WHOLE-WHEAT FLOUR MILLING AND THE EFFECT OF DURUM GENOTYPES AND TRAITS ON WHOLE-WHEAT PASTA QUALITY

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Program:
Cereal Science

June 2017

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

An ultra-centrifugal mill was evaluated by determining the effect of mill configuration and seed conditioning on particle size distribution and quality of whole-wheat (WW) flour. Ultra-centrifugal mill configured with rotor speed of 12,000 rpm, screen aperture of 250 µm, and seed conditioning moisture of 9% resulted in a fine WW flour where 82% of particles were <150 µm, starch damage was 5.9%, and flour temperature was below 35°C.

The single-pass and multi-pass milling systems were evaluated by comparing the quality of WW flour and the subsequent WW spaghetti they produced. Two single-pass mill configurations for an ultra-centrifugal mill were used (fine grind: 15,000 rpm with 250 µm mill screen aperture and coarse grind: 12,000 rpm with 1,000 µm mill screen aperture) to direct grind durum grain or to regrind millstreams from roller milling to make WW flour and WW spaghetti. Particle size, starch damage, and pasting properties were similar for direct fine grind WW flour and multi-pass reconstituted flour:fine bran blend and for direct coarse grind WW flour and multi-pass reconstituted semolina:coarse bran blend. Semolna:fine bran or semolina:coarse bran blends made spaghetti with high cooked firmness, while spaghetti made from direct coarse grind or from semolina:fine bran or coarse bran blends had low cooking loss.

Nineteen durum wheat (*Triticum turgidum* L. var. durum) cultivars and 17 breeding lines grown at 19 environments in North Dakota were evaluated for physical and cooking qualities of WW and traditional spaghetti. Of the 36 genotypes evaluated, 21 and 3 genotypes produced good and poor qualities of WW and traditional spaghettis, respectively, while other 12 genotypes produced good traditional spaghetti but produced poor quality WW spaghetti. These data indicate the need to select genotypes specifically for their WW pasta quality. Raw material traits (grain, semolina and WW flour characteristics) were evaluated to identify raw material traits

capable of predicting WW spaghetti quality. Grain protein content had significant positive correlation with cooking quality of WW spaghetti. Stepwise multiple regressions showed grain protein content and mixogram break-time and wet gluten were the predominant characteristics in predicting cooking quality of WW spaghetti.

ACKNOWLEDGMENTS

Most sincere gratitude goes to my adviser Dr. Frank Manthey, who has given me invaluable advice, assistance, guidance and continuous encouragement during the pursue of my Ph.D degree. His support also helped me developed skills and abilities for my professional career. My appreciation extends to my other research committee members, Dr. Senay Simsek, Dr. Julie Garden-Robinson and Dr. Elias Elias. I highly appreciate their technical assistance and guidance for my research.

My gratitude extends to Durum Wheat and Pasta Processing Lab technicians and students, Yu Liu, Hiroshi Ando, Jim Perleberg, Elena de la Peña and Claudia Cater, for their laboratory assistance and support. My special acknowledge expresses to Cereal Science Lipid Chemistry Lab, Dr. Clifford Hall and Mary Niehaus, for their laboratory assistance and technical support for part of my research study. My special acknowledge expresses to Cereal Science graduate students for their friendship and help.

My great appreciation expresses to my family, Fayong Deng, Dongying Liao and Yizhong Deng, for their endless love and continuous support.

DEDICATION

In memory of my uncle, Fahua Deng, your love and caring to the whole family are always remembered in my heart.

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GENERAL INTRODUCTION

There has been increased demand for food products that contain whole-wheat (WW) flour. As a result, WW flour production in the USA increased 13.7% from 2011 to 2015, reaching a total production of 1.08 million metric tons in 2015, which is 5% of total wheat milled (National Agricultural Statistics Service 2015).

Commercially, WW flour has been produced by milling wheat kernels using a single-stream one-pass system common with a stone mill, plate mill, and hammer mill or a multi-stream reconstitution system often associated with a roller mill (Miller Jones et al 2015; Posner and Hibbs 2009). A single-stream-one pass system is quick and simple but often generates excessive heat, which can adversely affect the functional properties of milled flour (Rao and Prabhasanker 2001; Miller Jones et al 2015). The multi-stream reconstitution system utilizing the roller mill generates less heat as it accomplishes size reduction slowly by using multiple passes. The bran and germ removed during roller milling is often ground using a hammer mill and blended back into the wheat flour at levels found in the intact kernel to make WW flour (AACC 1999).

Most mills used for laboratory research grind small sample sizes (25–250 g) and have a fixed mill configuration. To produce pasta using a semi-commercial pasta press requires milling 2-3 kg sample of grain. An ultra-centrifugal mill was found to be able to grind up to 3 kg wheat grain or bran. The mill has several possible mill configurations (rotor speed, screen aperture size, and feed rate). By adjusting mill configuration, the ultra-centrifugal mill could produce a WW flour with different particle sizes. Particle size can affect the processing properties and end-use quality of food products containing WW flour. For example, reduction of WW flour particle size increased the extensibility and maximum resistance to extension in dough and decreased appearance of bran specks (Wang et al 2016). Research has shown that fine particle size of flour can increase the firmness, cohesiveness and resilience, and reduce cooking loss of cooked WW noodles (Niu et al 2014b).

WW pasta is made from reconstituted blend of semolina, bran and germ fractions or made from whole-durum flour that is milled by direct grinding of the whole seed (Miller Jones et al 2015). WW pasta provides all the nutrients found in the endosperm, bran and germ. However, the bran is associated with many defects in WW pasta, such as dark appearance, reduced mechanical strength, and reduced cooking quality (Manthey and Schorno 2002; Chen et al 2011; Chillo et al 2008), which has impeded the growth in consumption of WW pasta. Limited research is available that compares the effects of milling system on whole durum flour and WW pasta qualities.

Durum industry is interested in identifying durum cultivars that possess traits that result in improved WW pasta quality. Published information concerning the genotype effect on WW pasta quality is limited. Previous research evaluated only a few genotypes for WW pasta and found that genotype had a significant effect on the end-use quality (Manthey and Schorno 2002; Padalino et al 2015).

The Durum Wheat Breeding Program at North Dakota State University has initiated efforts to identify or develop genotypes that produce high quality of WW pasta. Durum breeding programs have focused on improving traits associated with end-use quality, which is primarily pasta produced from semolina. Durum breeders screen their lines for improved pasta quality by screening for protein content, gluten/dough strength, and total yellow pigment or pasta color. Grain protein content and gluten/dough strength have been reported to be prerequisites for good cooking quality (Sissons et al 2005; AbuHammad et al 2012).

Developing a breeding program for WW pasta could stress available resources. Durum breeding programs routinely select genotypes that possess traits that result in improved quality of pasta made from semolina. The relationship between genotypes selected for their superior traditional, semolina pasta quality and their ability to make desirable WW pasta is not known. Identification of grain traits associated with improved WW pasta is needed.

The present study was undertaken with the following objectives:

1. To determine the configurations for a laboratory scale ultra-centrifugal mill that has a 2 to 3 kg capacity and can produce whole durum flour that has similar particle size and functionality as commercially available WW flour.
2. To determine the effect of milling system (single-pass vs multi-pass reconstituted) on the quality of WW flour and on the quality of WW pasta.
3. To determine importance of durum genotype on WW and traditional pasta qualities.
4. To identify traits that could be used by a breeding program to select durum genotypes that would produce high quality WW pasta.

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CHAPTER 1: LITERATURE REVIEW

Durum Grain

Durum (*Triticum turgidum* L. var. durum) grain has three main parts including endosperm, germ, and bran (Posner and Hibbs 2009). The endosperm is composed of starch granules embedded within a protein matrix; germ is composed of the embryo and scutellum; whereas, the bran surrounded with the endosperm includes aleurone, hyaline layer, seed coat, inner pericarp, and outer pericarp (Fig. 1). Whole grain contains 8-18% protein, 78-80% carbohydrate, 9-15% dietary fiber, 1-2% lipids, and 1-3% minerals (Miller Jones 2015). The hard texture, high protein and strong gluten strength, intense yellow color, and flavor (Sissons 2008) allowed durum to become best wheat for making semolina, pasta, bulgar, and couscous (Nachit 1998; Donnelly 1991; Qarooni 1994).

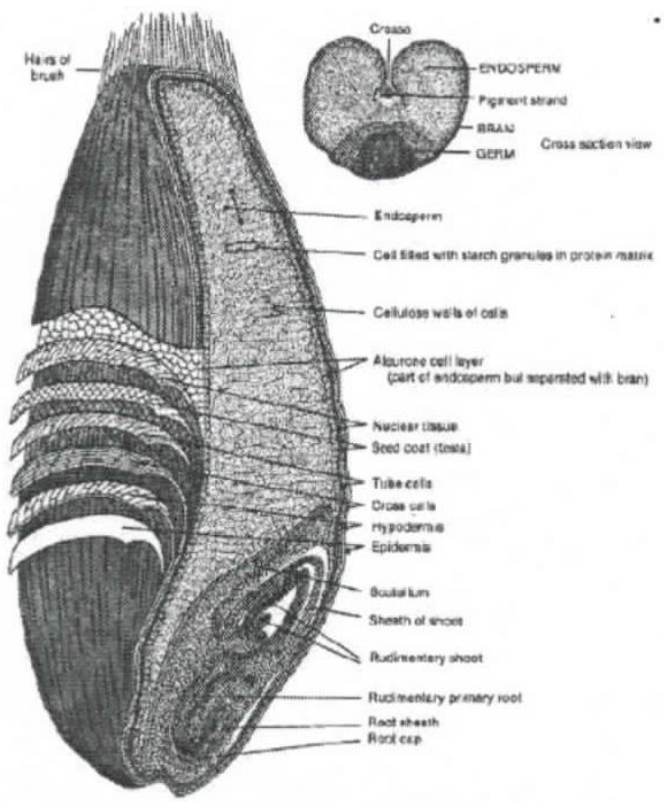

Figure 1. Wheat kernel longitudinal and cross sections (Reprinted from Delcour and Hoseney 2010).

Whole-Wheat Flour Milling

According to the definition by AACC (1999), “whole grain shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis”. Whole-wheat (WW) flour contains bran, germ, and endosperm of grain. Modern milling industry produces WW flour through single one-pass system where the grain is crushed between steel rollers or grooved millstones. All the parts of the original kernel stay together from the beginning to the end of the milling process. In multi-pass milling, bran, germ, and endosperm are separated after the grain is broken into small pieces. Small particles are sieved through sifters and large particles go back to the mill and further ground to obtain desirable particles. After the milling process, all the flour streams are blended together to make WW flour (Miller Jones 2015).

In the U.S code of the Federal Regulations (FDA 2016), WW flour is defined as “that at least 90% passed through 2.36 mm sieve and at least 50% passed through an 850 µm sieve”. However, the particle size distributions of the retail WW flours are smaller than would be required in the regulation. Doblado-Maldonado and Rose (2013) reported that the mean particle size of retail WW flours was 160-387 µm. Laboratory studies using different milling techniques produced WW flour with widely different particle sizes and functionalities (Kihlberg et al 2004; Niu et al 2014b; Inamdar et al 2015).

Mill Techniques and Flour Quality – Single Pass

Ultra-Centrifugal Mill

Ultra-centrifugal mill consists of feeding, grinding, and vacuum cooling system (Fig. 2). Wheat grain flows onto the rotor of the grinding chamber under a controllable rate. The high speed moving rotor creates centrifugal acceleration that throws grain outward and against a grating screen. Upon impact with the rotor blade, the grain fragments and the fragments are finely ground through shearing action between the rotor and screen (Fig. 2). In addition, a vacuum cooling system generates air flow that cools the ground material and protects flour from heat damage.

Figure 2. The ultra-centrifugal mill (left) and its grinding chamber (right).

The centrifugal mill is used for rapid size reduction of soft to medium-hard and fibrous materials (Retsch 2017). Feed rate is controlled by a vibratory feeder. When the rate is slow, great airflow occurs through the mill and moisture content is reduced. Thus, water activity of milled product is low (Schorno et al 2009). Under impact and shearing activities, the centrifugal milling can produce small particles (Naik and Chaudhuri 2015). Published studies used centrifugal mills to grind cereal grains and oilseeds. Schorno et al (2009) evaluated the milling configuration and flaxseed milled product characteristics on a centrifugal mill. The research found size reduction by shear force was more effective than size reduction by impact force. Doehlert and Wiessenborn (2007) reported the rotor speed affected the impact force and oats grain breakage increased with increasing rotor speed. The grain was accelerated to high velocity by the rotor and fracture into small particle at high rotor speed.

Stone Mill

Stone milling is achieved where the grains are crushed in the middle of stationary and moving stones and the broken material is pushed toward outside by the spoke-like pattern and finely ground occurs along the perimeter of stones (Gray's Grist Mill 2015). Stone ground produces large flour particles due to the compression of stones and the surface of flour particles were smooth and regular shaped (Bayram and Öner 2005). The gap between stationary and moving stones and grinding time affect the flour particle size. Fine particle size is achieved with the reduction in the gap and increased grinding time (Inamdar and Prabhasankar 2016).

Stone mill has high-energy consumption (Özdemir 2015) and the temperature generated during grinding of wheat can be high (Prabhasankar and Rao 2001). The generated heat can be absorbed by the milled flour and thus induce protein degradation or aggregation (Prabhasankar and Rao 2001). Aktan and Khan (1992) and Guerrieri et al (1997) reported the glutenin quality was greatly reduced as the product output temperature increased. The heat from grinding increased damaged starch (Özdemir 2015; INRA 2008) and decreased total amino acid, free lipid content, unsaturated fatty acid content (Prabhasankar and Rao 2001), vitamins, and minerals (Cook 1997), and bioavailability of iron, zinc, and calcium (Gibson 2010; Latunde-Dada 2014).

Hammer Mill

Hammer mill is a vertical grinder where the feed hopper is situated above the milling chamber where swinging hammers are fixed on a rotor. The ground material is discharged on the lower half of grinding chamber through a screen opening by gravity. The hammer mill can efficiently reduce particle size (Arámbula et al 1998) because the hammers rotate at high speed (5,000 to 7,000 m/min) crushing and shearing cereal grains into small fragments or fine particles. The hammer can have a flat (blunt) or sharp (knife) edge which affects the impact or crushing of the ground material. The end of a hammer has four times the energy of impact than a point half way between the end of a hammer and the center of the shaft (Earle 1987). The grains impacted with the end of one hammer are more finely ground than those impacted further down the shaft (Earle 1987). Thus, the hammer mill results in a wide range of particle size. The ground particle is also determined by the screen aperture size and small aperture size is preferred for fine and uniform flour products. In addition, the air flow induced from the inlet of the grinding chamber and vented at the discharged of the hammer mill removes heat generated during milling and improves mill efficiency and performance from 15 to 40% (Posner and Hibbs 2009).

Disc Mill

Disc mill consists of a pair of grinding disk surfaces, of which one is high rotating and the other is stationary. According to the physical position of surfaces, the disc mill has a horizontal-spindle, which has the disc rotating in a vertical plane (Bayram and Öner 2007). The material passes into a narrow gap between those two surfaces and the compression and shear actions result in comminution of the material. The disc mill readily grinds soft and non-abrasive materials into fine particles. For hard wheat, the compression along with shearing give uniform and granular particle size. In addition, the presence of disc could smooth the surface and improve appearance of particle (Bayram and Öner 2005). The scratching effect by the grooved surfaces may cause more damaged starch as compared to other mills imparting single reduction force. In contrast with high speed grinder such as hammer mill, the heat generation and damaged starch granule together with the rheological characteristics (farinograph water absorption, dough development time and stability) were decreased (Inamdar et al 2015).

Mill Techniques and Flour Quality – Multiple Pass

Roller Mill

WW flour can also be obtained with a multi-stream reconstitution system usually associated with roller milling (Miller Jones 2015). Roller mill has many pairs of break and reduction rolls to separate bran and germ from the endosperm and gradually reduce endosperm into small particles. Sifters and purifiers are used to segregate based on particle size. Large particles are passed through rolls until the desired particle size has been achieved. Bran, germ, and shorts are removed from the milling stream. Refined flour contains only ground endosperm. To make WW flour, the bran, germ, and shorts are reground often using a hammer mill and the reground material blended with the refined flour in the original proportion as found in the whole kernel. Roller mill has high milling efficiency by adjusting roll corrugation and differential speeds, which minimizes the length of time flour particles are in contact with the steel rolls and prevents the generation of heat. Flour made by roller milling has little to no denatured protein and high retention of α-amylase activity (Miller Jones 2015). The low temperature maintains flour components functionality (Inamdar et al 2015).

Particle Size and Product Quality

Particle size is an indication of the degree of milling of grain. Particle size influences functional properties of WW flour. Fine particle size decreased WW flour dough mixing tolerance and reduced dough mixing requirement (Zhang and Moore 1997). The starch granules are damaged by the high mechanical force during the grinding process. The rough and porous surface and agglomeration of damaged granules have been reported to alter starch gelatinization, swelling, solubility and digestibility, and thus affect flour and product end use quality (Tran et al 2011). Niu et al (2014a) studied fine grinding effect on WW noodle quality, and found the reduction of WW flour particle size (median diameter:141-206 μm) increased the texture of WW noodle (Niu et al 2014a). For WW cracker, fine particle size (median diameter:90-96 μm) gave good texture and geometry (Wang et al 2016). Reduction of particle size also can increase the bioaccessibility of phenolic acids such as sinapic and ferulic acids (Hemery et al 2011).

Genotype and Pasta Quality

Many studies have evaluated durum genotypes grown in different environments, and found genotype significantly affected cooked firmness and cooking loss of pasta or noodles made from durum semolina (Autran 1986; Mariani 1995; Kaur et al 2015). Other research on cooked durum pasta or noodles demonstrated genotype significantly affected pasta end-use quality assessed by viscoelasticity (Ames et al 1999), cohesiveness and adhesiveness (Kaur et al 2015); and resistance to compression and recovery texture parameters (Hatcher et al 2009).

Durum genotype is very important for pasta quality because grain traits (protein content and quality, vitreous kernel, hardness, and yellow pigment content) are associated with dough and pasta making properties. For example, grain protein content (Oak and Dexter 2006), semolina wet and dry gluten (Aalami et al 2007), and sedimentation value and gluten index (Liu and Rathjen 1994) are correlated with dough properties and pasta cooked texture. Durum yellow pigment content has been correlated with the semolina or pasta yellowness measured by Minolta colorimeter (Johnston et al 1980; Clarke et al 2000). Improving grain traits are necessary to achieve processing and cooking properties acceptable for pasta product (Peña and Pfeiffer 2008). Durum grain traits are under genetic control and durum breeders improve those grain traits through breeding to obtain high level of pasta end-use quality.

Grain Traits for Milling Quality

Test weight indicates bulk density of wheat kernels. Test weight is a grading factor of wheat quality (USDA 1988) and has been considered during commercial grain trading (Posner and Hibbs 2009). High test weight of grain indicates plump and mature kernels which are needed for high flour quality and price. In contrast, shriveled and immature kernels due to intolerance to weather (Czarnecki and Evans 1986), insect damage (Buntin et al 1992), defoliation (Blum et al 1991) or delayed harvesting (Pool et al 1958) have reduced test weight. Symons and Fulcher (1988) reported that grain size and shape affects test weight. Grain packing efficiency affects test weight. Uniform rounded kernels generally have high test weight due to improved packing (Yamazaki and Briggle 1969). Matsuo and Detxter (1980) studied the durum wheat physical characteristics and semolina milling properties and found test weight was significantly correlated with semolina yield ($r=0.52$, $P<0.01$). For milling efficiency and flour quality, low test weight wheat decreases the amount of flour produced in roller milling and increases the amount of ash released during the primary breaks, resulting in flour with high ash content (Gwirtz et al 1996).

Kernel weight is a measure of average kernel size. Schuler et al (1994) reported kernel weight was significantly correlated with kernel length ($r=0.88$), width ($r=0.90$), and volume ($r=0.99$, $P<0.01$). Flores et al (1991) studied the wheat grain physical and milling characteristics and found that kernel weight was the most important factor that affected flour extraction, and that the extraction increased rapidly as kernel weight increased from 24 to 28 mg for bread wheat. Similar results have been reported for durum wheat from 45 to 60 mg (Sissons and Hare

2002). Increased kernel size has a relative high proportion of endosperm relative to bran, which could improve milling yield by 2-3% (Marshall et al 1984).

In terms of the percent of hard and vitreous endosperm, durum wheat can be divided into hard amber (>75 %), amber (60-75 %) and durum wheat (<60 %), respectively. The hard and vitreous kernel has hard texture and a compact protein matrix surrounding starch granules. Non-vitreous kernels are opaque and starchy due to air spaces between starch granules. When milled, vitreous kernels produce high semolina yield. Matsuo and Dexter (1980), Dexter and Matsuo (1981), and Sissons et al (2000) reported that as vitreous kernel content decreases, kernel texture became softer, resulting in lower semolina yield and higher flour yield. Semolina protein content from vitreous kernel was higher than that from non-vitreous kernel (Dexter et al 1989). El-Khayat et al (2003) investigated starch content of vitreous and starchy grains and reported that a significantly lower starch content was evident in vitreous grains.

Grain Traits for Pasta Quality

Protein Quantity

Protein quantity is usually measured as grain or semolina protein content. High grain or semolina protein can improve pasta texture and reduce cooking loss with better retention of firmness in overcooking (Dexter and Matsuo 1983; Park et al 2003). Most wheat proteins are gliadin and glutenin that contribute to dough viscoelastic properties. When semolina mixes with water and kneads in extruder chamber, the viscoelastic of gluten network is fully developed (Wrigley and Békés 1999). Wet and dry gluten measure gluten quantity, and high level of those values are desirable for evaluated pasta quality.

Protein Quality

Gluten index, dough mixing parameters, and gluten subunit characterizations are measurements of protein quality. The protein quality determines dough or gluten strength. D'Egidio et al (1990) and Abuhammad et al (2012) found that the gluten index and mixograph tests can differentiate strong from weak genotypes. Gluten index and mixograph parameters had significant correlation with cooked pasta firmness and negative correlation with cooked weight (Park et al 2003). Gluten subunit characterizations such as combinations of high molecular weight and low molecular weight glutenin subunits, subunit molecular size, and number and distribution of disulfide bonding have great influence on gluten strength (Cubadda et al 2007; D'Ovidio and Masci 2004; Weegels et al 1996). The ratio of glutenin and gliadin was reported to directly affect the elasticity and viscosity of dough and high elasticity and viscosity results in high cooking stability and high cooked firmness of pasta (Delcour et al 2000; Liu et al 1996). Ohm et al (2006) predicted dough mixing and noodle texture by characterizing gluten subunits using HPLC.

Starch

Starch is a main component in durum wheat, which represents up to 80% db of semolina. During cooking, starch starts to swell and gelatinize which leads to amylose leaching and amylopectin displacement to the pasta surface (Lemlioglu-Austin and Jackson 2013). The starch content of semolina indicates a negative correlation with the cooking firmness of the obtained pasta (Porceddu 1995). Furthermore, the amylose/amylopectin ratio, starch damage, and distribution of granules influenced the viscoelastic behaviors of pasta dough, and solubilizing of starch and the interaction of starch and protein during pasta cooking, which resulted in poor texture to the cooked product (Sissons 2008).

Sprouted Grain

Pre-harvest sprouting reduced seed quality, specifically decreased test weight of the grain (Cabrera et al 1995) and increased the fine particles during milling (Dziki and Laskiwski 2010). Enzyme activities such as amylase and protease are very high in sprouted grains, provoking sufficient damage to the structural integrity of starch granules and storage proteins of wheat grains (Barbeau et al 2006). The breakdown of protein weakens gluten strength and decreases the dough tolerance index (Sekhon et al 1992) and a loss of starch gel viscosity in sprouted grains, which negatively affects pasta or noodle quality (Ingelbrecht et al 2001; Cato et al 2006).

Sprouting in wheat has been found to increase the overall concentration of phenolic acids and the proportion of free versus bound phenolic acids as compared to the sound seeds (Alvarez-Jubete 2010). The sprouted grain provides higher bioavailability and bioaccessibility of free phenolic acids. In contrast, the bound polyphenols are cross-linked with structural components of bran such as hydrolysable tannins, lignins, cellulose, and proteins, which may affect pasta digestibility (Dinelli et al 2011; van Huang et al 2011; Slavin 2004).

Bran and Pasta Quality

Color

Bran is the outer layers of wheat kernel that includes outer and inner pericarp, seed coat and nucellar epidermis. The oxidant enzymes in the bran layers are peroxidase, polyphenol oxidase, lipoxygenase and phenolase (Fraignier et al 2000; Okot-Kotber et al 2001; Gökmen et al 2007). The peroxidase performs oxidation on a wide variety of compounds in the presence of hydrogen peroxide, when the hydrogen peroxide is generated during the oxidation of phenolic compounds in polyphenol oxidase-catalyzed reactions (Sugai and Tadini 2006; Ndiaye et al 2009). Large variability of peroxidase activity was found between durum wheat cultivars

(Ndiaye et al 2009). Pasta products made from high peroxidase activity cultivars develop an undesirable brownish color during processing (Fraignier et al 2000). The polyphenol oxidase is the enzyme associated with the conversion of phenolic compounds to quinones and their product polymerization. The polyphenol oxidase oxidizes diphenols in the presence of molecular oxygen with resulting degradation of nutritionally valuable compounds and enzymatic browning (Schweiggert et al 2005). Whilst, in lipoxygenase-linoleate system, lipoxygenase catalyzes the stereospecific hydroperoxidation of polyunsaturated fatty acids, esters and glycerides containing a 1-cis, 4-cis-pentadiene structure, which can oxidize carotenoid pigment (MacDonald 1979, Schweiggert et al 2005). The oxidant enzymes can lead to the initiation of deterioration reactions, such as undesirable color and pigment loss (Goncalves et al 2010).

Mechanical Strength

Mechanical strength is an indicator of pasta handling and packaging ability. Pasta product containing bran had poor mechanical strength when compared to pasta made only from semolina (Manthey and Schorno 2002; Manthey et al 2004). The bran fragments in the pasta physically interferes with gluten matrix, which results in low mechanical strength. Kordonowy and Youngs (1985) reported that spaghetti containing 10% bran (w/w) had the most favorable rating for spaghetti containing bran; the breakage susceptibility of spaghetti decreased with bran addition at 15% and 20% (w/w) (Chillo et al 2008).

Cooking Quality

The presence of bran affects the hydration properties of semolina, because of its hydrophilicity that increases water absorption. With insufficient amount of water, the gluten development is inhibited and pasta cooked texture associated with gluten strength is decreased (Gallegos-Infante et al 2010). In addition, the complex material of bran induces physical disruption of the gluten network and starch granule are susceptible to leach from pasta during cooking (Chillo et al 2008; Petitot et al 2010).

The chemical compositions of bran are cellulose, arabinoxylan, fructan, β-glucan, starch, proteins, lipids, and minerals (Parker et al 2005; Barron 2011; Brouns et al 2012). Wang et al (2003) hypothesized that bran arabinoxylan cross-linked with proteinand the poor stiffness and extensibility of dough and low cooking quality might have been affected by the crosslinking. Moreover, the soluble nonstarch polysaccharides alter the internal pasta structure and form complex associations with protein and starch components that have caused low optimum cooking time (Mercier et al 2016; Vernaza et al 2012). The nonstarch polysaccharides increased moisture content and swelling index of pasta resulting in low cooked firmness (Brennan and Tudorica 2007).

