CAUSES OF DULL BROWN COLOR IN DURUM AND TRADITIONAL SEMOLINA PASTA

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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

Bleaching of durum wheat (*Triticum turgidum* L. ssp. *durum* [Desf.] Husn.) was evaluated by determining the effect of grain moisture content, temperature, and wet/dry cycles with bulk water and with high relative humidity on the hydration of durum wheat grain and their effect on some physical grain quality parameters. Low initial grain moisture, high temperature, and wet/dry cycles increased water gain. Scanning electron microscopy and light microscopy showed that the germ and ventral surface of grain were important for water absorption. A single exposure to moisture (bulk water or high relative humidity) seems to be enough to cause a decline in grain quality.

The effect of the environment on pasta color was evaluated by quantifying the relative importance of environment and genotype effects on pasta color and related traits. The relationship between environmental growing conditions, pasta color and semolina quality traits was determined. The environment had the highest relative proportion of variance for pasta color and related traits. Stepwise multiple linear regression indicated that the number of days with RH $\geq$ 80% diminished pasta color which could be related to increased speck count in semolina, soluble brown pigment content and an increased in semolina redness. The number of days $\leq$ 13°C enhanced pasta yellowness and pasta color score. However, why the positive effect occurred was not clear.

Milling and processing effects on pasta color were evaluated by determining the effect of milling and pasta processing on polyphenol oxidase (PPO) activity, peroxidase (POD) activity, soluble brown pigment content, and yellow pigment content. Milling caused a reduction in yellow pigment content, soluble brown pigment content, PPO activity and POD activity while pasta processing reduced yellow pigment content, and for some genotypes, increased soluble brown pigment content. Stepwise multiple linear regression indicated that yellow pigment

content had a positive effect and protein content, semolina ash content, and speck count had a negative effect on pasta color.

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GENERAL INTRODUCTION

Grain color and pasta color are two fundamental quality parameters that define durum wheat (*Triticum turgidum* L. ssp. *durum* [Desf.] Husn.) quality for producers, consumers, and related pasta industries such as milling and pasta processing companies. The method used to identify both grain color and pasta color is based on CIE or Hunter tristimulus values L measures the brightness of samples from black (0) to white (100), *a* measures the greenness (-60) and redness (60), and *b* measures blue (-60) to yellow (60). A pasta color score is based on Hunter L and *b* color values (AACCI approved method 14-22.01). For durum wheat grain, a vitreous translucent amber kernel color is expected by producers and millers. For pasta, a bright yellow color is desired by pasta companies and consumers (Troccolli et al., 2000).

One of the factors that can affect the color of the grain is known as grain bleaching. Grain bleaching results in bran discoloration due to the effect of moisture on the structure of the bran (Cazernecki & Evans, 1986; Cabas-Lühmann, 2017). Moisture necessary for grain bleaching comes from rainfall, heavy dew, and high relative humidity. When the grain reaches moisture content suitable for harvest (~12-13% moisture) bran layers can quickly absorb moisture. It does not take a lot of moisture to cause bleaching due to the relatively small amount of bran surrounding the kernel (Delcour & Hoseney, 2010; Paquet- Durand et al., 2015). Water absorption causes the bran to swell. The swollen bran does not contract when it dries. Each alternate wet and dry cycle causes slight swelling of the bran resulting in wrinkling of the kernel surface which changes its reflective properties (Bason et al., 1995; Debbouz et al., 1995).

Initial bleaching tends to cause a lightening of the seed coat appearance; however, as bleaching progresses the seed takes on a dull brown weathered appearance. It is not unusual for bleached kernels to be mistaken as non-vitreous kernels because the most common method used

to determine vitreousness is performed through visual analysis by an inspector. This sometimes causes an incorrect classification of some grains (Dowell, 2000; Neethirajan et al., 2006). It is possible for moisture to move into the bran layer causing it to swell and appear bleached but not reach or be absorbed by the endosperm. Research is needed to further understand how grain bleaching occurs, the range of genetic variability among durum genotypes, and the threshold in bleaching beyond which the functional quality (milling, processing, and pasta) of durum is impacted.

Consumers and pasta companies desire bright yellow pasta (Troccolli et al., 2000). Pasta color is a combination of three components: First, yellow color is due to yellow pigment content in semolina, which is the coarsely ground endosperm of durum wheat. The yellow pigment is primarily lutein which is a type xanthophyll from the carotenoids group (Ramachandran et al., 2010); second, a brown color that can be a consequence of enzymatic or non-enzymatic reactions (Kobrehel et al., 1974); and third, a red color formed during pasta drying (Feillet & Dexter, 1996).

Yellow pigment content in durum wheat is highly heritable (Clarke et al., 2006) mainly determined by the genotype (Borrelli et al., 2003) and less affected by the environment (Taghouti et al., 2010). Clarke et al. (2006) determined that yellow pigment content varied with environment. Schulthess et al. (2013) determined a heritability to 0.82 for semolina yellow pigment content while Clarke et al. (2006) determined a heritability ranged from 0.88 to 0.95 in data obtained from multi-location and multiyear trials.

Although the origin of pasta brownness still remains uncertain, pasta brownness has been reported to vary with genotype (Irvine & Anderson, 1952) and environment, with the environment having a greater effect than the genotype (Feillet et al., 2000; Harris et al., 1943).

The genotypic factors include both enzymatic and non-enzymatic browning. Non-enzymatic brownness can be a consequence of a soluble brown-cupric protein (Matsuo & Irvine, 1967) or enzymatic due to oxidative enzymes such as polyphenol oxidases (PPOs) and peroxidases (POD) (Kobrehel et al., 1974).

Processing has also been reported to affect pasta brownness and yellowness (Irvine, 1971; Dexter & Marchylo, 2001). Milling (Borrelli et al., 2008) can affect pasta color. Borrelli et al. (1999) determined that about 8% of carotenes are lost during milling to semolina. Dough kneading during pasta processing has been reported to promote oxidation and subsequent loss of yellow pigment (Dexter & Marchylo, 2001; Borrelli et al., 2003). The percentage of loss after processing can vary from 4 to 20% of yellow pigment content (Borrelli et al., 1999; DeSimone et al., 2010). Drying extruded pasta at high temperatures promotes, the Maillard reaction, which can mask yellow color of pasta (Marchylo & Dexter, 1989).

Understanding the causes of the changes in the durum grain color due to bleaching and pasta color during processing would be important information for breeding programs. In North Dakota it is not unusual to have bleached grain due to delayed harvest (Cabas-Lühmann, 2017). Limited scientific information about bleaching is available. In addition, it has been noticed that the pasta color scores in North Dakota have decreased through the years which would indicate a decline in brightness (L-value) and yellowness (*b*-value). Interestingly, yellow pigment content in grain, semolina, and subsequent pasta has increased. The reason for this phenomenon is uncertain.

The research presented in this dissertation addressed three questions:

1) what is the role of environmental factors such as temperature, initial grain moisture and consecutive wet/dry cycle exposures to bulk water and high relative humidity on the

hydration properties of durum wheat and their effect on some grain physical quality parameters related to bleaching;

2) what is the effect of growing environment on pasta color and suspected factors (yellow pigment content, brown pigment content, polyphenol oxidase activity and peroxidase activity) involved in pasta color;

3) what is the effect of processing (milling and pasta manufacturing) on pasta color and suspected factors (yellow pigment content, brown pigment content, polyphenol oxidase activity and peroxidase activity) involved in pasta color.

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CHAPTER 1: LITERATURE REVIEW

Durum Wheat

Durum wheat (*Triticum turgidum* ssp. *durum*) is a tetraploid species 2n=4x=28 with AA BB genome that belongs to the Poaceae family. Durum is the second most cultivated wheat on earth after *Tricticum aestivum* L.

Durum is an annual crop that grows best in temperate climates with hot-dry days and cool nights (Bozzini, 1988). The leaves are flat with parallel veins. The stem is cylindrical and can be hollow or solid (Clarke et al., 2002). The crop produces about three tillers from the auxiliary buds in addition to the main shoot (Bozzini, 1988). Each tiller in addition to the main stem produces one inflorescence known as a spike.

Wheat Inflorescence

The wheat inflorescence is a spike. Each spike has a rachis with spikelets. Each spikelet consists of two glumes and two-to-five florets. Each floret is a perfect flower that contains three anthers, a feathery stigma, and an ovary, enclosed by two glumes known as lemma and palea which cover the male and female reproductive organs (Sleeper & Poehlman, 2006). Every floret can produce one seed (caryopsis) (Bozzini, 1998). At anthesis, an organ located at the base of the floret called lodicule opens and the anthers and pistil are exposed for pollination (Whitford et al., 2013). Stiff glumes, lemmas, and paleas are often found in common wheat varieties and are associated with traits that prevent flower opening and kernel shattering (Vogel, 1941; Zhang et al., 2009).

Pollination and Fertilization

Pollination is known as the process where the pollen is deposited from the anthers onto the stigma of a floret (Evers & Millart, 2001). Wheat is a self-pollinated crop, although there is 1 to 2% cross-pollination (Sleeper & Poehlman, 2006). Pollen is formed in the anthers. To capture

pollen, stigmas use adhesive interactions to retain pollen grains. In wheat flowers, pollen is shed before or just after the flower starts to open. When the pollen is on the stigma, a pollen tube develops and two pollen grains (sperm) move down into the style and enter the ovary specifically to the embryo sac. Once in the embryo sac, one of the sperm (1N) fuses with the egg nuclei (1N) forming the zygote or embryo (2N). The second sperm fuses with two polar nuclei (1N each) forming the endosperm (3N) (Evers & Millart, 2001). The entire process is called double fertilization.

The endosperm develops through repeated divisions of the primary endosperm nucleus (Olsen, 2004). Next, the endosperm undergoes cellularization, where cell walls are formed starting from the micropylar area (Evers & Millart, 2001) which is an opening in the integument of an ovule located in the ventral side (Figure 1) (Bhatnagar & John, 1972). Cellularization is complete in about four days after pollination. The endosperm, including the aleurone layer and the germ arising from two separate fertilization events, contain genetic material from the male and the female, known as hybrid tissues (Millet, 1980).

The pericarp and testa are diploid maternal tissues that develop from integuments that surround the ovary. They represent most of the bran that is removed during roller milling. The origin of tissues is important to consider at the time of parental selection when breeding for certain traits such as bran characteristics. Millet (1980) determined that grain weight of a spring wheat cultivar was controlled mainly by the genotype of maternal tissues. It has been shown that rice grain shape is simultaneously controlled by triploid endosperm genes, cytoplasmic genes and maternal plant genes (Shi & Zhu, 1996).

Kernel Structure

The caryopsis or kernel of wheat has three major components: the bran, the germ, and the endosperm, each one is composed of many different layers (Figures 1 and 2). The endosperm is surrounded by bran. Germ is located at the basal region of the seed, close to the base of the floret. The germ is partially covered by some of the bran layers and it is exposed to the environment.

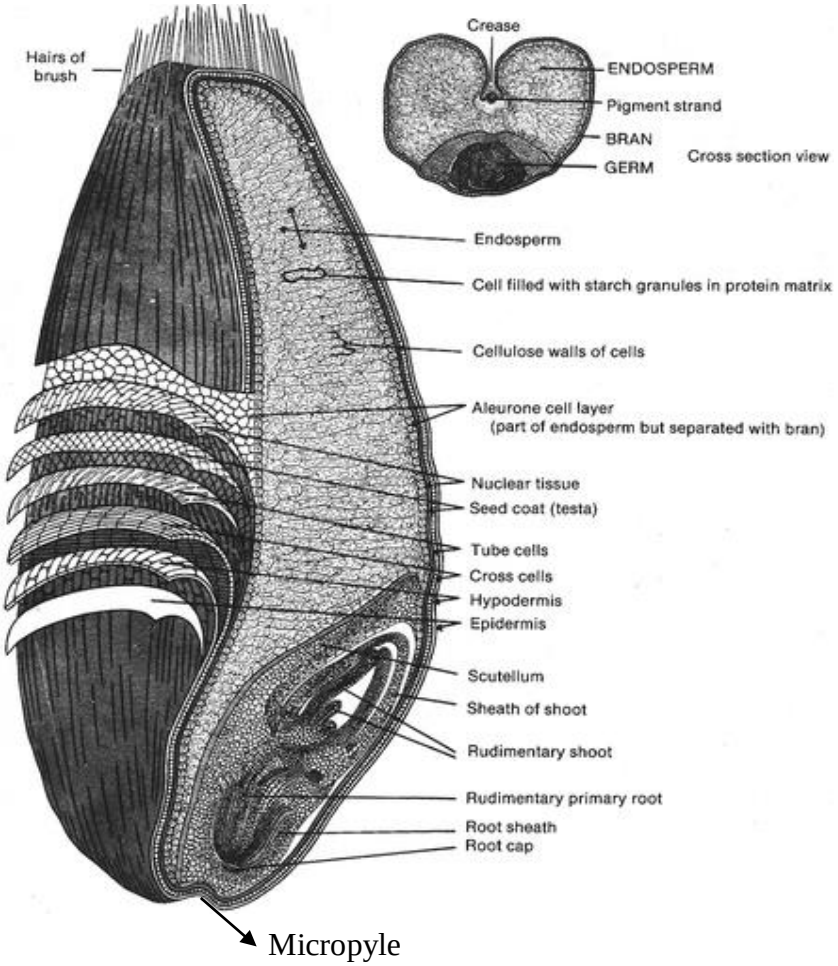

Figure 1. Longitudinal and cross section of wheat grain and its components (Delcour & Hoseney, 2010).

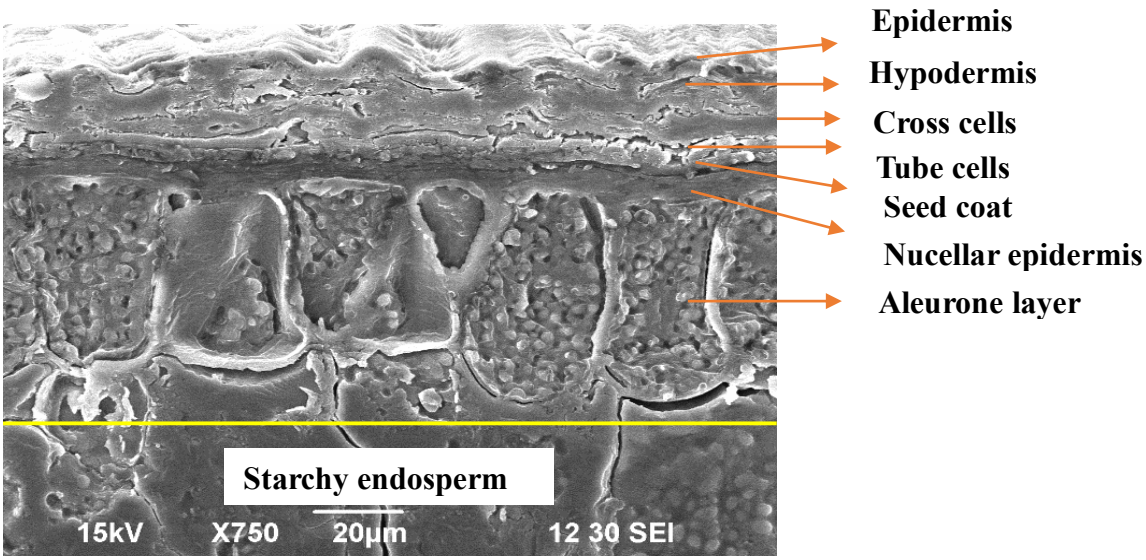

Figure 2. Electron microscope photos to indicate the bran layers and the starchy endosperm.

Wheat bran

Botanically, bran is a multilayered tissue made up of outer pericarp, testa, and hyaline layer all of which surrounds the endosperm. In the milling discipline, bran includes the aleurone layer because it is removed with the bran layer during the milling process (Hemery et al., 2011) (Figure 1 and 2). Bran represents about 14.5% of kernel dry weight. The bran is rich in complex non-starch polysaccharides (dietary fiber), essential fatty acids, protein, minerals, and vitamins. Physical properties of cereal brans are important in the final quality of the milling products (Chimna et al., 2015). Bran has good water and oil absorption capacities which range from 148 to 384% and 139 to 303%, respectively (Sharma et al., 2014).

The pericarp and testa are maternal tissues that develop from integuments that surround the ovary. The pericarp is composed of an inner and an outer layer. The outer pericarp is the epidermis followed by hypodermis and remnants of thin-walled cells. The inner pericarp consists of intermediate cells, cross cells, and tube cells. Pericarp makes up 5% of the kernel dry weight and contains about 6% protein, 2% ash, 20% cellulose, and 0.5% fat, the remainder percentage

are non-starch polysaccharides (Delcour & Hoseney, 2010). The pericarp is important to the protection of the seed.

Seed coat has a pigment layer that contains lipids. Its function at the later stages of development and at maturity is to control the water relations between the enclosed seed and its surroundings (Pomeranz, 1988). Pericarp and seed coat are composed of dead empty cells.

Aleurone layer, even though often associated with the bran by milling companies, is botanically a component of the endosperm. It is rich in proteins with high enzymatic activity, lipids, minerals including phosphorous, and niacin. The aleurone layer contains some hydrolytic enzymes that activate other hydrolytic enzymes that are mobilized to the starchy endosperm where they start the breakdown of storage proteins and carbohydrates into amino acids and sugars needed for germination and the growth of the seedling.

Wheat germ

The germ contains the embryo of the seed. It is the smallest part of the kernel (2-3 % of the total weight). The germ is composed of a single cotyledon (the scutellum) and an embryonic axis (coleoptile, primary root, and secondary roots) (Delcour & Hoseney, 2010). During germination, the embryonic axis grows into root and shoot. The scutellum is the storage part of the germ. The germ is rich in lipids (8-13%), proteins (25%) and sugars (18%) along with a small content of minerals (5%) and vitamins (B and E). The germ does not contain starch (Mattern, 1991). Due to high lipid content, oxidation of lipids, therefore rancidity, is a common problem in products that contain germ (Corke, 2016).

