4	20.7
ND113380AB-7Russ	Frozen processing	195.8	70.5	81.9	29.5
ND113387Ab-1y	Specialty	220.1	74.3	76.0	25.7
ND113386Ab-5	Chip processing	213.9	73.4	77.7	26.7
ND113397c-1	Chip processing	252.8	75.9	80.2	24.1
ND113398CB-1	Chip processing	193.0	68.6	88.4	31.4
ND113406B-3Russ	Frozen processing	232.8	74.4	80.3	25.7
ND113409b-2Russ	Frozen processing	272.9	71.6	108.1	28.4
ND113418CB-2RY	Specialty	159.4	70.3	67.5	29.8
ND113419CB-1R	Fresh	244.4	74.2	85.0	25.8
ND113421CB-1R	Fresh	134.3	69.2	59.6	30.8
ND113429CB-2RY	Specialty	194.9	70.5	81.8	29.6
ND113438CB-1R	Fresh	246.8	76.4	76.4	23.6
ND113438CB-8R	Fresh	117.9	68.6	54.0	31.4
ND113460c-3PS	Specialty	291.6	75.7	93.5	24.3
ND113461-1RS	Specialty	251.8	73.0	93.4	27.1
ND113461-2P	Specialty	256.6	73.4	93.0	26.6
ND113461-3R	Fresh	272.2	74.0	95.7	26.0
ND113461-5RCS	Specialty	307.9	75.2	101.6	24.8
ND113461-6P	Specialty	259.4	73.1	95.7	27.0
ND113461-8PCS	Specialty	221.8	73.0	82.0	27.0
ND113461-10RCS	Specialty	323.5	77.9	91.6	22.1
ND113470C-6	Chip processing	277.7	72.4	106.1	27.7
ND113470C-4	Chip processing	269.4	72.3	103.1	27.7
ND113477C-2Russ	Frozen processing	276.4	74.7	93.9	25.4
ND113484B-5R	Fresh	228.3	73.9	80.7	26.1
ND113484B-7R	Fresh	222.2	74.9	74.3	25.1
ND113486C-5	Chip processing	299.6	76.6	91.5	23.4
ND113487c-1	Chip processing	171.6	69.2	76.3	30.8
ND113491C-8	Germplasm	238.0	72.0	92.6	28.0

Table A.1. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Baker, MN, in 2014 (continued).

Clone	Market Class	Soluble Starch (mg/g)	Soluble Starch % of total	Resistant Starch (mg/g)	Resistant Starch % of total
ND113497B-1Russ	Dual purpose	177.4	72.4	67.7	27.6
ND113485C-3Russ	Frozen processing	248.0	76.3	77.2	23.7
ND113502AB-2Russ	Frozen processing	273.9	76.2	85.6	23.8
ND113503AB-3Russ	Frozen processing	275.3	71.7	108.9	28.3
ND113508C-4	Chip processing	337.4	73.4	122.5	26.6
ND113512ABC-4	Chip processing	199.3	72.2	76.8	27.8
ND113509C-2	Chip processing	242.8	76.7	73.9	23.3
ND113515ABC-6	Chip processing	327.9	73.8	116.2	26.2
ND113517ABC-4	Chip processing	299.0	70.1	127.7	29.9
ND113517ABC-6	Chip processing	285.8	69.1	127.9	30.9
ND113517ABC-9	Chip processing	284.5	65.2	152.1	34.8
ND113519ABC-5	Chip processing	294.3	71.4	118.0	28.6
ND113526CB-1Russ	Frozen processing	224.2	73.1	82.5	26.9
ND113526CB-8Russ	Frozen processing	242.8	68.6	110.9	31.4
ND113529CB-2	Chip processing	222.6	74.8	74.9	25.2
ND113533ABC-2	Chip processing	218.1	71.0	88.9	29.0
ND113541-1	Chip processing	221.2	75.4	72.3	24.6
NDD3375-112Y	Specialty	230.5	77.7	66.0	22.3
NDD3375-115Y	Specialty	200.3	67.3	97.4	32.7
Red Norland	Fresh	224.6	73.4	81.4	26.6
Russet Burbank	Dual purpose	230.8	75.5	75.0	24.5
Yukon Gold	Specialty	235.6	72.1	91.3	27.9
	Mean	246.6	74.0	85.8	26.1
	LSD (α=0.05)	18.6	na	na	na

Table A.2. Soluble, resistant, and percent soluble and resistant starch present within clones grown in Absaraka, ND, in 2014.