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CHAPTER 2: LABORATORY-SCALE MILLING OF WHOLE-DURUM FLOUR QUALITY: EFFECT OF MILL CONFIGURATION AND SEED CONDITIONING

Abstract

Research was conducted to develop a laboratory milling procedure to make whole-durum flour. An ultra-centrifugal mill was evaluated by determining the effect of mill configuration and seed conditioning on particle size distribution and quality of whole-wheat (WW) flour. The results showed particle size of WW flour decreased as screen aperture decreased from 1,000 to 250 µm; as rotor speed increased from 6,000 to 18,000 rpm; and as seed conditioning moisture content decreased from 15 to 9%. Feed rate during milling did not affect particle size distribution. Starch damage decreased as screen aperture increased; as rotor speed increased from 6,000 to 12,000 rpm; and as seed conditioning moisture content decreased from 15 to 9%. Flour temperature varied with milling parameters but did not exceed 34°C. Ultra-centrifugal mill configured with rotor speed of 12,000 rpm, screen aperture of 250 µm, and seed conditioning moisture of 9% resulted in a fine WW flour where 82% of particles were <150 µm, starch damage was 5.9%, and flour temperature was below 35°C.

Introduction

Commercially, WW flour has been produced by milling wheat kernels using a single stream one pass system common with a stone mill, plate mill, and hammer mill or a multi-stream reconstitution system often associated with a roller mill (Miller Jones et al 2015; Posner and Hibbs 2009). Single stream-one pass system commonly generates excessive heat, which can adversely affect the functional properties of milled flour. For example, Rao and Prabhasanker (2001) reported temperatures generated during grinding of wheat on a stone, plate, and hammer mill were 90, 85, and 55 °C, respectively. Miller Jones et al (2015) reported similar results with temperatures during milling of 85, 78, and 50 ˚C for the stone, plate, and hammer mill, respectively. These temperatures are capable of denaturing some protein and promoting lipid oxidation. Multi-stream reconstitution system generally involves the use of a roller mill. The bran and germ removed during roller milling is often ground using a hammer mill and blended back into the wheat flour at levels found in the intact kernel to make WW flour, according to the definition of whole grain (AACC 1999). Roller milling system generates less heat as it accomplishes size reduction slowly by using multiple passes. Flour temperatures of 32 to 35 ˚C are commonly reported (Miller Jones et al 2015; Rao and Prabhasanker 2001).

Currently, there are no standard methods for laboratory scale milling of WW flour. A single-stream milling system would be simpler than multi-stream reconstitution system. Most laboratory scale mills only mill a small sample size (25 to 250 g) and have a fixed mill configuration. An ultra-centrifugal mill was found to be able to mill a wide range of sample sizes (up to 3 kg) and had several possible mill configurations. By adjusting mill configuration, the centrifugal mill could produce a WWF with different particle sizes.

The ability of a milling system to mill to a particular particle size is very important attribute when selecting a mill for laboratory scale research. Particle size can affect the processing properties and end-use quality of food products containing WW flour. For example, reduction of WW flour particle size increased the extensibility and maximum resistance to extension in dough and decreased bran specks in appearance (Wang et al 2016). Nevertheless, fine bran particle in WW flour can decrease mixograph dough mixing time and water retention capacity during mixing (Cai et al 2014). Research has shown that fine particle size of flour can increase the firmness, cohesiveness and resilience, and reduce cooking loss of cooked WW noodle (Niu et al 2014).

Seed conditioning commonly is used in roller milling and affects mill performance and flour characteristics (Posner and Hibbs 2009). Water added to the seed toughens the bran and softens the endosperm. However, toughening the bran and softening the endosperm might be undesirable when direct milling grain into WW flour. The tough bran after conditioning may hamper endosperm breakage and subsequent size reduction in direct WW flour grinding. Therefore, the role of seed conditioning and the optimum conditioning level when direct milling grain into WW flour needs further be studied.

Research has been initiated by the Durum Wheat Quality/Pasta Processing Laboratory at North Dakota State University to evaluate durum genotypes for their WW flour and WW pasta quality characteristics based on the single pass grinding method of WW flour. To address this research, a simple laboratory scale milling system is needed that is capable of milling 2-3 kg samples into WW flour; that has particle size distribution and starch damage similar to that found in commercial WW flour; and that does not generate excessive heat. The objective of this research was to determine the effect of mill configuration (rotor speed, mill screen, and feed rate) and seed conditioning on particle size distribution and quality of WW flour produced on a laboratory scale ultra-centrifugal mill.

Materials and Methods

Durum Wheat

A bulk sample was obtained by blending durum wheat cultivars (‘Alzada’, ‘Carpio’, ‘Divide’, ‘Mountrail’, ‘Rugby’, and ‘Tioga’) grown in several locations (Casselton, Dickinson, Minot, and Williston) in ND, USA, 2012. AACC Approved methods (2010) were used to determine test weight (method 55-10.01), moisture content (method 44-11.01), protein content (method 39-25.01), ash content (method 08-01.01), and falling number (method 56-81.03).

Kernel size distribution was determined using the method described by Shuey (1960). 1000-Kernel weight was measured by counting 10 g clean intact wheat kernels on a seed counter (Model 77, Seedburo, Des Plaines, IL, USA), and calculated on (1,000/ number of wheat kernels)*10g. Vitreous kernel content was determined according to USDA standard method (1997) where the percentage (wt/wt) of kernels having vitreous endosperm was determined based on 15 g of cleaned intact wheat kernels.

Sample Preparation

Durum wheat had a moisture content of 9%. Seed moisture was adjusted by a conditioning process where distilled water was added to the grain in an amount necessary to achieve the different moisture contents used in the experiments. Seed moisture was allowed to equilibrate at least 48 h before milling. Each experimental unit consisted of 500 g of grain.

Mill Configuration

The ultra-centrifugal mill (ZM 200, Retsch, Germany) was used to mill WW durum flour. The ultra-centrifugal mill had three components: feeding, grinding, and cooling. The vibratory feeder was load controlled and gave uniform and constant feed and output rate. Grinding occurred by impacting with the rotor blade and mill screen and by shearing of wheat kernels between the rotor blade and mill screen. The mill was cooled by vacuum (GM80, Nilfisk, Morgantown, PA, USA) drawn air through the milling chamber, which cooled the finished flour and aided in a quick discharge from the grinding chamber into collecting container. Mill configuration was altered by varying feed rate, rotor speed, and screen aperture size.

Two experiments were conducted. In the first experiment, the grain was conditioned to 12% moisture and feed rate was 62 g min^{-1}. Rotor speeds evaluated were 6,000, 9,000, 12,000, 15,000, and 18,000 rpm and mill screen aperture sizes evaluated were 250, 500, and 1,000 µm.

In the second experiment, grain was conditioned to 9, 10, 12, 14, and 15% moisture content. The conditioned wheat was milled using two feed rates, 37.0 and 78.6 g min^{-1}. The rotor speed was 12,000 rpm and the mill screen aperture was 250 µm. Six commercial WW flours were evaluated for their particle size. Two WW flours were selected to be control flours. These flours represented the finest (brand 1) and coarsest (brand 2) particle size distribution.

Whole-Wheat Flour Tests

Rotor surface temperature and WW flour temperature were measured using an infrared thermometer (TN408LC, Metris, NJ, USA) immediately after milling. Flour moisture loss was determined by subtracting the flour moisture content (after milling) from the grain moisture content. Flour color was measured using a colorimeter (CR410, Minolta, NJ, USA) equipped with a D65 illuminant. The flour brightness was recorded using the *CIE* L-value. L-value varies on a scale of 0 to 100, where 0 is black and 100 is white. Particle size distribution test was conducted on a vibratory sieve shaker (AS 200, Retsch, Germany). The particle size distribution was based on the weight percentage retained on stacked sieves of 600, 500, 425, 250, 150, 100, 50, and <50 µm, respectively. Each sieve has 10 polyurethane balls that acted as sieve cleaners during sifting. Starch damage was measured using the AACC Approved Method (76-31.01). The geometric mean diameter (d_{gw}) and geometric standard deviation (s_{gw}) by mass of WW flour particle size were measured based on equation provided by ASABE S319.4.

$$d_{gw} = \log^{-1}\left[\frac{\sum_{i=1}^{n}(W_i \log \overline{d_i})}{\sum_{i=1}^{n} W_i}\right] \tag{1}$$

$$s_{log} = \left[\frac{\sum_{i=1}^{n} W_i(\log \overline{d_i} - \log d_{gw})^2}{\sum_{i=1}^{n} W_i}\right]^{1/2} \tag{2}$$

$$s_{gw} = \frac{1}{2} d_{gw}\left[\log^{-1} s_{log} - \left(\log^{-1} s_{log}\right)^{-1}\right] \tag{3}$$

Where d_{gw} is the geometric mean diameter of the particles by mass (µm); s_{log} is the geometric standard deviation of the log-normal distribution by mass; s_{gw} is the geometric standard deviation of the particle diameter by mass (µm); W_i is the mass on the ith sieve (g); n is the number of sieves; and d_i is the nominal sieve aperture size of the ith sieve (µm).

Statistical Analysis

The experimental design for the first experiment was a randomized complete block with a split-plot arrangement and three replications. The whole plot factor was mill screen aperture (three levels) and the subplot was rotor speed (five levels). Each block (replication) of treatments was milled on separate days. The experimental design for the second experiment was a randomized complete block with split-plot arrangement and three replications. The whole plot factor was seed moisture (five levels) and the subplot factor was feed rate (two levels). Each block (replication) of treatments was milled on separate days.

Data were subjected to analysis of variance using the Statistical Analysis System 9.4 (SAS Institute, Cary, NC, USA). *F*-Test was significant at $P \leq 0.05$. Treatment means were separated by *Fisher*'s Protected Least Significant Different (LSD) at $P=0.05$. Multiple regression was conducted using the Statistical Analysis System 9.4 (SAS Institute, Cary, NC, USA) at $P \leq 0.05$.

Results and Discussion

Durum Wheat Quality

Durum wheat used in this experiment was of good quality with good milling potential based on the high test weight (62 lb/bu) and vitreous kernel content (93% wt/wt) (Table 1). Kernel protein content, ash content, and kernel size and weight were typical for durum grown in the Northern Plains of USA (NDWC 2015). Falling number (682 s) is high and indicates that the grain was sound with low α-amylase activity.

Table 1. Durum wheat quality characteristics.

Sample	TW	1000-KWT	Protein	VK	Ash	FN	KS		
							L	M	S
	(lb/bu)	(g)	(%, 12% mb)	(%, wt/wt)	(%, 12% mb)	(s)		(%)	
Durum	62	37.9	13.4	93	1.7	682	58	38	4

TW = Test weight; 1000-KWT = 1000-Kernel weight;
VK = Vitreous kernel; FN = Falling number; KS = Kernel size; L = Large kernel (>2.92 mm); M = Medium kernel (2.24-2.92 mm); and S = Small kernel (<2.24 mm).

Rotor Speed and Screen Aperture Size

Particle size distribution

The effect of rotor speed and mill screen aperture on particle size distribution is shown in Fig. 3A-C. At each rotor speed, range in particle size distribution decreased as mill screen aperture decreased. As expected, particle size distribution range was greatest (1,000 to 50 μm) with 1,000 μm aperture mill screen, intermediate (425 to 50 μm) with 500 μm aperture mill screen, and least (250 to 50 μm) with 250 μm aperture mill screen, regardless of rotor speed. With each mill screen, particle size distribution was altered by varying rotor speed. The fraction of WW flour retained on each sieve was recorded as weight percentage. With the 250 μm aperture mill screen, the weight percentage of the >250-150 μm fractions declined 7 percentage units while the weight percentage of the <150-100 μm fractions increased 22 percentage units as rotor speed increased from 6,000 to 18,000 rpm. Similarly, with the 500 μm aperture mill screen, the weight percentage of the >425-250 μm fractions declined 12 percentage units while the weight percentage of the <250-150 μm fractions increased 28 percentage units, as rotor speed increased from 6,000 to 18,000 rpm. Finally, with the 1,000 μm aperture mill screen, weight percentage of the >600, 600-500, and 500-425 μm fractions declined 31, 9 and 5 percentage units, respectively, while the weight percentage on of the 250-150, 150-100, and 100-50 μm fractions increased 14, 18, and 10 percentage units, respectively.

Geometric mean diameter

Geometric mean provides a useful parameter that indicates the typical value of a set of numbers. The geometric mean diameter (d_{gw}) for particle size distribution was smallest with the 250 μm aperture mill screen and was greatest with the 1,000 μm aperture mill screen (Fig. 4A). As the rotor speed increased from 6,000 to 18,000 rpm, the geometric mean diameter of WW flour particles declined from 350 to 180 μm (170 μm) with 1,000 μm aperture mill screen; from 197 to 134 μm (63 μm) with 500 μm aperture mill screen; and from 117 to 84 μm (33 μm) with the 250 μm aperture mill screen.

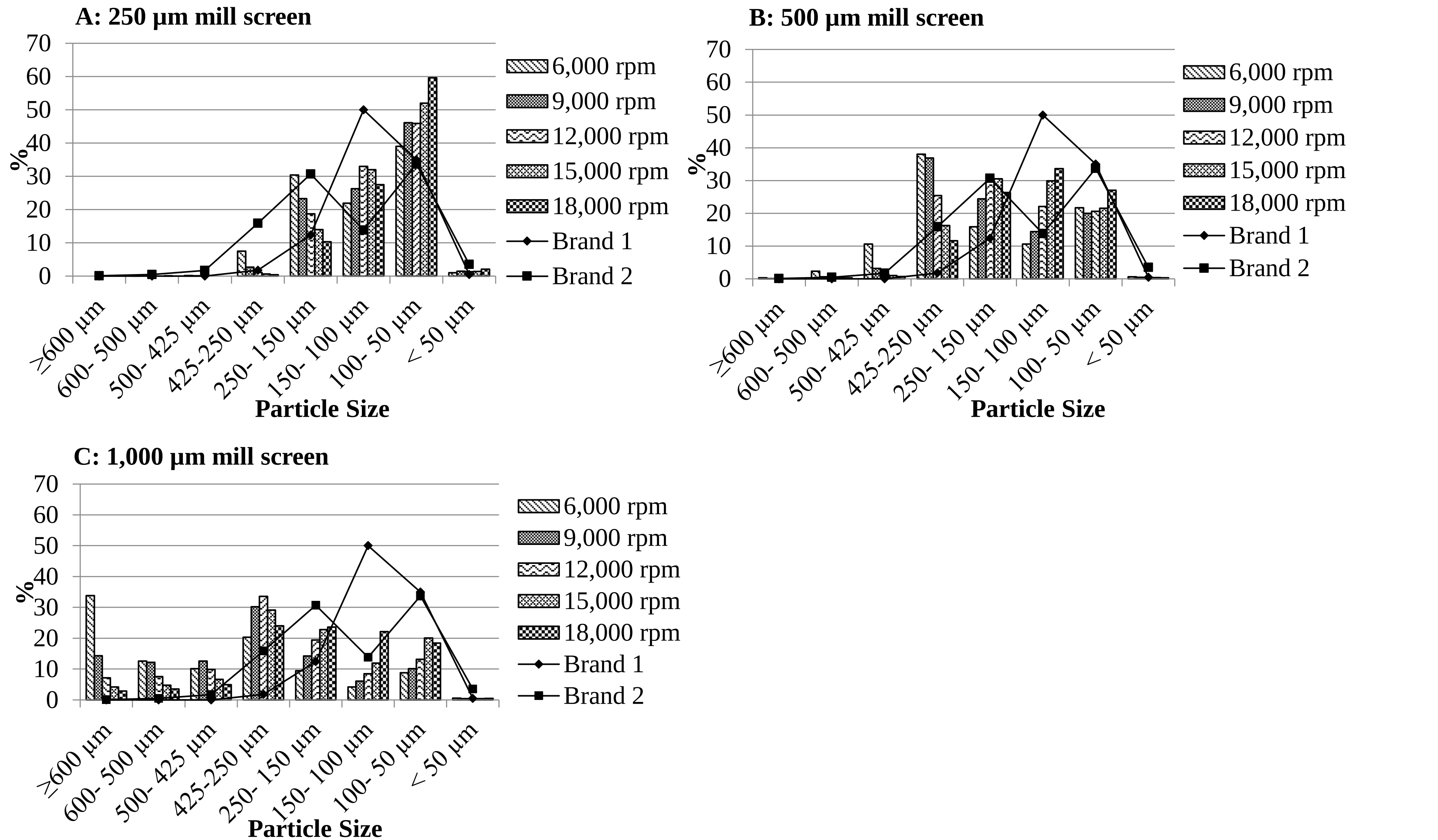

Figure 3. Particle size distribution of whole-wheat flours milled at different rotor speeds and mill screen aperture sizes. A = 250 µm mill screen; B = 500 µm mill screen; and C = 1,000 µm mill screen. LSD was compared on interaction effect of rotor speed and mill screen aperture at $P \leq 0.05$. LSD (≥600µm) = 3.5, LSD (600-500µm) = 2.4, LSD (500-425µm) = 3.0, LSD (425-250µm) = 6.3, LSD (250-150µm) = 10.0, LSD (150-100µm) = 20.3, LSD (100-50µm) = 23.4, LSD (<50µm) = 3.3.

The small geometric standard deviation of the particle diameter (s_{gw}) with the 250 µm aperture mill screen reflects a narrow particle distribution and inversely a high s_{gw} value with the 500 and 1,000 µm aperture mill screens indicated broad particle distribution (Fig. 4B). Geometric standard deviation of the particle diameter tended to decrease as rotor speed increased from 6,000 to 18,000 rpm with each mill screen; thus, high rotor speed resulted in a narrow particle size distribution.

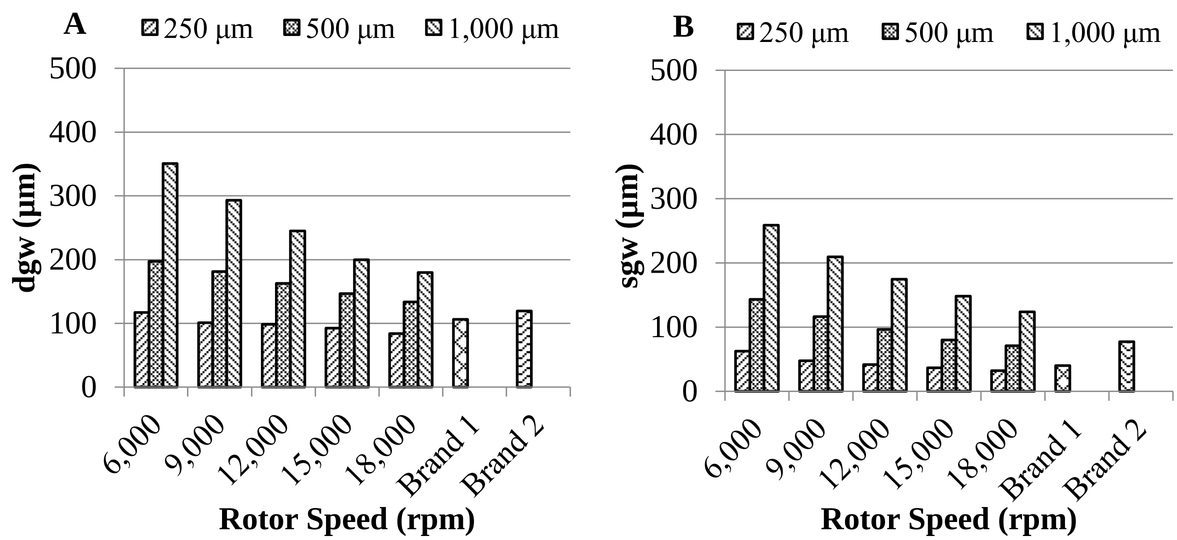

Figure 4. Geometric mean diameter (dgw) and geometric standard deviation (sgw) of whole-wheat flours milled at different rotor speeds and mill screen aperture sizes. A = dgw; and B = sgw. LSD was compared on interaction effect of rotor speed and mill screen aperture at $P \leq 0.05$. LSD (dgw) = 61.1.

In Table 2, the multiple regression equation (4) utilized rotor speed and mill screen aperture accounted for 89.7% of the variation in geometric mean diameter (d_{gw}) of WW flour. High rotor speed and small mill screen aperture produced fine particles and narrow particle size range. Conversely, low rotor speed and large mill screen aperture produced coarse particles with wide particle size distribution (Fig. 3 and 4). Hansen and Stewart (1965), Hansen and Henderson (1966), and Islam and Matzen (1988) have reported similar results using a hammer mill. These results are attributed to the centrifugal force on the grain generated by high rotor speed and enhanced impact between grain and wedge-shaped rotor blades. The 250 μm aperture screen size had more apertures (30 apertures/cm^2) and led to more shearing when grain fragments passed through the openings, compared with those of 500 (15 apertures/cm^2) and 1,000 μm screen aperture size (8 apertures/cm^2).

Table 2. Regression equations based on milling parameters (n_1=45 and n_2=30).

Regression equation	*P*	R^2	
dgw=143.1-0.007 RS+0.203 SAS	*P*<.0001	R^2=89.7 %	(4)
dgw=68.9+2.09 SM+0.22 FR	*P*<.0001	R^2=50.2 %	(5)
L=81.5+0.0003 RS-0.008 SAS	*P*<.0001	R^2=90.2 %	(6)
L=82.0-0.06 SM+0.03 FR	*P*<0.010	R^2=29.8 %	(7)
SD=13.3-0.0001 RS-0.01 SAS	*P*<.0001	R^2=85.1 %	(8)
SD=-4.22+0.79 SM+0.08 FR	*P*<.0001	R^2=86.4 %	(9)

dgw = Geometric mean diameter of whole-wheat flour particle size; RS = Rotor speed; SAS = Screen aperture size; SM = Seed moisture; FR = Feed rate; and SD = Starch damage.

Both rotor blade and mill screen are involved with size reduction; however, most of the direct size reduction was due to interaction of particles with the mill screen. For example, when the grain was exposed to only the rotor blades, most of the particles were ≥600 μm (Fig. 5). In this case the mill screen was removed and the collection pan was lined with sponge material to absorb energy of particle before hitting metal tray. As rotor speed increased from 6,000 to 18,000 rpm, the amount of particles ≥600 μm decreased from 95 to 80% and kernel breakage increased from 55 to 100%. With a mill screen, WW flour particles were varied around mill screen sizes, and particles reduced as rotor speed increased. Adjusting rotor speed and mill screen aperture size can result in WW flour with particle size distribution similar to that of commercial WW flour. The ultra-centrifugal mill configured with 9,000-18,000 rpm rotor speed and the 250 μm mill screen resulted in 74-89% of particle fraction retained on <150 μm and d_{gw} was 101-84 μm which was similar in fineness as available commercial brand 1 WWF (85% of particle fraction retained on <150 μm and d_{gw} was 106 μm) (Fig. 3A and 4A). Similarly, mill configured with rotor speed of 18,000 rpm and the 500 µm mill screen resulted in 87% of particle fraction retained on <250 µm and d_{gw} was 134 µm which was similar in fineness as commercial brand 2 WWF (82% of particle fraction retained on <250 µm and d_{gw} was 119 µm) (Fig. 3B and 4A).

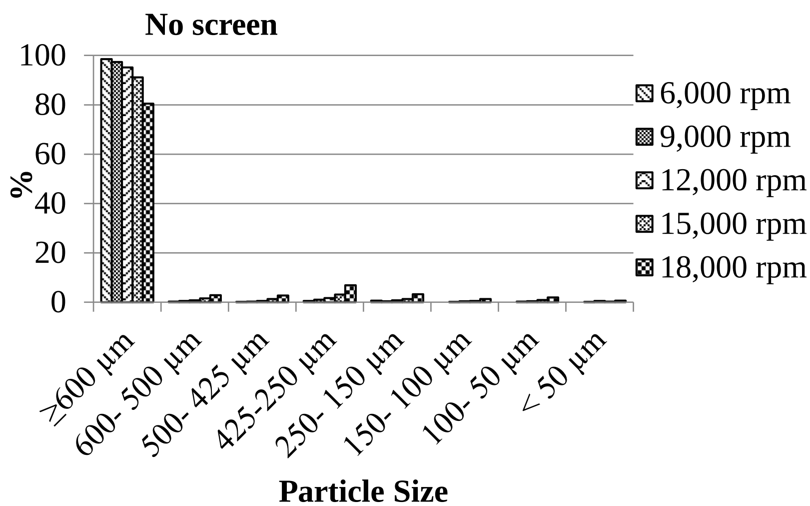

Figure 5. Particle size distribution of whole-wheat flours milled at different rotor speeds without a mill screen. LSD from rotor speed ($P \leq 0.05$). LSD (≥600µm) = 2.1.

Mill surface temperature

Mill surface temperature was significantly influenced by rotor speed and mill screen aperture ($P \leq 0.05$). For each rotor speed, mill surface temperature was greatest with 250 µm aperture mill screen; intermediate with 500 µm aperture mill screen; and least with 1,000 µm aperture mill screen (Fig. 6A). Mill surface temperature increased as rotor speed increased. The large increase in mill surface temperature was from 6,000 to 18,000 rpm with 250 and 500 µm aperture mill screens (5.6 and 6.9 °C, respectively).

Flour temperature

Flour temperature was significantly influenced by interaction between rotor speed and mill screen aperture. At 6,000, 9,000 and 12,000 rpm, flour temperature was greatest with 250 µm aperture mill screen; intermediate with 500 µm aperture mill screen; and least with 1,000 µm aperture mill screen; however, flour temperature from 15,000 to 18,000 rpm with 250 and 500 µm aperture mill screens were greater than that with 1,000 µm aperture mill screen (Fig. 6B). Overall, flour temperature increased 1.4 ˚C with 250 µm aperture mill screen from 6,000 to 18,000 rpm and 6.0 ˚C with both 500 and 1,000 µm aperture mill screens. Flour temperature was less than that of mill surface temperature because the vacuum created air flow that cooled and conveyed flour through the grinding chamber and screen.

Flour moisture loss

Flour moisture loss was significantly influence by rotor speed and mill screen aperture ($P \leq 0.05$). Moisture loss was greatest with 250 µm aperture mill screen; intermediate with 500 µm aperture mill screen; and least with 1,000 µm aperture mill screen. Moisture loss increased as rotor speed increased. Moisture loss was 1.1% increased with both 250 (from 2.6 to 3.7%) and 500 µm aperture (from 1.9 to 3.0%) mill screens and 8.0% increased with 1,000 µm aperture (0.2 to 1.0%) mill screen, as rotor speed increased from 6,000 to 18,000 rpm (Fig. 6C). Evaporative cooling helped prevent heat accumulation in the milling chamber and resulted in moisture loss in flour.