Wheat endosperm

Endosperm represents about 70% of the dry kernel weight (Mattern, 1991). It is composed mainly of starch granules (~80%) which consist in three types A-type (> 10 μm), B-

type (5–10 μm), and C-type (< 5 μm). Endosperm also contains 13 to 15% proteins that consists of glutenins and gliadins and small fractions of albumins and globulins (Hurkman & Tanaka, 2007), fats (1.5%), ash (0.5%), and dietary fiber (1.5%) (Belderok et al., 2000).

At physiological maturity, nutrients stop moving into the kernel (Schnyder & Baum, 1992; Calderini et al., 2000) and desiccation begins. During desiccation, the grain starts losing water until it reaches 12 to 13% moisture, when it is ready to harvest. At this point, there is a reduction in the kernel dimensions, due to contraction of bran layer and the protein network found in the endosperm (Lizana et al., 2010). The connective protein network of the endosperm contracts during desiccation eliminating open spaces resulting in a vitreous kernel (Anjum & Walker, 1991).

Grain Exposure to Moisture

Role of Spike

Spike morphology, especially awn length, spike angle, spike shape, and glume covering can affect the amount of water held by a spike and subsequently available for absorption into the wheat kernel. Wheat genotypes with spikes with bigger spaces between kernels are assumed to hold or collect more water during rain or a heavy dew than a more tightly packed spike. Glumes that tightly cover the grain can act as a barrier to restrict water absorption and wheat genotypes with glumes loosely adhered to the kernel allow more moisture in contact with seed (Thomason, 2009).

Moisture Absorption by Grain

Hydration kinetics of the wheat kernel is divided into two phases: Phase I, water is rapidly absorbed by bran layers and Phase II, water slowly enters endosperm (Bewely & Black, 1978). Water is unevenly distributed in the grain because the wheat grain has three major

components with very different water sorption kinetics: the bran, the endosperm, and the germ. Brooker et al. (1992) determined that the initial moisture content distribution in whole maize kernel at the moment of harvest was in average 36% moisture w.b. in the whole kernel, 48% moisture w.b. in the germ, 31% moisture w.b. in the endosperm, and 53% moisture w.b. in the pericarp. Based on mass, bran and germ absorb small amounts of water, but quickly, while the endosperm absorbs large amounts of water, but slowly.

The ratio of bran- to- endosperm in wheat is about 1:10 or 1:5 (Erling & Botterbrodt, 2008). Paquet-Durand et al. (2015) determined that the bran of wheat grain absorbed around 10% of water wt/wt and the absorption process finished within 22 min. Rathjen et al. (2009) found that the entrance of the water to the endosperm was around the scutellum and then the sub-aleurone started 7 h after imbibition. They assumed that the I_2/KI solution used to visualize the water uptake matched uptake of water. Paquet-Durand et al. (2015) reported that the endosperm absorbed the water slowly, but it absorbed ~ 50% of water wt/wt in 7 h, and the endosperm was still not completely saturated even after 48 h of soaking treatment. The starchy endosperm of wheat has a compact structure, which makes the hydration process take longer (Delcour & Hoseney, 2010).

Bran is an important barrier to water permeability (Finch-Savage & Leubner-Metzger, 2006). The main bran layers that could affect water permeability are seed coat and pericarp. Rathjen et al. (2009) found that the movement of water into the bran layer was more concentrated in the outer layers (pericarp and seed coat) and the crease (ventral surface) rather than bran covering the dorsal surface. It has been reported that the testa layer of the seed coat is resistant to water penetration in wheat (Moss, 1973). Testa has a pigment layer which has a suberized inner and outer cuticle (Black et al., 2006) that in combination with the nucellar

epidermis provides the greatest resistance to water diffusion into the endosperm (Moss, 1973). Black et al. (2006) further reported that water entered into the seed through micropyle area associated with the germ, which agrees with, Rathjen et al. (2009) who reported that the entrance of the water to the endosperm occurred around the scutellum.

Temperature and moisture are two factors that can affect the grain hydration process. As temperature or moisture increase, grain transitions from a glassy to a rubbery state, which makes the bran more permeable to water. However, it is important to consider that each bran component has a different transition temperature which would result in differences in their permeability (Miano & Augusto, 2015).

Impact of Moisture on Grain Quality

Damp conditions can result in reduced grain quality by promoting biotic stresses and abiotic stresses. Biotic stresses such as spike diseases (Blackpoint, Fusarium Head Blight, Pink Seed) and pre harvest germination of the grain and abiotic stress such as kernel bleaching and weathering.

Grain bleaching is the result of seed coat discoloration caused by damp conditions at harvest. This starts with changes in the reflective properties of the bran due to alternate wet and dry cycles, with each cycle causing slight swelling of the bran layer resulting in a wrinkling of the kernel (Bason et al., 1995; Debbouz et al., 1995). As the moisture in the bran evaporates, the bran does not contract causing it to become quite friable. Initial bleaching tends to cause a lightening of the seed coat appearance; however, as bleaching progresses the seed takes on a dull brown weathered appearance. Bleaching tends to become more pronounced as harvest is delayed (Cabas-Lühmann, 2017). Thus, a delay in harvesting durum can result in reduced grain/crop quality.

Factors such as low falling number, test weight and semolina extraction are associated with kernel bleaching (Debbouz et al., 1995; Cabas-Lühmann, 2017). The increase in kernel size, due to swollen bran layer, with no increase in weight, results in reduced test weight or density of the kernel. Low test weight can result in a decrease in the US grade classification and subsequently a reduction in value. Friable bran layer is difficult to remove during milling and often results in bran contamination in the semolina. Severe bleaching can occur with the onset of pre-harvest germination where there is an increase in activity of certain enzymes like α-amylase and proteases which affect dough and pasta rheological properties (Derera, 1989).

The appearance of bleached kernels can be similar to non-vitreous kernels. Moisture can enter the endosperm and cause it to expand and crack. These cracks cause the endosperm to appear non-vitreous. The most common method used to determine vitreous kernel content is performed through visual analysis by an inspector. This sometimes results in an incorrect classification of some grains that are bleached hard kernels but categorized as non-vitreous kernels (Dowell, 2000; Neethirajan et al., 2006).

Factors that Affect Pasta Color

Traditional pasta is known as a product made from durum wheat semolina. Semolina has a yellow color because of the carotenoids present in the endosperm. The color of durum endosperm is an important quality parameter to be measured since consumers and pasta companies desire pasta products with bright yellow color (Troccoli et al., 2000). Xanthophylls such as lutein are the most important carotenoids found in the durum wheat endosperm. Carotenoids are considered important antioxidants by quenching singlet oxygen and free radicals (Miller et al., 1996).

The color of pasta comes from more than just the yellow pigment content in the endosperm. Color is defined as a physical phenomenon light interacts with an object that the eye detects, and the brain perceives the color. In general, color is composed of hue, purity, and brightness. Hue is the spectral composition of the light leaving the object; purity is the amount in which the color is present also known as saturation; and brightness is the reflection of the light (Redlinger, 1993). Pasta color is a combination of three components yellow color, which is basically due to yellow pigments in the endosperm, brown color which is less known than yellow pigments; however, can be a consequence of enzymatic and non-enzymatic reactions (as discussed below); and finally a red color which has been reported to develop during pasta drying (Feillet & Dexter, 1996).

Dawe (2001) identified three important factors that need to be consider in pasta quality, the first was the raw material quality, the second was the production recipe, and the third was the processing conditions. In order to identify the real causes of the change in pasta color, it is relevant to consider factors such as the environment or the genotype as well as processing conditions.

Yellow Pigment Content

Yellow color in durum endosperm is caused primarily by carotenoids. Yellow pigments in durum are mainly affected by genotype (Borrelli et al., 2003). Taghouti et al. (2010) indicated that variation in yellow pigment content was due more to genotype effect than to the environment. However, the environmental conditions at the different stages of plant development can affect the final content (McCaig et al., 2006). Ramachandran et al. (2010) reported that lutein content increased from early grain filling to maturity for genotypes with high or medium yellow

pigment content. However, for genotypes with low yellow pigment content there was not a steady increase at all stages.

How the environment affects the yellow pigment content in durum wheat is not completely clear. Clarke et al. (2006) found that yellow pigment content varied in durum grown in different environments. However, it was not clear why this happened. They reported that in one environment the decline was due to frost and indirectly to kernel size and plumpness. Temperature and moisture effect is still not clear since some authors have indicated that cool temperatures with high moisture increase yellow pigment concentrations (Clarke et al., 2006) while others have reported that high yellow pigment contents were found in grain grown in environment with high temperature with no effect based on precipitation (Rharrabti et al., 2003).

The yellowness of the pasta has been related to the final yellow pigment content of the pasta (Feillet et al., 2000) and the lipoxygenase (LOX) activity during processing. Lipoxygenase indirectly causes the oxidation of α-tocopherol and carotenoid pigments, resulting in the bleaching of semolina (Sissons, 2008). Lipoxygenase oxidizes unsaturated free fatty acids to form hydro-peroxides through a free radical mechanism. Free radicals are quenched by carotenoid pigments which result in loss of color. Yemenicioglu & Ercan (1999) reported that LOX activity can be inactivated during processing by temperatures above 65°C.

Grain storage and milling can also affect the yellow pigment content in semolina (Borrelli et al., 2008). During storage, semolina ages resulting in increased fatty acid content and decreased stability of yellow pigments due to oxidation (Dahle, 1965). Borrelli et al. (1999) determined that about 8% of carotenes were lost during milling to semolina. Ash content, speck count, peroxidase activity, and polyphenol oxidase activity can increase brown hue and reduce yellow color in semolina (Kobrehel et al., 1974). Processing conditions (Dexter & Marchylo,

2001) such as kneading promotes oxidation by LOX (Borrelli et al., 2003), drying based on time and temperature, for instance, ultra-high temperature, promotes Maillard reaction which can mask yellow color of pasta (Marchylo & Dexter, 1989). The percentage of loss after processing can vary from 4 to 20% of yellow pigment content (Borrelli et al., 1999; DeSimone et al., 2010).

Brown Pigment Content

The dullness of pasta has been attributed to a brown appearance. Brightness refers to the capacity of an object to reflect the light (Redlinger, 1993). It can be measured by using colorimetric methods based on photoelectric reflectance. The colorimeter gives the brightness as L-value (AACCI methods, 2010). Brownness of pasta products has been defined as 100-L-value. Contrarily, brownness is not linked to any change in the reflectance curve. Research has indicated two possible origins of brown color, enzymatic and non-enzymatic. The non-enzymatic cause of brownness has been related to high total extraction and resulting bran contamination (Irvine, 1971), weather damage (Harris et al., 1943), and Maillard reaction. Bran contamination can lead to a brown color due to the presence of specks and high ash content. Maillard reaction is a non-enzymatic browning that occurs due to several reactions in presence of a reducing sugar and a free amino group associated with lysine which ultimately forms a brown pigment known as melanoidin. Enzymatic cause of brownness can be due to oxidative enzymes such as polyphenol oxidases (PPOs) and peroxidases (POD) (Kobrehel et al., 1974).

The genotypic variation in dull brown appearance can be due to enzymatic or non-enzymatic reaction (Irvine & Anderson, 1952). Genotypic brownness can be a consequence of a water-soluble brown-cupric protein (Matsuo & Irvine, 1967) or to polyphenol oxidases (PPOs) and peroxidases (POD) (Kobrehel et al., 1974). Feillet et al. (2000) cited Grignac (1970) who reported that the environment had a bigger impact on pasta brownness than on pasta yellowness,

while the genotype was more important for pasta yellowness than in brownness. For brightness they found that genotype was responsible for 12.6% of the response while environmental response was 67.9%.

Oxidative Enzymes

Enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) are related to color in durum wheat. Their presence can make the grain and pasta appear like dull brown color. They are unevenly distributed in the kernel. Their effect can be a consequence of the environment and/or genotype.

Polyphenol oxidase (PPO)

Polyphenol oxidases are ubiquitous copper-containing enzymes that are found in many different plants, including durum wheat (Nicolas et al., 1993). PPO catalyzes the hydroxylation of *o*-monophenols to *o*-diphenols and the oxidation of *o*-dihydroxyphenols to *o*-quinones (Van Gelder et al., 1997). The products of this oxidation react with some thiols, amines and phenolic acids such as ferulic acid and sinapic acid, which are important as substrates, forming melanins that are colored products responsible for brown discoloration.

In immature seeds, most PPO activity occurs in the endosperm, whereas when the kernel ripens, PPO activity is mostly located in the embryo and outer bran layer of the kernel, with decreasing activity in the endosperm (Marsh & Gilliard, 1986). During the milling process, PPO is removed almost totally. Although, when a portion of PPOs remain, they can cause serious problems in the color and quality of semolina pasta products (Rani et al., 2001; Demeke et al., 2001; Verlotta et al., 2010). In mature seeds about 3% of the total PPO activity of the grain is found in the wheat flour (Baik et al., 1994).

Although in durum wheat there is less PPO activity than in bread wheat, high PPO in semolina can cause the formation of brown pigment in pasta (Kobrehel et al., 1974). It has been identified that PPO activity is affected by genotype and the environment. These oxidative enzymes could be related to delayed harvest anomalies because their activity is largely affected by the environment (Baik et al., 1994). Different wheat cultivars present different levels of PPO activity (Demeke et al., 2001).

Peroxidase (POD)

Plant POD are hemeproteins that act as oxidoreductases enzymes which use the hydrogen peroxide (H_2O_2) as substrate. Hydrogen peroxide is formed during respiration. However, the origin of the required H_2O_2 is uncertain. One possibility is that during oxidation a radical phenol is formed releasing H_2O_2 (Heldt & Piechulla, 2011). The use of H_2O_2 allow them to oxidize a large number of components that act as hydrogen donors such as phenolic compounds, amines, ascorbic acid, indole, and certain inorganic ions (Gaspar et al., 1982). The general catalyzing reaction is:

$$ROOH+H_2O_2= H_2O+ ROH+{}^{1}/_{2}O_2 \quad (1.1)$$

Even though peroxidases have been considered important in plant pathogen defense; in the food they are considered negative because they can impart changes in color, flavor and nutritional value (Burnette, 1977). Pasta made from durum cultivars with high POD activity had a brownish color with a positive correlation with the brown index of pasta (Kobrehel et al., 1974). Peroxidases occur in different proportions throughout plant tissues. In wheat, POD isozymes and activity are high in the outer layers of the pericarp (epidermis and seed coat) with less activity in the starchy endosperm (Fraignier et al., 2000). Peroxidase activity depends more on genetic than on environmental factors (Feillet et al., 2000). They found that the same cultivar

grown at different locations had very similar POD activity with the real difference among cultivars with an averaged variation of 231 to 2920 A_{465}/min g among cultivars.

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CHAPTER 2: HYDRATION AND BLEACHING OF DURUM WHEAT

Abstract

Movement of moisture into dry grain can cause the grain to take on a weathered, bleached appearance. Bleaching is a concern for durum producers since the grain price is often discounted. A series of experiments were conducted to determine the effect of grain moisture content, temperature, and wet/dry cycles with bulk water and with high relative humidity on the hydration of durum wheat grain (*Triticum turgidum* L. ssp. *durum* [Desf.] Husn.) and their effect on some physical grain quality parameters related to bleaching. Water gain was greater for grain with low than high initial moisture content. At 24°C there was more water uptake than at 5°C. Exposure to a single bulk water wet/dry cycle caused durum kernels to increase in width and caused a rough and wrinkled bran surface which resulted in a decline in test weight and vitreous kernel content and an increase in kernel size and brightness. A single high relative humidity event caused the reduction of vitreous kernel content and an increase in kernel size. Additional cycles of high relative humidity were needed to cause a reduction in test weight. Micrographs from scanning electron microscope and light microscope showed that the germ and ventral surface of grain are important sites for water absorption. In conclusion, initial grain moisture, temperature, and wet/dry cycles affected water gain. A single exposure to moisture (bulk water or high relative humidity) was enough to cause a decline in grain quality. These data confirm anecdotal stories from durum producers that a single moisture event can cause bleaching and reduce grain quality and value.

Introduction

Grain bleaching is a result of bran discoloration that has been reported to happen when damp conditions prevail when the grain is ready to be harvested (Cazernecki & Evans, 1986;

Cabas-Lühmann, 2017). In the field, damp conditions exist due to rainfall, heavy morning dew, and high relative humidity. Initial exposure of grain to moisture often causes brightening of the outer bran layer which is described as being bleached. However, as bleaching progresses with prolonged exposure to moisture, the seed takes on a dull brown weathered appearance.

Bleached kernels often appear to be non-vitreous. This sometimes results in an incorrect classification of some grains by the grain inspector (Dowell, 2000; Neethirajan et al., 2006). It is important to consider that bleached grain can be vitreous if moisture has not penetrated into the endosperm. Moisture must reach and enter the endosperm in order to cause it to fracture and to become non-vitreous. Other quality factors that have been reported to be affected by damp conditions just before harvest include reduced test weight, increased kernel size and reduced falling number (Ferrer et al., 2006).

Bran protects the endosperm from biotic (disease and insects) and abiotic (moisture) stressors. Bran is composed of pericarp and seed coat. The pericarp is the outer bran layer. Pericarp represents about 5% of the kernel and it has around 6% protein, 2% ash, 20% cellulose and 0.5% fat and it is comprised of epidermis, hypodermis, thin-wall cells, intermediate, cross, and tube cells (Delcour & Hoseney, 2010). The inner bran layer is comprised of the seed coat (testa and nucellar epidermis). Aleurone layer is a single cell layer which botanically is part of the endosperm, but in the milling discipline, bran also includes the aleurone layer since the aleurone layer is tightly attached to the seed coat and is removed with the bran during milling. Aleurone layer completely surrounds the kernel, covering the starchy endosperm and the germ (Delcour & Hoseney, 2010).

Each bran layer has a different chemical composition and differs in its water permeability (Finch-Savage & Leubner-Metzger, 2006; Rathjen et al., 2009). Testa, which is a layer of the

seed coat, has a pigment layer with a suberized inner and outer cuticle (Black et al., 2006). The pigment layer in combination with the nucellar epidermis provides the greatest resistance to water diffusion into the endosperm (Moss, 1973). The main pathway for water uptake is lateral from the embryo where the outer cuticle is incomplete through the pericarp (Derera, 1989).