Clone	Market Class	Soluble Starch (mg/g)	Soluble Starch % of total	Resistant Starch (mg/g)	Resistant Starch % of total
ND102631AB-1	Chip processing	200.6	57.2	150.1	42.8
ND102642C-2	Chip processing	192.1	59.6	130.4	40.4
ND1026638-3R	Fresh	185.0	62.2	112.6	37.9
ND102775C-5RR	Specialty	187.2	55.4	150.6	44.6
ND102800ABC-1	Chip processing	288.6	64.3	160.3	35.7
ND102809AB-2	Chip processing	207.2	61.5	129.8	38.5
ND102858CB-4	Chip processing	215.3	63.4	124.4	36.6
ND102879C-1Russ	Dual purpose	219.8	69.6	96.2	30.5
ND102903-1R	Fresh	207.3	64.6	113.5	35.4
ND102908-2RR	Specialty	205.7	65.3	109.2	34.7
ND113192AB-1Russ	Dual purpose	233.3	67.6	111.6	32.4
ND102922C-3	Chip processing	190.0	63.5	109.0	36.5
ND102908-4R	Fresh	213.3	67.0	105.2	33.0
ND113096-1Russ	Dual purpose	241.9	67.4	117.0	32.6
ND113091B-2RY	Specialty	168.2	59.6	113.9	40.4
ND113065CB-1Russ	Dual purpose	152.6	64.2	85.2	35.8
ND113300C-3RSY	Specialty	196.0	66.8	97.3	33.2
ND113277-2	Chip processing	165.2	58.9	115.3	41.1
ND113256C-2R	Fresh	142.4	63.2	82.9	36.8
ND113224C-3Russ	Dual purpose	311.7	75.4	101.8	24.6
ND113286B-6	Chip processing	224.1	69.6	98.1	30.5
ND113207-1R	Fresh	291.0	73.0	107.5	27.0
ND113298-2RS	Specialty	210.0	65.8	109.0	34.2
ND113281B-2	Chip processing	214.2	65.6	112.3	34.4
ND113278-3	Chip processing	245.8	67.9	116.5	32.2
ND102597-3R	Fresh	184.3	65.9	95.2	34.1
ND113330-1Russ	Dual purpose	193.0	69.1	86.3	30.9
ND113337-4RS	Specialty	165.3	60.8	106.5	39.2
ND113356B-2PEY	Specialty	188.2	65.6	98.8	34.4
ND102745C-5R	Fresh	169.1	63.7	96.6	36.4
ND113338C-1R	Fresh	219.3	58.7	154.5	41.3
Inka Dawn	Specialty	136.1	68.1	63.7	31.9
Dakota Pearl	Chip processing	210.9	68.1	99.0	32.0
Dakota Ruby	Fresh	173.4	61.8	107.2	38.2
ND092150b-5pinto	Specialty	201.7	65.1	108.3	34.9
AH66-4	Dual purpose	200.7	71.4	80.4	28.6
ND7779c-1	Chip processing	257.2	62.9	151.8	37.1

Table A.2. Soluble, resistant, and percent soluble and resistant starch present within clones grown in Absaraka, ND, in 2014 (continued).

Clone	Market Class	Soluble Starch (mg/g)	Soluble Starch % of total	Resistant Starch (mg/g)	Resistant Starch % of total
NDJL19c-1	Germplasm	228.5	71.8	89.9	28.2
ATND99331-2PintoY	Specialty	154.2	66.0	79.5	34.0
Russet Norkotah	Fresh	198.9	64.2	111.0	35.8
Romanze	Fresh	191.9	69.3	85.1	30.7
ND7743C-2RS	Specialty	181.8	67.3	88.4	32.7
Dakota Russet	Dual purpose	230.8	74.0	81.2	26.0
Russet Burbank	Fresh	227.6	68.3	105.7	31.7
Red Norland	Fresh	192.5	62.6	115.0	37.4
Yukon Gold	Fresh	220.5	64.9	119.3	35.1
	Mean	205.1	65.4	108.3	34.6
	LSD (α=0.05)	na	na	na	na

Table A.3. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Absaraka, ND, in 2014.