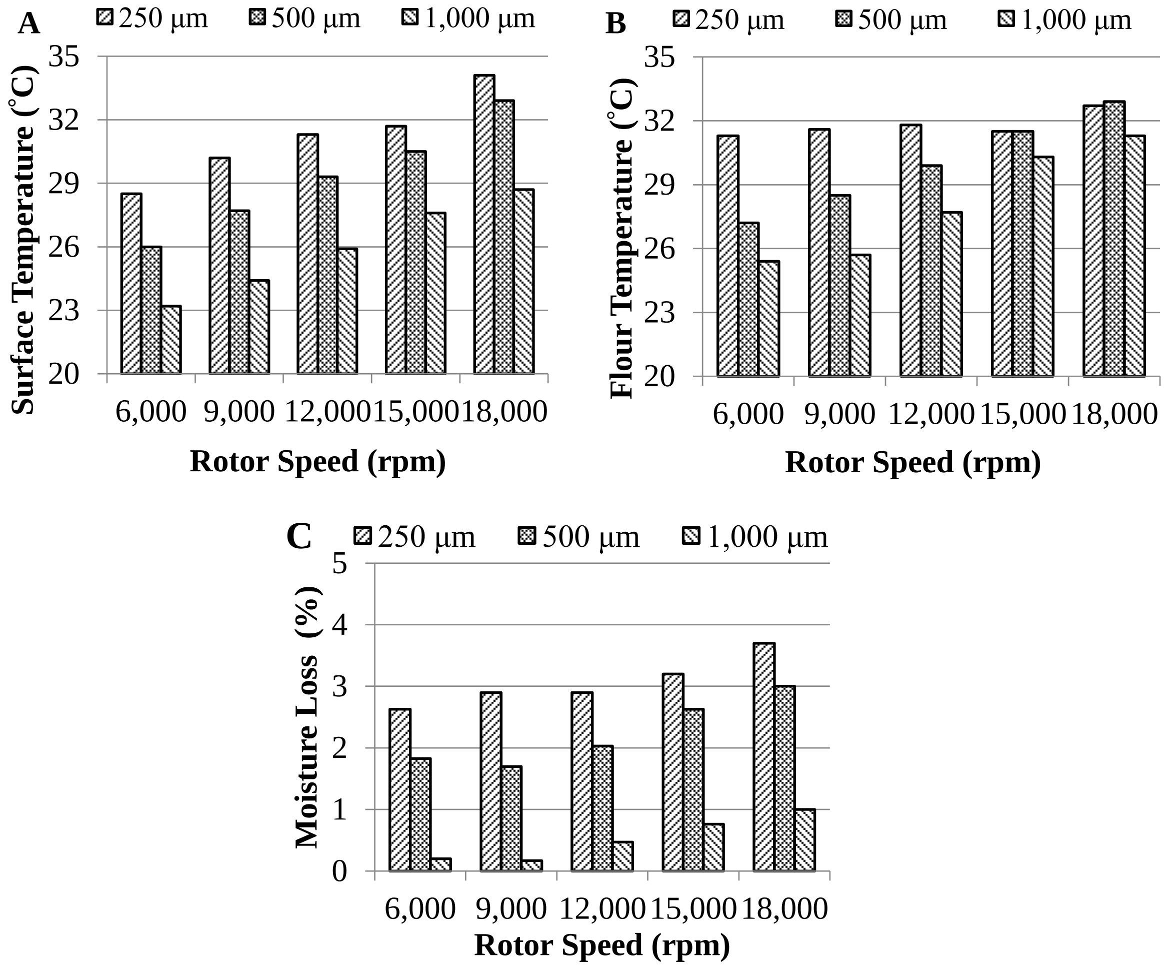

Figure 6. Whole-wheat flour characteristics milled at different rotor speeds and mill screen aperture sizes. A = Mill surface temperature; B = Flour temperature; and C = Moisture loss. LSD from mill screen aperture ($P \leq 0.05$): LSD (surface temperature) = 1.2, and LSD (moisture loss) = 0.3. LSD from rotor speed: LSD (surface temperature) = 0.7, and LSD (moisture loss) = 1. LSD from interaction between rotor speed and mill screen aperture: LSD (flour temperature) = 15.2.

Heat generated during milling grain on the ultra-centrifugal mill relates to: 1) the conversion and transfer of mechanical energy into strain energy into the grain and grain particles (Hansen and Stewart 1965); 2) friction associated with shearing of grain/particles through the mill screen opening in size reduction; and 3) moisture evaporation from particles (evaporative cooling) in the assembled vacuum system. Most energy input in grinding is converted into heat. Mechanical energy is greatest with high rotor speed and small screen aperture. Preliminary test showed flour temperature without vacuum system reached 76 ˚C with 12,000 rpm and 250 µm aperture mill screen of which flour temperature is close to those flour temperatures (90, 85, and 55 ˚C) reported for wheat ground on a stone mill, plate mill, and hammer mill, respectively (Rao and Prabhasankar 2001). With vacuum system, the highest flour temperature in the ultra-centrifugal was 33.8 ˚C, which is similar to flour temperature found during roller milling (Posner and Hibbs 2009). Evaporative cooling from vacuum system in the ultra-centrifugal milling prevented high flour temperatures which would affect starch, protein, and lipid properties.

Brightness

The brightness (L-value) of WW flour was significantly influenced by mill screen aperture and rotor speed ($P \leq 0.05$). Averaged across rotor speeds, L-value was the greatest with 250 µm aperture screen (82.40); intermediate with 500 µm aperture screen (79.68); and least with 1,000 µm aperture screen (76.12) (Fig. 7A). This probably relates to their corresponding particle sizes of WW flour with mill screens. Smaller perforated mill screen size effectively reduced and pulverized endosperm and bran into fine particle which subsequently had bright flour color. L-value increased 4.87 units (from 73.83 to 78.70) as rotor speed increased from 6,000 to 18,000 rpm when milling with the 1,000 µm aperture screen; however, rotor speed had little effect on brightness of WW flour made by using the 250 and 500 µm aperture screens (Fig. 7A).

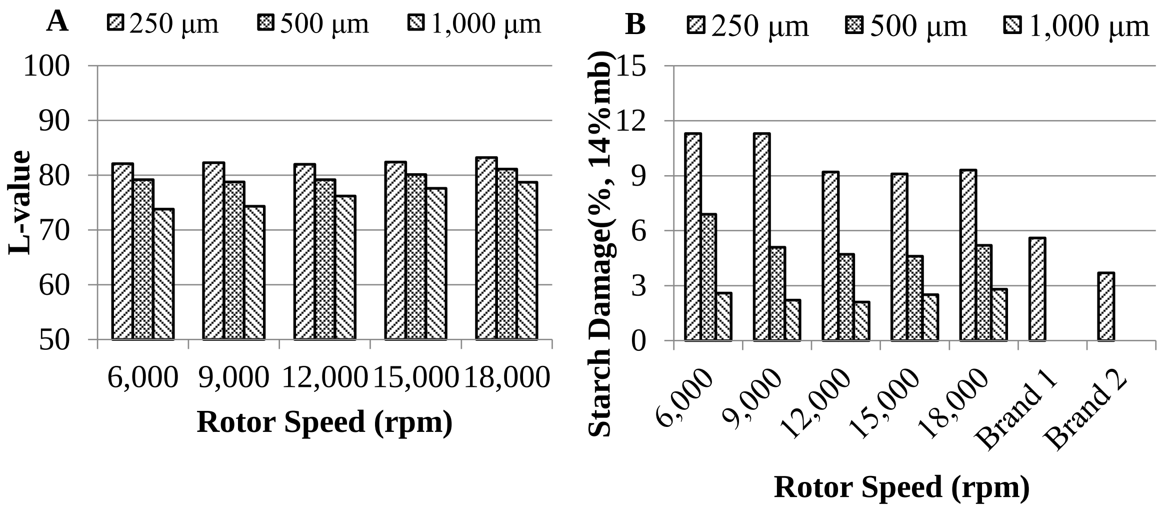

Figure 7. Whole-wheat flour characteristics milled at different rotor speeds and mill screen aperture sizes. A = L-value; and B = Starch damage. LSD from mill screen aperture ($P \leq 0.05$): LSD (L-value) = 0.8, and LSD (starch damage) = 0.7. LSD from rotor speed: LSD (L-value) = 0.4, and LSD (starch damage) = 0.3.

Rotor speed and mill screen aperture size affected flour brightness, an important quality characteristic, which can affect the appearance of final product. Multiple regression equation (6) could explain 90.2% of the variation in L-value by using rotor speed and mill screen aperture size (Table 2). Increased brightness of WW flour under configurations of high rotor speed and small mill screen aperture is attributed to the reduced particle size. Hidalgo et al (2014) also found that brightness increased as flour particle size decreased during roller milling. However, brightness had a larger increase from reduced mill screen aperture size (1,000 to 250 µm) than that from increased rotor speed (6,000 to 18,000 rpm), which indicates that mill screen had a more important role in brightness than rotor speed.

Starch damage

The effect of rotor speed and mill screen aperture on starch damage is shown in Fig. 7B. As expected, starch damage was greatest using the 250 µm aperture mill screen and was lowest using the 1,000 µm aperture mill screen, regardless of rotor speed. These results are attributed to greater shear being applied to milled product as it passed through the small aperture of mill screen. With the 250 and 500 μm aperture screens, starch damage was greater with 6,000 and 9,000 rpm than with 12,000, 15,000, or 18,000 rpm. It is speculated that starch damage was less with fast than slow rotor speed since at fast rotor speeds the wheat particles would pass through the mill screen apertures more quickly under greater centrifugal force derived from the greater rotor speed, and avoid extended shearing time between the rotor blades and the fixed screen. Khalid (2016) also reported greater starch damage with low than high rotor speed when milling hard red spring wheat into WW flour using an ultra-centrifugal mill. Starch damage can cause problems in mixing and dough handling, and subsequently result in negative effect on pasta and noodle color and texture (Hatcher et al 1999).

Starch damage of laboratory milled WW flour was greater than that of commercial WW flour, which probably reflects the harshness of milling using a single stream system compared to multiple stream-reconstitution system associated with roller milling. Roller milling uses separation and repeated size reduction by rollers and sifters to effectively remove bran and germ from endosperm and to obtain appropriate size reduction of endosperm (Campbell et al 2001). The progressive and gradual size reduction in roller milling is unlike the direct and fast size reduction (impact and shearing) in ultra-centrifugal mill, which leads to starch damage of milled products.

Rotor speed and mill screen aperture size affected starch damage of WW flour. Multiple regression equation (8) explained 85.1% of the variation in starch damage of WW flour using rotor speed and mill screen aperture size (Table 2). Severe physical forces from high rotor speeds, along with the small 250 μm aperture mill screen led to more damaged starch granules.

With the 250 µm mill screen aperture, starch damage decreased as rotor speed increased from 6,000 to 12,000 rpm and did not increase with rotor speeds above 12,000 rpm. With the 250 µm aperture mill screen, the starch damage with the rotor speed at 12,000 rpm (9.2%) was more similar to brand 1 (5.6%) than with lower rotor speeds (Table 3). The lower starch damage and lower energy input when milled at 12,000 rpm with 250 µm aperture mill screen would be used to produce fine particle of WW flour.

Table 3. The comparison of whole-wheat flour quality among milling configurations.

	9,000 rpm 250 µm	12,000 rpm 250 µm	18,000 rpm 250 µm	Brand 1
Particle size retained on <150 µm (%)	74a	80b	89c	85
dgw (µm)	101b	99b	84a	106
Starch damage (%)	11.3b	9.2a	9.3a	5.6

dgw = Geometric mean diameter of whole-wheat flour particle size.
LSD was compared on effect from milling configurations at $P \leq 0.05$.
Values followed by different letters in the row are significantly different at $P \leq 0.05$.

Seed Moisture and Feed Rate

Particle size distribution

For this experiment, the rotor speed (12,000 rpm) and the mill screen aperture (250 µm) were kept constant and seed conditioning moisture and feed rate were varied. The effect of seed conditioning moisture content on particle size distribution was most pronounced for the 150-100 and 100-50 µm fractions. Averaged across feed rates, the weight percentage of the 150-100 µm fraction increased from 33 to 42%, while the weight percentage of the 100-50 µm fraction decreased from 47 to 38%, respectively, when seed conditioning moisture content increased from 9 to 15% (Fig. 8A).

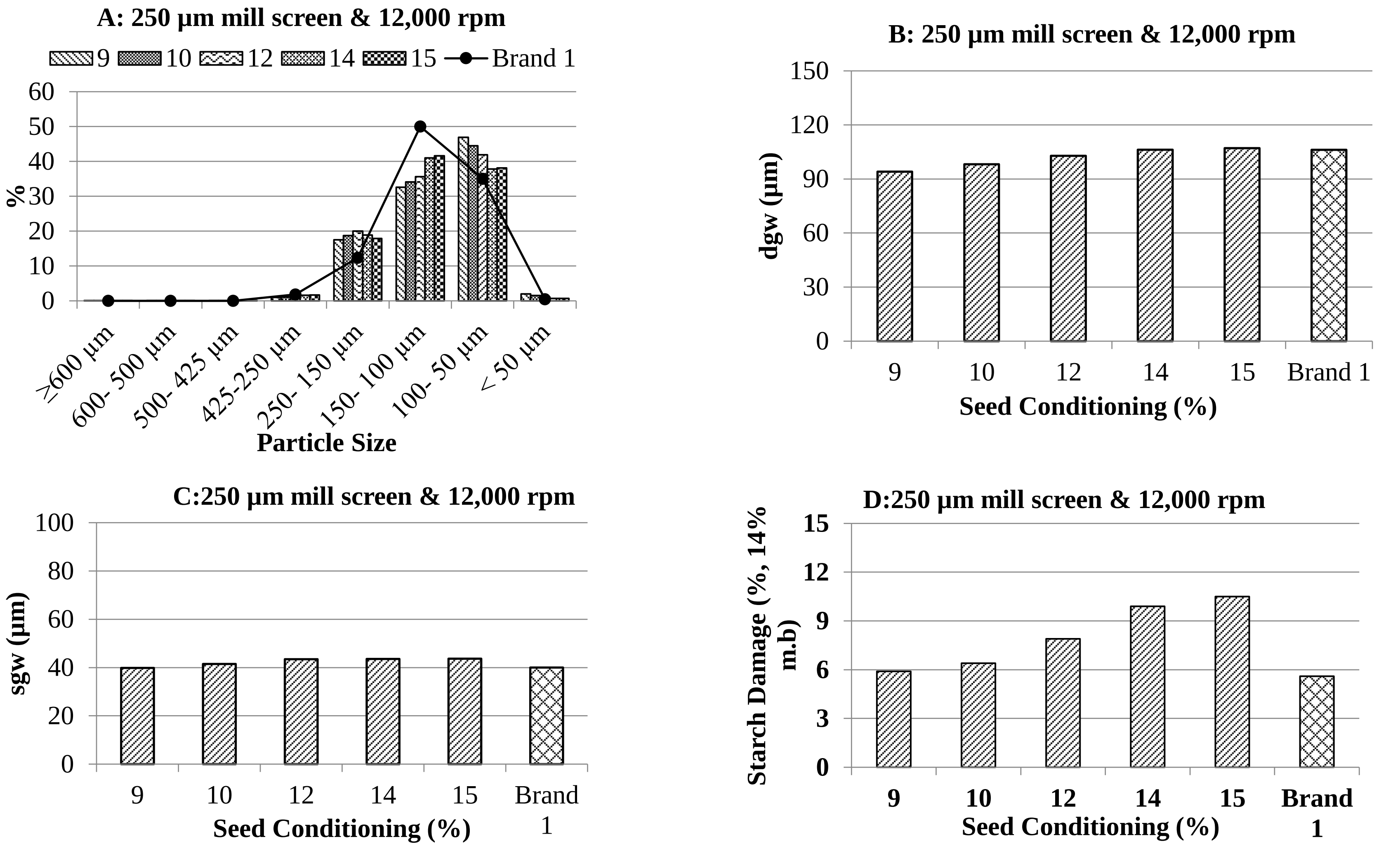

Figure 8. Whole-wheat flour characteristics milled at different seed conditioning moistures. A = Particle size distribution; B = dgw (geometric mean diameter); C = sgw (geometric standard deviation); and D = Starch damage. LSD from seed conditioning moisture ($P \leq 0.05$): LSD (500-425µm) = 0.02, LSD (425-250µm) = 0.2, LSD (250-150µm) = 1.3, LSD (150-100µm) = 10.4, LSD (100-50µm) = 8.5, LSD (<50µm) = 0.9, LSD (dgw) = 7.9, and LSD (starch damage) = 0.5.

Geometric mean diameter

Seed conditioning moisture content had little or no effect on weight percentages of the 425-250, 250-150, or <50 µm fractions. The geometric mean diameter (d_{gw}) was least (94 µm) with 9% seed moisture content and was greatest (107 µm) with 15% seed moisture content (Fig. 8B). The geometric standard deviation (s_{gw}) by mass of WW flour particle size values did not vary much with seed conditioning moisture content (Fig. 8C), which indicated that the particle size distribution was similar (250-50 µm range) regardless of seed conditioning moisture. Feed rate did not affect the particle fraction on each sieve, d_{gw}, and s_{gw} (Table 4).

Table 4. Particle size and starch damage of whole-wheat flour milled at different feed rates.

Feed rate (g min^{-1})	425-250 µm	250-150 µm	150-100 µm	100-50 µm	< 50 µm	dgw	sgw	Starch damage
			(%)			(µm)		(%, 14% m.b.)
37.0	1.2ns	18.1ns	36.9ns	42.5ns	1.3ns	100.6ns	41.6	7.7a
78.6	1.4ns	19.0ns	37.1ns	41.2ns	1.1ns	102.9ns	43.1	8.5b
Brand 1	1.8	12.4	50.0	35.0	0.5	106.2	40.0	5.6

dgw = Geometric mean diameter and sgw = Geometric standard deviation of whole-wheat flour particle size.
Particles on ≥600, 600-500, and 500-425 µm were not detected. LSD was compared on main effect from feed rate at $P \leq 0.05$. Values followed by different letters and by 'ns' in the columns are significantly and non-significantly different at $P \leq 0.05$, respectively.

Seed conditioning moisture and feed rate had less effect on particle size of WW flour than rotor speed and mill screen aperture size factors. In this study, feed rate did not affect particle size reduction. Multiple regression equation (5) to predict the d_{gw} of WW flour could only explain 50.2% of the variation in d_{gw} by using conditioning moisture and feed rate (Table 2).

The variation in particle size seems to be primary due to the seed conditioning moisture. Seed conditioning moisture is associated with endosperm brittleness and bran plasticity. Hsieh et al (1980), Glenn et al (1991), Shellenberger (1980), and Delwiche (2000) reported that endosperm brittleness decreased and bran plasticity increased with increased seed conditioning moisture, which together resulted in coarse particle size during milling. Silver (1932) demonstrated that more energy was required in comminution of moist conditioned seed than dry seed. With the same energy input, the moist conditioned seed would fracture less and produce large particles.

Bran tends to be more brittle/friable and less pliable at low than at high conditioning moisture. Seed with 9-12% conditioning moisture produced WW flour, 82-79% of particles were retained on <150 µm, and d_{gw} was 94-103 µm, respectively, which was similar in particle fineness as the commercial brand 1 WW flour, with 85% of particle fraction retained on <150 µm and d_{gw} was 106 µm (Fig. 8A and B).

Flour temperature and moisture loss

Seed conditioning moisture main effect was significant for mill surface temperature and WW flour moisture content (Table 5). For example, mill surface temperature significantly increased from 27.7 to 31.1 °C and moisture loss increased from 0.4 to 5.6% as seed conditioning moisture increased from 9 to 15%. However, seed conditioning moisture did not affect WW flour temperature. The vacuum cooling system was sufficient to prevent severe increase in flour temperature but was responsible for moisture loss. Evaporative cooling aided in temperature control.

Feed rate main effect was significant for mill surface temperature and WW flour temperature. For example, mill surface temperature and WW flour temperature increased from 28.9 to 30.1 and 30.7 to 33.8 °C, respectively, when feed rate increased from 37.0 to 78.6 g min^{-1} (Table 5). More grain entered the milling chamber with the high feed rate, which generated more heat due to increased collisions with rotor blades, screen and other grain and grain fragments. Feed rate did not affect the final WW flour moisture content.

Table 5. Whole-wheat flour milling characteristics at different seed conditioning moistures and feed rates.

	Surface temperature (°C)	Flour temperature (°C)	Moisture loss (%)	L
Seed moisture (%)				
9	27.7a	32.0ns	0.4a	82.6ns
10	28.4ab	32.6ns	1.1b	82.7ns
12	29.1b	32.2ns	2.4c	82.3ns
14	31.2c	32.0ns	4.4d	82.4ns
15	31.1c	32.5ns	5.6e	82.2ns
Feed rate (g min^{-1})				
37.0	28.9a	30.7a	2.7ns	82.3a
78.6	30.1b	33.8b	2.8ns	82.6b

LSD was compared on main effect from rotor speed and mill screen aperture at $P \leq 0.05$.
Values followed by different letters in the columns are significantly different at $P \leq 0.05$.
Values followed by 'ns' in the columns are not significantly different at $P \leq 0.05$.

Brightness

The brightness (L-value) of WW flour was not affected by seed conditioning moisture. Brightness of WW flour was 82.6 with 78.6 than 82.3 with 37.0 g min^{-1} feed rate. Feed rate and seed conditioning moisture had little or no effect on WW flour L-value in ultra-centrifugal milling. Multiple regression equation (7) can only explain 29.8% of the variation in L-value using conditioning moisture and feed rate (Table 2).

Starch damage

Seed conditioning moisture content and feed rate main effects were significant for starch damage. Starch damage decreased 4.6% as seed conditioning moisture content declined from 15 to 9% (Fig. 8D), and it also decreased 0.8% as feed rate declined from 78.6 to 37.0 g min^{-1} (Table 4). Regression equation (9) was established to predict the starch damage of WW flour by adjusting conditioning moisture and feed rate when milling with rotor speed of 12,000 rpm and

250 μm mill screen aperture. The equation (9) could explain 86.4% of the variation in starch damage using conditioning moisture and feed rate (Table 2). Starch damage was decreased with decreased seed conditioning moisture and feed rate. Seed with 9% conditioning moisture produced WW flour with similar starch damage (5.9%) as brand 1 (5.6%, Fig. 8D) and as the 4.7 – 7.7% starch damage reported by Doblado-Maldonado and Rose (2013) for four commercial WW flours.

Conclusions

The results of this research indicate that the ultra-centrifugal mill can grind durum wheat in a single pass resulting in particle size distribution similar to that of commercial WW flour without generating excessive heat. The results of this research indicate that rotor speed, screen aperture, seed conditioning moisture, and feed rate can affect particle size, temperature and starch damage of WW flour. A bright (L-value = 83.20) fine WW flour was obtained under a configuration of 18,000 rpm and 250 μm mill screen with 89% of granulation <150 μm, without exceeding 35 °C. However, starch damage was relatively high at 9.3%. Starch damage was reduced and fine particle size was maintained by reducing the rotor speed to 12,000 rpm and seed conditioning moisture to 9%. Durum grain with 9% seed conditioning moisture milled at 12,000 rpm with a 250 μm mill screen produced WW flour that had 82% of particles retained on <150 μm and 5.9% starch damage.

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CHAPTER 3: EFFECT OF SINGLE-PASS AND MULTI-PASS MILLING SYSTEMS ON WHOLE-WHEAT DURUM FLOUR AND WHOLE-WHEAT PASTA QUALITY

Abstract

Single-pass and multi-pass milling systems were evaluated by comparing the quality of whole-wheat (WW) durum flour and the subsequent WW spaghetti they produced. Two single-pass mill configurations for a centrifugal mill were used (fine grind: 15,000 rpm with 250 µm mill screen aperture and coarse grind: 12,000 rpm with 1,000 µm mill screen aperture) to direct grind durum grain or to regrind millstreams from roller milling to make WW flour and WW spaghetti. Particle size, starch damage, and pasting properties were similar for direct fine grind WW flour and multi-pass reconstituted flour:fine bran blend and for direct coarse grind WW flour and multi-pass reconstituted semolina:coarse bran blend. Semolina:fine bran blend had low starch damage and had desirable pasting properties for pasta cooking. WW spaghetti was better when made with WW flour produced using the multi-pass than single-pass milling system. Mechanical strength was greatest with spaghetti made from semolina:fine bran or durum flour:fine bran blends. Semolna:fine bran or semolina:coarse bran blends made spaghetti with high cooked firmness while spaghetti made from direct coarse grind or from semolina:fine bran or coarse bran blends had low cooking loss.

Introduction

WW flour is a ground whole grain product that contains bran, germ, and endosperm. WW flour can be produced using a single-pass system often using a stone, hammer, or disc mill or a multi-stream reconstitution system using a roller mill (Miller Jones 2015; Posner and Hibbs 2009). In the single-pass system, stone mill and hammer mill are the most common single-pass systems. Stone mill consists of rotating and stationary stones, which break the entire wheat grain between the center of the two stones and finely grinds particles along the perimeter of the stones (Gray's Grist Mill 2015). Hammer mill is a high-speed grinder that impacts and fractures wheat grain with fast rotating hammers. Mill configuration determines milling performance and the particle size distributions of milled product (Bayram and Öner 2005). For the stone mill, the milling configuration includes stone gap and the speed of the rotating stone; and for the hammer mill, the number and design of hammers, hammer-tip speed and screen aperture size. Single-pass milling systems are known to generate a lot of heat that can adversely affect flour quality (Miller Jones et al 2015; Rao and Prabhasankar 2001). These mills often use air flow to remove heat from the system.

Roller mill has a series of paired corrugated break rolls and a series of reduction rolls that fractionate wheat grain into semolina/flour, bran, germ, and shorts. Bran, germ, and shorts fractions are blended together and reground into varying particle sizes (mean size: 100-420 μm) using a single-pass milling system (Zhang and Moore 1997; Cai et al 2014). Fine regrinding techniques increase bran surface area, speck appearance, and functional properties in cereal based products (Zhang and Moore 1999; Campbell 2008; Noort et al 2010; Coda et al 2014). The reground material is blended with flour or semolina in the same ratio as found in grain to make a reconstituted WW flour.

An ultra-centrifugal mill has been evaluated for its ability to mill 2 to 4 kg samples of wheat into WW flour, and for milling bran/germ removed by roller milling into coarse and fine bran flour (Deng and Manthey 2016; Khalid 2016). Ultra-centrifugal mill grinds wheat grain by impact with a rotor blade and by shear between rotor blade and screen. A vacuum cooling system assembled with the mill can avoid excessive heat damage generated within milled product. The milling capacity is suitable for laboratory-scale productions of WW flour and WW pasta.

In this study, the ultra-centrifugal mill was used as a single-pass milling system to finely and coarsely grind durum wheat into WW flour which was then used to make WW spaghetti. A roller mill (Bühler model MLU 202) was used as a multi-pass milling system. The bran/germ/shorts collected during roller milling was reground with the ultra-centrifugal mill and the reground material was blended with semolina or durum flour to make WW flours which were used to make WW spaghettis. The objective was to compare the WW flour and WW spaghetti made from durum wheat milled using the single-pass and multiple-pass milling systems.

Materials and Methods

Samples

A bulk sample of durum wheat grown at Casselton, ND 2015 was used in this study. The durum sample had grain moisture (9.1%), protein (13.3%), test weight (77.0 kg/hL), 1000-kernel weight (36.0 g), and percentage of vitreous (90%). The kernel size had 53% large kernels retained on a Tyler No. 7 (2.92 mm opening), 43% medium kernels retained on a Tyler No. 9 (2.24 mm opening) and 4% small kernels retained on the bottom pan, respectively. The wheat samples were sound with a falling number of 451 sec. Sample was stored at 12 $^{\circ}$C until used.

Whole-Wheat Flour Milling

Durum was milled into WW flour by single and multi-pass milling systems. In the single-pass milling system, the wheat sample (1kg) was air dried to 9.0% moisture and directly ground using an ultra-centrifugal mill (ZM 200, Retsch, Germany) which consisted of a vibratory feeder and a vacuum cooling system. Fine and coarse particle size WW flours were milled with rotor speed of 15,000 and 12,000 rpm and screen aperture size of 250 and 1,000 µm, respectively. Both fine and coarse milling configuration used feed rate of 62 g/min.