Initially, a small amount of moisture is absorbed rapidly by the bran layers, reflecting the bran-endosperm mass difference (1:10) (Erling & Botterbrodt, 2008). Conversely, the endosperm slowly absorbs a large amount of water (Delcour & Hoseney, 2010; Paquet-Durand et al., 2015). Hydrated kernels undergo rapid expansion of the bran layer and the slow formation of tiny fissures in the endosperm. During dry periods following damp conditions, the fissures remain in the endosperm and the bran layer does not contract to its original size, which results in an increase in kernel size without an effect on kernel weight (Debbouz et al., 1995). Drying causes the bran to become friable, which can reduce the milling quality of the grain.

During harvest time, there are different factors that could be involved in the rate of moisture penetration into the kernel such as initial grain moisture, temperature, and wet/dry events due to rainfall, dew, or high relative humidity. There is limited scientific information about the causes of bleaching and since grain bleaching can be detrimental for both durum growers and end-use industry it is important to determine what are the most probable causes. This research was conducted to evaluate the hydration of the grain as affected by initial grain moisture, temperature, and consecutive wet/dry cycles using bulk water and high relative humidity, as well as their effect on some grain physical quality parameters related to bleaching.

Materials and Methods

Plant Material and General Procedure

To understand the hydration of durum wheat grain, five experiments were conducted using three unique bulk samples of durum wheat. The samples were a blend of good quality grain (test weight ≥ 80 kg/hL; vitreous kernel content ≥ 80%) collected from advanced genotype trials that were conducted in 2017 and 2018 and from durum survey harvest samples.

The general procedure consisted of soaking grain in distilled water. Grain weight depended on the experiment. Initially, the grain was gently stirred for 5 sec to eliminate air pockets that could form and restrict exposure of grain to water. At the appropriate time, the grain was removed from water using a colander. The surface water was eliminated by using a paper towel. The sample was weighed immediately and the water gain was calculated using the following formula:

$$\textbf{Water gain (\%)} = [(\text{sample}_{wet} - \text{sample}_{dry,\ as\ is})/\ \text{dry sample}_{dry,\ as\ is}] \times 100 \qquad (2.1)$$

The water absorbed by the grain was estimated from the water gained as a g/100 g of kernels. Soaking times were determined based on preliminary experiments. Samples were allowed to dry at room temperature to ~12%. Quality analyses were performed on dried grain.

Experiment 1. Initial Grain Moisture by Soaking Time-Bulk Water

Samples from newly harvested grain were collected during the annual durum crop quality survey. Moisture content was determined on all samples as they were received. A set of durum samples were collected that had harvest grain moisture contents of 13, 14, 15, 16 and 17%. These samples were stored in sealed plastic containers at 4°C. The experiments were conducted soon after receiving the samples to minimize moisture loss and the chance of spoilage. An individual grain sample was prepared per each soaking time. Grain (5 g) was soaked in distilled water (25

mL) 20 times (from 0 to 3 h). Because of the small sample size grain, quality analysis could not be performed but water gain was determined.

Experiment 2. Temperature by Soaking Time - Bulk Water

The experiment was designed to determine the effect of the temperature and soaking time on the rate of water absorption into the wheat kernel. The initial kernel moisture content was ~ 12%. Soaking temperatures were 5 and 24 °C. These temperatures were selected because they reflect air temperatures that commonly occur during the harvest season in North Dakota. Soaking times (20) ranged from 0 to 30 min. The grain quality parameters performed for this experiment were: kernel moisture content determined by FOSS Infratec™ 1241 Grain Analyzer (FOSS Tecator, Hogonas, Sweden); test weight (kg/hL) determined by AACCI approved method 55-10.01; 1000-kernel weight was determined by counting the number of kernels in 10 g of cleaned grain with an electronic seed counter (Seedburo Equipment Co., Chicago, IL); vitreous kernel content was determined by cutting 100 kernels using a farinator; kernel color determined by Minolta CR410 colorimeter (Konica Minolta, Ramsey, NJ) L*, *a**, and *b** color values; and kernel size distribution determined using the method described by Shuey (1960); where kernels were classified as large when remained on Tayler No 7 sieve with 2.92 mm opening (top sieve); medium when they remained on Tayler No 9 sieve with 2.24 mm opening (middle sieve); and small kernels passed directly through both sieves.

Experiment 3. Wet/dry Cycles – Bulk Water

A series of wet/dry cycles were conducted to determine the effect of multiple exposures on moisture absorption into the kernel. Grain was subjected to four wet/dry cycles. For each cycle, grain (350 g) was soaked in distilled water (0, 15 sec, 1 min, 6 min, 1 h, 3 h, 6 h, 9 h, and 12 h) and allowed to dry to about 12% moisture. An individual grain sample was prepared per

each soaking time. The grain quality parameters, kernel moisture content, test weight, vitreous kernel content, 1000-kernel weight, kernel color, and kernel size distribution were determined as described above; Additional tests included yellow pigment content (whole wheat, bran, and semolina) determined by modified AACCI approved method 14-50.01 using 2 g of sample instead of 8 g. Polyphenol oxidase activity (whole wheat, bran, and semolina) determined by AACCI approved method 25-85.01; and peroxidase activity determined by Honold & Stahmann (1968) and Fraignier et al. (2000) modified methods.

Experiment 4. Wet/dry Cycles - High Relative Humidity

Grain was subjected to four wet/dry cycles. For each cycle grain (350 g) was exposed to relative humidity (80 to 85%) in a humidity chamber (Standard, Model 3 5-0 M, Oklahoma, USA) kept at constant 25°C for 6 and 12 h using 0 h as control. An individual grain sample was prepared per each exposure time. During each cycle the grain was allowed to dry to about 12% moisture. The grain quality parameters performed for this experiment were: kernel moisture content; test weight; 1000-kernel weight; vitreous kernel content; kernel color; and kernel size distribution.

Morphological Changes in the Kernels after Moisture Exposure

Scanning electron microscope was used to compare the effect of wet/dry cycles using bulk water and relative humidity on kernel morphology. Kernels selected randomly from untreated, kernels soaked in bulk water for 15 sec and soaked for 12 h for 1 cycle and 4 cycles and kernels that were exposed to high relative humidity for 12 h for 1 cycle and 4 cycles were used. Images were taken of the surface, cross section, and germ of the kernel and two kernels were evaluated per treatment. Kernels were mounted on cylindrical aluminum mounts with colloidal silver paste (Structure Probe Inc., West Chester PA, USA). Images were obtained using

JEOL JSM-6490LV Scanning electron microscope (JEOL USA, Peabody MA, USA) at an acceleration voltage of 15 KV.

After moisture treatment described above, kernels (50) were selected randomly for each treatment to measure the kernel length, width and thickness using a digital caliper (model 147, General Tools & Instruments, New York, NY) with an accuracy of 0.01 mm on the images obtained from a microscope camera (model OT-M, Opti-TekScope, Chandler, AZ) (Figure 3). The microscope camera was placed 10 cm from the base which captured the 10-kernel image with adjustable focus lens. The volume of the grain was calculated through the equation described by Al-Mahasneh & Rababah (2007):

$$V = \frac{\pi B^2 L^2}{6(2L-B)} \quad (2.2)$$

Where:

L= kernel length (mm); W= kernel width (mm); T= kernel thickness (mm); and B= $\sqrt{WT}$

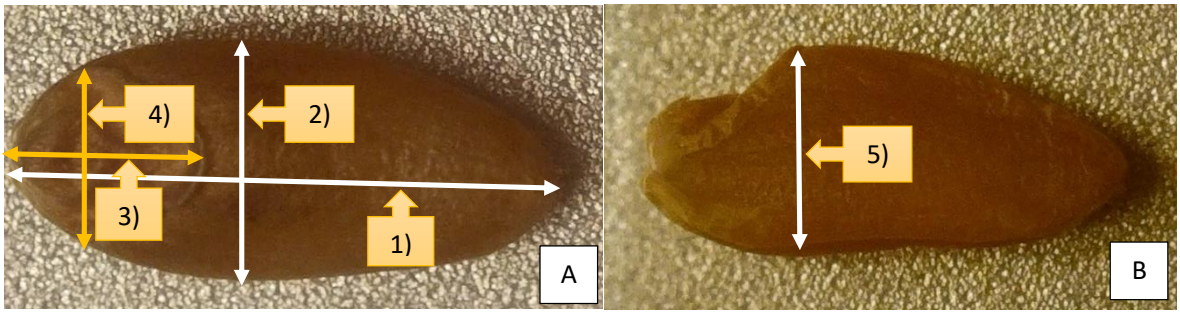

Figure 3. Kernel measurements. A1) kernel length. A2) kernel width. A3) germ length. A4) germ width. B5) kernel thickness.

Experimental Design and Statistical Analysis

The experimental design for all four experiments was a randomized complete block with three replicates in split plot in time arrangement. Main plots were: Experiment 1, moisture content; Experiment 2, temperature; Experiments 3 and 4, wet cycles. For all experiments, subplot was soaking time.

Temperature, moisture, cycles and time were considered fixed effects. All data collected were subjected to analysis of variance (ANOVA) at 95% level of confidence (F tests: $P \leq 0.05$). Means were separated by Fisher's-Protected LSD at $P=0.05$. Statistical analysis was performed using SAS version 9.4 (SAS Inst., Cary, NC) was used to evaluate significance and interaction effects.

Results

Moisture Content at Harvest by Soaking Time

Initial kernel moisture content × soaking time interaction was not significant for water gain as observed by the similarities of the curves in Figure 4.

Initial kernel moisture content and soaking time main effects were significant for water gain at $P \leq 0.05$. In general, as initial kernel moisture content increased, the amount of moisture absorbed by the kernel decreased. When averaged over soaking time, greatest amount of water (7.5 g/100 g of kernels) was absorbed by kernels with 13 and 14% moisture content, intermediate amount (6.9 and 6.3 g/100 g of kernels) with 15 and 16% moisture, respectively and least amount (5.6 g/100 g of kernels) by kernels with 17% moisture content ($LSD_{0.05}= 0.5$). This is equal to an increase of 0.9% of moisture content at 13% moisture content at harvest and 0.7% at 17% moisture content at harvest. For all initial kernel moisture contents, there was a very rapid increase in moisture content within the first 0.25 min with the rate of moisture uptake declining

with soaking time (Figure 4). When averaged over initial kernel moisture content, weight increased 1.5 g/100 g of kernels after 0.25 min soaking, 4.8 g/100 g of kernels after 5 min, 8.6 g/100 g of kernels after 30 min, and 19.3 g/100 g of kernels after 180 min ($LSD_{0.05}$ = 1.1).

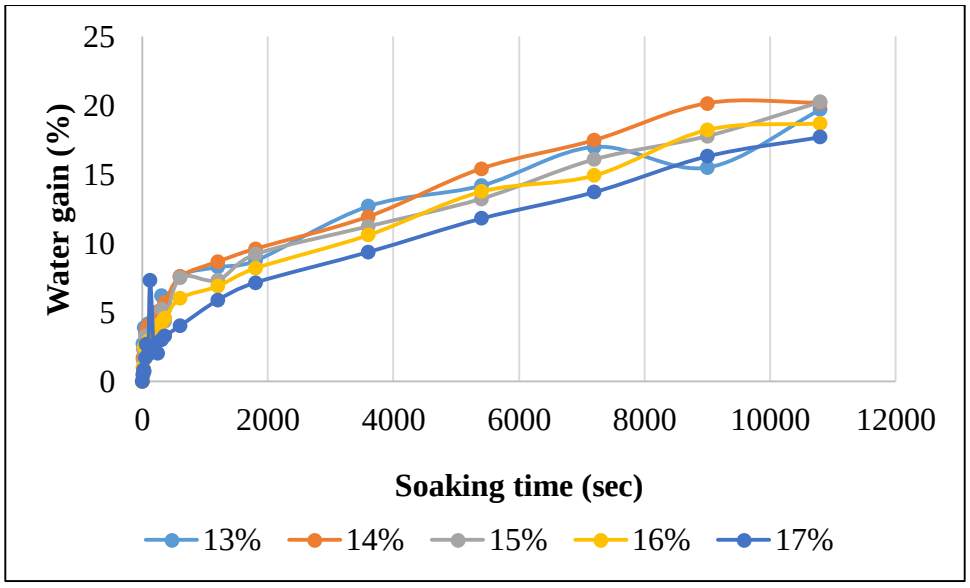

Figure 4. Water gain percent due to moisture content at harvest × soaking time interaction.

Temperature by Soaking Time

Temperature × soaking time interaction was not significant at $P \leq 0.05$ for water gain or any grain quality parameter evaluated. Temperature and soaking time main effects were significant (Table 1).

When averaged over soaking time, moisture gain was greater at 24 than at 5 °C (Table 1). Figure 5 shows the similar behavior of the water absorbed by the grain at 5 and 24 °C. At both temperatures, there was rapid initial water absorption followed by a slower rate. When averaged over temperature, kernels gained 5.6 g/100 g in weight after 0.25 min of soaking which

represents 52% of the total weight gain (10.8 g/100 g) after 30 min. From 10 to 30 min the weight increase was quite constant with 1.7 g/100 g increased.

Table 1. Means for water gain and grain quality parameters related to bleaching averaged across temperature and soaking times.

Treatment	Water gain	Test weight	KWT	VK	CIE L	Large grain
T° (°C)	g/100 g	kg/hL	g	%		%
5	6.8	79.9	48.7	70	53.82	78
24	7.2	79.8	49.0	73	54.21	83
LSD (0.05)	0.2	0.1	0.3	1	0.35	1
Control	0.0	82.4	48.3	80	53.48	78
Soaking time (sec)						
15	5.6	79.8	48.9	77	54.91	80
300	7.9	79.3	49.0	71	53.32	81
600	9.1	79.2	49.6	68	53.99	82
1200	10.1	79.2	48.9	67	53.60	82
1800	10.8	79.3	49.4	67	54.32	83
LSD (0.05)	0.5	0.2	0.9	2	1.11	2

KWT= thousand kernel weight; VK= vitreous kernel content; LSD represents significant differences at 95% level of confidence.

Temperature main effect was significant ($P \leq 0.05$) for the quality parameters evaluated except for 1000- kernel weight (Table 1). The effect of temperature was relatively small, particularly in comparison with effect of soaking time. In general, vitreous kernel content, CIE L*, *a**, and *b** values, and kernel size were greater at 24 than 5°C. Conversely, test weight was lower at 24 than 5 °C. Soak time main effect was significant ($P \leq 0.05$) for test weight, vitreous kernel content, CIE *b**-value, and large kernel size and not significant for CIE L* and *a**-values. Test weight and vitreous kernel content decreased and CIE *b**-value and large kernels increased with soaking time. The effect of soaking time on 1000-kernel weight was variable and did not

consistently increase with time. When compared to the untreated control, it is evident that most of the effect of soaking occurred with 15 sec of soaking. This is particularly true for test weight which decreased from 82.4 to 79.8 kg/hL ($LSD_{0.05}$= 0.2), vitreous kernel content from 80 to 77% ($LSD_{0.05}$= 2.2) and large kernel from 78 to 80% ($LSD_{0.05}$= 1.7) (Table 1).

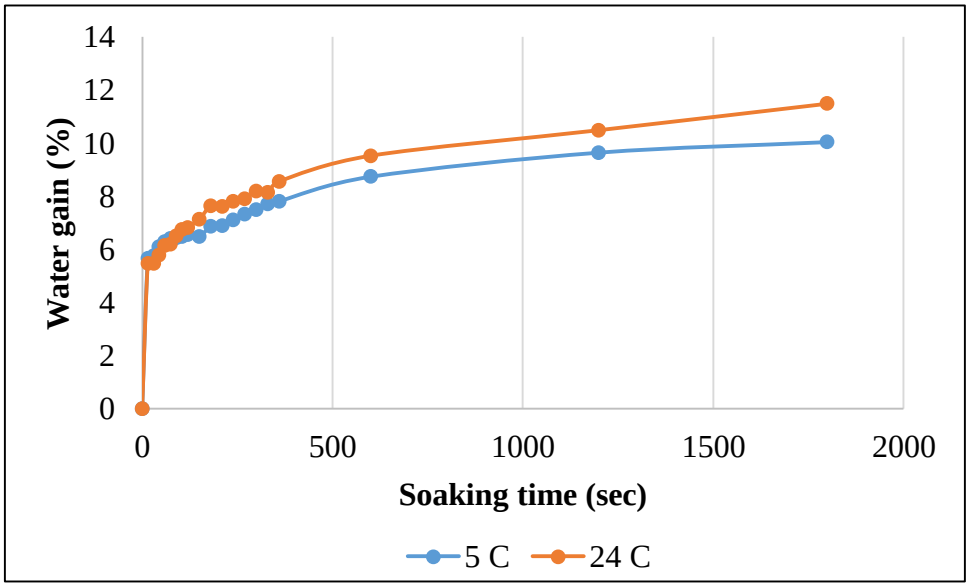

Figure 5. Water gain percent due to temperature x soaking time interaction.

Wet/dry Cycles- Bulk Water

Bleaching has been reported after consecutive wet/dry cycle events (Bason et al., 1995). During preliminary experiments changes were noticed in the quality of the grain, for this reason, additional quality parameters were added. Means due to main effects are summarized in Table 2.

Wet/dry cycle × soaking time interaction was significant for water gain. At all cycles, the shape of the curves was similar, with a rapid initial water absorption followed by a slower rate (Figure 6). There was a rapid increase in weight from 0 to 0.25 min ~ 8 g/100 g of initial kernel weight with cycles 1 and 2, 9 g/100 g of kernels with cycle 3 and 11 g/100 g of kernels with cycle 4. With short soaking times, water absorption was greater with more wet/dry cycles. The

rate of water uptake diminished over time. For example, the rate of water uptake was 0.06 g/min at 12 h (gain of 45 g of water in 720 min) of soaking compared to 9.7 g/min after 1 min of soaking.