Clone	Soluble Starch (mg/g)	Soluble Starch % of Total	Resistant Starch (mg/g)	Resistant Starch % of Total
ND102642C-2	184.2	63.1	107.6	36.9
ND113207-1R	347.0	76.4	107.3	23.6
ND113278-3	301.8	72.2	116.2	27.8
Dakota Pearl	200.5	66.7	99.9	33.3
Dakota Ruby	163.1	60.1	108.1	39.9
Dakota Russet	202.8	69.9	87.2	30.1
ND102775C-5RR	179.3	58.4	127.8	41.6
ND113224C-3Russ	367.7	78.4	101.6	21.6
ND7743C-2RS	153.8	62.0	94.4	38.0
Romanze	164.0	64.3	91.1	35.7
Russet Norkotah	170.9	59.4	117.0	40.6
Mean	220.8	66.5	110.7	33.6
LSD (α=0.05)	66.9	na	28.0	na

Table A.4. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Baker, MN, in 2014.

Clone	Soluble Starch (mg/g)	Soluble Starch % of Total	Resistant Starch (mg/g)	Resistant Starch % of Total
90245.1	309.3	73.5	111.4	26.5
95043.11	216.4	67.3	105.3	32.7
463-4	224.8	76.6	68.5	23.4
Dakota Jewel	250.3	82.0	54.9	18.0
Dakota Pearl	238.0	75.5	77.4	24.5
Dakota Ruby	261.0	77.9	74.0	22.1
Dakota Russet	273.0	75.0	90.9	25.0
Dakota Trailblazer	246.7	83.3	49.4	16.7
Etb-6-21-3	288.0	77.8	82.2	22.2
Etb-6-21-4	180.9	71.8	71.2	28.2
Etb-6-5-5	249.6	78.6	68.1	21.4
Gala	177.4	73.3	64.5	26.7
J103-K7	269.1	81.3	62.0	18.7
J138-A12	219.2	76.7	66.5	23.3
ND060735-4Russ	305.1	91.0	30.0	9.0
ND081557c-5P	281.9	89.0	34.8	11.0
ND081571-3R	313.6	82.8	65.2	17.2
ND081577-1R	240.4	76.7	73.2	23.3
ND092019C-4Russ	213.4	75.5	69.2	24.5
ND102663B-3R	240.9	73.3	87.6	26.7
ND102775C-5RR	220.7	70.1	94.3	29.9
ND102921C-3	227.3	63.4	131.4	36.6
ND102990B-2R	243.1	57.6	179.1	42.4
ND113060-1	179.6	56.6	138.0	43.4
ND113113B-1PSY	292.8	83.6	57.5	16.4
ND113224C-3Russ	203.6	76.8	61.5	23.2
ND113230C-1	255.8	77.4	74.9	22.6
ND113289C-1	214.8	70.2	91.1	29.8
ND113338C-3R	215.9	54.9	177.5	45.1
ND113460c-3PS	319.0	79.1	84.2	20.9
ND113461-1RS	279.2	76.9	84.1	23.1
ND113461-2P	284.0	77.2	83.7	22.8
ND113508C-4	335.1	66.6	168.1	33.4
ND113526CB-1Russ	223.8	62.3	135.4	37.7
ND113541-1	220.8	63.8	125.3	36.2
ND4100C-19	277.5	78.0	78.4	22.0
ND7743C-2RS	279.9	78.4	77.2	21.6
ND8068-5Russ	228.0	75.2	75.2	24.8

Table A.4. Soluble, resistant, and percent soluble and resistant starch present within clones grown at Baker, MN, in 2014 (continued).