The multi-pass system utilized a roller mill (MLU 202, Bühler, Switzerland) with two Miag purifiers. Wheat sample (1 kg) was tempered in three steps: 1) wheat was tempered to 12% moisture at least 72 h before milling, 2) the grain was tempered to 14.5% moisture 24 h before milling, and 3) the grain was tempered to 17.5% moisture 45 min before milling. The wheat was milled into three fractions: semolina, bran/germ, and shorts. The bran/germ and shorts fractions were blended together and ground into fine and coarse flours using the ultra-centrifugal mill as described above. Semolina was milled into durum flour with the ultra-centrifugal mill using the fine particle size configuration. WW flour was reconstituted from semolina:bran or flour:bran and blended in a cross-flow blender (Vitamix Co., Cleveland, OH) for 5 min. Thus, four reconstituted WWFs were prepared: 1) SFB (semolina blended with finely ground bran, germ, and shorts); 2) SCB (semolina blended with coarsely ground bran, germ, and shorts; 3) FFB (durum flour blended with finely ground bran, germ, and shorts); and 4) FCB (durum flour blended with coarsely ground bran, germ, and shorts). All the WW flours are shown in Fig. 9.

Whole-Wheat Flour Quality

Flour brightness (*CIE* L^*-value) was measured using a colorimeter (CR410, Minolta, NJ, USA) equipped with a D65 illuminant. The flour was poured into a black cell (1 cm deep) that had a quartz glass window. Flour brightness was recorded as a mean value of three readings. L^*-value varies on a scale of 0 to 100, where 0 is black and 100 is white.

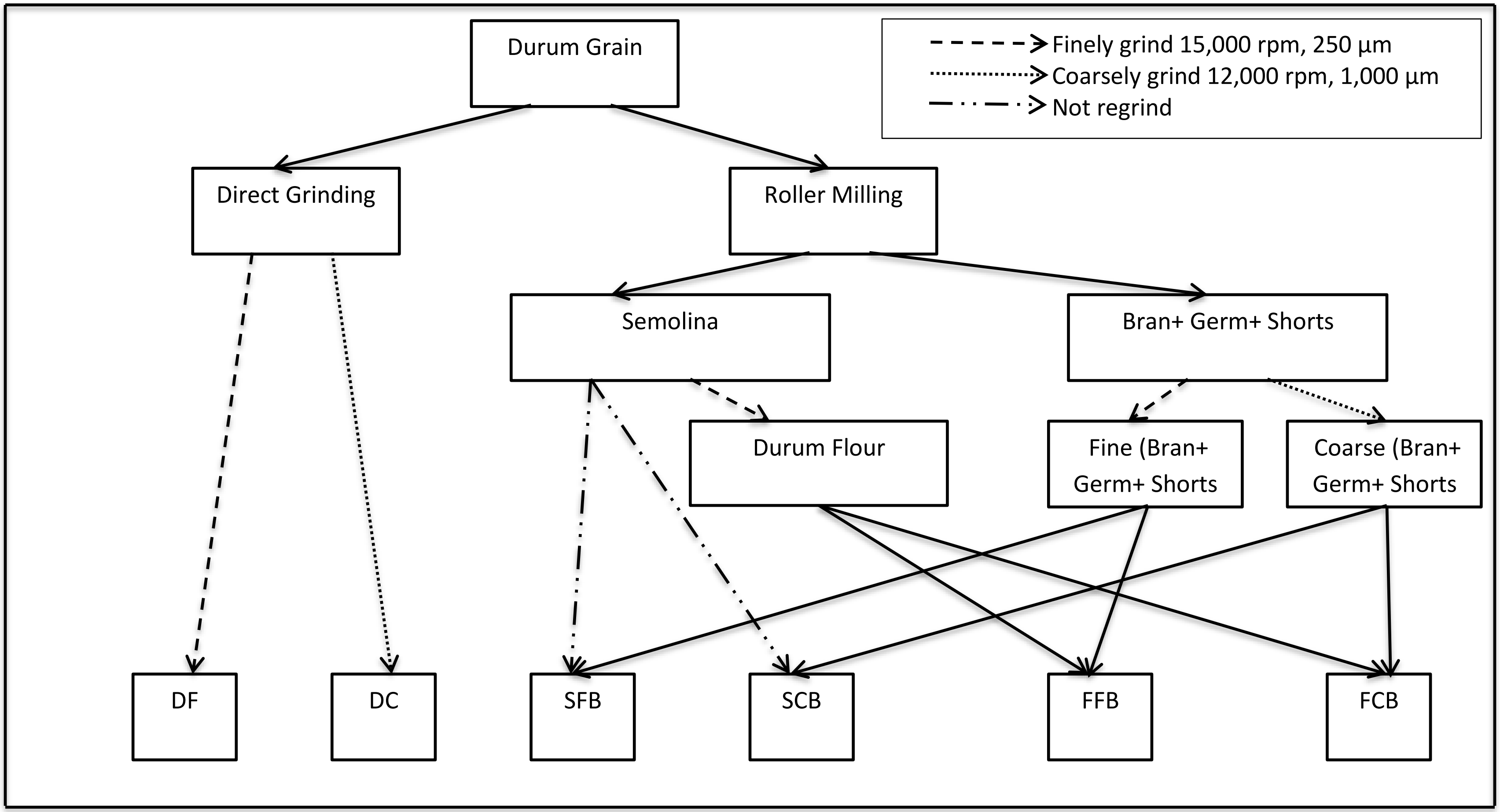

Figure 9. Direct ground and reconstituted whole-wheat flours. DF = Fine direct ground flour; DC = Coarse direct ground flour; SFB = Semolina blended with finely ground bran, germ, and shorts; SCB = Semolina blended with coarsely ground bran, germ, and shorts; FFB = Durum flour blended with finely ground bran, germ, and shorts; and FCB = Durum flour blended with coarsely ground bran, germ, and shorts.

Particle size distribution test was conducted using a vibratory sieve shaker (AS 200, Retsch, Germany). The particle size distribution was recorded based on the weight percentage retained on stacked sieves of 600, 500, 425, 250, 150, 100, 50, and <50 µm. Each sieve had 10 polyurethane balls that acted as sieve cleaners during sifting. The geometric mean diameter (d_{gw}) and geometric standard deviation (s_{gw}) by mass of WW flour particle size were measured based on equations provided by ASABE S319.4.

$$d_{gw} = \log^{-1}\left[\frac{\sum_{i=1}^{n}(W_i \log \overline{d_i})}{\sum_{i=1}^{n} W_i}\right] \tag{1}$$

$$s_{log} = \left[\frac{\sum_{i=1}^{n} W_i(\log \overline{d_i} - \log d_{gw})^2}{\sum_{i=1}^{n} W_i}\right]^{1/2} \tag{2}$$

$$s_{gw} = \frac{1}{2} d_{gw}\left[\log^{-1} s_{log} - \left(\log^{-1} s_{log}\right)^{-1}\right] \tag{3}$$

Where d_{gw} is the geometric mean diameter of the particles by mass (µm); s_{log} is the geometric standard deviation of the log-normal distribution by mass; s_{gw} is the geometric standard deviation of the particle diameter by mass (µm); W_i is the mass on the ith sieve (g); n is the number of sieves; and d_i is the nominal sieve aperture size of the ith sieve (µm).

Starch damage was measured using AACC International Approved Method 76-31.01. Pasting properties were determined using a Rapid Visco Analyzer (RVA) (4SA, Newport Scientific, Warriewood NSW, Australia) according to the AACC International Approved Method 76-21.01. The heating and cooling rates were both 12 °C/min. Starch gelatinization parameters were reported as peak time, and peak, trough, and final viscosities, and their viscosity units were reported in rapid visco units (RVU). Dough properties were evaluated using a mixograph according to AACC International Approved Method 54-40.02 with modifications (Deng 2017). Mixogram was run by adding 7.8 mL distilled water to 10 g (14 % mb) WW flour. The test ran for 10 min. Parameters recorded were peak time and peak height, where peak time (min) was measured as the time for the dough to reach maximum consistency, which corresponds to peak height of the mixogram curve. Peak height (cm) was measured as the distance from the base to the center point of the curve.

Whole-Wheat Spaghetti Extrusion

Each WW flour sample (1 kg) was hydrated to 33% moisture (wb) and extruded into WW spaghetti using a semi-commercial pasta extruder (DeMaCo, Melbourne, FL). Extrusion conditions were: extrusion temperature, 45ºC; mixing chamber vacuum, 46 cm of Hg; and an auger extrusion speed: 25 rpm. The extrusion auger has a length to diameter ratios of 8.1:1, a constant root diameter, and uniform pitch the entire length of the auger. After extrusion, the spaghetti was dried in a laboratory dryer (Standard Industries, Fargo, ND) using the high temperature drying cycle (length: 10 h and peak temperature: 73°C) as described by Yue et al (1999).

Whole-Wheat Spaghetti Qualities

WW spaghetti brightness was determined using a CR410 Minolta colorimeter according to AACC International Approved Method 14-22.01. The spaghetti was placed on a black plastic template that ensured a uniform background color and depth (1 cm) during testing. The spaghetti brightness was measured by *Hunter* L-value and color measurements were taken at three different places of the dry spaghetti and mean color value was determined. *Hunter* L-value varies on a scale of 0 to 100, where 0 is black and 100 is white. Mechanical strength of spaghetti was determined by the spaghetti flexure test using a TA-XT2 texture analyzer with a Spaghetti Flexure Rig (Texture Technologies Corp., Scarsdale, NY). The amount of force (g) required to break one spaghetti strand (20 cm) was recorded on five strands per treatment. Test speed was 2.5 mm/sec. Cooking quality including firmness, cooking loss, and cooked weight were determined based on AACC International Approved Method 66-50.01. Cooking quality was determined on samples that were cooked 2, 4, 6, 8, 10, 12, 14, and 16 min. Cooked weight was determined as the increase in pasta product weight after cooking and was expressed as the percentage increase in pasta weight before cooking.

Statistical Analysis

The experimental design was randomized complete block design. The experiment had six different treatments of fine and coarse direct ground WW flours and semolina/durum flour and fine and coarse bran blends. Each treatment had three replicates. Each replicate was done on a separate day. Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System v 9.4 (SAS Institute, Cary, NC, USA). Treatment means were separated by Fisher's Protected Least Significant Different (*LSD*) at $P \leq 0.05$. Pearson's correlation coefficients were estimated between whole durum flour and WW spaghetti quality parameters.

Results and Discussion

Whole-Wheat Flour Qualities

Particle size distribution

The particle size distribution of WW flour was recorded as weight percentage of fraction retained on stacked sieve (600-50 µm mesh). d_{gw} is the geometric mean diameter of flour particles and a high value of d_{gw} indicates large mean particle size, and s_{gw} is the standard deviation of geometric mean diameter and a small s_{gw} value reflects a narrow particle distribution and conversely large s_{gw} reflects broad particle distribution. The particle size distribution of WW flours from direct grind and from reconstituted blends were consistent with their d_{gw} and s_{gw} results (Table 6).

As expected, direct fine grind had produced fine particles with a narrower size distribution (49.9% particle between 100 to 50 μm and d_{gw}=92 and s_{gw}=42 μm) than did direct coarse grind (18.4% particle between 100 to 50 μm and d_{gw}=216 and s_{gw}=163 μm, Table 6). Semolina is coarsely ground endosperm of durum wheat and had a coarser mean particle size (d_{gw}=225 μm) than did durum flour (d_{gw}=56 μm) or did the direct fine grind (d_{gw}=92 μm). Direct fine grind had similar coarse particle size as durum flour:fine bran blend with the amount above 250 μm screen being 2.1% for direct fine and 1.7% for durum flour:fine bran blend. However, direct fine grind had more fine particles 100 to 50 μm (49.9%) than did durum flour:fine bran (39.1%). Even though milled with the same mill configuration, fine bran had more coarse particles (28.5% >150 μm) than did durum flour (11.7% >150 μm), which indicates that compared to semolina, bran (being fibrous and flexible) was harder to mill into fine particles.

Direct coarse grind had similar coarse particle size as semolina:coarse bran with the amount above 250 μm screen being 49.5% for direct coarse and 49.0% for semolina coarse bran (Table 6). The large aperture mill screen would result in the coarse particles of direct coarse grind. Coarse milled bran had 22.7% on 600 μm sieve under the 1,000 μm aperture mill screen which was much more than what was found with any other milled products. The standard deviation of geometric mean diameter was very large (216 μm) which indicates a wide distribution of particle sizes. The large aperture size (1,000 μm) allowed a wide range of particle sizes to pass. The high quantity of large bran particles and wide range in particle size reflects the difficulty in milling bran to a uniform particle size.

Table 6. Particle size distribution of whole-wheat flours.

	≥600μm	600-500μm	500-425μm	425-250μm	250-150μm	150-100μm	100-50μm	<50μm	dgw	sgw	Bright-ness
	(%)								(μm)		
Single-Pass											
DF	0.4c	0.5c	0.3e	0.9e	13.9c	32.0b	49.9a	2.1a	92e	42e	82.11a
DC	5.8a	6.4a	7.6a	29.7c	21.8b	9.9d	18.4c	0.3b	216a	163a	76.01bc
Multi-Pass											
SFB	0.2c	0.2d	0.7d	37.2b	26.6a	18.2c	16.9c	0.2b	183b	108c	77.55b
SCB	2.8b	1.6b	2.7b	41.9a	27.3a	10.9cd	12.7c	0.2b	215a	131b	72.91c
FFB	0.3c	0.3cd	0.2e	0.9e	14.0c	44.7a	39.1b	0.5b	105d	43e	81.66a
FCB	2.9b	1.8b	1.9c	7.2d	14.5c	34.1b	37.4b	0.2b	125c	81d	81.57a
Control											
S	0.1	0.1	0.7	49.7	36.6	8.3	3.9	0.8	225	94	84.40
F	0.5	0.3	0.2	0.5	10.2	20.3	56.5	11.4	56	37	89.20
FB	0.8	0.1	0.2	3.4	24.0	31.5	39.9	0.0	116	57	-
CB	22.7	6.3	6.9	20.0	17.6	15.1	11.4	0.0	267	216	-

DF = Direct fine ground flour; DC = Direct coarse ground flour; SFB = Semolina blended with fine reground bran, germ, and shorts; SCB = Semolina blended with coarse reground bran, germ, and shorts; FFB = Durum flour blended with fine reground bran, germ, and shorts; FCB = Durum flour blended with coarse reground bran, germ, and shorts; S = Semolina; F = Durum flour; FB = Fine reground bran; and CB = Coarse reground bran; dgw = Geometric mean diameter of flour particles; and sgw = Standard deviation of geometric mean diameter.

Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Brightness

The presence of bran in direct grind and in the reconstituted blends reduced brightness compared to durum flour and semolina alone (Table 6). Fine grinding associated with direct grinding (L^*-value=82.11) and with durum flour:fine bran (L^*-value=81.66) and durum flour:coarse bran blends (L^*-value=81.57) resulted in small particles that resulted in relatively high brightness (Table 6). Semolina blends having larger particle size had lower brightness (L^*-value=77.55 and 72.91). Hidalgo et al (2014) suggested that as flour particle size increased, the surface became more heterogeneous which resulted in a decrease in brightness. Significant negative correlations were found between brightness and d_{gw} (r=-0.84, $P \leq 0.05$) and between brightness and s_{gw} (r=-0.80, $P \leq 0.05$, Table 7). Direct fine grind was similar to durum flour:fine bran for geometric mean (92 vs 105 μm), s_{gw} (42 vs 43 μm) and brightness (L^*-value =82.11 vs 81.66). Direct coarse grind was similar to semolina:coarse bran for d_{gw} (216 vs 215 μm), s_{gw} (163 vs 131 μm) but had greater brightness (L^*-value=76.01 vs 72.91).

Table 7. The Pearson Correlation between quality parameters.

	Flour Brightness	RVA			Mixograph	
		PT	PV	FV	PT	PH
dgw	-0.84**	-0.91**	-0.03	0.16	0.73**	-0.59**
sgw	-0.80**	-0.88**	-0.21	-0.01	0.65**	-0.56**
SD	0.80**	-0.72**	0.10	-0.20	-0.72**	0.56**

dgw= Geometric mean diameter of flour particles; sgw = Standard deviation of geometric mean diameter; SD = Starch damage; RVA = Rapid Visco Analyzer; PT = Peak time; PV = Peak viscosity; FV = Final viscosity; and PH = Peak height.

Starch damage

Starch damage was greater with direct fine grind and durum flour:bran samples (7.5-7.9%) than with direct coarse grind (2.3%) and semolina:bran samples (3.3-3.6%, Table 8). Significant negative correlation between starch damage and particle d_{gw} (r=-0.97, $P \leq 0.05$) and between starch damage and s_{gw} (r=-0.94, $P \leq 0.05$, data not shown) were observed. Durum flour is known to have greater starch damage than semolina (Pasqualone et al 2004). During regrinding, the energy needed to reduce particle size from coarse semolina to fine flour results in damaged starch (Deng and Manthey 2016).

Table 8. RVA and Mixograph parameters of whole-wheat flours.

	SD	RVA				Mixograph	
		PT	PV	TR	FV	PT	PH
	(%, 14%mb)	(min)		(RVU)		(min)	(cm)
Single-Pass							
DF	7.6ab	6.0a	190d	110c	249de	3.7c	4.2
DC	2.3e	5.5b	168e	112bc	246e	5.9ab	3.5
Multi-Pass							
SFB	3.6c	5.6b	225a	136a	435a	7.0a	3.5
SCB	3.3d	5.5b	216b	130a	303c	6.8a	3.5
FFB	7.9a	6.0a	208c	116b	334b	3.7c	4.0
FCB	7.5b	5.9a	202c	113bc	257d	4.5bc	3.8
Control							
S	3.5	5.6	180	117	237	3.3	6.1
F	10.2	6.0	187	110	215	3.2	7.6

DF = Direct fine ground flour; DC = Direct coarse ground flour; SFB = Semolina blended with fine reground bran, germ, and shorts; SCB = Semolina blended with coarse reground bran, germ, and shorts; FFB = Durum flour blended with fine reground bran, germ, and shorts; FCB = Durum flour blended with coarse reground bran, germ, and shorts; S = Semolina; and F = Durum flour. SD = Starch damage; RVA = Rapid Visco Analyzer; PT = Peak time; PV = Peak viscosity; TR = Trough viscosity; FV = Final viscosity; and PH = Peak height.
Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Pasting properties

Direct fine grind and samples containing durum flour had a longer peak time (5.9-6.0 min) than did direct coarse grind or samples containing semolina (5.5-5.6 min, Table 8). Significant negative correlations were found between peak time and d_{gw} (r=-0.91, $P \leq 0.05$), s_{gw} (r=-0.88, $P \leq 0.05$), and starch damage (r=-0.72, $P \leq 0.05$, Table 7), respectively. The long time to reach peak viscosity probably relates to the low level of starch damage associated with large particles. Hydration of intact starch granules would be slower than that of damaged starch.

Peak viscosity was greater with reconstituted blends (202-225 RVU) than with direct ground samples (168-190 RVU, Table 8). For reconstituted samples, peak viscosity was greatest with semolina:fine bran (225 RVU), intermediate with semolina:coarse bran (216 RVU) and least with durum flour:fine or coarse bran blends (202-208 RVU). Peak viscosity did not correlate with d_{gw}, s_{gw} or starch damage.

RVA trough viscosity indicates the holding strength of starch paste at constant heating and mechanical shearing stage. Trough viscosity was similar for direct fine and direct coarse grinds and durum flour:coarse bran blend (110-112 RVU, Table 8). In reconstituted blends, the trough viscosity was greater with semolina:fine bran blend (136 RVU) and semolina:coarse bran (130 RVU) than with durum flour:fine bran (116 RVU) or durum flour:coarse blend (113 RVU).

Final viscosity was similar for direct fine and direct coarse grinds (246-249 RVU) which was lower than the final viscosity with semolina:fine bran (435 RVU), semolina:coarse bran (303 RVU) or durum flour:fine bran (334 RVU, Table 8). For the reconstituted samples, final viscosity was greater with fine bran than coarse bran and with semolina than flour. Overall, final viscosity was greatest with semolina:fine bran (435 RVU), intermediate with durum flour:fine bran (334 RVU), and lowest with direct fine grind (249 RVU). For samples with coarse bran particles, final viscosity was greatest with semolina:coarse bran (303 RVU), intermediate with durum flour:coarse bran (257 RVU) and least with direct coarse grind (246 RVU). Final viscosity did not correlate with d_{gw}, s_{gw} or starch damage.

Direct fine and direct coarse grinds had lower peak, trough and final viscosities relative to reconstituted blends. Direct coarse grind had coarse bran particles that could interfere with the swelling of starch granules; the ability to withstand heating and shear; and the re-association between starch molecules to form a gel (Becker et al 2001; Cai et al 2014). Semolina:fine bran had high peak, trough and final viscosities (225, 136, and 435 RVU, respectively). The greater particle size of semolina relative to durum flour was associated with higher viscosity, which was in accordance with the results reported by Becker et al (2001).

Dough properties

Dough properties were evaluated using a mixograph where peak time (time to maximum dough consistency) was greater for direct coarse grind (5.9 min) than direct fine grind (3.7 min, Table 8). For the reconstituted blends, mixogram peak time was greater for samples that contained semolina (6.8-7.0 min) than those that contained durum flour (3.7-4.5 min). Compared to flour, the large semolina particles would take longer to hydrate, which would delay gluten formation and result in prolonging the time needed to reach the maximum dough consistency. These results are supported by the positive correlation between mixogram peak time and d_{gw} (r=0.73, $P \leq 0.05$) or s_{gw} (r=0.65, $P \leq 0.05$, Table 7). Mixogram peak time was reduced with increased starch damage as indicated by the negative correlation between starch damage and mixogram peak time (r=-0.72, $P \leq 0.05$). Flour particles must be fully hydrated before maximum dough consistency can be achieved (Cai et al 2015). High starch damaged associated with direct fine and durum flour:bran blends resulted in increased rate of water absorption and decreased the time to fully hydrate the flour particles which subsequently reduced mixogram peak time. Compared with semolina or durum flour without bran, the presence of bran, whether fine or coarse, caused an increase in peak time and the effect of bran was similar whether fine or coarse. The bran has greater water binding than semolina or flour (de la Peña et al 2016). Thus, bran would reduce the amount of water available to hydrate flour or semolina particles, which would slow the rate of hydration and increase the mixogram peak time. Peak height was greatest with durum flour (7.6 cm) and semolina (6.1 cm) alone (Table 8). The presence of bran whether fine or coarse caused a reduction in dough strength as indicated by peak height. Bran was also reported to reduce dough strength (Özboy and Köksel 1997; Zhang and Moore 1997). No differences occurred in peak height with samples containing bran.

Whole-Wheat Spaghetti Qualities

Appearance

The surface of WW spaghetti was smoother when made with fine than with coarse bran (Fig. 10). Brightness was greater for spaghetti made from durum flour (L-value=51.92) or semolina (L-value=51.91) than for whole-wheat spaghettis (L-value=31.14 to 34.21, Table 9). These results indicate that the presence of bran reduced overall brightness of spaghetti. Durum flour and semolina lose their original particle structure during pasta processing, so particle size is no longer important. However, bran particles remain as distinct particles in WW spaghetti (Manthey and Schorno 2002). The distinct bran particles still impact how light reflects off particles in WW spaghetti.

WW spaghettis containing coarse bran were brighter (L-value=33.99-34.01) than those with fine bran (L-value=31.14-31.20, Table 9). The large and rough bran particles were apparent on the surface of coarse bran WW spaghettis (Fig. 10). Different particles absorb the spectral energy from the light source and has their own reflectance pattern (Kennamer et al 2017). Reduced light absorption by large bran particles results in an increase in light reflection, which is attributed to the increased brightness of WW spaghetti containing coarse bran. Although statistically different, the difference in brightness between direct fine grind (L-value=32.71) and semolina:fine bran (L-value=31.20) or flour:fine bran (L-value=31.14) was small and probably is not of practical importance.

Table 9. Brightness and mechanical strength of whole-wheat spaghettis.

	Brightness	Mechanical Strength (g)
Single-Pass		
DF	32.71b	36.0b
DC	34.21a	35.5b
Multi-Pass		
SFB	31.20c	40.7a
SCB	34.01a	35.9b
FFB	31.14c	40.4a
FCB	33.99a	36.8b
Control		
S	51.91	44.2
F	51.92	47.5

DF = Direct fine ground flour; DC = Direct coarse ground flour; SFB = Semolina blended with fine reground bran, germ, and shorts; SCB = Semolina blended with coarse reground bran, germ, and shorts; FFB = Durum flour blended with fine reground bran, germ, and shorts; FCB = Durum flour blended with coarse reground bran, germ, and shorts; S = Semolina; and F = Durum flour.

Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Mechanical strength

Spaghetti made from direct fine grind and direct coarse grind had similar mechanical strengths (35.5-36.0 g, Table 9) which were less than when made from semolina or durum flour with fine bran (40.4-40.7 g) but similar when made from semolina or durum flour with coarse bran (35.9-36.8 g). Overall, bran reduced mechanical strength regardless if direct ground or a reconstituted blend. Mechanical strength was greater with fine than coarse bran. Shiau et al (2012) reported similar results concerning the effect of particle size of wheat bran on dry noodle strength. They reported that dry noodle strength was greater with small than with large particle sizes.

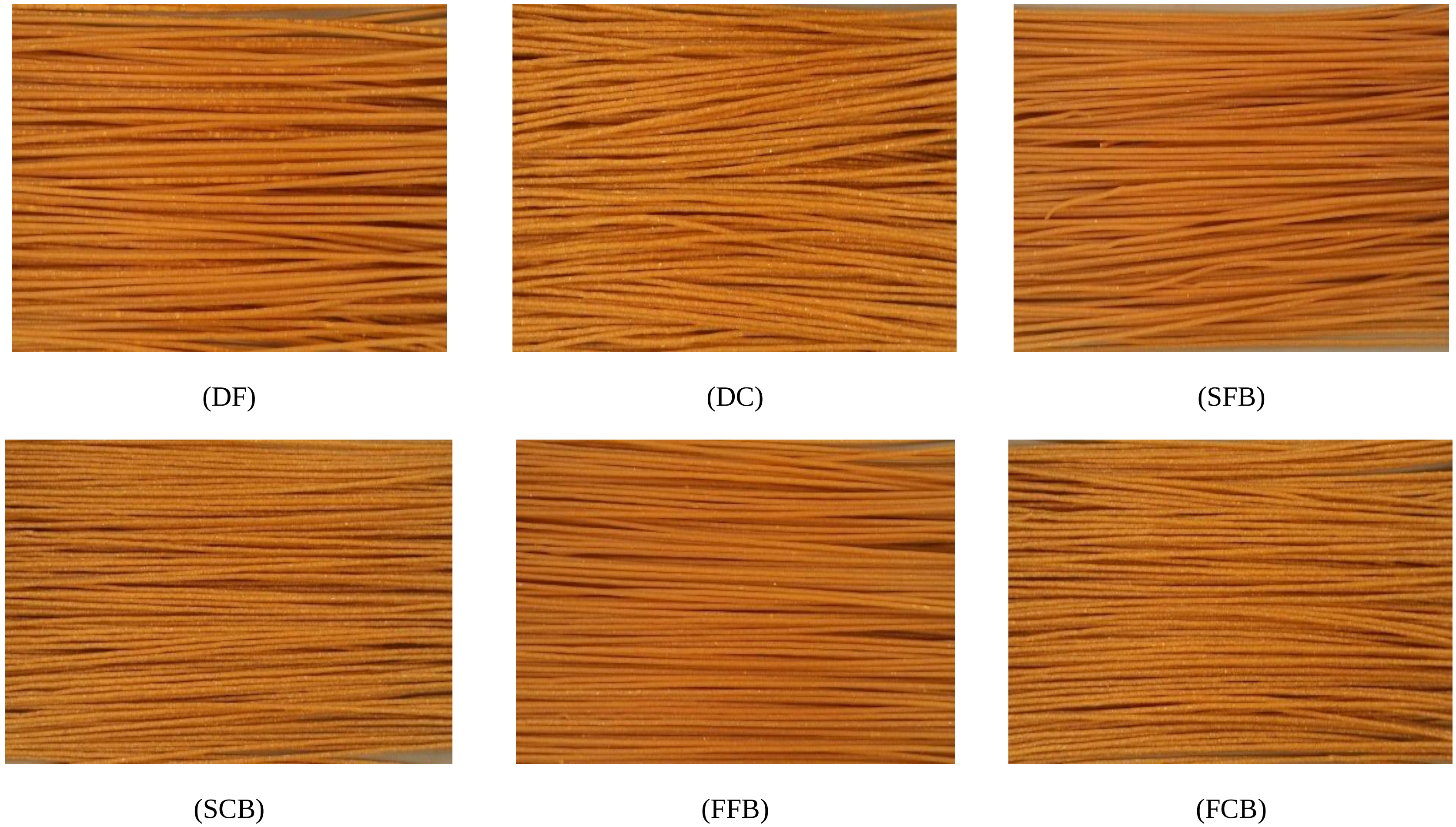

Figure 10. The appearance of whole-wheat spaghettis. DF = Direct fine ground flour; DC = Direct coarse ground flour; SFB = Semolina blended with fine reground bran, germ and shorts; SCB = Semolina blended with coarse reground bran, germ and shorts; FFB = Durum flour blended with fine reground bran, germ and shorts; and FCB = Durum flour blended with coarse reground bran, germ and shorts.