Table 2. Means for water gain and grain quality parameters due to bulk water wet/dry cycles and soaking time main effects.

Treatment	Water gain	Test weight	KWT	VK	CIE L	Large grain
Cycle	g/100 g	kg/hL	g	%		%
1	20.8	78.0	41.1	68	54.70	57
2	20.7	77.5	40.8	65	54.56	56
3	21.3	77.3	41.0	62	55.19	58
4	22.5	76.9	41.2	62	55.87	59
LSD(0.05)	0.5	0.2	0.6	2	0.62	1
Control	0.0	81.5	41.8	95	52.80	51
Soaking time (min)						
0.25	8.5	78.7	41.0	89	54.07	54
1	9.7	78.6	40.9	88	53.89	56
6	12.0	78.5	40.7	85	53.96	58
60	17.2	78.0	41.6	82	54.20	59
180	26.8	77.0	41.4	55	55.40	58
360	33.6	76.0	41.0	45	56.76	60
540	39.6	75.0	40.3	26	57.61	59
720	44.5	73.9	40.3	13	57.05	63
LSD(0.05)	0.7	0.3	0.9	2	0.92	1

KWT= thousand kernel weight; VK= vitreous kernels content; LSD represents significant differences at 95% level of confidence.

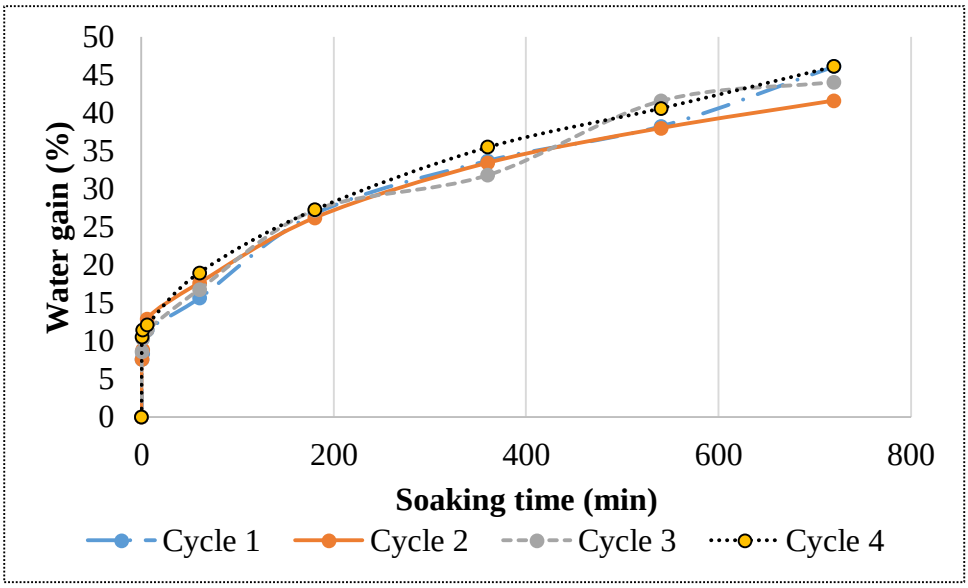

Figure 6. Water gain percent due to wet/dry cycles x soaking time interaction.

Wet/dry cycle x soaking time interaction was significant for test weight, vitreous kernel content, and kernel brightness but not for 1000-kernel weight. For all wet/dry cycles, test weight decreased with soaking time. Similar to temperature experiment above, test weight was reduced after only 0.25 min of soaking (Figure 7A). The differences in test weight among wet/dry cycles did not begin until 180 or more min of soaking, after which test weight was generally lower as the number of wet/dry cycles increased. Regardless of wet/dry cycle, vitreous kernel content declined slowly with soaking time up to 60 min, after which there was a sharp decline in vitreous kernel content. It appears that at some time between 60 min to 180 min, moisture entered the endosperm causing it to fracture and become non-vitreous. At 60 min vitreous kernel content was similar with 1, 2, and 3 wet dry cycles but there was a pronounced decline in vitreous kernel content after four wet/dry cycles (Figure 7B). Kernel brightness (L*-value) generally increased with soaking time. Kernel brightness was greater after four wet/dry cycles than after one, two, or three cycles, which tended to result in similar brightness (Figure 7C). Kernel size increased with

soaking time with noticeable increase in size after only 0.25 min of moisture, regardless of wet/dry cycle. With 180 min or more soaking, kernel size was greater as the number of wet/dry cycles increased (Figure 7D).

Soaking time had little or no effect on POD activity in whole wheat, bran, or semolina. In general, POD activity in whole wheat, bran and semolina was lower after four wet/dry cycles (Figure 8 A, B, C). For PPO activity, soaking time had little effect in whole wheat or bran but activity in semolina increased with soaking time. Wet/dry cycles did not affect PPO activity in whole wheat or semolina but activity in bran seemed to be greater after four than one cycle (Figure 9 A, B, and C).

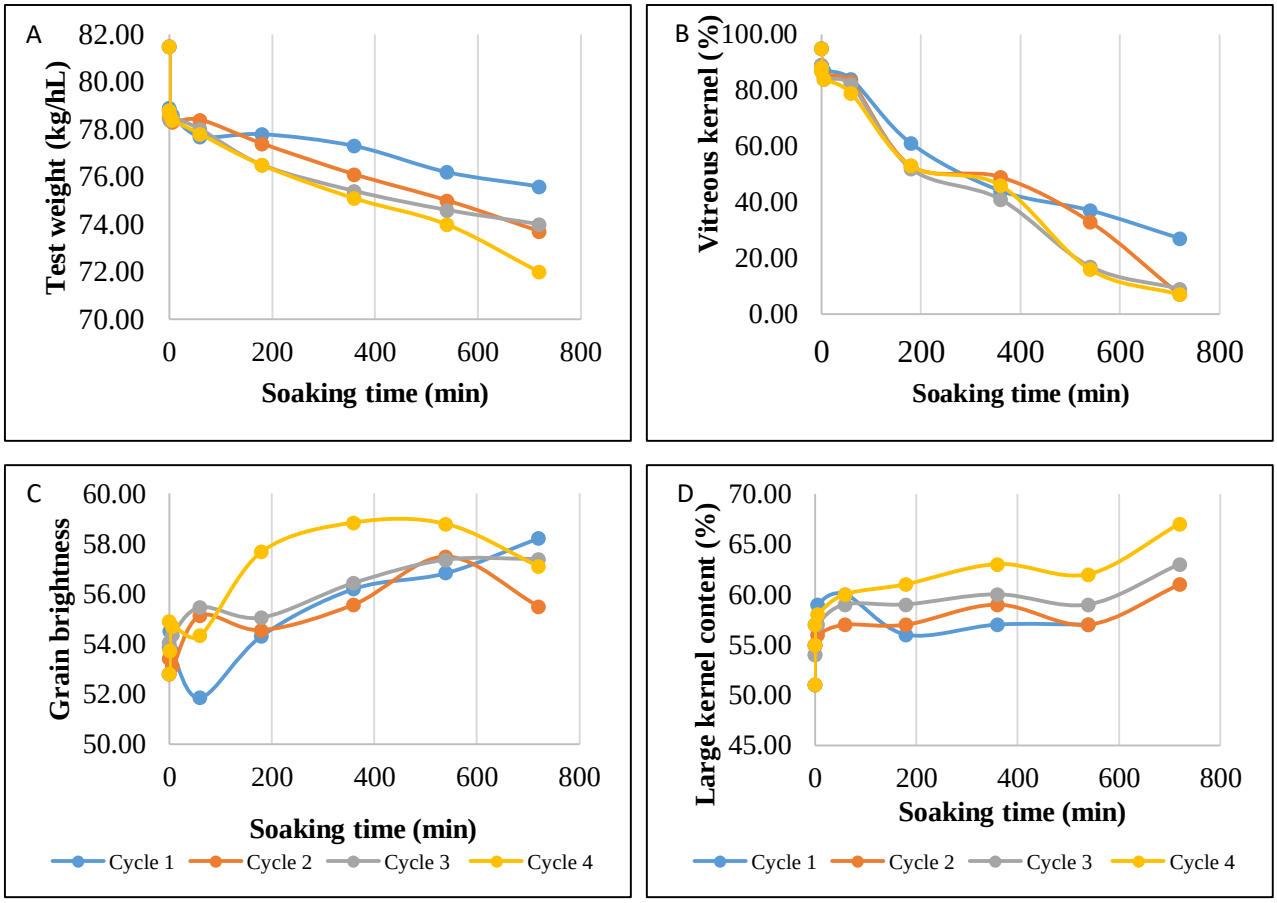

Figure 7. Effect of bulk water wet/dry cycles x soaking time interaction in: A) test weight; B) vitreous kernel content; C) grain brightness; D) large kernel content.

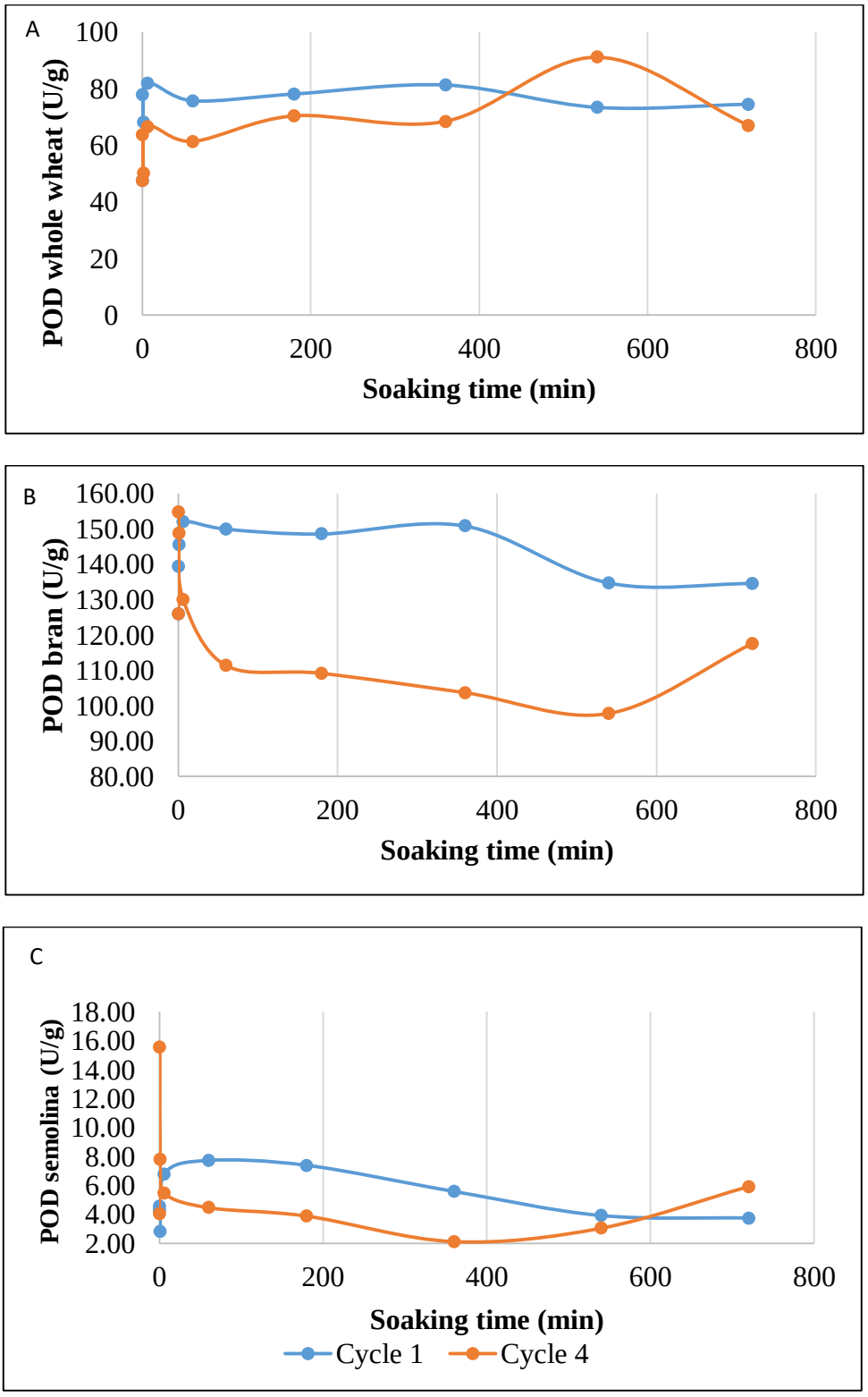

Figure 8. Effect of bulk water wet/dry cycles x soaking time interaction in POD activity in A) whole wheat; B) bran; C) semolina.

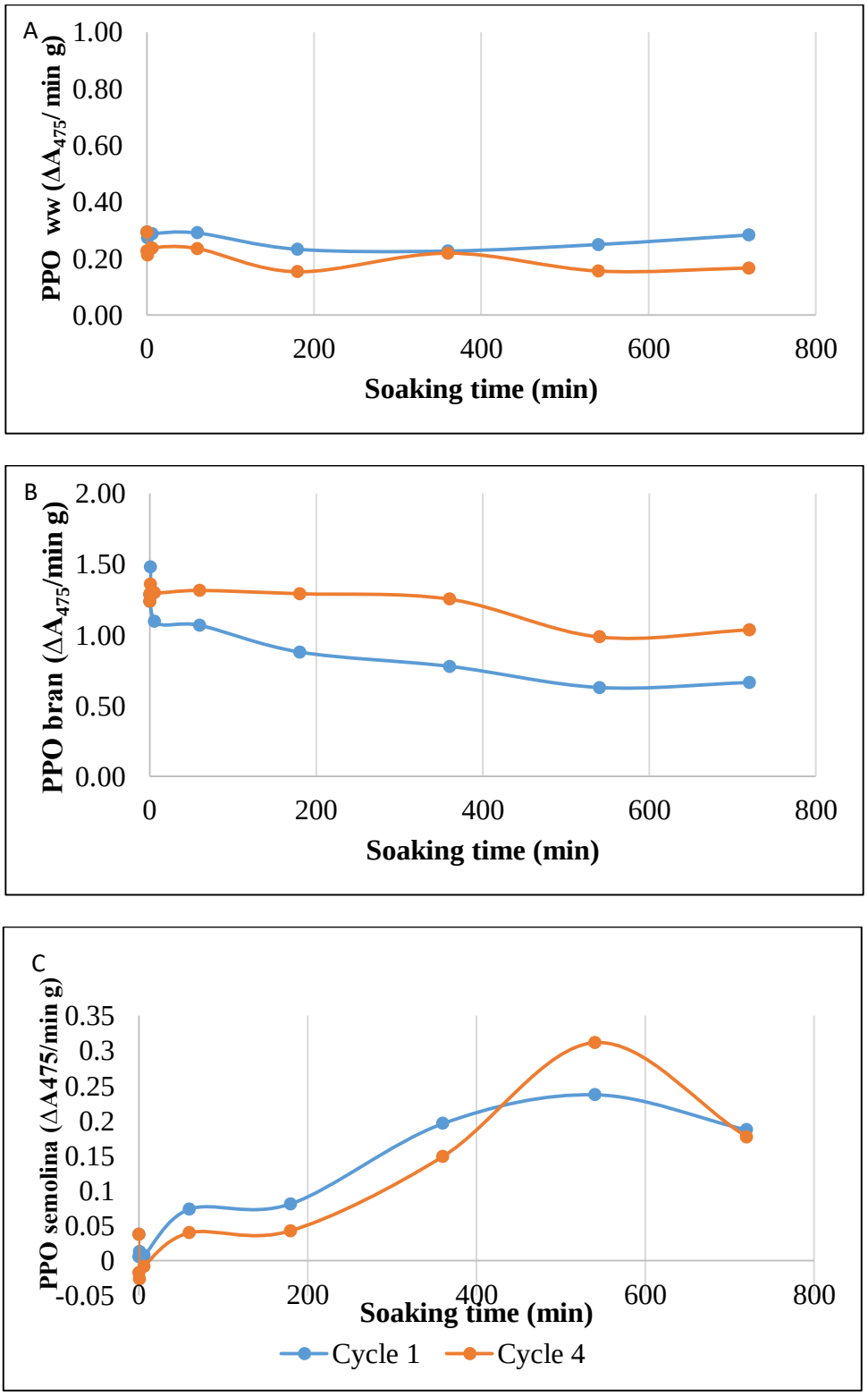

Figure 9. Effect of bulk water wet/dry cycles x soaking time interaction in PPO activity in A) whole wheat; B) bran; C) semolina.

Wet/dry Cycles-High Relative Humidity

Wet/dry cycles based on high relative humidity × exposure time interaction was significant for water gain and test weight ($P \leq 0.05$). Moisture absorbed increased with increased exposure time to high relative humidity. Moisture gained was greater with 3 and 4 wet dry cycles than with 1 and 2 wet/dry cycles (Figure 10). Cycles 1 and 2 were similar (averaged increase = 2.8 g/100 g of kernels and 3.1 g/100 g of kernels, respectively) and cycles 3 and 4 were similar in moisture absorption with exposure time as well as cycles 3 and 4 (5.1 and 5.7 g/100 g of kernels, respectively). The average difference in water gain between cycles 1 and 4 was 2.8 percentage units. With cycles 1 and 2, the weight gained from 0 to 6 h was (~4 g/100 g of kernels) while the weight gained with cycles 3 and 4 was (~7 g/100 g of kernels). From 6 to 12 h the increase in weight was similar for all cycles (~ 1 g/100 g of kernels). Test weight was similar for control and one cycle and for all exposure times during cycle 1 and ranged from 81.1 to 81.3 kg/hL. After cycle 2, 3 and 4, there was a reduction in test weight from 0 to 6 h equal to 0.7, 0.3, and 0.3 kg/hL, respectively. While from 6 to 12 h the reduction was similar for all cycles ~0.3 kg/hL (Table 3).