Clone	Soluble Starch (mg/g)	Soluble Starch % of Total	Resistant Starch (mg/g)	Resistant Starch % of Total
ND8331Cb-2	260.8	77.4	76.3	22.6
ND8527B-94Y	217.3	72.8	81.1	27.2
NDJL64BV-1R	257.1	81.0	60.4	19.0
Romanze	224.5	84.6	41.0	15.4
Russet Norkotah	231.9	80.3	56.7	19.7
Shepody	224.0	74.2	77.8	25.8
WND8625-2Russ	248.4	75.5	80.5	24.5
Mean	247.3	75.0	86.2	25.1
LSD (α=0.05)	67.4	na	28.3	na

Table A.5. Soluble, resistant, and percent soluble and resistant starch present within clones grown in the greenhouse.

Clone	Soluble Starch (mg/g)	Soluble Starch % of Total	Resistant Starch (mg/g)	Resistant Starch % of Total
90245.1	136.1	89.0	16.8	11.0
95043.11	110.9	90.1	12.2	9.9
463-4	118.8	85.5	20.2	14.5
Dakota Jewel	99.3	91.4	9.4	8.6
Dakota Pearl	171.5	84.9	30.5	15.1
Dakota Ruby	177.5	88.0	24.3	12.0
Dakota Russet	171.5	80.7	40.9	19.3
Dakota Trailblazer	188.5	81.5	42.7	18.5
Etb-6-21-3	153.9	38.8	242.6	61.2
Etb-6-21-4	85.3	37.5	142.1	62.5
Etb-6-5-5	171.1	40.3	253.5	59.7
Gala	126.0	90.4	13.4	9.6
J103-K7	216.4	44.5	270.1	55.5
J138-A12	184.5	44.8	227.0	55.2
ND060735-4Russ	186.6	42.2	255.8	57.8
ND081557C-5P	134.2	88.8	16.9	11.2
ND081571-3R	149.1	84.5	27.3	15.5
ND081577-1R	81.1	82.2	17.6	17.8
ND092019C-4Russ	173.1	82.8	36.0	17.2
ND102642C-2	158.2	42.6	212.9	57.4
ND102663B-3R	75.8	91.0	7.5	9.0
ND102775C-5RR	91.6	86.9	13.8	13.1
ND102921C-3	153.8	83.4	30.6	16.6
ND102990B-2R	120.4	74.7	40.7	25.3
ND113060-1	182.2	76.1	57.3	23.9
ND113113B1PSY	90.6	40.0	136.1	60.0
ND113207-1R	129.1	38.2	208.7	61.8
ND113224C-3Russ	132.0	70.2	56.0	29.8
ND113230C-1	203.4	41.2	290.8	58.8
ND113278-3	191.0	40.8	277.5	59.2
ND113289C-1	153.5	41.8	214.0	58.2
ND113338C-3R	209.2	48.8	219.7	51.2
ND113460C-3PS	130.0	90.9	13.0	9.1
ND113461-1RS	88.2	90.5	9.2	9.5
ND113461-2P	75.9	88.5	9.9	11.5
ND113508C-4	257.3	73.5	92.8	26.5
ND113526CB-1Russ	159.2	91.2	15.4	8.8
ND113541-1	115.0	72.0	44.7	28.0

Table A.5. Soluble, resistant, and percent soluble and resistant starch present within clones grown in the greenhouse (continued).

Clone	Soluble Starch (mg/g)	Soluble Starch % of Total	Resistant Starch (mg/g)	Resistant Starch % of Total
ND4100C-19	119.4	41.6	167.6	58.4
ND7743C-2RS	104.6	66.0	53.9	34.0
ND8068-5Russ	127.4	88.2	17.1	11.8
ND8331Cb-2	178.9	77.2	52.9	22.8
ND8527B-94Y	196.0	39.5	300.5	60.5
NDJL64BV-1R	112.4	63.7	64.2	36.3
Romanze	183.2	87.3	26.7	12.7
Russet Norkotah	132.0	87.6	18.7	12.4
Shepody	116.7	89.3	14.0	10.7
WND8625-2Russ	149.7	83.9	28.7	16.1
Mean	145.9	70.3	94.6	30.0
LSD (α=0.05)	33.4	na	35.6	na