Cooked firmness

Cooked firmness of WW spaghetti decreased with prolonged cooking time (Table 10). However, the magnitude of change in cooked firmness was different with differing WW flours and cooking time. At 2 min, WW spaghetti made from direct coarse grind had higher cooked firmness (9.0 g cm) than spaghetti made from direct fine grind (6.5 g cm). Similarly, spaghetti made with coarse bran:durum flour/semolina blends had higher cooked firmness than blends with fine bran. Cooked firmness ranged from 6.5 g cm for direct fine grind to 11.0 g cm for durum flour:coarse bran. Unlike durum flour and semolina particles that lose their integrity during dough development and spaghetti processing, bran particles remain basically intact (Manthey and Schorno 2002). Manthey and Dick (2012) attributed the occurrence of elevated cooked firmness of WW spaghetti with the bran particles acting as a physical barrier to the cutting action of the pasta blade used to measure cooked firmness.

At 4 min, cooked firmness of all samples greatly decreased because most of the starch gelatinization and protein denaturation have occurred (de la Peña et al 2015). Cooked firmness ranged from 5.0 g cm for direct fine to 6.7 g cm for semolina:coarse bran, difference of 1.7 g cm (Table 10). By 6 min, the difference between high and low cooked firmness (0.8 g cm) stabilized and remained relatively constant as cooking progressed to 16 min (0.7 g cm).

At 8 min, WW spaghetti was cooked as indicated by the disappearance of central white starch core as described in AACC International Approved Method 66-50.01 From 6-16 min, difference in cooked firmness became more pronounced between flour and semolina, while at 2 and 4 min the differences were greater between coarse and fine bran (Table 10).

Table 10. Cooked firmness of whole-wheat spaghettis from 2 to 16 min of cooking.

	Cooked Firmness (g cm)							
	2min	4min	6min	8min	10min	12min	14min	16min
Single-Pass								
DF	6.5c	5.0c	4.9c	4.5c	4.0b	3.7c	3.4b	3.3c
DC	9.0ab	5.4bc	4.9c	4.6bc	4.2ab	3.9bc	3.6b	3.4c
Multi-pass								
SFB	7.3bc	5.9ab	5.6a	5.1a	4.6a	4.6a	4.3a	4.0a
SCB	9.8a	6.7a	5.7a	5.0ab	4.6a	4.1b	4.1a	3.9ab
FFB	6.9c	5.5bc	5.1bc	4.4c	4.0b	3.8c	3.6b	3.4c
FCB	11.0a	6.1ab	5.4ab	4.6c	4.0b	4.0bc	3.7b	3.5bc
Control								
S	9.4	5.1	4.7	4.0	3.9	3.8	3.5	3.3
F	9.5	5.3	4.7	4.0	3.8	3.6	3.5	3.1

DF = Direct fine ground flour; DC = Direct coarse ground flour; SFB = Semolina blended with fine reground bran, germ, and shorts; SCB = Semolina blended with coarse reground bran, germ, and shorts; FFB = Durum flour blended with fine reground bran, germ, and shorts; FCB = Durum flour blended with coarse reground bran, germ, and shorts; S = Semolina; and F = Durum flour.
Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

The high starch damage of durum flour:bran blends (7.5-7.9%) might have caused more starch to leach from the spaghetti and result in reduced cooked firmness. The negative correlations between starch damage and cooked firmness at 8 and 16 min (r=-0.57 and -0.49, $P \leq 0.05$; respectively, Table 11) support the explanation. In general, cooked firmness was greater or equal with spaghetti made from semolina:fine bran or semolina:coarse bran compared to spaghetti made from semolina or durum flour alone or durum flour:bran blends or direct grinding (Table 10).

Table 11. The Pearson Correlation between whole durum flour and whole-wheat spaghetti qualities.

Whole Durum Flour	Cooked Firmness			Cooking Loss			Cooked Weight		
	2min	8min	16min	2min	8min	16min	2min	8min	16min
dgw	0.33	0.59**	0.50**	-0.64**	-0.86**	-0.94**	0.26	0.22	-0.21
sgw	0.41	0.47**	0.38**	-0.56**	-0.83**	-0.89**	0.36	0.20	-0.10
SD	-0.20	-0.57**	-0.49**	0.61**	0.84**	0.93**	-0.26	-0.22	0.19

dgw = Geometric mean diameter of flour particles; sgw = Standard deviation of geometric mean diameter; and SD = Starch damage.

Cooking loss

Cooking loss for all WW spaghettis increased as cooking time progressed from 2 to 16 min (Table 12). The bran in WW spaghetti has been shown to physically disrupt gluten matrix which has been associated with increased cooking loss when compared with spaghetti made from semolina or durum flour (Manthey and Schorno 2002).

Cooking loss was similar for all WW spaghettis at 2 min of cooking (3.6-4.2%, Table 12). By 4 min, cooking loss was high with direct fine grind and durum flour:fine bran (5.9-6.2%). High amount of damaged starch from those samples probably allowed water to easily penetrate causing starch gelatinization. Fragments from ruptured starch granules would leach from the spaghetti and result in increased cooking loss. At 8 min, the water penetrated into the center of the spaghetti strand and the core of spaghetti sample has been cooked. Spaghetti made from semolina:fine bran and semolina:coarse bran blends had similar cooking loss at each cooking time. Similarly, spaghetti made from durum flour:fine bran and durum flour:coarse bran had similar loss (except at 4 min) at each cooking time. When overcooked to 16 min, spaghetti made from direct fine grind had the greatest cooking loss (12.3%), followed by spaghetti made from durum flour:bran blends (11.3-11.5%). Spaghetti made from direct coarse grind and semolina:bran blends had relatively low cooking loss (9.4-9.6%). This further reflects the importance of high amount of damaged starch associated with direct fine grind and durum flour samples contributing to cooking loss. These results are further supported by the significant positive correlation between starch damage and cooking loss at 8 and 16 mins (r=0.84 and r=0.93, $P \leq 0.05$, Table 11). The d_{gw} and s_{gw} had significant negative correlation with cooking loss at 8 min (r=-0.86 and -0.83, $P \leq 0.05$) and 16 min (r=-0.94 and -0.89; respectively, $P \leq 0.05$, Table 11). Thus, small d_{gw} and s_{gw} of flour particles was associated with high starch damage that increased cooking loss.

Table 12. Cooking loss of whole-wheat spaghettis from 2 to 16 min of cooking.

	Cooking Loss (%)							
	2min	4min	6min	8min	10min	12min	14min	16min
Single-Pass								
DF	4.2	5.9ab	7.0a	8.4a	9.6a	10.4a	11.2a	12.3a
DC	3.8	5.2bc	6.1bc	7.0b	7.6b	8.3b	8.9c	9.4c
Multi-Pass								
SFB	3.6	5.1bc	5.6c	7.0b	7.5b	8.3b	8.7c	9.6c
SCB	3.6	4.8c	5.5c	6.9b	7.5b	8.6b	8.6c	9.6c
FFB	4.1	6.2a	7.0a	8.4a	9.4a	10.6a	10.4b	11.5b
FCB	4.0	5.1bc	6.7ab	7.8a	9.0a	9.9a	10.7ab	11.3b
Control								
S	2.8	3.7	4.7	5.4	6.0	6.7	7.3	7.5
F	3.3	4.6	4.8	6.3	6.7	7.8	8.0	8.9

DF = Direct fine ground flour; DC = Direct coarse ground flour; SFB = Semolina blended with fine reground bran, germ, and shorts; SCB = Semolina blended with coarse reground bran, germ, and shorts; FFB = Durum flour blended with fine reground bran, germ, and shorts; FCB = Durum flour blended with coarse reground bran, germ, and shorts; S = Semolina; and F = Durum flour.

Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Cooked weight

Cooked weight increased with cooking time (Table 13). Cooked weight was greatest for spaghetti without bran, and spaghetti made from semolina and durum flour (without bran) had similar cooked weights at each cooking time. The presence of bran (fine or coarse) reduced cooked weight. Cooked weight was similar for all direct grind and reconstituted blends, regardless of durum flour or semolina.

Table 13. Cooked weight of whole-wheat spaghettis from 2 to 16 min of cooking.

	Cooked Weight (% gain)							
	2min	4min	6min	8min	10min	12min	14min	16min
Single-Pass								
DF	178	208ab	226a-c	249	264ab	282ab	292	304
DC	180	211a	233a	250	272a	286a	302	309
Multi-Pass								
SFB	174	200d	217c	242	253c	265c	277	290
SCB	178	207a-c	221bc	256	266ab	279ab	288	302
FFB	175	203cd	224a-c	244	259bc	276b	284	300
FCB	176	204b-d	231ab	245	266ab	277ab	291	298
Control								
S	190	224	250	276	298	321	344	359
F	182	222	243	265	295	314	332	352

DF = Direct fine ground flour; DC = Direct coarse ground flour; SFB = Semolina blended with fine reground bran, germ, and shorts; SCB = Semolina blended with coarse reground bran, germ, and shorts; FFB = Durum flour blended with fine reground bran, germ, and shorts; FCB = Durum flour blended with coarse reground bran, germ, and shorts; S = Semolina; and F = Durum flour.
Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Conclusions

Particle size, starch damage, and pasting properties were similar for direct fine grind WWF and multi-pass flour:fine bran and for direct coarse grind WW flour and multi-pass semolina:coarse bran flour. The semolina:fine bran blend had low starch damage and had the most desirable pasting properties (highest peak viscosity and final viscosity) for pasta cooking.

WW spaghetti was better when made with WW flour produced using a multi-pass than a single-pass milling system. The surface of WW spaghetti was smoother when made with fine than with coarse bran. Although the brightness was less, spaghetti made from semolina:fine or durum flour:fine bran had better mechanical strength than WW spaghetti made from the other WW flours. Reconstituted semolina:fine or coarse bran blends made spaghetti with higher cooked firmness than spaghetti made from durum flour:bran blends or direct fine or coarse grinding flour. Spaghetti made from direct coarse grind or from reconstituted semolina:fine or coarse bran blends had the lowest cooking loss. Thus, the best physical quality of WW spaghetti was made from semolina:fine bran blend. WW spaghetti with the best cooking quality was made from a reconstituted blend either semolina:fine bran or semolina:coarse bran.

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CHAPTER 4: INFLUENCE OF DURUM GENOTYPE ON WHOLE-WHEAT AND TRADITIONAL SPAGHETTI QUALITIES

Abstract

This research was conducted to determine if genotypes selected for their superior traditional semolina pasta quality would also make the best whole-wheat (WW) pasta. Results from 19 durum wheat cultivars and 17 breeding lines grown at 19 different environments in North Dakota showed that physical and cooking qualities varied differently for WW and traditional spaghettis, respectively. Ward's clustering segregated the 36 genotypes into five groups based on WW spaghetti quality. Group 1 and 2 (21 genotypes) produced good to high quality WW pasta which displayed high mean values for cooked firmness (4.3 and 4.1 g cm), mechanical strength (31.3 and 31.0 g) and color (brightness: 34.92 and 34.54), respectively. Group 4 and 5 produced poor quality WW pasta which had low cooked firmness (both 3.5 g cm) and high cooking loss (10.1 and 10.4%). Grain protein content (≥13.9%) was found with high quality of WW spaghetti. Of the 36 genotypes evaluated, 21 and 3 genotypes produced good and poor qualities of WW and traditional spaghettis, respectively, while other 12 genotypes produced good traditional spaghetti but produced poor quality WW spaghetti. These data indicate the need to select genotypes specifically for their WW pasta quality.

Introduction

Whole-wheat (WW) pasta is made from reconstituted blend of semolina, bran, and germ fractions or made from whole-durum flour that is milled through direct grinding of the whole seed (Miller Jones et al 2015). WW pasta provides all the nutrients found in the endosperm, bran, and germ. However, the bran is associated with many defects in WW pasta, such as dark appearance, reduced mechanical strength, and reduced cooking quality, which has impeded the growth in consumption of WW pasta. Wheat bran particles have been shown to interfere with dough development and cause a decrease in cooked firmness and an increase in cooking loss of WW pasta (Manthey and Schorno 2002). Mechanical strength is an indicator of a pasta’s ability to withstand stresses associated with handling, packaging, and shipping. Chen et al (2011) and Chillo et al (2008) reported that mechanical strength was reduced by bran, especially coarse bran particles. Many approaches have been employed to reduce the adverse effects of bran and improve the WW product quality, i.e. bran size reduction (Steglich et al 2015), bran fermentation, enzymatic and heat treatments (Salmenkallio-Marttila et al 2001; Hartikainen et al 2014), and processing modification (high temperature drying) (Manthey and Schorno 2002; Bock et al 2015).

In addition to developing and utilizing bran treatments, pasta processors are interested in identifying durum cultivars that possess traits that result in improved WW pasta quality. Published information concerning the variability in WW pasta quality among large number of durum cultivars and experimental breeding lines is limited. Previous research evaluated only a few genotypes for WW pasta and found that genotype had a significant effect on the end-use quality (Manthey and Schorno 2002).

End-use quality would be similar for traditional and WW pasta, with the exception being pasta color. WW pasta will lack the yellow translucent appearance characteristic of traditional pasta. Otherwise, dough properties and pasta mechanical strength and cooking characteristics would have similar desirable traits. It is not known if quality factors for traditional pasta would be similar to those for WW pasta.

Developing a breeding program for WW pasta could stress available resources. Durum breeding programs routinely select genotypes that possess traits that result in improved quality of pasta made from semolina. However, it is not known if genotypes selected for their superior traditional, semolina pasta quality would also make the best WW pasta.

Cluster analysis is widely used in wheat breeding program to analyze multiple traits. The purpose of hierarchical clustering is finding the similarity of genotypes with regard to the end use quality traits, which is maximum within the clusters, and difference of genotypes, which is maximum between clusters (Johnson and Wichern 2002). The clustering method is performed by merging two genotypes together to form a new cluster, and then further merging based on the distance until only one cluster remains from previous clusters.

The objectives of this study were: 1) to determine variability of durum genotypes on WW and traditional pasta qualities; 2) to identify genotypes in terms of their pasta qualities using the cluster analysis; and 3) to determine if all genotypes that make excellent traditional, semolina pasta would also make excellent WW pasta.

Materials and Methods

Wheat Samples

Thirty-six genotypes of durum wheat were grown at 19 environments in North Dakota for evaluation of their traditional and WW pasta qualities. The 36 genotypes included 19 released cultivars and 17 experimental breeding lines (Table 14).

Table 14. The origin and PI number for 36 durum genotypes.

Genotype	Origin	PI No.	Reference
Alkabo	NDSU	642020	Elias and Manthey 2007a
Alzada	Westbred, USA	n.a	n.a
Ben	NDSU	596557	Elias and Miller 1998
Carpio	NDSU	670039	Elias et al 2015
Commander	Canada	641222	Clarke et al 2006a
Divide	NDSU	642021	Elias and Manthey 2007b
Grenora	NDSU	642022	Elias and Manthey 2007c
Joppa	NDSU	673106	Elias and Manthey 2016
Lebsock	NDSU	613620	Elias and Manthey 2001
Maier	NDSU	607531	Elias and Miller 2000a
Mountrail	NDSU	607540	Elias and Miller 2000b
AC Navigator	Canada	610666	Clarke et al 2001
Normanno	Italy	n.a	n.a
Pierce	NDSU	632366	Elias et al 2004
Rugby	NDSU	CI 17284	Quick et al 1975
Strongfield	Canada	641223	Clarke et al 2006b
Tioga	NDSU	660664	Elias and Manthey 2013
VTPeak	NDSU	n.a	n.a
Verona	NDSU	n.a	n.a
D041708	NDSU	Breeding line	-
D04586	NDSU	Breeding line	-
D06587	NDSU	Breeding line	-
D06707	NDSU	Breeding line	-
D06855	NDSU	Breeding line	-
D06886	NDSU	Breeding line	-
D06932	NDSU	Breeding line	-
D071016	NDSU	Breeding line	-
D071022	NDSU	Breeding line	-
D071579	NDSU	Breeding line	-
D07726	NDSU	Breeding line	-
D07892	NDSU	Breeding line	-
D08900	NDSU	Breeding line	-
D09555	NDSU	Breeding line	-
D09557	NDSU	Breeding line	-
D09690	NDSU	Breeding line	-
D09970	NDSU	Breeding line	-

n.a. = Not available.

The 19 environments in North Dakota were Carrington, Dickinson, Langdon, Minot, Williston in 2012, 2013, and 2014; and Casselton, Hettinger in 2013 and 2014. The genotypes were grown in long strip-plots (75 ×1.2 m) at each environment. Harvested durum samples were cleaned and stored at 12 °C until needed.

The NDSU Breeding Program has historically evaluated genotype performance on traditional spaghetti. The quality traits evaluated for semolina included semolina protein content, wet gluten, gluten index and mixogram score and for traditional spaghetti included color, cooked firmness, cooking loss and cooked weight. Additional quality evaluations included WW (grain) protein content, WW dough mixing properties and WW spaghetti mechanical strength and cooking quality.

Grain Quality

Samples of individual durum genotypes were measured for grain protein content and falling number according to AACC International Approved Methods (39-25.01) and (56-81.03), respectively.

Grain Milling

WW flour was obtained by direct grinding individual grain samples from the 36 genotypes grown in 19 environments using an ultra-centrifugal mill (ZM 200, Retsch, Germany) configured with a 12,000 rpm rotor speed, 250 μm mill screen aperture size, and 62 g/min feed rate. The grain was conditioned to 12.5% moisture 72 h before milling.

Semolina was produced from individual grain samples that were conditioned from 12.5 to 14.5% moisture 24 h before milling and further conditioned from 14.5 to 17.5% moisture, 45 min before milling. The conditioned wheat grain was milled into semolina using a Bühler MLU-202 experimental mill fitted with two Miag laboratory-scale purifiers (Bühler-Miag, Minneapolis, MN, USA).

Whole-Wheat Flour and Semolina Qualities

Dough strength, as determined by mixograph, was evaluated differently for semolina and WW flour. Breeding programs assess many phenotypic traits. Often to simplify selection for a given trait, such as dough strength, a single measurement is determined which best reflects the trait being considered.

For WW flour, dough strength was evaluated using a modified mixograph procedure AACC International Approved Method (54-40.02). Mixograph was run by adding 7.8 mL distilled water to 10 g (14% mb) WW flour for 10 min. Mixogram break-time was measured as the length of time before the dough began to rapidly breakdown (Fig. 11). A prolonged break-time indicates a strong dough.

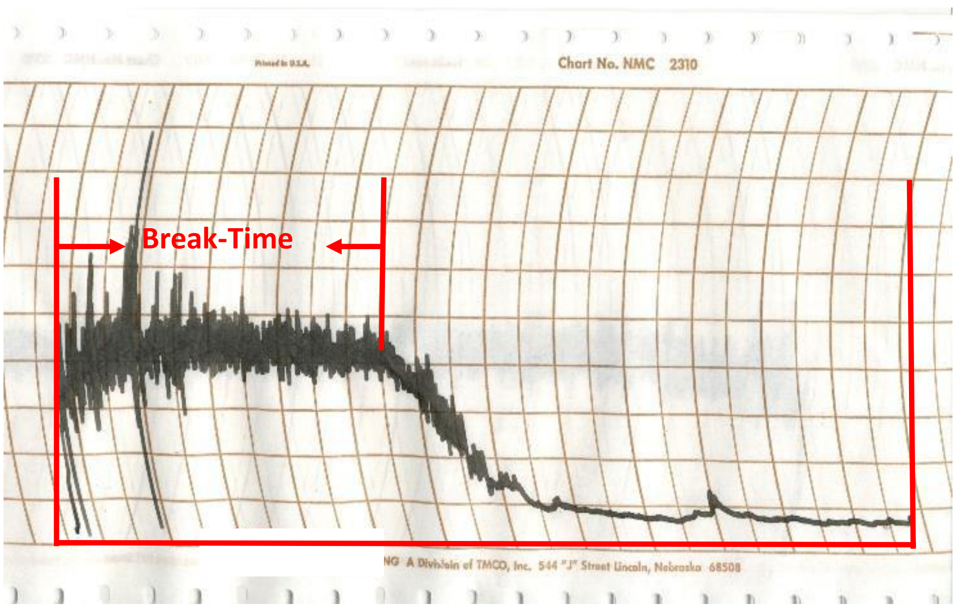

Figure 11. Break-time in whole-wheat flour dough mixogram.

For semolina, dough strength was also evaluated using a modified mixograph procedure. Mixograph was run by adding 5.8 mL distilled water to 10 g (14% mb) semolina and allowed to run for 8 min. The resulting mixogram was assigned a score from 1-8, where 1 corresponded to a weak dough and 8 corresponded to a very strong dough. The wet gluten and gluten index were determined for semolina using AACC International Approved Method (38-12.02).

Pasta Processing

Spaghetti was made from individual samples (36 genotypes grown at 19 environments). The WW flour and semolina samples (1,200 g each) were hydrated (33% moisture, wb, for WW flour and 32% for semolina), mixed, and extruded into WW spaghetti and traditional spaghetti using a semi-commercial pasta extruder (DEMACO, Melbourne, FL, USA). Extrusion conditions were: extrusion temperature, 45°C; mixing chamber vacuum, 46 cm of Hg; an auger (length to diameter ratios of 8.1:1); and extrusion speed, 25 rpm. The WW spaghetti was dried in a laboratory dryer (Standard Industries, Fargo, ND, USA) using a high temperature drying cycle (length 10 h; peak temperature 73°C). Commercially, WW spaghetti is dried typically using a high temperature drying cycle. The traditional spaghetti was dried at low temperature (length 18 h; peak temperature 40°C), which is the drying cycle used to evaluate traditional pasta quality characteristics of durum lines in the durum breeding program at NDSU.

Pasta Quality

The WW and traditional spaghetti samples were evaluated for their cooking qualities (cooked firmness and cooking loss) using AACC International Approved Method (66-50.01). The Standard Operating Procedure of the Durum Breeding Program at NDSU for evaluating cooking quality of traditional spaghetti requires that the dried spaghetti be cooked to 12 min. Cooked weight was determined as the increase in weight after cooking 12 min and was expressed as the percentage increase in pasta weight before cooking.

Spaghetti was placed on a black plastic template that ensured a uniform background color and depth (1 cm). Spaghetti color was evaluated using a colorimeter (CR410, Minolta, NJ, USA) to measure the Hunter L, *a* and *b* color scale as described in AACC International Approved Method (14-22.01). Hunter L-value is black to white based on a scale from 0 to 100; *a*-value is redness when positive and greenness when negative; and *b*-value is yellowness when positive and blueness when negative. Mechanical strength of the dry WW spaghetti was determined by the flexure test using a TA-XT2 texture analyzer with a Spaghetti Flexure Rig (Texture Technologies Corp., Scarsdale, NY, USA). The amount of force (g) required to break one spaghetti strand (20 cm) was recorded on six strands per treatment. Test speed was 2.5 mm/sec.

Experimental Design and Data Analysis

The experimental design was an unbalanced randomized complete block design. Genotypes were grown in a randomized complete block and each growing environment was treated as block. Genotype was considered a fixed effect and was unbalanced at each environment (block). All the data were subjected to analysis of variance in SAS 9.3 software (SAS Institute, Cary, NC, USA) using PROC GLM procedures. Least Significant Difference (LSD) was conducted for significantly difference at $P \leq 0.05$ by using multiple comparisons (lsmeans genotype/lines) in PROC GLM.

The PROC ACECLUS was used to transfer the raw pasta quality data (dry spaghetti color and mechanical strength, cooked firmness, cooking loss and cooked weight) to canonical variables. A hierarchical cluster analysis was conducted to group genotypes according to those transformed and standardized canonical variables. Clustering was performed following Ward's method, where the distance was the sum of square between two clusters, and the R^2 value was the proportion of variance accounted for by the clusters.

Boxplot analysis of data was conducted using Microsoft Excel 2010. Boxplots provide an overview of the distribution of data by grouping data into quartiles.

Results and Discussion

Descriptive Statistics

Whole-wheat flour and semolina

The mean, SD, range, and genotype significance for WW flour and semolina quality parameters are summarized in Table 15. Falling number of WW flour did not vary significantly with genotype. Falling number test is an indirect measure of α-amylase activity in the grain and of grain soundness (Finney 2001). Falling number ranged from 367-498 sec among the 36 genotypes, which indicates that there was little α-amylase activity in the grain and that the grain was sound.

Among the 36 genotypes, grain protein content varied from 12.4% for Joppa to 15.3% for Ben. The mean grain protein content was 13.8%. Fifty percent of the genotypes surrounding the median value (13.7%) fell within a relatively narrow range of 13.3 to 14.1% protein (Fig. 12A). The first and fourth quartiles individually had relatively wide ranges of 0.9 and 1.2 percentage units, respectively when compared to the second and third quartiles, both being 0.4 percentage units. Semolina protein content and wet gluten content ranged from 11.3 to 13.7% and 30 to 41%, respectively (Table 15). The mean semolina protein content was 12.4%, and for semolina wet gluten content was 34%. Both semolina protein content and wet gluten content had similar distribution patterns, where the second quartile had the narrowest range (Fig. 12D and E).

Table 15. The descriptive statistics of quality parameters (n=506).

Parameter	Mean	SD	Range	Genotype
Whole-Wheat Flour				
GPC (%, 12% mb)	13.8	0.6	12.4-15.3	**
FN (sec)	422	37	367-498	ns
BT (sec)	446	74	231-561	**
Semolina				
SPC (%, 14% mb)	12.4	0.5	11.3-13.7	**
WG (%, 14% mb)	34	2	30-41	**
GI	67	17	12-95	**
MS	6.5	1.1	3.0-8.0	**
Whole-Wheat Spaghetti				
HL	34.69	1.0	30.30-36.19	**
Ha	9.10	0.2	8.81-9.46	ns
Hb	12.72	0.6	10.08-13.69	**
Mechst (g)	30.7	1.1	27.7-33.0	**
Firm (g cm)	3.9	0.3	3.2-4.5	**
Ckloss (%)	9.8	0.4	8.9-10.7	**
CWT (% gain)	286	2	281-290	ns
Traditional Spaghetti				
HL	54.73	1.2	49.28-56.02	**
Ha	1.89	0.6	1.31-4.47	**
Hb	26.48	0.9	23.12-28.12	**
Firm (g cm)	4.4	0.3	3.7-5.1	**
Ckloss (%)	6.9	0.2	6.3-7.4	ns
CWT (% gain)	312	3	305-320	**

SD = Standard deviation; GPC = Grain protein content; FN = Falling number; BT = Break-time; SPC = Semolina protein content; WG = Wet gluten; GI = Gluten index; MS = Mixograph score; HL= Hunter-L; Ha = Hunter-*a*; Hb = Hunter-*b*; Mechst = Mechanical strength; Firm = Firmness; Ckloss = Cooking loss; and CWT = Cooked weight.
** = Genotype had significant effect on quality parameter $P \leq 0.05$.
ns = Genotype had non-significant effect on quality parameter at $P \leq 0.05$.