A single wet/dry cycle did not reduce test weight but did reduce vitreous kernel content, and increase kernel brightness and kernel size (Table 3). Cycle main effect was significant for kernel brightness $P \leq 0.05$. Although significant, there was no clear trend observed throughout the cycles. High relative humidity wet/dry cycles did not further affect vitreous kernel content, kernel brightness or kernel size. Compared to untreated control, a six-hour exposure to high relative humidity reduced test weight, and vitreous kernel content and increased kernel brightness and size. Time of exposure main effect reduced vitreous kernel content from 89 to

73%, increased kernel brightness from 52.81 to 54.66, and increased large kernel size content from 54 to 57%.

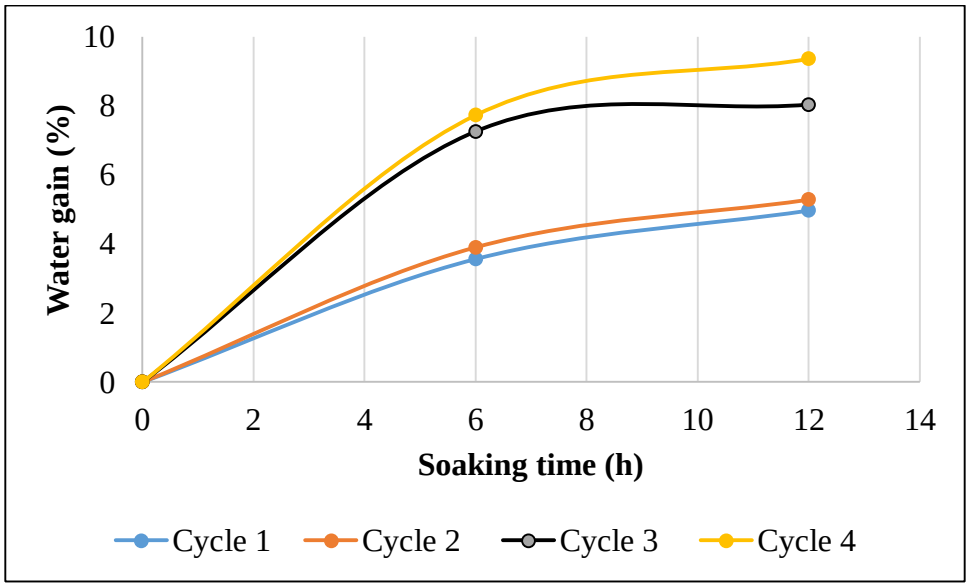

Figure 10. Water gain percent due to wet/dry cycles x relative humidity time of exposure interaction.

Table 3. Means for water gain and grain quality parameters related to bleaching averaged across cycles and relative humidity exposure times.

Treatment	Water gain	Test weight	VK	CIE L*	Large grain
Cycle	%	kg/hL	%		%
1	2.9	81.2	81	53.75	56
2	3.1	80.7	80	52.86	56
3	5.1	80.9	80	54.19	55
4	5.7	80.9	80	54.11	55
LSD (0.05)	0.3	0.2	2	1.12	0.8
Control	0.0	81.3	89	52.81	54
Exposure to high relative humidity (h)					
6	5.6	80.9	79	53.71	55
12	6.9	80.6	73	54.66	57
LSD (0.05)	0.3	0.2	2	0.97	1

VK= vitreous kernels content; LSD represents significant differences at 95% level of confidence.

Kernel Dimension After Wet/Dry Cycles Treatment

Electron microscope images showed that control samples had organized bran layers with almost no space between bran layers and endosperm (Figure 11 A1 and A2), although there was a small opening in the aleurone layer surrounded the germ (Figure 11 A3). The bran on the dorsal surface was quite smooth (Figure 11 A4). Samples treated with four wet/dry cycles for 15 sec in bulk water showed signs of bran swelling (Figure 11 B2, B3, and B4). There was slight separation of pericarp from the seed coat along the endosperm (Figure 11 B2) and more pronounced separation along the germ (Figure 11 B3). Bran on the dorsal side showed some surface roughening (Figure 11 B4). Samples treated with four wet/dry cycles for 12 h in bulk water had greater separation of pericarp from seed coat along the endosperm (Figure 11 C2 vs B2) and between pericarp and seed coat and between seed coat and embryo (Figure 11 C3 vs B3) and had much rougher bran on the dorsal surface (Figure 11 C4 vs B4) compared to samples exposed for only 15 sec. Samples exposed to four wet/dry cycles of high relative humidity also had some separation of pericarp from the seed coat along the endosperm (Figure 11 D2); separation between pericarp and seed coat and between seed coat and germ (Figure 11 D3); and roughening of the bran on the dorsal surface (Figure 11 D4). Comparing the SEMs, it appears that 15 sec of bulk water and 12 h of high relative humidity had a similar effect on the bran.

The swelling of bran layers and roughening of bran surface when exposed to high relative humidity or bulk water was reflected in the increase in large kernel content (Tables 2 and 3). The effect of exposure time was significant and was greater with 12 than 6 h of high relative humidity and with 12 h than 15 sec of bulk water. Swelling of bran layers and roughening of bran surface when exposed to bulk water resulted in an increase in kernel width, kernel surface area and kernel volume (Table 4). When compared to the untreated control kernels, there was

little to no effect of bulk moisture on kernel length or kernel thickness. The effect of wet/dry cycle was manifested after the first exposure of either high relative humidity or bulk water with little or no further increase in large kernels, kernel width, surface area or volume with additional cycles (Tables 2 and 3).

Table 4. Means for kernel dimensions as affected by cycles and soaking time main effects.

Treatment	Length	Width	Thickness	SA	Volume
		mm		mm^3	mm^3
Cycle					
1	8.0	3.3	3.4	53.1	3.3
2	7.9	3.3	3.4	53.4	3.4
3	8.0	3.4	3.4	54.5	3.4
4	8.0	3.3	3.3	52.4	3.3
LSD (0.05)	0.1	0.1	0.2	2.1	0.1
Control	7.9	3.1	3.3	49.6	3.2
Soaking time (min)					
0.25	7.9	3.2	3.3	50.8	3.2
720	8.0	3.4	3.4	54.3	3.4
LSD (0.05)	0.1	0.1	0.1	1.4	0.1

L/W= Length/width; SA= surface area; LSD represents significant differences at 95% level of confidence.

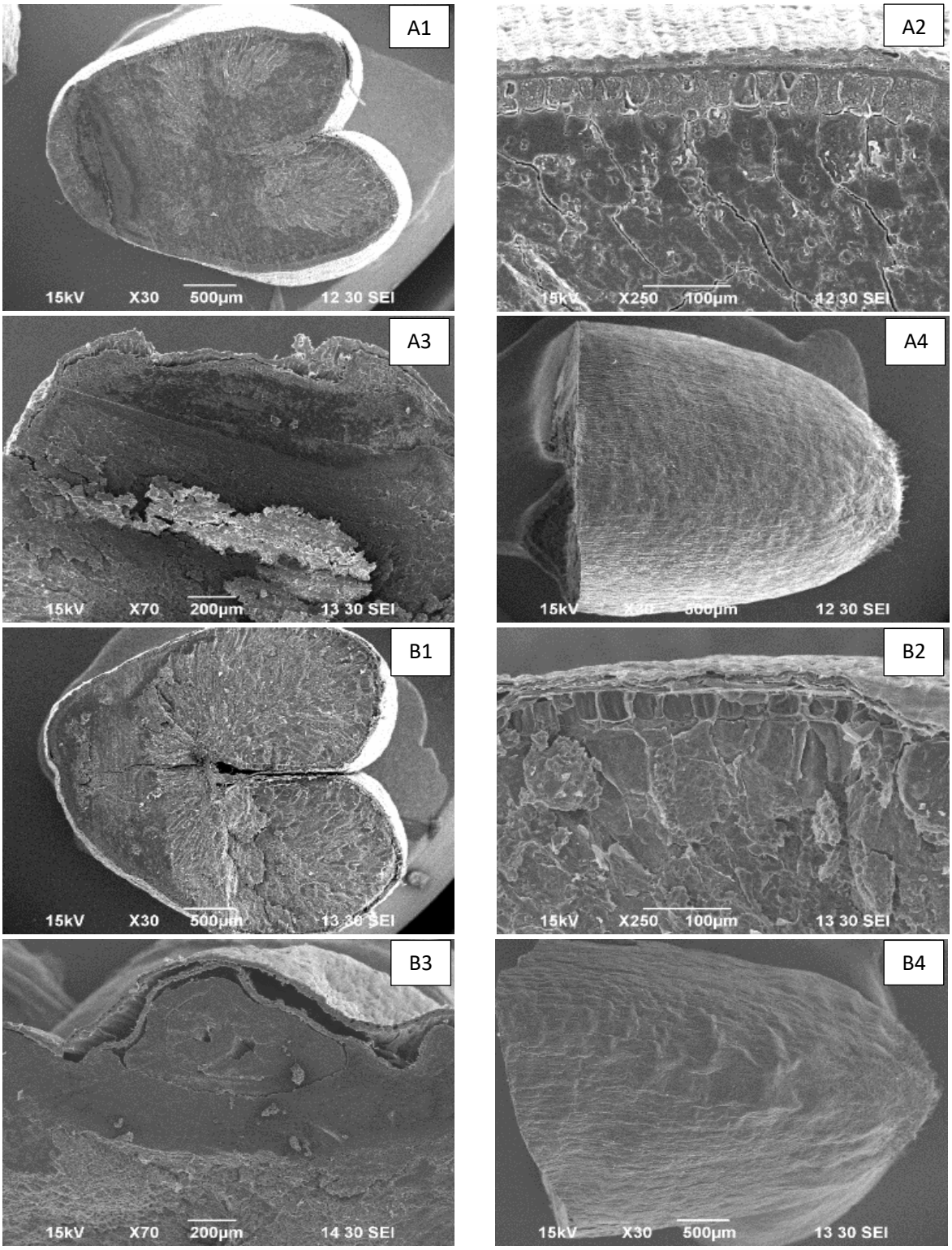

Figure 11. Electron microscope images. A1) control half section, A2) control bran layer, A3) control germ, A4) control surface; B1) 15 sec-C4 half section B2) 15sec-C4 bran layers, B3) 15sec-C4 germ, B4) 15 sec-C4 surface.

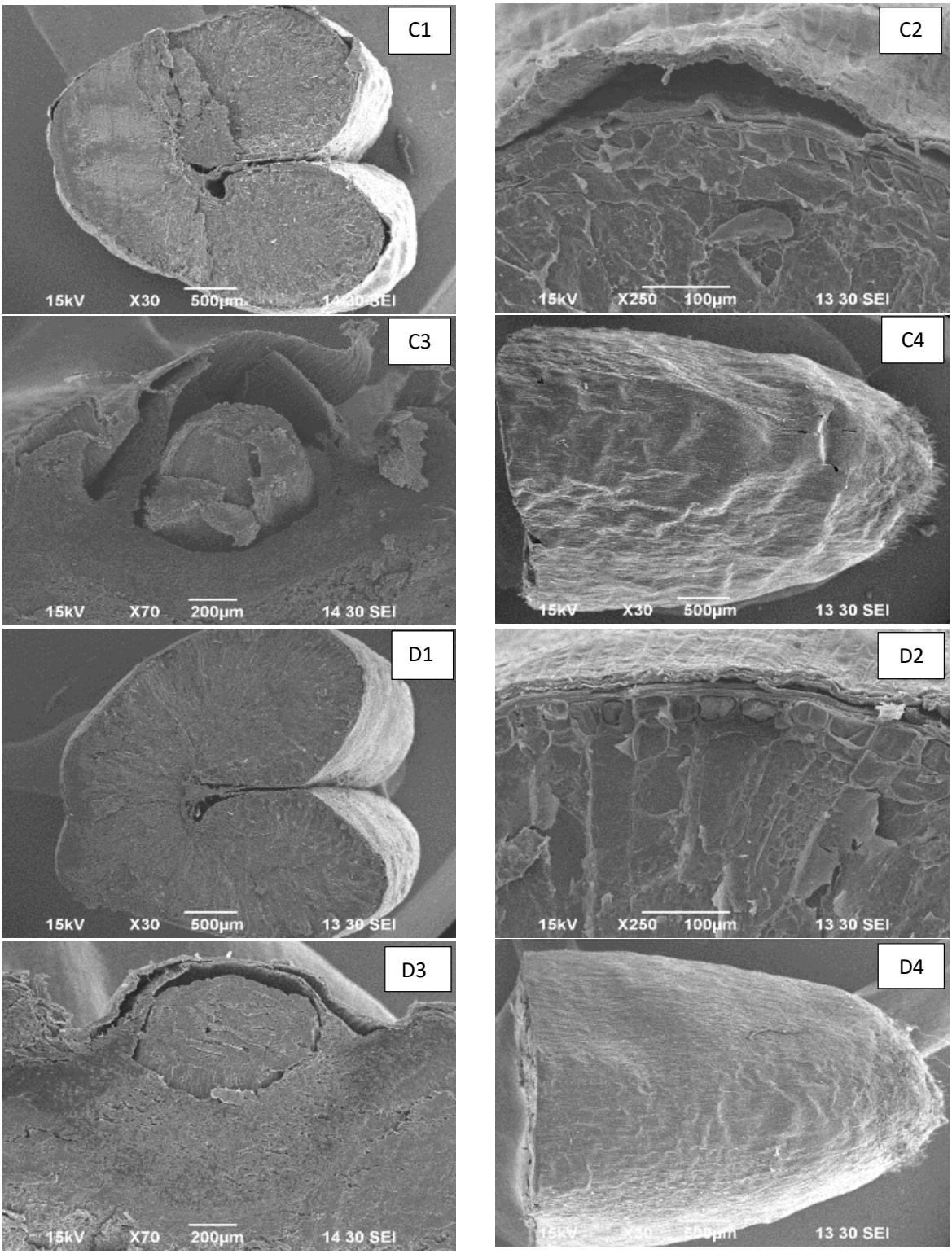

Figure 11. Electron microscope images (continued). C1) 12h-C4 half section; C2) 12h-C4 bran layer, C3) 12h-C4 germ, C4) 12h-C4 surface; D1) 12h-C4RH half section, D2) 12h-C4RH bran layer, D3) 12h-C4RH germ, D4) 12h-C4RH surface.

Discussion

Grain is composed of three main structures which differ in hydrophilicity: the bran, the germ, and the endosperm. Based on mass, bran and germ absorb small amounts of water, but quickly, while the endosperm absorbs large amounts of water, but slowly. The ratio of bran- to-endosperm in wheat is about 1:10 to 1:5 (Erling & Botterbrodt, 2008). Paquet-Durand et al. (2015) determined that the bran of wheat grain absorbed around 10% of water wt/wt and the absorption process was finished within 22 min. The seed coat is found under the pericarp layer. Moss (1973) reported that the testa layer of the seed coat is resistant to water penetration. Testa has a pigment layer that has a suberized inner and outer cuticle (Black et al., 2006) that in combination with the nucellar epidermis provides resistance to water diffusion into the endosperm. Because bran is hydrophilic and readily absorbs water the results of this study showed that even grain harvested at 17% moisture content was still found to absorb water (Figure 4). The amount of water absorbed increased as initial grain moisture content decreased. Regardless of conditions tested in this research, there was a rapid initial absorption rate of moisture by the seed, as seen in Figures 4 to 6.

At the end of the germ, there is a small gap or hole in the bran coverage of the kernel called the micropyle. The micropyle area is an important site of moisture movement into the wheat kernel (Black et al., 2006). Micropyle is a very small opening that is found just beneath the hilum, which is the point of attachment of the seed to the rachis of the spike. Light microscope images were taken to visualize the movement of water after 12 h moisture exposure (Figure 12B) using zero exposure as control (Figure 12A). The loss of vitreous kernel content of the endosperm shows that water moved into the endosperm at the basal ventral side of the kernel where the crease is located rather than the dorsal surface which agreed with the results found by

Rathjen et al. (2009). SEM micrographs also show that the biggest effect of moisture was associated with the germ area of the kernel (Figure 11 B3, C3, D3) and other reports of the germ area being the primary site of moisture absorption into the wheat kernel which comes in contact with the endosperm (Rathjen et al., 2009).

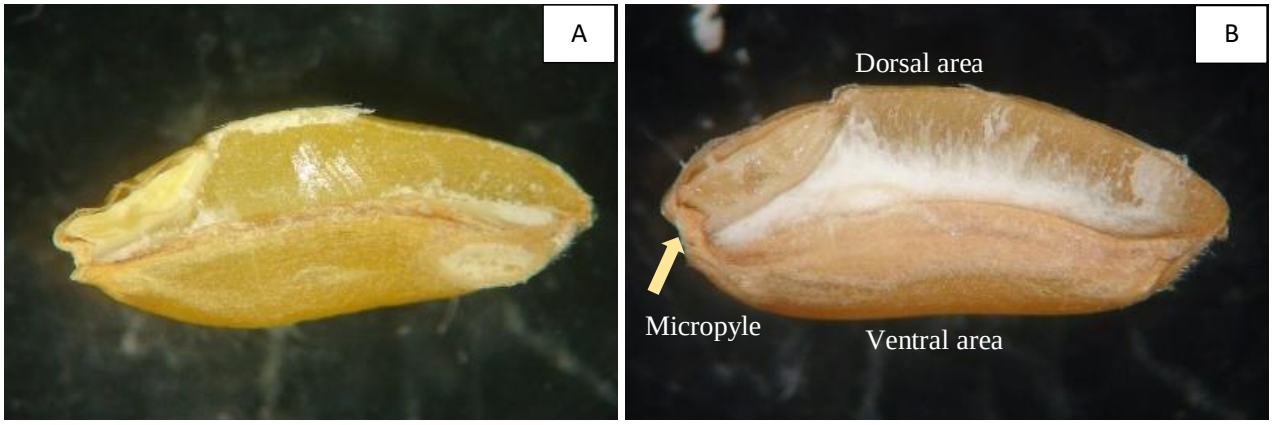

Figure 12. Light microscope images to determine the water into the grain: A) untreated kernel; B) kernel expose 12 h to water.

The starchy endosperm of wheat has a compact structure, which slows hydration into the endosperm (Delcour & Hoseney, 2010) and is seen by the low slope of curves in Figures 4 to 6. Rathjen et al (2009) found that the entrance of the water to the endosperm was around the scutellum and then the sub-aleurone and could be detected after 7 h after imbibition. Paquet-Durand et al. (2015) reported that the endosperm absorbed the water slowly, and was still not completely saturated even after 48 h of soaking. In this study, absorption of moisture caused the bran to expand and moisture entered the endosperm, causing the endosperm to fracture and become non-vitreous. At some time between 1 to 3 h of water soaking there was a big decline in vitreous kernel content (Figure 5B). This provides an indication as to how fast water moves through micropyle entering to the endosperm causing fractures and loss of vitreous kernel

content. Bason et al. (1995) and McCaigh et al. (2006) determined that the formation of air spaces in the endosperm after a hydration event caused the reduction of vitreous kernels. When the kernel dried, the bran layer did not contract leaving a rough outer surface and space between pericarp and seed coat and ultimately between the seed coat and endosperm as seen in SEM micrographs (Figure 11). McCaigh et al. (2006) suggested that the bleaching of grain by moisture absorption is related to physical changes on the seed coat structure due to wrinkling, which results in changes in the visible reflective properties of the wheat. In this study, the swelling of the kernel without contraction when dried resulted in an increase in kernel size as seen by the increase in large kernels after moisture treatments (Tables 1, 2, and 3). The increase in kernel size seems to be most associated with kernel width as there was little or no increase in kernel thickness or length measured (Table 4). The increase in width also resulted in an increase in kernel surface area and volume. The increase in kernel volume without an increase in weight resulted in a reduction in test weight (Table 2).

It is not unusual that night temperatures ranged from 5 to 10 ^{o}C during harvest season in North Dakota. As these results indicate, lower temperatures (5 ^{o}C) slowed the penetration of water into grain compared with warmer temperatures (24 ^{o}C). The increased in water gain due to temperature could be related to an expansion in the pores of the grain as well as dilatation of tissues, making the water penetration faster because of the increase in water diffusion (Oliveira et al., 2013).

In the field, a wet/dry event can happen multiple times before harvest. Farmers have commented that just a single moisture event can cause bleaching and the reduction in test weight and vitreous kernel content. The results of this research clearly confirm their observations. Based on the results a single bulk moisture event (rainfall or heavy morning dew) caused the decline in

test weight (3.5 kg/hL declined) and vitreous kernel content (27% declined) and the increase in large kernel size (6% increased) (Table 4). In this research, the initial moisture exposure caused the greatest effect with consecutive wet/dry cycles having less impact. In similar experiments, Swanson (1941) and Gan et al. (2000) also determined a reduction in test weight from 4.2 kg/hL in the first soak –dry cycle with just 0.7 kg/hL decline for the three added soak-dry cycles for a total of 4.9 kg/hL. The results suggested that only one relative humidity event was needed to cause the reduction of vitreous kernels (6% declined) and an increase in large kernel content (2%). However, more wet/dry cycles were needed to cause a reduction in test weight. Sandhu et al. (2009) determined a reduction in durum wheat vitreous kernel content after 1 day of exposure at 88% relative humidity.

Conclusions

Overall, lower initial grain moisture content, higher temperature and the addition of wet/dry cycles caused an increase in water gain by the grain ($P \leq 0.05$). It seems that a single bulk water event (rainfall event or heavy morning dew) would be enough to cause weather bleaching by the decline in test weight and vitreous kernel content and the increase in kernel size, and brightness. The results suggested that a single high relative humidity event was able to cause the reduction of vitreous kernel content and the increase in large kernel content. However, more wet/dry cycles were needed to cause a reduction in test weight. Electron microscope images showed that untreated kernels had a more compact and dense bran layers before soaking treatment than samples treated 15 sec and 12 h. After soaking, the area around the germ appeared to be the first section that changed its structure. After 12 h exposure to relative humidity the kernel morphology was similar with 15 sec soaking. Width was the most affected kernel dimension after bulk water events.

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CHAPTER 3: ENVIRONMENT DURING GRAIN FILLING AFFECTS PASTA COLOR

Abstract

Pasta color is an important quality parameter for pasta companies and consumers. This research was carried out to determine the relative importance of environment and genotype effects on pasta color and related traits using durum lines grown in North Dakota, USA; to determine the relationships between pasta color and semolina quality traits; and to evaluate the relationship between environmental growing conditions and pasta color. The environment had the highest relative proportion of variance for pasta color (ranged from 89 to 94%) and related traits. Stepwise multiple linear regression analyses suggested that the number of days with RH ≥ 80% diminished pasta brightness, yellowness and enhanced pasta redness while the number of days with ≤ 13°C enhanced pasta yellowness and pasta color score. The negative effect of high relative humidity could be related to speck counts and soluble brown pigments while the positive effect of low temperature was not clear. Therefore, more research needs to be done. In conclusion, the number of days with RH ≥ 80% diminished pasta color while the number of days with ≤ 13°C enhance pasta color. Speck count, soluble brown pigment and semolina redness negatively affect pasta color while yellow pigment content and semolina yellowness had a positive effect. Since weather cannot be controlled, this information will be helpful to select environments, growing locations, zones with more favorable climatic conditions to achieve better quality and pasta color traits. Understanding relationship between climatic variables and quality traits aid in developing cropping systems that favor high quality durum wheat.

Introduction

Pasta appearance can vary from bright yellow to a dull brown. Brightness and yellowness of pasta are important quality parameters for pasta companies and consumers (Troccoli et al.,

2000). People consider bright yellow color of semolina and pasta as an indicator of high quality and nutritional value. Pasta color is basically the combination of yellowness and brownness (Kobrehel et al., 1974) and a red coloration that occurs during certain drying conditions (Feillet & Matsuo, 1996). The yellowness of semolina and pasta relies mainly on lutein, a type of carotenoid. In durum wheat, lutein corresponds to ~80% of the yellow pigments found in the endosperm with the remainder being small amounts of zeaxanthin, beta carotene and other unidentified compounds (Ramachandran et al., 2010).

Research has indicated two origins of pasta brownness, enzymatic and non-enzymatic. Non-enzymatic brownness can be a consequence of brown-cupric soluble protein (Matsuo & Irvine, 1967) and/or Maillard reaction products. Brown pigments associated with Maillard reaction are a consequence of multiple reactions between free amino groups and reducing sugars. Enzymatic origins of brown pigment include products from oxidative enzymes such as polyphenol oxidases (PPOs) and peroxidases (POD) (Kobrehel et al., 1974). PPO reaction forms melanins that are colored products responsible for brown discoloration e.g. browning associated with cut surface of apples and potatoes. During the milling process, PPOs are removed almost totally (Baik et al., 1994). Although, when a portion of PPOs remain, they can cause serious problems in the color and quality of semolina pasta products (Demeke, 2001; Rani et al., 2001; Verlotta, 2010). Peroxidases use hydrogen peroxide (H_2O_2) as a substrate to catalyze the oxidation of a large number of phenols and aromatic compounds which result in brown color. (Gaspar et al., 1982). Previous research found that pasta products made from genotypes with high POD activity were browner than the ones with low POD activity (Kobrehel et al., 1974; Taha & Sagi, 1987).

Yellow pigment content is mainly affected by genotype (Borrelli et al., 2003) and less affected by environment (Taghouti et al., 2010). Semolina yellowness which has been related to pasta color (Borrelli et al., 2003) is highly influenced by genotype (Digesu et al., 2009). However, environmental conditions at different stages of kernel development can also affect the final content. Ramachandran et al. (2010) reported that lutein content increased from early grain filling to maturity for genotypes with medium to high yellow pigment content. However, for genotypes with low yellow pigment content there was not a constant increase at all stages. Clarke et al. (2006b) determined that yellow pigment content in durum varied with growing environment but it was not clear how the environment affected the final yellow pigment content.

Pasta brownness has been reported to vary with genotype (Irvine & Anderson, 1952) and environment, with the environment having a greater effect than the genotype (Harris et al., 1943; Feillet et al., 2000); although the relative effect of the genetic and environmental factors is still unclear (Feillet et al., 2000). Matsuo & Irvine (1967) reported that the inherited brownness was the result of non-enzymatic soluble proteins. Contrarily, Fraignier et al. (2000) identified that enzymatic brownness based on a POD isoform in the endosperm of durum wheat could be responsible for genotypic differences in brownness of pasta. On the other hand, PPO activity levels had been more associated to the growing environment than genotype (Park et al., 1997).

Over time, pasta color scores have declined even though yellow pigment content of new durum cultivars has improved (personal observation). The reason for the decline is not apparent. The objectives of this research were: to quantify the relative importance of environment and genotype effects on pasta color and related traits using cultivars and experimental lines grown in the Northern plains; to determine the relationships between pasta color and semolina quality traits; and to evaluate the relationship of environmental growing conditions on pasta color.

Materials and Methods

Plant Material and Environments

Grain samples were obtained from 24 genotypes grown at 12 environments in North Dakota, USA. The 24 genotypes included 11 released cultivars [‘Alkabo’ (Elias & Manthey, 2007a), ‘Alzada’, ‘Carpio’ (Elias et al., 2014), ‘ND Grano’, ‘ND Riveland’ (Elias & Manthey, 2019), ‘Divide’ (Elias & Manthey, 2007b), ‘Joppa’ (Elias & Manthey, 2016), ‘Maier’ (Elias & Miller, 2000a), ‘Mountrail’ (Elias & Miller 2000b), ‘Strongfield’ (Clarke et al., 2006), and ‘Tioga’ (Elias & Manthey, 2013] and 13 experimental breeding lines (‘D09555’, ‘D111068’, ‘D111156’, ‘D12846’, ‘D12863’, ‘D13344’, ‘D134003’, ‘D13500’, ‘D13526’, ‘D13541’, ‘D13720’, ‘D13750’, and ‘D13899’). Twelve growing environments consist of locations and years identified in Table 5.

Durum was harvested from long strip plots (75 ×1.2 m) grown in each environment. Harvested durum samples were cleaned and stored at 12°C until needed. Planting, harvest dates, total days to harvest, and average heading days per environment were summarized in Table 5.

Meteorological Data

Daily maximum temperature, minimum temperature, average temperature, total rainfall, average dew point, daily total solar radiation (TSR), and daily potential evapotranspiration (PET) for one month before harvest (grain filling) was obtained from North Dakota Agricultural Weather Data (NDAWN, 2019). Relative humidity (RH) was calculated by using the equation described by Alduchov & Eskridge (1996) based on Magnus equation obtained from (http://bmcnoldy.rsmas.miami.edu/Humidity.html).

$$\mathbf{RH} = 100*(\mathrm{EXP}((17.625*TD)/(243.04+TD))/\mathrm{EXP}((17.625*T)/(243.04+T))) \quad (3.1)$$

Where EXP is the Exponential function in Excel; TD is dew-point temperature (ºC); and T is average temperature (ºC). The number of days with temperature ≥ 30 ºC, ≤ 13 ºC, and ≥ 80% relative humidity were also determined.

Table 5. Planting, harvest dates, and total days to harvest for 12 environments in North Dakota.

Environment	Planting date	Harvest date	Total days[†]
Carrington-18	5/14/18	8/20/18	98
Casselton-17	4/27/17	8/18/17	113
Casselton-18	5/1/18	8/7/18	99
Dickinson-17	5/1/17	8/18/17	110
Dickinson-18	5/4/18	8/21/18	109
Hettinger-17	4/6/17	8/10/17	126
Hettinger-18	4/27/18	8/29/18	124
Langdon-17	5/12/17	9/12/17	123
Langdon-18	5/7/18	8/22/18	107
Minot-17	5/2/17	8/20/17	111
Minot-18	4/19/18	8/14/18	117
Williston-17	4/12/17	8/2/17	112

[†] Total days, refers to amount days from planting to harvest; Heading days, were in averaged 63 and refers to an average of the amount of days from planting to 50% heading out obtained from Elias & Manthey (2019).

Grain Quality Traits

Test weight (kg/hL) was determined by AACCI approved method 55-10.01 (AACC International, 2010). 1000-Kernel weight was determined by counting the number of kernels in 10 g of cleaned grain with an electronic seed counter (Seedburo Equipment Co., Chicago, IL). The total number of kernels was adjusted to the weight of 1,000 kernels. Kernel size distribution was determined using the method described by Shuey (1960); where kernels were classified as large when remained on Tyler No 7 sieve with 2.92 mm opening (top sieve); medium when they remained on Tyler No 9 sieve with 2.24 mm opening (middle sieve); and small kernels passed directly through both sieves. Vitreous kernel content was determined by cutting 100 intact grains

with a farinator, starchy and opaque kernels were classified as non-vitreous. Grain protein content was determined using NIR technology from FOSS InfratecTM 1241 Grain Analyzer (FOSS Tecator, Hogonas, Sweden). Falling number was determined by AACCI approved method 56-81.03.

Grain Milling Procedure

Grain samples (2 kg) were tempered to 14.5% moisture 24 h before milling and further conditioned from 14.5 to 17.5% moisture 45 min before milling. The tempered grain was milled into semolina with a Bühler MLU-202 experimental mill fitted with two Miag laboratory-scale purifiers (Bühler-Miag, Minneapolis, MN, U.S.A.). Additional grain samples (75 g) were ground into whole wheat meal using a UDY cyclone sample mill (UDY Corp., Fort Collins, CO, USA).

Semolina Quality Traits

Semolina color was determined by Minolta CR410 colorimeter (Konica Minolta, Ramsey, NJ) configured to measure Commission Internationale d' Eclairage (CIE) L*, a*, and b*-color values. L* measures the lightness or brightness of samples from black (0) to white (100), a* measures the greenness (-60) and redness (60), and b* measures blue (-60) to yellow (60). Sample was placed in a black cell that was 1 cm deep and covered with a quartz glass. Ash content was determined by AACCI approved method (08-01.01). Semolina protein was determined using NIR technology from Foss Infratec 1241 Grain Analyzer. Speck count was determined by counting the number of specks on a flat surface under a constant light source. The number of specks in three different 6.5 cm^2 areas was converted to the number of specks/dm^2.

Pasta Processing

Semolina hydrated to 32% moisture was extruded into spaghetti using a semi-commercial pasta extruder (DEMACO, Melburne, FL, U.S.A.). Extrusion conditions were: 45 ^oC, 25 rpm, 46

cm of hg, and an auger ratio of 8.1:1 Spaghetti was dried in a laboratory dryer (Standard Industries, Fargo, ND, U.S.A.) using a low temperature drying cycle (length, 18 h; peak temperature, 40°C) as describe by Yue et al. 1998. Dry spaghetti (70 g) was ground into flour using a UDY cyclone sample mill (UDY Corp., Fort Collins, CO, USA).

Pasta Color Score

Pasta color score was determined by using AACCI approved method 14-22.01. The spaghetti was placed on a black plastic background. Color was recorded three times in the top, medium, and bottom part of the pasta by using Minolta CR410 colorimeter (Konica Minolta, Ramsey, NJ) configured to measure Hunter L, *a*, and *b*-color values. Pasta color score was determined by using the color map for Minolta Color Difference Meter, model CR310 described in for AACCI approved method 14-22.01 and Debbouz (1994) where L and *b* values are compared. Dry spaghetti samples (50 g) were ground into flour using a UDY cyclone sample mill (UDY Corp., Fort Collins, CO, USA).

Yellow Pigment Content

Yellow pigment content was determined by using a modified AACCI approved method 14-50.01. The sample size was 4 g of ground material (whole wheat meal, semolina, and spaghetti flour) instead of 8 g. The solvent was prepared in 5:1 ratio, which corresponds to 20 mL of water saturated n-butanol reagent (WSB) added to 4 g of ground sample. The mixture was shaken on a vortex mixer for 2 min followed by a 30 min rest; after which the samples were centrifuged (Eppendorf 5810R centrifuge, Rotor: F-34-6-38, Radius: 11.5cm) for 5 min at 18,514 x *g* relative centrifuge force (RCF). Absorbance of the supernatant was measured in a spectrophotometer (Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer) at

436 nm. Measurements per extracted sample were converted to yellow pigment concentration (μg/g or ppm) using β-carotene extinction coefficient 1.6632.

Soluble Brown Pigment Content

The soluble brown pigments were determined using a modified procedure described by Matsuo & Irvine (1967). Ground material (2 g, whole wheat meal, semolina, and spaghetti flour) was added to 4 mL deionized distilled water. The mixture was shaken twice on a vortex mixer for 5 min followed by a 5 min rest; after which the samples were centrifuged (Eppendorf 5810R centrifuge, Rotor: F-34-6-38, Radius: 11.5cm) for 4 min at 18,514 x *g* relative centrifuge force (RCF). Supernatant (1.5 mL) was placed into an Eppendorf tube and centrifuged for 6 min. Absorbance of the supernatant was measured in a spectrophotometer (Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer) at 400 nm. Final measurement was registered as OD_{400}.

Peroxidase (POD) Activity

Peroxidase activity was determined by using the method described by Honold & Stahmann (1968) and Fraignier et al. (2000) with some modifications. Ground material (150 mg, whole wheat meal, semolina, and spaghetti flour) was suspended in 1.5 mL of 50 mM phosphate citrate buffer pH 4.6 (1:10 w/v) and rotated for 2 h at room temperature in an orbital shaker (Glas-Col, Terre Haute, IN, USA). After rotation, samples were centrifuged for 10 min at 14,000 × *g*. Then, 150 µL of the supernatant was mixed with 1.5 mL of solution contained 5 mM guaiacol, 10 mM H_2O_2, and 50 mM citrate phosphate buffer (pH 5). POD activity was determined spectrophotometrically (Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer) at 470 nm by measuring the oxidation of guaiacol to tetrahydroguaiacol in the presence of H_2O_2 for a min measuring every 15 sec. The total activity was expressed in units

per gram (U/g) of dry weight. One unit of enzyme activity was defined as a change in absorbance unit per min.