Dough strength of WW flour was measured by mixogram break-time. The mean break-time among the 36 genotypes was 446 sec. Normanno had the greatest break-time (561 sec); whereas Rugby, known as weak gluten/dough cultivar (AbuHammad et al 2012), had the shortest break-time (231 sec). The distribution of break-time values was skewed to the right, with 75% of the values being 397 sec or higher (Fig. 12B). The semolina dough strength was evaluated based on an overall mixogram score, which had a mean value of 6.5 on a scale of 1.0 to 8.0. Mixogram scores for the 36 genotypes ranged from 3.0 to 8.0 and were skewed to the right indicating that 75% of the genotypes had strong dough properties with mixogram scores greater than 6.0 (Fig. 12G).

The mean value for semolina gluten index was 67. Gluten index values for 36 genotypes ranged from 12 to 95 and were skewed to the right that indicating that 75% of the genotypes had strong gluten properties with gluten index values greater than 62 (Fig. 12F). Ames et al (1999) investigated 10 durum genotypes and lines grown in Canada and found a similar range with gluten index (9-77). Similar results were also reported by Marchylo et al (2001) and AbuHammad et al (2012). Break-time, mixogram score, and gluten index all had distributions that were skewed to the right. Strong gluten is known to result in strong doughs (AbuHammad et al 2012).

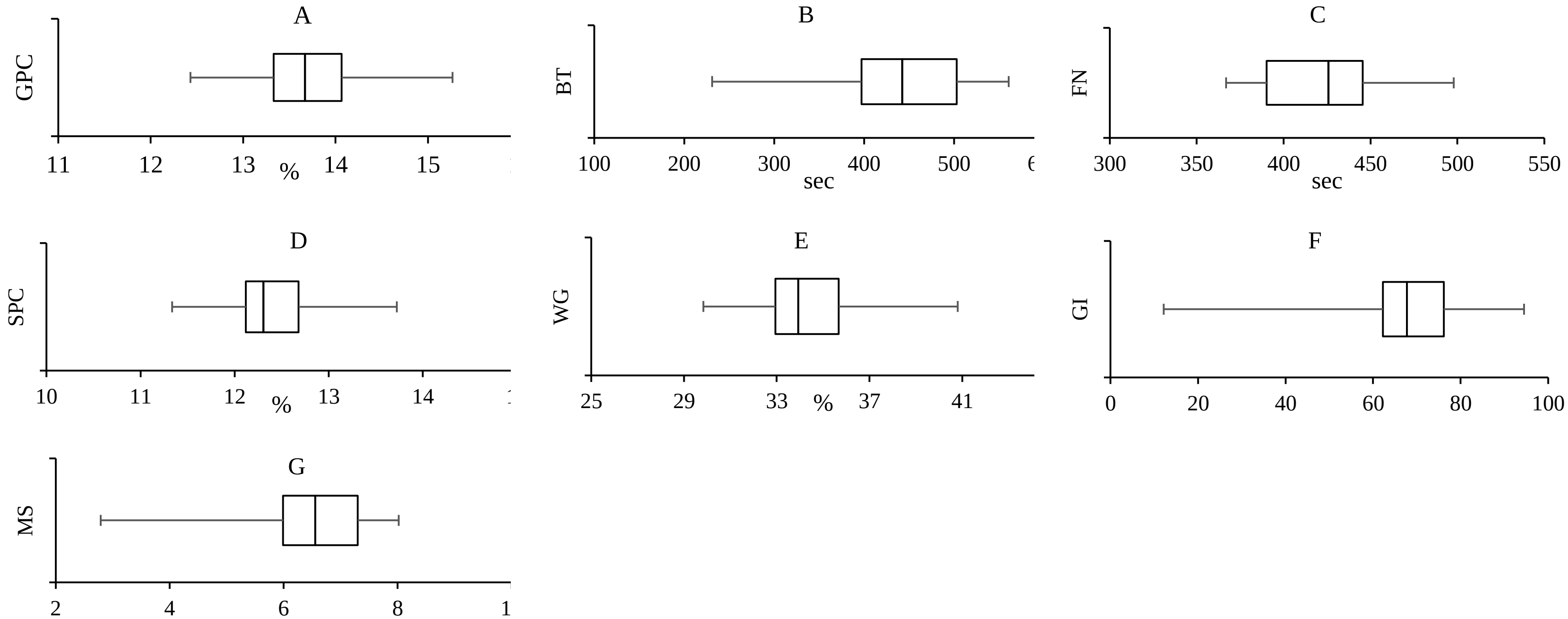

Figure 12. Boxplots of whole-wheat flour and semolina quality parameters. Two extremes indicate minimum and maximum values, and left, middle and right lines of the box indicate 1st quartile, median, and 3rd quartile values, respectively. (A) GPC = Grain protein content, (B) BT = Break-time, and (C) FN = Falling number of whole-wheat flour; and (D) SPC = Semolina protein content, (E) WG = Wet gluten, (F) GI = Gluten index, and (G) MS = Mixogram score of semolina.

Whole-wheat and traditional spaghetti

The mean, SD, range, and genotype significance of WW and traditional spaghetti quality parameters are summarized in Table 15. Appearance of WW and traditional spaghetti was evaluated by assessing the Hunter L, *a* and *b*-values. Genotype affected WW spaghetti L-values (brightness) and *b*-values (yellowness) but not their *a*-values (redness) (Table 15). The variation in brightness could be dependent on the polyphenol oxidase (Lamkin et al 1981; McCallum and Walker 1990), and peroxidase (Kobrehel et al 1972; Taha and Sagi 1987) activities in bran, which catalyze the oxidation of endogenous phenolic acids to quinones and quinones react with amines and thiol groups to produce dark pasta product. The grain carotenoid concentration determines the yellowness (Beleggia et al 2011). L-value and *b*-value for WW spaghetti had mean value of 34.69 and 12.72. L-values for WW spaghetti made from 36 genotypes ranged from 30.30 to 36.19 and were skewed to the right that indicating that 75% of the genotypes produced WW spaghetti with L-values of 34.45 or higher (Fig. 13A) and similarly, *b*-values ranged from 10.08 to 13.69 and were skewed to the right that indicating that 75% of the genotypes produced WW spaghetti with *b*-value of 12.54 or higher (Fig. 13C).

Genotype affected traditional spaghetti L, *a*, and *b*-values (Table 15). The mean L-value (brightness) and *b*-value (yellowness) of traditional spaghetti (54.73 and 26.48, respectively) were greater than means for WW spaghetti (34.69 and 12.72, respectively). Conversely, mean *a*-value (redness) of traditional spaghetti (1.89) was lower than that for WW spaghetti (9.10), indicating the negative effect of bran on spaghetti color. Similar to that found for WW spaghetti, the distribution of L-values and *b*-values were skewed to the right with 75% of the genotypes having L-value of 54.63 or higher (Fig. 13D) and *b*-value of 26.06 or higher (Fig. 13F). The distribution of *a*-values for traditional spaghetti was skewed to the left, with 75% of the values were 1.92 or less (Fig. 13E). In contrast, *a*-values did not significantly differ with genotype for WW spaghetti (Table 15).

Genotype affected mechanical strength of WW spaghetti and ranged from 27.7-33.0 g (Table 15). Rugby had the lowest value of mechanical strength (27.7 g), followed by Mountrail (28.7 g). D071022 had the greatest mechanical strength (33.0 g) and most genotypes had mechanical strength between 30.0-33.0 g (data not shown). Boxplot indicated that mechanical strength values were skewed to the right with 75% of the values 30.0 g or higher (Fig. 13G).

Cooking quality was assessed by determining cooked firmness, cooking loss, and cooked weight of WW and traditional spaghetti. For WW spaghetti, genotype affected cooked firmness and cooking loss but cooked weight, with its narrow range of 281-290% gain, did not vary with genotype (Table 15). Cooked firmness of WW spaghetti made from the 36 genotypes ranged from 3.2-4.5 g cm and had mean value of 3.9 g cm.

Cooking loss from WW spaghetti varied from 8.9-10.7% among the 36 genotypes (Table II). Boxplot indicated that the narrowest range occurred with the third quartile, indicating greater variability occurred with the first, second, and fourth quartiles (Fig. 13I).

For traditional spaghetti, genotype affected cooked firmness and cooked weight but not cooking loss (Table 15). The cooked firmness of traditional spaghetti ranged from 3.7-5.1 g cm among the 36 genotypes and had a mean value of 4.4 g cm which was higher than that for WW spaghetti (3.9 g cm). WW and traditional spaghetti had similar magnitude of range in cooked firmness (1.3 and 1.4 g cm) (Fig. 13H and K). However, the range within the second and third quartiles was smaller for traditional (Fig 13K) than for WW spaghetti (Fig. 13H).

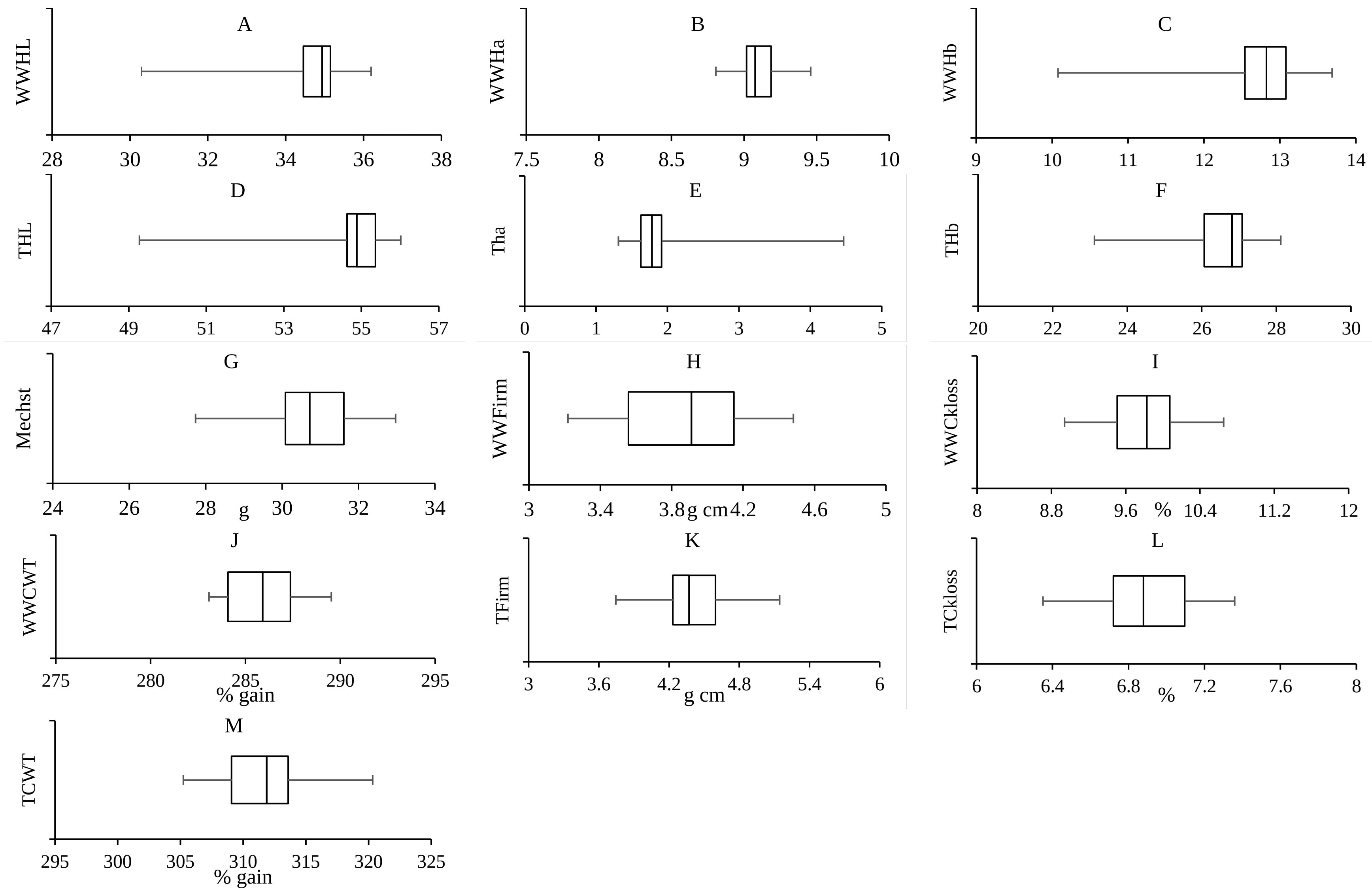

Figure 13. Boxplots of whole-wheat and traditional spaghetti quality parameters. Two extremes indicate minimum and maximum values and left, middle and right lines of the box indicate 1st quartile, median, and 3rd quartile values, respectively.

(A) WWHL= Hunter-L, (B) WWHa=Hunter-*a*, (C) WWHb=Hunter-*b* of whole-wheat spaghetti; (D) THL=Hunter-L, (E) THa= Hunter-*a*, (F) THb= Hunter-*b* of traditional spaghetti; (G) Mechst= Mechanical strength of whole-wheat spaghetti, (H) WWFirm=Firmness, (I) WWCkloss= Cooking loss, and (J) WWCWT=Cooked weight of whole-wheat spaghetti; and (K) TFirm= Firmness, (L) TCkloss= Cooking loss, and (M) TCWT= Cooked weight of traditional spaghetti.

Unlike WW spaghetti, genotypes did not significantly differ in their cooking loss of traditional spaghetti which ranged from 6.3-7.4% (Table 15), and the magnitude of the range (1.1 percentage units) being smaller than that occurred within the WW spaghetti (1.8 percentage units). Conversely, genotypes did differ in their cooked weight of traditional spaghetti which ranged from 305 to 320% gain (Table 15), and the magnitude of the range (15 percentage units) was greater than that for WW spaghetti (9 percentage units). The traditional spaghetti cooked weight ranged from 305-320% gain, which was greater than that found for WW spaghetti (281-290% gain).

Clustering of Genotypes Based on Overall Pasta Quality

Whole-wheat spaghetti

Based on WW spaghetti quality (cooked firmness, cooking loss, cooked weight, mechanical strength and Hunter L-value, *a*-value and *b*-value), the 36 genotypes were classified into five clusters as seen in the dendrogram (Fig. 14). The proportion of variance accounted for by the clusters was 75%. The WW spaghetti physical and cooking qualities of each clustered group are as follows.

Group 1 included three breeding lines (D06587, D06932 and D071579). This group had the greatest mean values for grain protein content (14.8%), cooked firmness (4.3 g cm), mechanical strength (31.3 g), Hunter L and *b*-values (34.92 and 13.16) and less cooking loss (9.1%) than the genotypes in the other three groups (Table 16). Group 1 had average values for dough strength, based on mixogram break-time (431 sec).

Group 2 included 13 of the 19 released cultivars and five breeding lines which altogether accounts for 50% of the total genotypes evaluated. This group showed high mean values for mixogram break-time (470 sec), cooked firmness (4.1 g cm), mechanical strength (31.0 g), and Hunter L and *b*-values (34.54 and 12.61), and showed mean values for grain protein content (13.9%) and cooking loss (9.8%), similarly as their average values of the 36 genotypes.

Group 3 included three released cultivars and three breeding lines (17% of the total genotypes), and they showed mean values for cooked firmness (3.6 g cm), cooking loss (9.7%), mechanical strength (29.3 g), and mixogram break-time (439 sec), which was similar to the average values of the 36 genotypes, but showed mean values above average values for Hunter L and *b*-values (35.18 and 12.87), and a mean value below average for grain protein content (13.4%).

Group 4 included two released cultivars and six breeding lines (22% of the total genotypes), and they showed means for cooking loss (10.1%), Hunter L and *b*-values (35.11 and 13.01), and mechanical strength (30.9 g) that were above their average values of the 36 genotypes, and showed a mean value for mixogram break-time (439 sec) similar to average, and mean values for cooked firmness (3.5 g cm), and grain protein content (13.4%) below averages.

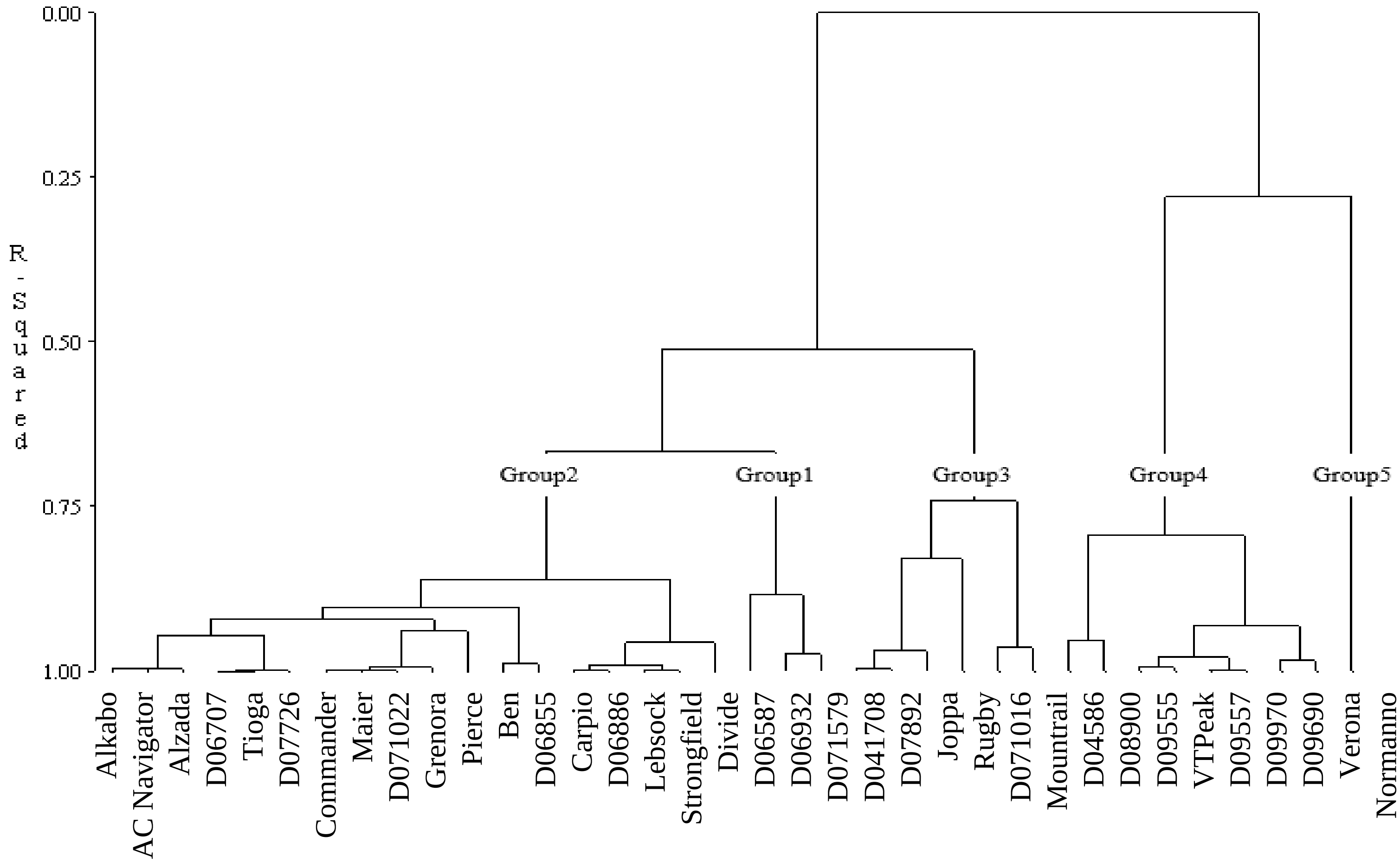

Figure 14. Dendrogram of 36 durum genotypes on whole-wheat spaghetti quality.

Table 16. The clustered groups among 36 genotypes and their grain and whole-wheat spaghetti quality.

			Grain Protein (%, 12%mb)	Falling Number (sec)	Break -Time (sec)	Hunter-L	Hunter-*a*	Hunter-*b*	Firm-ness (g cm)	Cook-ing loss (%)	Cooked Weight (% gain)	Mech-anical strength (g)
Group 1	D06587, D06932, D071579	Range Mean	14.6-15.2 14.8	450-498 480	375-504 431	34.18-35.46 34.92	8.95-9.07 9.02	13.11-13.19 13.16	4.2-4.5 4.3	8.9-9.3 9.1	283-288 285	30.7-31.7 31.3
Group 2	AC Navigator, Alkabo, Alzada, Ben, Carpio, Commander, Divide, Grenora, Lebsock, Maier, Pierce, Strongfield, Tioga, D06707, D06855, D06886, D071022, D07726	Range Mean	13.4-15.3 13.9	367-492 423	383-557 470	33.40-35.30 34.54	9.02-9.46 9.16	11.97-13.07 12.61	3.8-4.3 4.1	9.3-10.6 9.8	281-288 285	29.5-33.0 31.0
Group 3	Joppa, Mountrail, Rugby, D041708, D07892, D071016	Range Mean	12.4-14.5 13.4	373-484 419	231-520 439	34.72-36.11 35.18	8.95-9.37 9.16	12.52-13.50 12.87	3.2-3.8 3.6	9.1-10.3 9.7	287-290 288	27.7-30.8 29.3
Group 4	D04586, D08900, D09555, D09557, D09690, D09970, Verona, VTPeak	Range Mean	12.9-14.0 13.4	387-431 419	380-503 439	33.47-36.19 35.11	8.81-9.09 8.95	11.95-13.69 13.01	3.4-3.6 3.5	9.7-10.7 10.1	281-287 285	29.7-31.7 30.9
Group 5	Normanno		13.1	381	561	30.30	8.89	10.08	3.5	10.4	281	28.8
Aver-age			13.8	422	446	33.94	9.03	12.28	3.7	9.8	284	30.0

Group 5 included only one genotype (Normanno) which displayed means for cooked firmness (3.5 g cm), cooked weight (281% gain) and mechanical strength (28.8 g) that were below averages of the 36 genotypes, and displayed a mean for cooking loss (10.4%) above average. Normanno had low grain protein content (13.1%) and Hunter L and *b*-values (30.30 and 10.08), and high break-time (561 sec). Normanno originated from Italy and displayed significant dark color and poor cooking quality which may be attributed to not being well adapted to grow in North Dakota.

When grain protein content was ≥13.9%, the WW spaghetti had high cooked firmness (≥4.1 g cm, Table 16). Matsuo et al (1972), Ross et al (1997), and Hou et al (2013) previously reported similar positive effects of increasing grain protein content on firmness or cutting stress of cooked traditional pasta and noodles. According to the data of Group 1 and 2 (Table 16), genotypes with high grain protein quantity (≥13.9%) also had high mechanical strength (≥31.0 g), suggesting that the high grain protein content increased the resistance to breakage. High protein content did not guarantee low cooking loss as some of the high protein genotypes in Group 2 had above average cooking losses (Table 16). For example, six genotypes (Alzada, Commander, Divide, Strongfield, Tioga and D06707) displayed cooking loss of ≥10.0%.

Traditional spaghetti

Dendrogram contained four clusters that grouped the 36 genotypes based on traditional spaghetti quality (cooked firmness, cooking loss, cooked weight, and Hunter L-value, *a*-value and *b*-value) (Fig. 15). The proportion of variance accounted for by the clusters was 75%. The summarized physical and cooking quality of traditional spaghetti of each clustered group are as followed.

Group 1 included one released cultivar and four breeding lines (Table 17 and Fig. 15). Traditional spaghetti made from these genotypes had above average cooked firmness (4.9 g cm) and Hunter L and *b*-values (55.28 and 27.25), and below average cooking loss (6.7%) and cooked weight (308% gain).

Group 2 included 15 released cultivars and 13 breeding lines, accounting for 78% of the total genotypes. This group had mean values for cooked firmness (4.4 g cm), cooking loss (6.9%), and cooked weight (312% gain) that were similar to the corresponding average values of the 36 genotypes; but had above average values for Hunter L-value and *b*-value (54.82 and 26.54) (Table 17).

Group 3 included two released cultivars and they had low means for cooked firmness and cooking loss (3.8 g cm and 6.7%) below averages of the 36 genotypes, but had mean values similar to the averages for Hunter L-value and *b*-value (54.78 and 25.31) (Table 17).

However, Normanno in Group 4 displayed below average cooked firmness (4.3 g cm), and Hunter L and *b*-values (49.28 and 23.12) and above average cooking loss (7.3%) (Table 17).

Thirty-three (92%) of the total 36 genotypes were categorized in Group1 and Group 2 and produced traditional spaghetti that had high physical and cooking attributes, as reflected by values for Hunter L-value ≥54.82 and cooked firmness ≥4.4 g cm (Table 17). These results reflect the durum breeding efforts that resulted in cultivars with desirable end use quality. As expected, the cooking quality of traditional spaghetti increased when the semolina protein and gluten strength increased (Table 17). Strong gluten and high protein semolina have been reported to produce traditional spaghetti with firm texture (AbuHammad et al 2012).