Polyphenol Oxidase (PPO) Activity

Polyphenol oxidase activity was determined using AACCI approved method 25-85.01. Solution (1.5 mL) composed of 5 mM of L-3, 4 dihydroxyphenylalanine (L-DOPA) in 50 mM of 3-(N-morpholino) propanesulfonic acid (MOPS) buffer pH 6.5, 0.02% Tween-20 was added to micro-centrifuge tube containing ground material (200 mg, whole wheat meal, semolina, and spaghetti flour). The tubes were placed on an orbital shaker (Glas-Col, Terre Haute, IN, USA) and were rotated for 1 h at room temperature to allow the reaction. Then samples were centrifuged for 2 min at 14,000 x *g*. Supernatant absorbance was measured at 475 nm (Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer). A control sample was prepared for each sample but treated with a solution without L-DOPA. The absorbance of the control was subtracted from each corresponded sample. The enzyme activity was expressed ΔA_{475}/ min g of sample. One unit of enzyme activity was defined as a change in absorbance unit per min.

Experimental Design and Statistical Analysis

The field experiment was a randomized complete block design (RCBD) with unbalance data. Each location-year was treated as a separate environment (12 environments) with 24 genotypes per environment. Environments were considered fixed effects and genotypes random effects. To evaluate the environmental effect, data was analyzed by considering genotypes as blocks. Data analyses were performed by Statistical Analysis System (SAS) version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). All data collected were subjected to analysis of variance (ANOVA) at 95% level of confidence (F tests: $P \leq 0.05$) using PROC MIXED method. LSMeans were separated by Fisher's-protected LSD at $P=0.05$. The environment and/or

genotype relative proportion of variance was reported based on the mean squares. The intra-class correlation was calculated based on the relative proportion of variance of the genotype to estimate the heritability (Eagles et al., 2002). Values less than 0.5 indicated poor reliability, 0.5 to 0.75 means moderate reliability, 0.75 to 0.9 means good reliability, and higher than 0.9 means excellent reliability of heritability. Stepwise multiple linear regression was performed to identify meteorological data that can predict pasta color score, pasta brightness, pasta redness, and pasta yellowness. A significance level of $P \leq 0.05$ was used for forward inclusion of quality traits in the regression model. Pearson correlations were computed for whole grain, semolina, and pasta parameters n= 12.

Results

Descriptive Statistics

The median, mean, standard deviation (SD), and range for grain, semolina, and pasta traits averaged across genotypes n=12, along with crop survey 5 yr average for these traits are summarized in Table 6. Quality traits ranged from poor to excellent quality. Median and mean values were similar in most of the studied parameters except for large kernel content where the median was 66% while the mean was 58%. This indicates that more than 50% of the samples had larger kernel size than the mean value. The mean values for grain traits were similar to or greater than the 5 yr crop average except for vitreous kernel content which averaged 83% compared to 87% for the 5 yr crop average. However, the median value for vitreous kernel content (88%) was similar to the 5 yr average. Median and mean values for semolina traits were similar for all traits tested. Median and mean values for semolina extraction and protein content were similar to the 5 yr crop average. However, median and mean values for semolina brightness and yellowness were lower and for *a*-value, speck count and ash content were greater than the 5 yr averages.

Similarly, median and mean values for pasta quality traits were similar but all were lower than the 5 yr crop average.

Table 6. Descriptive statistics for quality parameters for twelve environments averaged across genotypes.

Parameter	Median	Mean	SD†	Range	Crop survey 5 yr avg
Grain traits					
Test weight (kg/hL)	79.9	79.6	2.12	75.5-83.0	78.8
Vitreous kernel content (%)	88	83	13.9	54-96	87
1000-kernel weight (g)	43.5	41.7	5.5	32.0-51.0	39.2
Protein content (%)	14.5	14.4	1.5	11.7-16.8	13.9
Large kernel size (%)	66	58	19	23-81	49
Falling Number (sec)	530	500	106	275-626	384
Semolina traits					
% Semolina extraction (%)	67.1	67.5	2.4	64.4-71.8	67.1
CIE L*	83.65	83.58	0.63	82.64-84.63	84.0
CIE *a**	-2.55	-2.58	0.17	-2.22-2.88	-2.9
CIE *b**	28.77	29.02	1.51	26.81-31.49	29.5
Protein content (%)	13.2	13.1	1.4	10.6-15.1	13.0
Ash content (%)	0.77	0.76	0.11	0.61-0.88	0.70
Speck count (specks/dm^2)	31	33	7.57	24-44	27
Yellow pigment content (ppm)	7.2	7.3	0.68	6.1-8.8	NA
PPO activity (ΔA_{475}/min g)	0.10	0.11	0.04	0.04-0.20	NA
POD activity (U/g)	16.4	16.2	3.35	8.82-20.87	NA
Soluble brown pigments content (OD_{400})	0.18	0.19	0.05	0.13-0.31	NA
Pasta traits					
Hunter L	53.03	52.94	1.40	50.39-55.34	53.7
Hunter *a*	2.96	3.06	0.51	2.43-4.29	NA
Hunter *b*	25.37	25.35	1.56	22.29-27.91	26.6
Pasta color score	8.2	8.2	0.68	6.7-9.3	8.7
Yellow pigment content (ppm)	5.4	5.7	1.11	4.38-7.87	NA
PPO activity (ΔA_{475}/min g)	0.06	0.06	0.01	0.04-0.08	NA
POD activity (U/g)	12.1	10.7	2.95	5.19-15.83	NA
Soluble brown pigment content (OD_{400})	0.15	0.18	0.09	0.12-0.42	NA

†SD= standard deviation; CV= coefficient of variation; WW =whole wheat meal; PPO= polyphenol oxidases; POD= peroxidases; NA= no data

The median and mean values for pasta color score were similar (8.2) but lower than the 5 yr average (8.7). The lower pasta color score reflects the lower values for L and *b* values for pasta and for semolina. Pasta color score ranged from 6.7 which is very poor quality and not acceptable commercially to 9.3 which is quite good and is commercially acceptable.

Semolina Quality Traits Related to Pasta Color

Pearson correlations between semolina quality traits and pasta color parameters were computed using environments averaged over genotypes (n=12) and are summarized in Table 7. Semolina extraction did not correlate with any grain, semolina, or pasta quality traits tested even though it varied from 64.4% at Hettinger-17 to 71.8% at Carrington-18 and Langdon-18 (Table 8). Pasta brightness correlated positively with semolina CIE L* (r= 0.82, $P \leq 0.0001$) and negatively with semolina CIE *a** (r= -0.71, $P \leq 0.0001$) and speck count (r= -0.62, $P \leq 0.05$). Pasta redness was positively correlated with CIE *b** (r= 0.65, $P \leq 0.05$), yellow pigment content (r= 0.68, $P \leq 0.01$), and soluble brown pigment content (r= 0.71, $P \leq 0.01$). Pasta yellowness was negatively correlated with semolina CIE *a** (r= -0.66, $P \leq 0.05$) and speck count (r= -0.81, $P \leq 0.0001$) and positively correlated with semolina CIE L* (r= 0.64, $P \leq 0.05$), CIE *b** (r= 0.64, $P \leq 0.05$), and yellow pigment content (r= 0.58, $P \leq 0.05$).

Pasta color score was negatively correlated with semolina CIE *a** (r= -0.74, $P \leq 0.0001$) and speck count (r= -0.78, $P \leq 0.0001$) and positively correlated with CIE L* (r= 0.69, $P \leq 0.01$), CIE *b** (r= 0.57, $P \leq 0.05$), and yellow pigment content (r= 0.56, $P \leq 0.05$). Pasta color score was positively correlated with pasta brightness (L-value, r= 0.76, $P \leq 0.05$) and pasta yellowness (*b*-value, r= 0.99, $P \leq 0.05$). Interestingly, the amount of yellow pigment in pasta did not relate to pasta color score. For example, pasta made from durum wheat grown at Casselton-17, Casselton-18, and Hettinger-18 had similar yellow pigment contents of 4.9, 4.9, and 4.8 ppm but differed in

their pasta color scores of 8.8, 7.7 and 6.7, respectively. Similarly, Casselton-17 and Williston-17 had the same pasta color score (8.8) but differed in their yellow pigment content of 4.9 and 7.8 ppm, respectively (Table 8).

Neither PPO activity nor POD activity in semolina showed strong correlations to pasta color parameters (Table 7). However, several studies have linked the peroxidases and polyphenol oxidases activities in semolina with pasta color (Matsuo & Irvine, 1967; Kobrehel & Gautier, 1974; Kobrehel et al., 1974; Baik et al., 1994).

Table 7. Pearson Correlations computed to quality traits vs pasta color for environments, n=12

	Pasta Color Parameters			
Parameter	**Pasta Hunter L**	**Pasta Hunter *a***	**Pasta Hunter *b***	**Pasta color Score**
Semolina traits				
% Semolina extraction	0.09	0.21	-0.06	-0.04
CIE L*	0.82 ***	-0.35	0.64 *	0.69 **
CIE *a**	-0.71 ***	0.22	-0.66 *	-0.74 ***
CIE *b**	0.11	0.65 *	0.64 *	0.57 *
Protein	-0.38	0.17	-0.12	-0.24
Speck count	-0.62 *	0.16	-0.81 ***	-0.78 ***
Ash content	-0.51	0.52	-0.53	-0.55
Yellow pigment content	0.10	0.68 **	0.58 *	0.56 *
PPO activity	-0.17	0.50	-0.17	-0.16
POD activity	-0.21	0.51	0.13	0.04
Soluble Brown pigment content	-0.31	0.71 **	0.25	0.23
Pasta traits				
Yellow pigment content	0.05	0.68 **	0.53	0.47
PPO activity	-0.42	0.25	-0.34	-0.25
POD activity	-0.09	0.21	-0.26	-0.28
Soluble brown pigment content	-0.36	0.39	0.13	0.14

*Significant at the 0.01 probability level.
** Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.

Table 8. Means for grain, milling, and semolina traits related to pasta color per environment, n=12.

Parameter	Carr-18	Cass-17	Cass-18	Dic-17	Dic-18	Hett-17	Hett-18	Lang-17	Lang-18	Min-17	Min-18	Will-17	LSD 0.05
Grain traits													
Test weight	91.0	54.4	71.8	82.3	93.5	62.0	84.3	91.2	96.0	76.0	94.2	96.4	4.2
VK	91	54	72	82	94	62	84	91	96	76	94	96	4.2
1000-KW	37.7	45.1	43.3	37.4	46.4	37.2	45.9	35.8	51.0	44.6	43.8	32.0	1.1
Protein cont	13.4	12.5	11.7	16.4	15.1	13.5	16.8	13.5	14.2	15.4	14.7	15.0	0.39
Large grain	47	77	66	31	70	37	73	52	81	70	66	23	3.9
FN	597	275	471	486	526	626	576	457	562	328	535	561	29.8
Semolina traits													
% semolina extraction	71.8	68.1	68.7	64.9	65.7	64.4	65.5	66.8	71.7	68.3	66.2	67.5	0.8
CIE L*	82.95	84.63	84.01	83.84	83.52	84.37	82.64	82.73	83.44	83.77	83.13	83.89	0.37
CIE *a**	-2.55	-2.88	-2.70	-2.51	-2.50	-2.75	-2.22	-2.56	-2.43	-2.67	-2.53	-2.62	0.11
CIE *b**	31.50	26.97	27.98	30.08	28.81	30.13	26.80	29.14	28.64	28.13	28.72	31.34	0.56
Protein cont	12.4	11.3	10.6	14.9	13.8	12.2	15.1	12.1	12.9	14.2	13.4	13.9	0.32
Ash content	0.88	0.70	0.88	0.62	0.76	0.68	0.88	0.80	0.78	0.62	0.75	0.82	0.03
Speck count	35	28.5	43.5	25.5	32.6	24.4	42.3	43.6	40.8	26.3	28.2	26.9	3.1
YPC	8.25	6.89	7.39	7.14	6.92	7.90	6.10	7.78	6.60	6.38	7.21	8.76	0.38
PPO activity	0.13	0.10	0.20	0.12	0.11	0.04	0.08	0.10	0.01	0.07	0.10	0.15	0.03
POD activity	18.0	8.8	13.1	17.6	17.6	14.4	14.6	15.2	18.5	20.9	15.1	20.4	2.3
SBPC	0.22	0.14	0.19	0.18	0.14	0.22	0.14	0.31	0.17	0.17	0.16	0.27	0.05
Pasta traits													
Hunter L	53.32	54.72	53.19	52.99	52.86	55.34	50.65	50.39	53.08	53.56	52.79	52.35	0.48
Hunter *a*	3.60	2.43	3.03	3.03	2.81	2.60	3.06	3.49	2.89	2.80	2.76	4.29	0.16
Hunter *b*	26.46	26.38	23.87	26.34	24.94	27.91	22.29	24.22	24.33	25.79	24.74	26.91	0.30
PC score	8.6	8.8	7.7	8.6	8.1	9.3	6.7	7.8	7.8	8.3	8.0	8.8	0.24
YPC	7.9	4.9	4.9	5.4	5.8	6.3	4.8	5.5	5.1	4.4	5.7	7.8	0.3
PPO activity	0.07	0.05	0.07	0.05	0.06	0.04	0.04	0.08	0.06	0.04	0.07	0.05	0.01
POD activity	12.7	5.2	12.3	8.9	12.6	7.9	9.5	7.3	15.8	12.0	12.7	12.3	2.1
SBPC	0.18	0.13	0.16	0.22	0.12	0.24	0.12	0.42	0.13	0.13	0.14	0.21	0.04

Test weight (kg/hL); VK= vitreous kernel content (%); 1000-KW= thousand kernel weight (g); Protein cont= protein content (%); Large grain= large kernel size content (%); FN= falling number (sec); YP= yellow pigment content (ppm); SBPC= soluble brown pigment content (OD_{400}); PPO activity (ΔA_{475}/min g); POD activity (U/g); PC score= pasta color score; LSD represents significant differences at 95% level of confidence.

Relative Proportion of Variance

Analysis of variance indicated that the environment effect was significant ($P \leq 0.05$) for all traits tested. Genotype main effect was significant for all traits tested except for semolina speck

count, semolina PPO activity, and pasta soluble brown pigment content which were not significant for the genotype main effect (data not presented).

The relative proportion of variance and intra-class correlation for tested grain, semolina, and pasta quality traits are summarized in Table 9. Relative proportion of variance indicates that environment had more influence on quality traits than did genotype, except for falling number in grain and POD activity in semolina and pasta where environment counted for 54, 39, and 46%, respectively while genotype counted for 45, 59, and 51%, respectively. The relative proportion of variance for environment by genotype interaction based on residuals was confounded within experimental error and was relatively small indicating that the interaction did not greatly impact quality traits.

The intra-class correlation coefficient (ICC) provides an estimate of broad-sense heritability (Koo & Li, 2016). Intra-class correlation coefficient was determined by the proportion of variance attributed to genotype relative to that of genotype x location interaction and error variance, so traits with higher intra-class correlation coefficient would have more response to genotype (Caffe-Treml et al., 2011). The intra-class correlations showed excellent reliability (>0.9) for large kernel content, falling number, whole wheat PPO and POD, semolina CIE *a**-value and *b**-value, semolina ash content, semolina POD, and yellow pigment content in semolina and pasta. For pasta color parameters, Hunter L and *b* values showed good reliability (0.75 and 0.86, respectively) while pasta color score had medium reliability (0.74) (Table 9). Traits with high intra-class correlation are considered traits with high heritability, therefore they are parameters that can be used when breeding for a specific characteristic, even if the environment has a high impact on the response.

Table 9. Relative proportion of variance and intra-class correlation for tested grain, milling, semolina and pasta quality traits.

Parameter	Relative Proportion of Variance (%)			ICC	
	Environment	Genotype	Residual		
Grain traits					
Test weight (kg/hL)	93	6	1.0	0.86	*
Vitreous kernel content (%)	96	3	1.2	0.69	
1000-kernel weight (g)	96	3	0.5	0.86	*
Protein content (%)	97	3	0.8	0.77	*
Large kernel size (%)	94	5	0.5	0.91	**
Falling Number (sec)	54	45	0.5	0.99	**
Semolina traits					
% Semolina extraction	95	3	1.4	0.70	
CIE L*	86	10	3.8	0.72	
CIE *a**	62	35	2.8	0.93	**
CIE *b**	77	22	1.4	0.94	**
Protein content (%)	97	2	0.6	0.80	*
Ash content (%)	93	7	0.7	0.91	**
Speck count (specks/dm^2)	96	2	2.0	0.53	
Yellow pigment content (ppm)	74	25	1.7	0.94	**
PPO activity (ΔA_{475}/min g)	83	11	6.4	0.63	
POD activity (U/g)	39	59	2.4	0.96	**
Soluble brown pigment content (OD_{400})	86	9	5.2	0.63	
Pasta traits					
Hunter L	94	4	1.4	0.75	*
Hunter *a*	89	10	1.2	0.89	*
Hunter *b*	94	5	0.8	0.86	*
Pasta color score	94	4	1.6	0.74	
Yellow pigment content (ppm)	81	18	1.1	0.94	**
PPO activity (ΔA_{475}/min g)	73	18	9.1	0.67	
POD activity (U/g)	46	51	2.9	0.95	**
Soluble brown pigment content (OD_{400})	94	3	2.8	0.50	

ICC= intra-class correlation coefficient, parameter with * is good (0.75-0.90); with ** is excellent (>0.90). Koo & Li (2016).

Description of Climatic Variables During Grain Filling

The relative proportion of variance described above indicated the importance of environment on quality traits including pasta color. Weather is an important part of

environmental effects. Means for meteorological data averaged a month before harvest for 12 environments in North Dakota are summarized in Table 10.

Table 10. Means for meteorological data averaged a month before harvest (grain filling) for 12 environments in North Dakota.