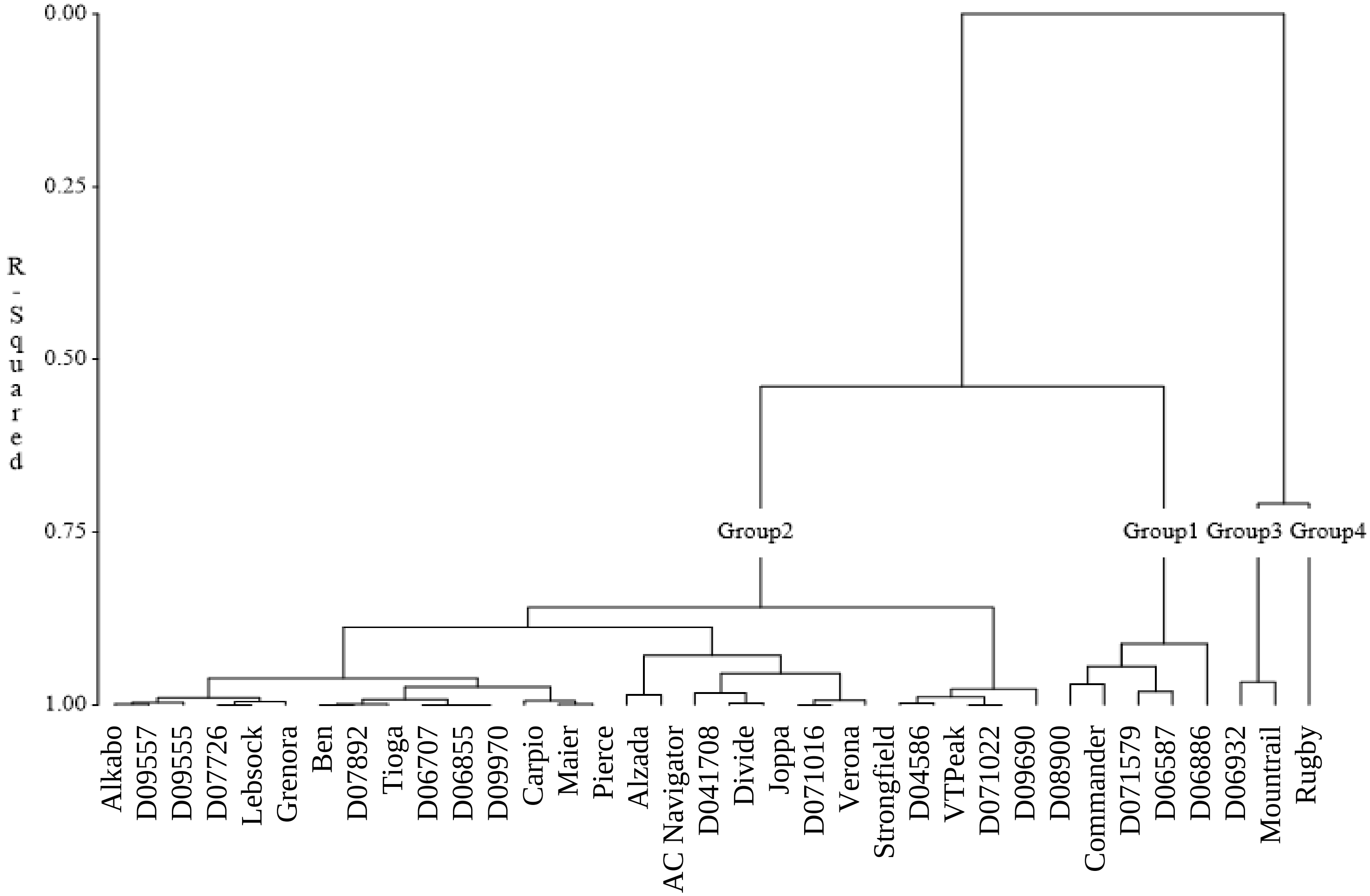

Figure 15. Dendrogram of 36 durum genotypes on traditional spaghetti quality.

Table 17. The clustered groups among 36 genotypes and their semolina and traditional spaghetti quality.

			Semolina Protein (%, 14%mb)	Wet Gluten (%)	Gluten Index	Mixogram Score	Hunter -L	Hunter *-a*	Hunter *-b*	Firm-ness (g cm)	Cooking loss (%)	Cooked Weight (% gain)
Group 1	Commander, D06587, D06932, D06886, D071579	Range Mean	12.2-13.7 13.1	32-39 37	66-89 75	6-8 7	54.10-55.84 55.28	1.52-2.63 1.89	26.87-28.12 27.25	4.6-5.1 4.9	6.3-7.2 6.7	305-310 308
Group 2	AC Navigator, Alkabo, Alzada, Ben, Carpio, Divide, Grenora, Joppa, Lebsock, Maier, Pierce, Strongfield, Tioga, Verona, VTPeak, D041708, D04586, D06707, D06855, D071016, D071022, D07726, D07892, D08900, D09555, D09557, D09690, D09970	Range Mean	11.3-13.6 12.3	30-41 34	46-94 68	5-8 7	53.15-54.87 54.82	1.31-2.82 1.82	25.42-27.28 26.54	3.9-4.8 4.4	6.5-7.4 6.9	308-318 312
Group 3	Mountrail, Rugby	Range Mean	12.3-12.9 12.6	34-35 35	12-20 16	3-5 4	54.65-54.92 54.78	1.31-1.67 1.49	24.82-25.79 25.31	3.7-3.8 3.8	6.6-6.7 6.7	316-320 318
Group 4	Normanno		12.1	31	86	8	49.28	4.47	23.12	4.3	7.3	312
Aver-age			12.4	34	67	6	54.73	1.89	26.48	4.4	6.9	312

Twenty-one genotypes listed in Group 1 and Group 2 of Table 16 displayed both good qualities for WW spaghetti and traditional spaghetti. However, some genotypes (Joppa, D041708, D07892 and D071016 in Group 3 and Verona, VTPeak, D04586, D08900, D09555, D09557, D09690, D09970 in Group 4, Table III) performed well when used to make traditional spaghetti (Table 17) but performed poorly when used to make WW spaghetti (Table 16). Best genotypes for WW spaghetti are listed in Group 1 of Table 16. The best genotypes listed in Group 1 of Table 16 had greater cooked firmness (4.3 vs 3.6 and 3.5 g cm), lower cooking loss (9.1 vs 9.7 and 10.1%), and greater mechanical strength (31.3 vs 29.3 and 30.9 g) when compared to the genotypes that produced poor quality WW spaghetti listed in Group 3 and Group 4, respectively. Differences in cooking quality is attributed in part to the higher grain protein content (14.8 vs 13.4%) associated with the best genotypes compared to the genotypes that produce poor quality WW spaghetti.

Conclusions

Results indicated that selecting for traditional spaghetti quality does not always concurrently select for WW spaghetti quality. Among the overall quality parameters, the cluster analysis segregated more than half of genotypes for their good WW or traditional spaghetti quality. Twenty-one of the 36 genotypes produced good quality WW and traditional spaghettis. In general, genotypes with the grain protein content ≥13.9% produced WW spaghetti with high cooking quality. Low grain/semolina protein content and dough strength produced low quality WW and traditional spaghettis. The inconsistent performances of 12 genotypes on WW and traditional spaghetti, suggests that genotypes that make good traditional spaghetti do not necessarily make good WW spaghetti. Khalid (2016) reported similar findings for bread wheat and end-use products. Future work needs to focus on bran chemistry impact on WW pasta quality.

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CHAPTER 5: RELATIONSHIP BETWEEN GRAIN, SEMOLINA AND WHOLE-WHEAT FLOUR PROPERTIES AND PHYSICAL AND COOKING QUALITIES OF WHOLE-WHEAT SPAGHETTI

Abstract

Durum breeding programs need to identify raw material traits capable of predicting whole-wheat (WW) spaghetti quality. Nineteen durum wheat (*Triticum turgidum* L. var. durum) cultivars and 17 breeding lines grown at 19 environments in North Dakota were evaluated for physical and cooking qualities of WW spaghetti. Raw material traits evaluated included grain, semolina and WW flour characteristics. Grain protein content had significant positive correlation with cooking quality of WW spaghetti. Stepwise multiple regressions showed grain protein content and mixogram break-time and wet gluten were the predominant characteristics in predicting cooking quality of WW spaghetti.

Introduction

Durum wheat breeding program at North Dakota State University has initiated efforts to identify or develop genotypes that produce high quality whole-wheat (WW) pasta. Durum breeding programs have focused on improving traits associated with end-use quality, which is primarily pasta produced from semolina, the coarsely ground durum wheat endosperm. Durum breeders screen their lines for improved pasta quality by screening for protein content, gluten/dough strength, and total yellow pigment or pasta color. Grain protein content and gluten/dough strength have been reported to be prerequisites for good cooking quality (Sissons et al 2005; AbuHammad et al 2012). D'Egidio et al (1990) used grain protein (GP) and gluten quality (GQ) to predict pasta texture (PT) using the following equation: PT=13.29+2.42 GP+1.57 GQ. Landi (1995) reported the following equation to predict cooked pasta quality: PV = K +

$2GLU + 0.04W + 8.5 P/L - 2(P/L)^2$, where PV = pasta value, K= factor for pasta drying temperature, P/L =alveogram extensibility value, W= alveogram strength value and GLU=semolina dry gluten. This equation incorporates the impact of drying temperature K, gluten/dough strength, P/L, W and dry gluten (protein) content (GLU).

Previous research found that of the durum genotypes tested, 40% produced good quality traditional spaghetti but made poor quality WW spaghetti (Deng 2017). These data indicate that the traits selected for good traditional pasta quality do not necessarily result in selection of genotypes that produce acceptable WW pasta. The objectives of this study were to identify traits that could be evaluated by a breeding program that would select durum genotypes that would produce high quality WW pasta.

Materials and Methods

Wheat Samples

Thirty-six genotypes of durum wheat were grown at 19 environments in North Dakota. The 36 genotypes included 19 released cultivars and 17 experimental breeding lines (Table 14). The 19 environments in North Dakota were Carrington, Dickinson, Langdon, Minot, Williston in 2012, 2013 and 2014; and Casselton, Hettinger in 2013 and 2014. Harvested durum samples were cleaned and stored at 12 °C until needed for quality analysis. The above data set was used to evaluate interrelationship of quality parameters and build predictive equations for end use pasta qualities. Another data set consisted of 45 genotypes grown at Dickinson, Langdon, Minot and Williston in 2015 was used to assess the accuracy of regression equations.

Sample Processing

Durum wheat was milled into semolina using a Bühler MLU-202 experimental mill fitted with two Miag laboratory-scale purifiers (Bühler-Miag, Minneapolis, MN, USA). Whole durum grain was ground into WW flour using an ultra-centrifugal mill (ZM 200, Retsch, Germany) configured with a 12,000 rpm rotor speed, 250 μm mill screen aperture size, and 62 g min^{-1} feed rate as described by Deng and Manthey (2016).

WW spaghetti was made by hydrating WW flour to 33% moisture and extruding into spaghetti with a DEMACO semi-commercial laboratory extruder and then dried using a high temperature drying cycle as described by de la Peña and Manthey (2017).

Quality Tests

AACC International approved methods 55-10.01, 39-25.01, 08-01.01 and 56-81.03 were used to determine test weight, protein content, ash content and falling number, respectively. Thousand-kernel weight was measured as described by Deng and Manthey (2016). Vitreous kernel content was determined according to USDA standard method (1997) where the percentage (w/w) of kernels having vitreous endosperm was determined based on 15 g of cleaned intact wheat kernels. Semolina wet gluten and gluten index were measured according to the AACC International Approved Methods (38-12.02). A colorimeter (CR410, Minolta, NJ, USA) was used to measure *CIE* L, a and b-values on a tristimulus scale for WW flour (Deng and Manthey 2016) and spaghetti (AACC International Approved Method 14-22.01). Dough strength of WW flour was evaluated using a modified mixograph procedure described by AACC International Approved Method (54-40.02). Mixogram was run for 10 min after adding 7.8 mL distilled water to 10 g (14% mb) WW flour. The mixogram parameters, peak time and peak height, were measured. Break-time was measured as the time up to the point that the dough begins to break down as indicated by an abrupt drop in the mixogram curve (Deng et al 2017).

Mechanical strength of WW spaghetti was determined by the flexure test using a TA-XT2 texture analyzer with a Spaghetti Flexure Rig (Texture Technologies Corp., Scarsdale, NY, USA) as described by de la Peña and Manthey (2017). Cooking qualities (cooked firmness and cooking loss) of WW spaghetti at 12 min were evaluated using AACC International Approved Method (66-50.01). Cooked weight was determined as the increase in pasta product weight after 12 min cooking and expressed as the percentage increase in pasta weight before cooking.

Statistical Analysis

The experimental design was a randomized complete block with unbalanced design. Genotype was considered a fixed effect and environment was treated as replication. Genotype was unbalanced at each environment (block). All the data were analyzed using SAS 9.3 software (SAS Institute, Cary, NC, USA). Pearson correlation and partial correlation analyses determined the interrelationship between grain, semolina, WW flour and WW spaghetti quality. Stepwise multiple regression was performed to identify the important quality traits to predict WW spaghetti quality. A significance level of $P \leq 0.05$ was used for forward inclusion or exclusion of quality traits in the regression model.

Results and Discussion

Relationship of Quality Parameters

The mean, SD and range for durum grain, semolina, WW flour and WW spaghetti quality parameters are summarized in Table 15. All parameters, except for falling number and cooked weight of WW spaghetti, ranged in quality from poor/fair to excellent. Falling number ranged from 367-498 sec, indicating that all samples were sound and had low α-amylase activity (Finney 2001). The range for cooked weight was quite narrow (281-290% gain).

The *CIE* L-value is associated with brightness or whiteness. The *CIE* L-value of WW flour was positively correlated with *CIE* L-value of WW spaghetti (r=0.42, $P \leq 0.05$, data not shown). Stepwise multiple regression was performed using grain and flour traits to predict WW spaghetti brightness. The flour brightness was the first variable included in the regression model with a R^2 contribution of 0.18 (Table 18), which indicates its contribution to WW spaghetti brightness. The relatively low R^2 value indicates that other factors not evaluated contributed to the brightness of WW spaghetti. These factors might include those associated with Maillard products and polyphenol oxidase (Liu et al 2016). These and other traits need to be evaluated for their role in WW spaghetti appearance.

Mechanical strength is important in keeping pasta intact during handling and shipping. Protein (grain protein content and wet gluten content) and protein/dough quality traits (gluten index and mixogram break-time, peak height and peak time) were positively correlated with mechanical strength of WW spaghetti (Table 19). Stepwise regression equation included mixogram break-time and peak height and test weight. The final R^2 was low for this three-variable equation (R^2=0.13, Table 18) and indicates that other factors influenced mechanical strength of WW spaghetti. Shiau et al (2012) reported that the particle size of wheat fiber impacted the dry noodle strength and that noodle strength was greater with small than with large fiber size. Furthermore, bran dilution of the gluten network has been associated with lower mechanical strength (Manthey and Schorno 2002). Further study is needed to determine the mechanism of action between bran, gluten and mechanical strength of pasta.

Table 18. Stepwise multiple regressions of whole-wheat spaghetti quality parameters with grain, semolina and whole-wheat flour quality traits (n=506).

Spaghetti Quality	Independent Variables					
	1st	R^2	2nd	R^2	3rd	Final R^2
CIE L	(+)*CIE* FL	0.18	(-)Ash	0.21	(+)VK	0.22
Mechst	(+)BT	0.07	(+)PH	0.10	(+)TW	0.13
Firm	(+)GPC	0.64	(+)BT	0.69	(+)GI	0.70
Ckloss	(-)WG	0.33	(+)KWT	0.40	(-)TW	0.48
CWT	(-)GPC	0.14	(+)WG	0.20	(-)KWT	0.23

TW = Test weight; VK = Vitreous kernel; KWT = 1000-Kernel weight; GPC = Grain protein content; WG = Wet gluten; PH = Peak height; BT = Break-time; *CIE* FL = Brightness of whole-wheat flour; *CIE* L = Brightness of whole-wheat spaghetti; Mechst = Mechanical strength; Firm = Firmness; Ckloss = Cooking loss; and CWT = Cooked weight.
+/- = Coefficient value.

Cooked quality includes cooked firmness, cooking loss and cooked weight, and is important to consumer acceptance of WW spaghetti. Cooked firmness was strongly correlated with grain protein content (r=0.80, $P \leq 0.05$) and wet gluten content (r=0.70, $P \leq 0.05$, Table 19), suggesting that high protein and wet gluten contents would promote increased cooked firmness of WW spaghetti. These results are similar to that reported for traditional pasta (Dexter and Matsuo 1977; Samaan et al 2006). Test weight and kernel weight were negatively correlated with cooked firmness (r=-0.42 and -0.45, respectively, $P \leq 0.05$, Table 15), which probably reflects their inverse relationship with protein content (Table 19). Plump heavy kernels associated with high test weight and high kernel weight tend to have a lower proportion of protein relative to starch.

Table 19. The Pearson Correlation between grain, semolina, whole-wheat flour and spaghetti qualities (n=506).

	Mechanical Strength	Cooked Firmness	Cooking Loss	Cooked Weight
Grain Quality				
TW	0.05	-0.42**	0.04	0.30**
VK	0.16**	0.38**	-0.48**	0.01
KWT	0.02	-0.45**	0.48**	0.03
GPC	0.22**	0.80**	-0.54**	-0.37**
Semolina Quality				
WG	0.15**	0.70**	-0.57**	-0.25**
GI	0.23**	0.30**	-0.15**	-0.13**
Whole-Wheat Flour Quality				
PT	0.19**	0.13**	-0.03	-0.06
PH	0.20**	0.54**	-0.43**	-0.22**
BT	0.27**	0.37**	-0.03	-0.26**

TW = Test weight; VK = Vitreous kernel; KWT = 1000-Kernel weight; GPC =Grain protein content; WG = Wet gluten; GI = Gluten index; PT = Peak time; PH = Peak height; and BT = Break-time.

Partial correlation was conducted to remove the quantitative variation of grain protein content between grain, semolina and WW spaghetti quality parameters. Break-time had similar Pearson and partial correlations with cooked firmness (r=0.37 and partial r=0.34, $P \leq 0.05$, Table 19 and 20). The results indicate the association of break-time and cooked firmness was independent of grain protein content. However, the low correlation coefficient (r) values indicate that other factors (perhaps related to bran) affect dough strength and cooked firmness.

Stepwise multiple regression indicated that grain protein content was the most important parameter to be included in the model of cooked firmness, with a R^2 contribution of 0.64 (Table 18). The addition of mixograph break-time and gluten index to the regression model increased the R^2 value to 0.70. Thus, cooked firmness was impacted by traits related to protein content, gluten strength and dough strength stability. These results are similar to those reported for traditional spaghetti (Sissons 2005).

Table 20. The Partial Correlation between grain, semolina, whole-wheat flour and spaghetti qualities (n=506).

	Mechanical Strength	Cooked Firmness	Cooking Loss	Cooked Weight
Grain Quality				
TW	0.19^{**}	-0.05	-0.32^{**}	0.14^{**}
VK	0.06	-0.06	-0.29^{**}	0.25^{**}
KWT	0.15^{**}	-0.13^{**}	0.31^{**}	-0.18^{**}
Semolina Quality				
WG	-0.14^{**}	-0.15^{**}	-0.22^{**}	0.26^{**}
GI	0.21^{**}	0.33^{**}	-0.09^{**}	-0.08
Whole-Wheat Flour Quality				
PT	0.18^{**}	0.16^{**}	-0.03	-0.05
PH	0.09^{**}	0.10^{**}	-0.15^{**}	0.01
BT	0.23^{**}	0.34^{**}	0.10^{**}	-0.19^{**}

TW = Test weight; VK = Vitreous kernel; KWT = 1000-Kernel weight; GPC = Grain protein content; WG = Wet gluten; GI = Gluten index; PT = Peak time; PH = Peak height; and BT = Break-time.

Cooking loss of WW spaghetti was negatively correlated with grain protein content, wet gluten content and mixogram peak height (r=-0.54, -0.57 and -0.43, respectively, $P \leq 0.05$, Table 19). High grain protein content has been associated with low cooking loss (Kaur et al 2012; Bagdi et al 2014). The more protein undergoes coagulation and transformation during cooking, the more starch that is trapped within the protein-starch matrix which results in reduced loss of solids in the cooking water. The absolute partial r value decreased from basic correlation (r=-0.57 and partial r=-0.22, $P \leq 0.05$, Table 19 and 20, respectively), which indicates that wet gluten had a high correlation with cooking loss interdependent with grain protein content. Stepwise regression indicated that wet gluten content contributed the most to cooking loss. The wet gluten content included in the model with a R^2 contribution of 0.33 that best predicted the loss of solid mass during cooking (Table 18).

Cooked weight did not vary greatly among the genotypes tested (281-290% gain, Table 15). The low level of variation probably made it difficult to detect strong relationships with traits tested and suggests that cooked weight may not be a quality factor to focus on in the initial stages of the breeding program.

Model Validation

Plots of actual and predicted values for spaghetti quality parameters are shown in Fig 16. Among all the plots, WW spaghetti cooked firmness was the most accurately modeled with a coefficient of determination (R^2)=0.70, followed by cooking loss (R^2=0.32).

High grain protein content, mixogram break-time and gluten index were associated with high cooked firmness. The grain protein content combined with wet gluten were associated with decreased cooking loss. Thus, grain protein content, wet gluten content and mixogram break-time would be useful to screen lines in large breeding program for improved WW pasta quality. Low R^2 for color and mechanical strength indicate the need to identify traits other than those tested that impact color and mechanical strength. A bran quality test probably needs to be identified that would help in selecting genotypes that would produce good WW pasta.

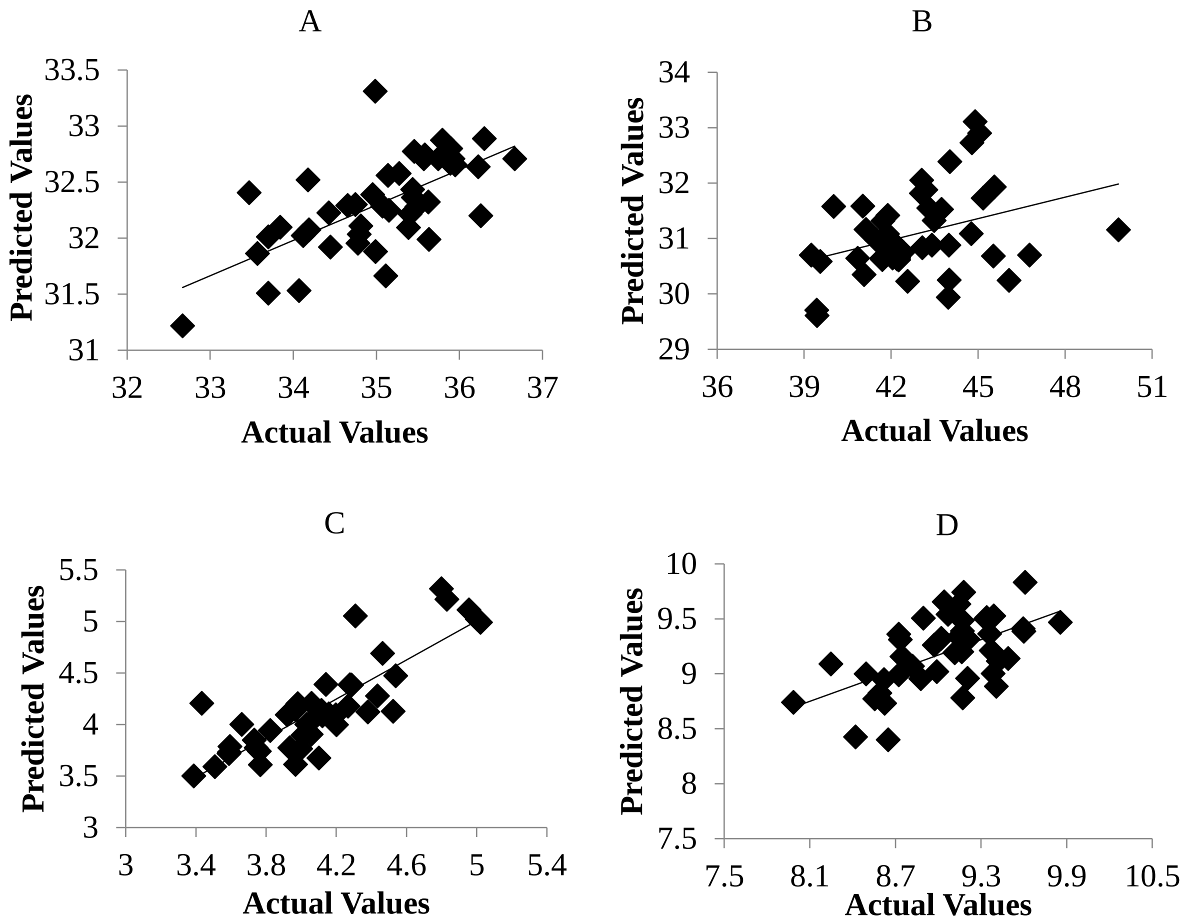

Figure 16. The validation of prediction models of whole-wheat spaghetti physical and cooking qualities. A = Brightness (R^2=0.44); B = Mechanical strength (R^2=0.12); C = Cooked firmness (R^2=0.70); and D = Cooking loss (R^2=0.32).

Conclusions

These results indicate that *CIE* L-value for WW flour, grain protein content, wet gluten, and mixogram break-time and peak height would be important traits for WW spaghetti quality. Low r and R^2 values indicate that traits other than those evaluated in this study impact spaghetti brightness and mechanical strength. These traits are probably associated with bran properties. Cooked firmness was associated with grain protein content, mixogram break-time and gluten index. Cooking loss was correlated with grain protein content and wet gluten content. The grain protein content and quality traits were shown to predict WW spaghetti quality using the regression equations. These traits would be useful in selecting WW spaghetti with desired cooking qualities.

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CHAPTER 6: OVERALL CONCLUSIONS

The results of this research indicate that rotor speed, screen aperture, seed conditioning moisture and feed rate can affect particle size, temperature and starch damage of whole-wheat (WW) flour. Durum grain with 9% seed conditioning moisture milled at 12,000 rpm with a 250 µm mill screen produced WW flour that had 82% of particles retained on <150 µm and 5.9% starch damage. The ultra-centrifugal mill can grind durum wheat in a single pass resulting in particle size distribution similar to that of commercial WW flour without generating excessive heat.

Particle size, starch damage, and pasting properties were similar for direct fine grind WW flour and multi-pass flour:fine bran and for direct coarse grind WW flour and multi-pass semolina:coarse bran flour. The semolina:fine bran blend made from multi-pass milling system had low starch damage and had the most desirable pasting properties (highest peak viscosity and final viscosity) for pasta cooking. The best physical quality of WW spaghetti was made from semolina:fine bran blend. WW spaghetti with the best cooking quality was made from a reconstituted blend either semolina:fine bran or semolina:coarse bran.

Genotypes with the grain protein content ≥13.9% produced WW spaghetti with high cooking quality. Low grain/semolina protein content and dough strength produced low quality WW and traditional spaghettis. Twenty-one of the 36 genotypes produced good quality WW and traditional spaghettis. Twelve genotypes preformed good on traditional but poorly on WW spaghetti. Selecting for traditional spaghetti quality does not always concurrently select for WW spaghetti quality.

Cooked firmness of WW spaghetti was associated with grain protein content, mixogram break-time and gluten index. Cooking loss was correlated with grain protein content and wet gluten content. The grain protein content and quality traits were shown to predict WW spaghetti quality using the regression equations. These traits would be useful in selecting WW spaghetti with desired cooking qualities.

CHAPTER 7: FUTURE RESEARCH AND APPLICATIONS

Further research is needed to determine the mill configuration and seed conditioning effects on flowing ability and agglomeration of whole-wheat (WW) flour that confirm the optimal configurations for producing good quality of WW flour.

Bran particle size varied with milling system and mill configuration. Scanning electron microscopic imaging could be used to evaluate bran physical and structural characteristics of direct grinds and semolina and bran blends. Differences in bran properties could further explain milling system effect on WW flour and spaghetti quality.

Genotype performed inconsistently with regard to their WW and traditional spaghetti. More intense tests could be evaluated on those genotypes to understand the bran component and structure chemistry effects on WW spaghetti quality.

APPENDIX

Table A1. Analysis of variance for whole-wheat flour particle size distributions milled at different rotor speeds and mill screen apertures.

	Source	DF	Type III SS	MS	F Value
≥600μm	Rep	2	0.67	0.34	4.28**
	SAS	2	1527.02	763.51	9698.78**
	SAS(rep)	4	1.33	0.33	4.22**
	RS	4	648.89	162.22	2060.68**
	SAS*RS	8	1275.82	159.48	2025.83**
600-500μm	Rep	2	0.69	0.34	13.41**
	SAS	2	594.74	297.37	11585.80**
	SAS(rep)	4	0.68	0.17	6.58**
	RS	4	92.99	23.25	905.77**
	SAS*RS	8	124.63	15.58	606.95**
500-425μm	Rep	2	0.80	0.40	4.58**
	SAS	2	580.29	290.14	3303.35**
	SAS(rep)	4	0.73	0.18	2.07
	RS	4	153.32	38.33	436.40**
	SAS*RS	8	162.82	20.35	231.72**
425-250μm	Rep	2	0.68	0.34	0.55
	SAS	2	5748.09	2874.04	4605.43**
	SAS(rep)	4	0.68	0.17	0.27
	RS	4	798.18	199.54	319.75**
	SAS*RS	8	1320.81	165.10	264.56**
250-150μm	Rep	2	2.82	1.41	4.54**
	SAS	2	462.97	231.48	745.92**
	SAS(rep)	4	13.10	3.28	10.55**
	RS	4	94.87	23.72	76.42**
	SAS*RS	8	1465.39	183.17	590.25**
150-100μm	Rep	2	49.91	24.96	3.60**
	SAS	2	2353.02	1176.51	169.94**
	SAS(rep)	4	3.00	0.75	0.11
	RS	4	1423.96	355.99	51.42**
	SAS*RS	8	525.04	65.63	9.48**
100-50μm	Rep	2	82.72	41.36	5.39**
	SAS	2	9563.38	4781.69	623.59**
	SAS(rep)	4	18.86	4.71	0.61
	RS	4	802.90	200.72	26.18**
	SAS*RS	8	287.41	35.93	4.69**
≤50μm	Rep	2	0.47	0.23	2.64
	SAS	2	10.82	5.41	61.06**
	SAS(rep)	4	0.94	0.23	2.65
	RS	4	0.51	0.13	1.44**
	SAS*RS	8	1.87	0.23	2.63**

SAS = Screen aperture size; RS = Rotor speed; DF = Degree of freedom; SS = Sum of square; MS = Mean square; and ** = Significant at $P \leq 0.05$.

Table A2. Analysis of variance for whole-wheat flour characteristics milled at different rotor speeds and mill screen apertures.

	Source	DF	Type III SS	MS	F Value
dgw	Rep	2	448.42	224.21	9.61**
	SAS	2	182150.54	91075.27	3903.44**
	SAS(rep)	4	386.70	96.67	4.14
	RS	4	45530.29	11382.57	487.85**
	SAS*RS	8	22000.97	2750.12	117.87**
Starch Damage	Rep	2	0.25	0.12	1.60
	SAS	2	498.11	249.06	3220.55**
	SAS(rep)	4	2.05	0.51	6.63**
	RS	4	17.12	4.28	55.35**
	SAS*RS	8	11.66	1.46	18.85**
Mill Surface Temperature	Rep	2	4.99	2.49	5.04**
	SAS	2	206.85	103.42	209.13**
	SAS(rep)	4	5.75	1.44	2.91**
	RS	4	192.70	48.18	97.41**
	SAS*RS	8	4.90	0.61	1.24
Flour Temperature	Rep	2	0.20	0.10	0.08
	SAS	2	110.98	55.49	43.46**
	SAS(rep)	4	25.45	6.36	4.98**
	RS	4	108.83	27.21	21.31**
	SAS*RS	8	43.90	5.49	4.30**
Moisture Loss	Rep	2	0.04	0.02	2.04
	SAS	2	50.64	25.32	2560.27**
	SAS(rep)	4	0.39	0.10	9.83**
	RS	4	6.76	1.69	170.87**
	SAS*RS	8	0.49	0.06	6.17**
L	Rep	2	2.74	1.37	9.94**
	SAS	2	296.13	148.07	1075.54**
	SAS(rep)	4	2.57	0.64	4.66**
	RS	4	44.13	11.03	80.14**
	SAS*RS	8	20.82	2.60	18.90**

SAS = Screen aperture size; RS = Rotor speed; DF = Degree of freedom; SS = Sum of square; MS = Mean square; and ** = Significant at $P \leq 0.05$.

Table A3. Analysis of variance for whole-wheat flour particle size distributions milled at different seed conditioning levels and feed rates.

	Source	DF	Type III SS	MS	F Value
≥600 µm	Rep	2	0.00007	0.00004	0.26
	SC	4	0.00025	0.00006	0.47
	SC(rep)	8	0.00102	0.00013	0.95
	FR	1	0.00003	0.00003	0.20
	SC*FR	4	0.00026	0.00006	0.48
600-500 µm	Rep	2	0.00022	0.00011	3.23
	SC	4	0.00002	0.00001	0.18
	SC(rep)	8	0.00047	0.00006	1.77
	FR	1	0.00014	0.00014	4.24
	SC*FR	4	0.00057	0.00014	4.29
500-425 µm	Rep	2	0.00041	0.00020	3.71
	SC	4	0.00124	0.00031	5.63**
	SC(rep)	8	0.00137	0.00017	3.13**
	FR	1	0.00149	0.00149	27.12**
	SC*FR	4	0.00036	0.00009	1.65
425-250 µm	Rep	2	0.32	0.16	57.61**
	SC	4	2.26	0.56	203.26**
	SC(rep)	8	0.17	0.02	7.82**
	FR	1	0.63	0.63	228.25**
	SC*FR	4	0.15	0.04	13.77**
250-150 µm	Rep	2	19.30	9.65	13.89**
	SC	4	22.78	5.70	8.20**
	SC(rep)	8	7.89	0.99	1.42
	FR	1	6.52	6.52	9.38**
	SC*FR	4	6.79	1.70	2.44
150-100 µm	Rep	2	33.05	16.52	0.67
	SC	4	405.36	101.34	4.13**
	SC(rep)	8	483.95	60.49	2.47
	FR	1	0.38	0.38	0.02
	SC*FR	4	135.23	33.81	1.38
100-50 µm	Rep	2	16.43	8.22	0.49
	SC	4	382.59	95.65	5.70**
	SC(rep)	8	329.78	41.22	2.46
	FR	1	13.29	13.29	0.79
	SC*FR	4	123.33	30.83	1.84
≤50 µm	Rep	2	0.88	0.44	1.06
	SC	4	8.17	2.04	4.91**
	SC(rep)	8	3.88	0.48	1.16
	FR	1	0.14	0.14	0.34
	SC*FR	4	0.72	0.18	0.43

SC = Seed conditioning; FR = Feed rate; DF = Degree of freedom; SS = Sum of square; MS = Mean square; and ** = Significant at $P \leq 0.05$.

Table A4. Analysis of variance for whole-wheat flour characteristics milled at different seed conditioning levels and feed rates.

	Source	DF	Type III SS	MS	F Value
dgw	Rep	2	19.10	9.55	0.40
	SC	4	725.79	181.45	7.57**
	SC(rep)	8	285.01	35.63	1.49
	FR	1	37.75	37.75	1.57
	SC*FR	4	120.94	30.24	1.26
Starch Damage	Rep	2	6.06	3.03	4.75**
	SC	4	97.49	24.37	38.22**
	SC(rep)	8	1.14	0.14	0.22
	FR	1	5.13	5.13	8.05**
	SC*FR	4	1.66	0.42	0.65
Mill Surface Temperature	Rep	2	47.26	23.63	31.38**
	SC	4	59.07	14.77	19.61**
	SC(rep)	8	7.17	0.90	1.19
	FR	1	10.56	10.56	14.03**
	SC*FR	4	2.57	0.64	0.85
Flour Temperature	Rep	2	20.23	10.12	18.41**
	SC	4	1.83	0.46	0.83
	SC(rep)	8	4.07	0.51	0.93
	FR	1	72.70	72.70	132.26**
	SC*FR	4	2.05	0.51	0.93
Moisture Loss	Rep	2	0.76	0.38	1.91
	SC	4	113.90	28.48	142.38**
	SC(rep)	8	1.68	0.21	1.05
	FR	1	0.16	0.16	0.81
	SC*FR	4	0.11	0.03	0.14
L	Rep	2	1.38	0.69	7.49**
	SC	4	1.01	0.25	2.74
	SC(rep)	8	0.83	0.10	1.12
	FR	1	0.88	0.88	9.52**
	SC*FR	4	0.10	0.02	0.26

SC = Seed conditioning; FR = Feed rate; DF = Degree of freedom; SS = Sum of square; MS = Mean square; and ** = Significant at $P \leq 0.05$.

Table A5. Analysis of variance for whole-wheat flour characteristics milled under single and multi-pass milling systems.

	Source	DF	Type III SS	MS	F Value
≥600μm	Rep	2	0.21	0.10	6.68**
	Trt	5	74.48	14.90	963.33**
600-500 μm	Rep	2	0.03	0.01	0.60
	Trt	5	84.50	16.90	766.03**
500-425 μm	Rep	2	0.00	0.00	0.07
	Trt	5	118.44	23.69	3919.81**
425-250 μm	Rep	2	31.95	15.98	2.59
	Trt	5	5280.47	1056.09	171.21**
250-150 μm	Rep	2	10.96	5.48	2.07
	Trt	5	606.61	121.32	45.90**
150-100 μm	Rep	2	12.00	6.00	0.36
	Trt	5	2974.89	594.98	35.91**
100-50 μm	Rep	2	14.80	7.40	0.66
	Trt	5	3404.70	680.94	60.45**
≤50 μm	Rep	2	0.23	0.11	1.97
	Trt	5	8.38	1.68	29.22**
dgw	Rep	2	168.51	84.26	3.20
	Trt	5	46355.18	9271.04	352.20**
sgw	Rep	2	52.53	26.27	2.39
	Trt	5	35481.03	7096.21	647.04**
Brightness	Rep	2	9.74	4.87	1.23
	Trt	5	211.80	42.36	10.70**
Starch	Rep	2	0.27	0.14	4.58**
Damage	Trt	5	99.13	19.83	668.79**
RVA					
Peak Time	Rep	2	0.05	0.02	3.38
	Trt	5	0.91	0.18	26.26**
Peak	Rep	2	1674.45	837.23	55.81**
Viscosity	Trt	5	6213.74	1242.75	82.84**
Trough	Rep	2	1851.90	925.95	86.22**
Viscosity	Trt	5	1788.22	357.64	33.30**
Final	Rep	2	5213.61	2606.80	82.64**
Viscosity	Trt	5	80015.02	16003.00	507.34**
Mixogram					
Peak-Time	Rep	2	8233.33	4116.67	0.81
	Trt	5	121983.33	24396.67	4.82**
Peak Height	Rep	2	0.18	0.09	0.54
	Trt	5	1.24	0.25	1.47

DF = Degree of freedom; SS = Sum of square; MS = Mean square; and
** = Significant at $P \leq 0.05$.

Table A6. Analysis of variance for whole-wheat spaghetti characteristics milled under single and multi-pass milling systems.

	Source	DF	Type III SS	MS	F Value
Brightness	Rep	2	2.10	1.05	5.85**
	Trt	5	30.46	6.09	33.92**
Mechanical	Rep	2	10.66	5.33	1.49
Strength	Trt	5	82.03	16.41	4.59**
Cooked Firmness					
2 min	Rep	2	3.97	1.99	1.56
	Trt	5	46.74	9.35	7.36**
4 min	Rep	2	0.38	0.19	1.14
	Trt	5	5.32	1.06	6.35**
6 min	Rep	2	0.21	0.10	2.06
	Trt	5	2.00	0.40	7.88**
8 min	Rep	2	0.05	0.02	0.37
	Trt	5	1.28	0.26	4.00**
10 min	Rep	2	0.04	0.02	0.27
	Trt	5	1.22	0.24	3.78**
12 min	Rep	2	0.25	0.12	4.29
	Trt	5	1.38	0.28	9.51**
14 min	Rep	2	0.22	0.11	4.62**
	Trt	5	1.64	0.33	13.54**
16 min	Rep	2	0.13	0.06	1.42
	Trt	5	1.34	0.27	6.02**
Cooking Loss					
2 min	Rep	2	0.03	0.02	0.18
	Trt	5	1.15	0.23	2.42
4 min	Rep	2	0.81	0.40	1.90
	Trt	5	4.21	0.84	3.95**
6 min	Rep	2	0.08	0.04	0.28
	Trt	5	6.66	1.33	9.29**
8 min	Rep	2	0.07	0.04	0.25
	Trt	5	7.28	1.46	10.49**
10 min	Rep	2	0.17	0.08	0.37
	Trt	5	15.07	3.01	13.60**
12 min	Rep	2	0.50	0.25	1.25
	Trt	5	17.77	3.55	17.68**
14 min	Rep	2	0.55	0.28	1.26
	Trt	5	19.43	3.89	17.68**
16 min	Rep	2	0.23	0.12	1.04
	Trt	5	23.44	4.69	42.06**

DF = Degree of freedom; SS = Sum of square; MS = Mean square; and
** = Significant at $P \leq 0.05$.

Table A6. Analysis of variance for whole-wheat spaghetti characteristics milled under single and multi-pass milling systems (Continued).

	Source	DF	Type III SS	MS	F Value
Cooked Weight					
2 min	Rep	2	0.16	0.08	1.29
	Trt	5	0.93	0.19	3.01
4 min	Rep	2	0.50	0.25	3.21
	Trt	5	2.29	0.46	5.91**
6 min	Rep	2	0.95	0.47	1.52
	Trt	5	5.50	1.10	3.55**
8 min	Rep	2	1.94	0.97	1.15
	Trt	5	4.03	0.81	0.96
10 min	Rep	2	0.13	0.07	0.35
	Trt	5	6.53	1.31	6.84**
12 min	Rep	2	1.27	0.64	2.34
	Trt	5	8.99	1.80	6.62**
14 min	Rep	2	23.19	11.59	1.57
	Trt	5	47.97	9.59	1.30
16 min	Rep	2	23.63	11.82	2.29
	Trt	5	29.81	5.96	1.16

DF = Degree of freedom; SS = Sum of square; MS = Mean square; and
** = Significant at $P \leq 0.05$.

Table A7. Analysis of variance for grain and semolina quality characteristics among genotypes.

	Source	DF	Type III SS	MS	F Value
Grain Protein Content	Rep	18	407.96	22.66	9.76**
	Geno	35	162.32	4.64	2.00**
Falling Number	Rep	18	1795445.22	99746.96	6.90**
	Geno	35	710294.65	20294.13	1.40
Break-Time	Rep	18	670986.19	37277.01	2.88**
	Geno	35	2521735.16	72049.58	5.57**
Semolina Protein Content	Rep	18	334.71	18.59	10.48**
	Geno	35	113.98	3.26	1.84**
Wet Gluten	Rep	18	3467.62	192.65	9.23**
	Geno	35	2463.15	70.38	3.37**
Gluten Index	Rep	18	8862.10	492.34	3.05**
	Geno	35	135482.08	3870.92	23.99**
Mixogram Score	Rep	18	27.91	1.55	2.52**
	Geno	35	480.81	13.74	22.29**

Geno = Genotype; DF = Degree of freedom; SS = Sum of square; MS = Mean square; and ** = Significant at $P \leq 0.05$.

Table A8. Analysis of variance for whole-wheat and traditional spaghetti quality characteristics among genotypes.

	Source	DF	Type III SS	MS	F Value
Whole-Wheat Spaghetti					
HL	Rep	18	776.17	43.12	10.37**
	Geno	35	290.77	8.31	2.00**
Ha	Rep	18	21.56	1.20	5.47**
	Geno	35	10.14	0.29	1.32
Hb	Rep	18	234.40	13.02	8.35**
	Geno	35	108.04	3.09	1.98**
Mechanical Strength	Rep	18	380.77	21.15	2.60**
	Geno	35	510.15	14.58	1.79**
Cooked Firmness	Rep	18	39.29	2.18	3.04**
	Geno	35	42.75	1.22	1.70**
Cooking Loss	Rep	18	113.21	6.29	4.52**
	Geno	35	82.94	2.37	1.70**
Cooked Weight	Rep	18	22.54	1.25	2.22**
	Geno	35	21.80	0.62	1.10
Traditional Spaghetti					
HL	Rep	18	357.29	19.85	5.89**
	Geno	35	341.68	9.76	2.90**
Ha	Rep	18	32.51	1.81	5.53**
	Geno	35	85.02	2.43	7.44**
Hb	Rep	18	169.96	9.44	6.00**
	Geno	35	254.91	7.28	4.63**
Cooked Firmness	Rep	18	84.02	4.67	8.13**
	Geno	35	39.88	1.14	1.99**
Cooking Loss	Rep	18	71.54	3.97	6.19**
	Geno	35	25.40	0.73	1.13
Cooked Weight	Rep	18	101.85	5.66	7.73**
	Geno	35	48.44	1.38	1.89**

Geno = Genotype; DF = Degree of freedom; SS = Sum of square; MS = Mean square; and ** = Significant at $P \leq 0.05$.

Table A9. The durum grain and whole-wheat spaghetti qualities among 19 cultivars.

Cultivar	Protein Content (%, 12%mb)	Break-Time (sec)	Falling Number (sec)	Hunter-L	Hunter-*a*	Hunter-*b*	Mechanical Strength (g)	Cooked Firmness (g cm)	Cooking Loss (%)	Cooked Weight (% gain)
AC Navigator	13.8bc	462a-e	467	33.78bc	9.04	12.21c	31.0a-e	4.3ab	9.8abc	285
Alkabo	13.7bc	391efg	381	35.00ab	9.33	13.02abc	30.1c-f	3.9abc	9.9abc	286
Alzada	13.8bc	557a	466	33.40c	9.05	12.07c	32.4a	4.3ab	10.3ab	285
Ben	15.3a	422b-g	431	34.14bc	9.12	12.30bc	30.1b-f	4.1ab	9.4bc	283
Carpio	13.4bcd	538a	459	35.02ab	9.29	12.74abc	30.2b-f	4.2ab	9.7abc	284
Commander	13.6bc	556a	492	34.17bc	9.03	12.45bc	32.1ab	4.2ab	10.0ab	286
Divide	13.5bcd	492abc	426	34.54abc	9.46	12.54bc	30.9a-e	3.9abc	10.0abc	286
Grenora	13.6bc	436b-g	390	35.06ab	9.14	12.86abc	30.0def	4.1ab	9.8abc	284
Joppa	12.4d	520a	374	36.11a	9.25	13.50a	29.5def	3.6bcd	10.3ab	289
Lebsock	14.7ab	383e-h	427	34.59abc	9.02	12.55abc	30.4a-e	4.3ab	9.3bc	286
Maier	14.3ab	435b-g	367	34.79abc	9.12	12.56abc	30.7a-e	4.2ab	9.7abc	284
Mountrail	13.6bc	307hi	394	34.97ab	9.30	12.73abc	28.7ef	3.5bcd	9.5bc	288
Normanno	13.1bcd	561a	381	30.30d	8.89	10.08d	28.8def	3.5bcd	10.4ab	281
Pierce	13.8bc	481a-e	390	35.30ab	9.18	12.94abc	30.8a-e	4.0ab	9.5bc	281
Rugby	14.5ab	231i	377	35.25ab	8.95	12.79abc	27.7f	3.2d	9.8abc	290
Strongfield	14.2ab	508ab	394	33.73bc	9.23	12.11c	31.7a-d	4.1ab	10.6a	286
Tioga	13.4bcd	553a	377	33.63bc	9.20	11.97c	32.0ab	4.1ab	10.4a	283
VTPeak	13.9abc	380e-h	431	34.57abc	8.90	12.87abc	31.1a-e	3.6bcd	10.0abc	285
Verona	14.0abc	443a-g	387	33.47bc	9.03	11.95c	31.7a-d	3.5bcd	10.7a	281

Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Table A10. The durum grain and whole-wheat spaghetti qualities among 17 breeding lines.

Breeding Lines	Protein Content (%, 12%mb)	Break-Time (sec)	Falling Number (sec)	Hunter-L	Hunter-*a*	Hunter-*b*	Mechanical Strength (g)	Cooked Firmness (g cm)	Cooking Loss (%)	Cooked Weight (% gain)
D041708	13.3bcd	356gh	373	35.14ab	9.10	12.94abc	29.6def	3.7bcd	9.8abc	288
D04586	13.3bcd	415b-g	425	36.19a	8.81	13.69a	30.5a-e	3.6bcd	9.7abc	287
D06587	15.2a	504abc	491	35.46ab	8.95	13.17abc	31.6a-d	4.5a	9.1bc	284
D06707	13.7bc	481a-d	392	34.73abc	9.18	12.77abc	30.2b-f	3.9a-d	10.2ab	287
D06855	13.7bc	449a-f	402	34.91ab	9.26	13.03abc	29.5def	3.8bcd	9.5bc	284
D06886	14.6ab	399efg	444	35.02ab	9.12	13.04abc	32.3ab	4.0abc	9.3bc	288
D06932	14.6ab	375fgh	450	35.11ab	9.07	13.19abc	30.7a-e	4.2ab	8.9c	283
D071016	13.5bcd	425b-g	484	34.72abc	9.37	12.52bc	29.6def	3.8bcd	9.1bc	288
D071022	13.7bc	538a	425	35.20ab	9.15	13.07abc	33.0a-d	4.0abc	9.8abc	287
D071579	14.8ab	415c-g	498	34.18bc	9.04	13.11abc	31.7a-d	4.2ab	9.3bc	288
D07726	13.7bc	389efg	449	34.98ab	9.06	12.94abc	30.6a-e	3.9a-d	9.6abc	288
D07892	13.2bcd	376fgh	440	34.87ab	8.98	12.71abc	30.8a-e	3.6bcd	9.5bc	287
D08900	13.3bcd	472a-e	428	36.04a	8.83	13.35ab	30.9a-e	3.6bcd	10.1ab	287
D09555	13.3bcd	442a-g	429	35.17ab	9.01	13.12abc	31.7a-d	3.5bcd	9.9abc	285
D09557	13.2bcd	403d-g	430	35.41ab	9.06	13.25ab	29.7def	3.5bcd	9.8abc	286
D09690	13.2bcd	503abc	416	34.87abc	8.89	12.73abc	30.5a-e	3.5bcd	10.1ab	283
D09970	12.9cd	456a-g	404	35.15ab	9.09	13.14abc	31.0a-e	3.4cd	10.4ab	287

Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Table A11. The semolina and traditional spaghetti qualities among 19 cultivars.

Cultivar	Semolina Protein (%, 14%mb)	Wet Gluten (%)	Gluten Index	Mixogram Score	Hunter-L	Hunter-*a*	Hunter-*b*	Cooked Firmness (g cm)	Cooking Loss (%)	Cooked Weight (% gain)
AC Navigator	12.4bcd	34.6cd	69ef	7c-f	53.57gh	2.58bc	26.75b-g	4.6a-d	7.1	308cde
Alkabo	12.2b-e	33.9cde	51i	6mn	54.58b-h	1.88de	26.94bcd	4.2d-g	7.1	313a-d
Alzada	12.6bcd	33.0def	90ab	8ab	53.15h	2.82b	26.26c-g	4.6a-d	7.2	310b-e
Ben	13.6a	40.8a	53hi	6lmn	54.83a-g	1.55e-h	25.42gh	4.6a-d	6.7	308cde
Carpio	12.3b-e	32.0def	95a	8a	54.76a-g	1.95de	27.10bcd	4.5bcd	6.9	311b-e
Commander	12.2b-e	32.2def	89ab	8abc	54.10d-h	2.63bc	27.12bcd	4.8abc	7.2	309cde
Divide	12.3bcd	32.6def	76cd	7bcd	54.86a-g	1.79d-g	26.18d-g	4.1d-g	7.0	313a-d
Grenora	12.3bcd	34.0cde	67ef	6h-l	54.72a-h	1.64e-h	26.31c-g	4.4b-f	7.1	312a-d
Joppa	11.3e	29.8f	84bc	7c-f	55.42a-d	1.78d-g	27.28ab	3.9efg	7.1	317ab
Lebsock	13.1abc	38.9ab	46i	5n	54.88a-g	1.54e-h	25.90efg	4.5a-d	6.7	312b-e
Maier	12.9a-d	37.1bc	52hi	6i-m	54.15c-h	2.07d	26.50b-g	4.6a-d	6.9	309cde
Mountrail	12.3b-e	35.1cd	20j	4o	54.65a-h	1.67d-h	24.82h	3.7g	6.7	316ab
Normanno	12.1b-e	31.0def	86abc	8abc	49.28i	4.47a	23.12i	4.3b-g	7.3	312a-e
Pierce	12.5bcd	35.0cd	62fgh	7h-l	54.78a-g	1.91de	26.46b-g	4.4b-e	6.8	309cde
Rugby	12.9a-d	33.9cde	12j	3p	54.92a-g	1.31h	25.79fgh	3.8fg	6.6	320a
Strongfield	12.6a-d	34.9cd	62fgh	7d-h	53.97fgh	2.11d	25.99efg	4.4b-e	7.1	312b-e
Tioga	12.1b-e	32.8def	76cd	7cde	53.98e-h	2.06d	26.09efg	4.5bcd	7.4	309cde
VTPeak	12.6a-d	36.0bcd	68def	7g-l	54.74a-h	1.54e-h	25.42gh	4.7a-d	7.0	312b-e
Verona	12.8a-d	35.7bcd	63e-h	6h-m	53.26gh	2.24cd	25.44gh	4.8a-d	7.2	309b-e

Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Table A12. The semolina and traditional spaghetti qualities among 17 breeding lines.

Breeding Lines	Semolina Protein (%, 14%mb)	Wet Gluten (%)	Gluten Index	Mixogram Score	Hunter-L	Hunter-*a*	Hunter-*b*	Cooked Firmness (g cm)	Cooking Loss (%)	Cooked Weight (% gain)
D041708	12.0de	34.2cde	55ghi	6mn	55.19a-f	1.45fgh	25.64gh	4.3b-g	6.7	317ab
D04586	12.2b-e	33.6c-f	74cde	6mn	55.69abc	1.64d-h	27.17a-d	4.3b-g	6.8	318ab
D06587	13.7a	39.0ab	78cd	7d-h	55.32a-e	1.76d-h	27.05bcd	5.1a	6.4	305e
D06707	12.5bcd	33.3def	68def	7g-k	54.72a-h	1.70d-h	26.27c-g	4.5a-d	6.9	310b-e
D06855	12.4bcd	34.3cd	64efg	6j-m	54.88a-g	1.81d-g	27.10bcd	4.3b-f	7.0	313a-d
D06886	13.0abc	37.4abc	66ef	6lm	55.71ab	1.52e-h	27.08bcd	4.8abc	6.7	309cde
D06932	13.2ab	37.2abc	69def	6klm	55.84ab	1.69d-h	28.12a	4.6a-d	6.3	308de
D071016	12.2b-e	33.1def	68def	6h-l	55.54abc	1.85def	27.25ab	4.2d-g	6.6	316ab
D071022	12.3bcd	34.0cde	76cd	7c-g	55.30a-e	1.67d-h	27.07bcd	4.3b-f	6.9	314abc
D071579	13.3ab	37.6abc	72cde	7f-j	55.45a-d	1.84d-g	26.87b-e	4.9ab	6.7	310b-e
D07726	12.6a-d	35.7bcd	55ghi	6mn	55.27a-e	1.63e-h	26.94bcd	4.3b-f	6.8	315abc
D07892	12.0cde	32.6def	65ef	6h-l	55.28a-e	1.82d-g	27.05bcd	4.2d-g	6.9	313a-d
D08900	12.0cde	33.2def	77cd	7b-e	56.02a	1.31h	26.52b-g	4.6a-d	6.5	312a-d
D09555	11.9de	33.0def	76cde	7e-i	55.54abc	1.41gh	26.91b-e	4.2d-g	6.8	315abc
D09557	11.8de	33.0def	64efg	6h-l	55.36a-d	1.62e-h	27.17abc	4.3b-g	6.7	313a-d
D09690	11.9de	30.7ef	85abc	8abc	55.40a-d	1.82d-g	26.93b-e	4.2d-g	7.0	311b-e
D09970	11.8de	31.7def	73cde	7e-i	55.11a-f	1.78d-h	27.12a-d	4.2c-g	6.9	312a-d

Values followed by different letters in the columns are significantly different at $P \leq 0.05$.