	Meteorological data								
Environment	**Daily T° Max**	**Days T° ≥30**	**Daily T° Min**	**Days T° ≤13**	**TR**	**RH**	**Days RH≥80**	**TSR**	**PET**
	°C	days	°C	days	mm	%	days	MJ/m^2	mm
Grain filling†									
Carrington-18	27.9	12	11.4	23	0.14	68	3	21.8	5.9
Casselton-17	26.4	6	13.1	16	2.48	77	7	20.7	4.7
Casselton-18	26.8	8	13.0	18	0.91	79	9	21.6	5.1
Dickinson-17	27.9	13	13.6	14	1.52	57	2	20.7	6.4
Dickinson-18	29.6	14	12.6	22	0.86	51	0	21.8	6.9
Hettinger-17	30.9	21	14.1	12	1.99	54	1	23.2	7.7
Hettinger-18	28.3	14	11.3	25	1.51	57	2	19.9	5.9
Langdon-17	24.3	1	10.5	27	0.54	65	4	18.3	4.9
Langdon-18	25.9	6	11.2	25	0.13	67	3	20.8	5.5
Minot-17	26.9	8	13.2	21	2.30	64	4	22.3	6.1
Minot-18	28.8	11	12.8	20	0.47	59	0	24.2	6.8
Williston-17	32.2	24	16.3	2	1.65	46	0	23.8	8.6
Means	28.0	12	12.7	19	1.2	62	3	21.6	6.2

† Meteorological data for grain filling was considered a month before harvest; T° ≥30= number of days with temperatures equal or higher than 30°C; Days T° ≤13= number of days with temperatures equal or lower than 13°C; TR= total rainfall; RH= daily average relative humidity; Days with RH≥80= number of days with relative humidity equal or higher than 80%; Daily T° Max and Daily T° Min, are averages daily maximum and minimum temperatures; TSR= daily average total solar radiation; PET= daily average total evapotranspiration.

During grain filling, Langdon-17 and Langdon-18 had the lowest average temperature (17.3 and 18.5 °C, respectively) while Hettinger-17 had the highest average temperature (22.4 °C). In general, environments had more days with daily minimum temperatures ≤ 13 °C (19 days) than days with temperatures ≥ 30 °C (12 days). Williston-17 and Hettinger-17 had the most days with temperatures ≥ 30 °C while Langdon-17 had the most days with temperatures ≤ 13 °C. With

respect to total rainfall, Casselton-17 (2.48 mm) and Minot-17 (2.30 mm) had the highest precipitation, while lowest precipitation occurred at Langdon-18 (0.13 mm) and Carrington-18 (0.14 mm). Daily average relative humidity was higher at Casselton-17 (77%) and Casselton-18 (79%) with the greatest number of days (7 and 9, respectively) with RH ≥ 80%. In contrast, daily average relative humidity was lowest at Williston-17 (46%) with no days with relative humidity above 80%. Daily total solar radiation (TSR) was quite similar across the environments while potential evapotranspiration (PET) which considers the amount of water lost by the plant during transpiration and the evaporation of water from the earth surface which is influenced by the wind, humidity, sunlight, and temperature was highest at Williston-17 (8.6 mm) and lowest at Casselton-17 (4.7 mm). Overall PET increased with increased maximum daily temperature and number of days above 30°C and decreased with increased daily average relative humidity.

Effect of Climatic Variables During Grain Filling on Semolina Quality Traits

The effect of climatic variables on semolina traits that correlated to pasta color was determined by stepwise multiple linear regression and summarized in Table 11. The climatic variables explained 33% of variability in the response of CIE L*, 19% of the variability in the response of CIE *a**, 30% of the variability in the response of CIE *b**, 50% of the variability in the response of speck count, 31% of the variability in the response of yellow pigments and 24% of the variability in the response of soluble brown pigments.

Table 11. Stepwise multiple linear regression for semolina quality traits correlated to pasta color and all climatic variables, n= 287.

Parameter	Climatic variables	Effect†	Partial R^2	R^2
Semolina traits				
CIE L*	Daily average minimum T°	(+)	0.19	0.33
	Daily average relative humidity	(+)	0.11	
	Total rainfall	(+)	0.02	
CIE *a**	Total rainfall	(-)	0.07	0.19
	Daily average minimum T°	(-)	0.07	
	Daily average relative humidity	(-)	0.05	
CIE *b**	PET	(+)	0.17	0.30
	Total rainfall	(-)	0.07	
	Number of days with ≤ 13°C	(-)	0.06	
Speck count	Daily average minimum T°	(-)	0.34	0.50
	Total rainfall	(-)	0.07	
	Daily average relative humidity	(-)	0.05	
	Number of days with RH ≥ 80%	(+)	0.04	
Yellow pigment content	Number of days with ≤ 13°C	(-)	0.15	0.31
	Total rainfall	(-)	0.14	
	Daily average minimum T°	(-)	0.02	
Soluble brown pigment content	Daily average minimum T°	(-)	0.08	0.24
	Daily average relative humidity	(+)	0.08	
	Number of days with ≤ 13°C	(-)	0.05	
	Total rainfall	(-)	0.03	

† Effect (+) means positive effect on the quality traits, (-) means negative effect on the quality traits, symbols were obtained from the intercept value of the regression equation; PET= daily potential evapotranspiration.

For each semolina trait, the results indicate that semolina L* (brightness) was positively affected by daily average minimum temperature (partial R^2= 0.19) and daily average relative humidity (partial R^2= 0.11) (Table 10). Semolina *a** (redness) was diminished the most by total rainfall (partial R^2= 0.07) and daily average minimum temperature (partial R^2= 0.07). Semolina *b** (yellowness) was affected positively by PET (partial R^2= 0.17) but was diminished the most by total rainfall (partial R^2= 0.07) and number of days with ≤ 13 °C (partial R^2= 0.06). Yellow

pigment content was diminished the most by the number of days with ≤ 13 °C (partial R^2= 0.15) and total rainfall (partial R^2= 0.14). Speck count was diminished the most by daily average minimum temperature (partial R^2= 0.34). Soluble brown pigment content was diminished the most by the daily average minimum temperature (partial R^2= 0.08) and the number of days with ≤ 13 °C (partial R^2= 0.05) and positively affected by daily average relative humidity (partial R^2= 0.08).

Effect of Climatic Variables During Grain Filling on Pasta Color

The effect of climatic variables on pasta color traits was determined using stepwise multiple linear regression and is summarized in Table 12. In general, climatic variables explained the most overall variability for pasta yellowness, pasta brightness, and pasta color score and explained the least for pasta redness.

Pasta brightness was diminished the most by TSR (partial R^2= 0.18). The climatic variables that enhanced pasta brightness the most were, daily average relative humidity (partial R^2= 0.13) and daily average minimum temperature (partial R^2= 0.13), and by PET (partial R^2= 0.06). Altogether climatic variables contributed to explain 65% of the variability in the response of pasta brightness.

Pasta redness was diminished by daily average relative humidity (partial R^2= 0.09), number of days with ≤ 13°C (partial R^2= 0.06), and total rainfall (partial R^2= 0.06). Number of days with RH ≥ 80% (partial R^2= 0.17) had a positive effect on pasta redness. Altogether, climatic variables contributed to explain 0.41% of the variability in the response of pasta redness.

Pasta yellowness was diminished the most by the number of days with RH ≥ 80% (partial R^2= 0.15). Conversely, pasta yellowness was enhanced the most by the number of days with ≤ 13 °C (partial R^2= 0.36) and to a lesser extent by total rainfall (partial R^2= 0.05), daily average RH

(partial R^2= 0.05), and PET (partial R^2= 0.05), Altogether, climatic variables contributed to explain 72% of the variability in the response of pasta yellowness.

Table 12. Stepwise multiple linear regression for pasta color traits and all climatic variables, n= 283.

Color parameter	Climatic variables	Effect[†]	Partial R^2	R^2
Pasta brightness	TSR	(-)	0.18	0.65
	Daily average relative humidity	(+)	0.13	
	Daily average minimum T^o	(+)	0.13	
	PET	(+)	0.06	
	Number of days with RH ≥ 80%	(-)	0.03	
	Number of days with ≥ 30^{o}C	(+)	0.01	
	Number of days with ≤ 13^{o}C	(+)	0.01	
Pasta redness	Number of days with RH ≥ 80%	(+)	0.17	0.41
	Daily average relative humidity	(-)	0.09	
	Number of days with ≤ 13^{o}C	(-)	0.06	
	Total rainfall	(-)	0.06	
	Daily average minimum T^o	(-)	0.03	
Pasta yellowness	Number of days with ≤ 13^{o}C	(+)	0.36	0.72
	Number of days with RH ≥ 80%	(-)	0.15	
	Total rainfall	(+)	0.05	
	PET	(+)	0.05	
	Daily average relative humidity	(+)	0.05	
	Daily average maximum T^o	(+)	0.03	
	Daily average minimum T^o	(+)	0.02	
Pasta color score	Number of days with ≤ 13^{o}C	(+)	0.28	0.65
	Number of days with RH ≥ 80%	(-)	0.13	
	Daily average maximum T^o	(+)	0.06	
	Total rainfall	(+)	0.04	
	PET	(+)	0.04	
	Daily average relative humidity	(+)	0.04	
	TSR	(-)	0.02	
	Daily average minimum T^o	(+)	0.02	
	Number of days with ≥ 30^{o}C	(-)	0.01	

[†] Effect (+) means positive effect on the quality traits, (-) means negative effect on the quality traits, symbols were obtained from the intercept value of the regression equation; TSR= daily total solar radiation; PET= daily potential evapotranspiration.

Pasta color score was diminished the most by the number of days with RH ≥ 80% (partial R^2= 0.13), while pasta color score was enhanced the most by the number of days with ≤ 13 °C (partial R^2= 0.28) and to a lesser extent, enhanced by daily average maximum temperature (partial R^2= 0.06) and daily average RH (partial R^2= 0.04). Altogether climatic variables contributed to explain 65% of the variability in the response of pasta color score. Number of days with ≤ 13°C and number of days with RH ≥ 80% were the top two climatic variables explaining pasta color score and pasta yellowness. For both pasta color score and pasta yellowness number of days ≤ 13°C was associated with enhanced color and number of days with RH ≥ 80% was associated with diminished color. Number of days with RH ≥ 80% also explained the most variability in pasta redness and was associated with increased redness.

Discussion

In this study, grain harvested in twelve environments and averaged across genotypes ranged from excellent to poor quality (Table 5). The overall means for grain, semolina, and pasta traits were similar to or greater than those for the crop survey 5 yr average data. Environments such as Casselton-17, Casselton-18, Hettinger-17, and Minot-17 had the lowest test weights, vitreous kernel contents, and falling numbers. This might be related to the total rainfall of these environments during grain filling, especially at Casselton-17 (2.48 mm), Minot-17 (2.30 mm), and Hettinger-17 (1.99 mm) (Table 10). Reductions in test weight, vitreous kernel content, and falling number due to rainfall have been found in studies performed by Gan et al. (2000); McCaig et al. (2006); and Ferrer et al. (2006). In addition, the regression analysis performed in this study determined that total rainfall diminished vitreous kernel content, and falling number (partial R^2= 0.39 and 0.14, respectively) (Data not shown).

The environment is made up of biotic and abiotic factors. This research focused on climatic factors. Results showed that environment greatly affected factors associated with semolina quality and pasta color. For example, pasta color score was the lowest for pasta made from durum wheat grown at Hettinger-18, Langdon-17, Langdon-18, and Casselton-18, intermediate from durum wheat grown at Minot-17, Minot-18, and Dickinson-18; and highest from durum wheat grown at Williston-17, Casselton-17, Carrington -18, Dickinson-17, and Hettinger-17 (Table 8).

It is known that durum wheat requires warm days and cool nights to grow properly (Bozzini, 1988). During grain filling, it is not unusual to have low temperatures during the night (≤ 13ºC) and high temperature and relatively high relative humidity during the day. According to the results, the number of days with ≤ 13°C enhanced pasta yellowness and pasta color score while the number of days with RH ≥ 80% diminished pasta color. The number of days with RH ≥ 80% was also explained the most variability in pasta redness and was associated with increased redness (Table 12).

Environments with the lowest pasta color score had the highest speck counts values (Table 8). The negative effect of speck counts on pasta color was corroborated on the computed correlations where: pasta brightness (r= -0.62, $P \leq 0.05$), pasta yellowness (r= -0.81, $P \leq 0.0001$), and pasta color score (r= -0.78, $P \leq 0.0001$) were negatively correlated to speck counts. In addition, semolina speck count increased with an increase in the number of days with RH ≥ 80% (Table 11). High relative humidity can have a detrimental effect on grain quality, reducing vitreous kernel content (Sandhu et al., 2009), test weight, and grain brightness (see Chapter 2 of this thesis). A decline in grain quality can affect the milling properties of the grain as expressed by a reduction in semolina yield and an increase of bran contamination. Specks represent

contamination of bran and germ particles into the semolina which are detrimental to the pasta appearance due to the presence of brown spots in the pasta (Symons et al., 2009).

An increase in soluble brown pigment after pasta processing was reported in Chapter 4 of this thesis. The positive effect of RH ≥ 80% in pasta redness could be related to the soluble brown pigment content which was enhanced by daily average relative humidity (Table 11). It is known that brown pigments can mask yellowness by decreasing the brightness (Matsuo & Irvine, 1967; Feillet et al., 2000). In this case, soluble brown pigments were positively correlated with pasta redness (r= 0.71, $P \leq 0.01$); however, no association with pasta yellowness or pasta brightness was determined. This could happen since pasta brownness is a complex process that can be related to soluble brown pigments from Maillard reaction during processing and impurities in the semolina (see Chapter 4 and review of Feillet et al., 2000).

According to the results, the number of days with ≤ 13°C enhanced pasta yellowness (*b*-value) and pasta color score. The effect of the number of days with ≤ 13°C on pasta yellowness (*b*-value) might not be related to yellow pigment content since results based on stepwise multiple linear regression showed that the number of days with ≤ 13°C had a negative effect on yellow pigment content in grain (data not shown) and semolina (Table 11). Yellow pigment content did not correlate with pasta color. As mentioned above, pasta made from durum wheat grown at Casselton-17, Casseslton-18, and Hettinger-18 had similar yellow pigment content of 4.9, 4.9, and 4.8 ppm but differed in their pasta color scores of 8.8, 7.7 and 6.7, respectively (Table 8). Similarly, Casselton-17 and Williston-17 had the same pasta color score (8.8) but differed in their yellow pigment content of 4.9 and 7.8 ppm, respectively.

The results of this research confirm those of Dexter et al. (1994) who determined that it was unlikely that PPO or POD had an effect on pasta color. This because the amount of PPO in

semolina is very small and there is no substrate (H_2O_2) available for POD during processing (Feillet et al., 2000).

Conclusions

The results from this study suggest that the environment main effect had a major influence on pasta color, intermediate by genotype and to a lesser extent by the G×E interaction. The majority of studied parameters showed good to excellent intra-class correlation to estimate heritability. According to the results, the number of days with RH ≥ 80% diminished pasta color while the number of days with ≤ 13°C enhanced pasta yellowness and pasta color score. The negative impact of high relative humidity on grain quality and therefore milling properties could be related to high speck counts in semolina and soluble brown pigments which had a detrimental effect on pasta color. Contrary to what was expected, pasta yellowness (*b*-value) was not strongly related to yellow pigment content. In addition, it is unlikely that PPO or POD had an effect on pasta color. Durum breeders strive to improve pasta color. Since weather cannot be controlled this information will be helpful to select environments, growing locations, zones with more favorable climatic conditions to achieve better quality and pasta color traits. Understanding relationship between climatic variables and quality traits aid in developing cropping systems that favor high quality durum wheat.

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CHAPTER 4: MILLING AND PASTA PROCESSING AFFECTS PASTA COLOR

Abstract

Enzymes, yellow pigment and brown pigment have been associated with color of dry pasta. This research was conducted to determine the effect of milling and pasta processing on polyphenol oxidase (PPO) activity, peroxidase (POD) activity, soluble brown pigment content, and yellow pigment content and to relate these results and other quality traits to the final dry pasta color. Milling caused a reduction in yellow pigment, soluble brown pigment, PPO and POD activities (22, 64, 71, and 62%, respectively) while pasta processing reduced yellow pigment content, and for some genotypes, increased soluble brown pigment content. Overall, the loss due to milling for yellow pigment content, POD activity, PPO activity, and soluble brown pigment content was 1.3X, 4.4X, 6.2X, and 17.5X greater than loss due to processing. Stepwise multiple linear regression analysis determined that the most important factors that determined pasta color were yellow pigment content (positive effect) and protein content, semolina ash content, and speck counts (all with negative effects). In conclusion, milling had a greater effect than did pasta processing on pasta color. For the studied parameters, only yellow pigment content had an important positive impact on pasta color. Additional quality traits such as speck count, protein content, and ash content had significant negative impact on pasta color. Results indicate the importance of milling on end-product quality of pasta and identified semolina quality traits that can be used to select semolina that would produce desired pasta color. For durum breeders, these results show the importance in selecting genotypes that have high yellow pigment content and excellent milling qualities.

Introduction

Color is an important aesthetic parameter for traditional dry pasta made from durum wheat semolina. Pasta color can vary from dull brown to bright yellow. The desired final color is bright yellow without specks or cracks. Pasta color is a combination of a yellow hue, brown hue, (Kobrehel et al., 1974), and red color (Feillet & Dexter, 1996). Pasta color often is measured using a reflectance colorimeter method that determines Hunter L, *a, b* color scale, where L-value indicates brightness on a scale of 0 to 100, *a*-value indicates redness when positive and greenness when negative, and *b*-value indicates yellowness when positive. A pasta color score can be determined using AACCI approved method 14-22.01 where a score is assigned from 1 to 12 based on Hunter L and *b* color values. Pasta color scores over 8 are commercially acceptable.

Polyphenol oxidase (PPO) and peroxidase (POD) activities and yellow and brown pigments have been reported to affect pasta color (Matsuo & Irvine, 1967; Kobrehel & Gautier, 1974; Kruger, 1976; Clarke et al., 2006a). The yellowness of pasta is due to lutein which is a type of xanthophyll that belongs to the carotenoid group of pigments. It has been reported that about 80% of lutein is found in the endosperm (Ramachandran et al., 2010). The cause of the pasta brownness is unclear. Matsuo & Irvine (1967) reported that durum genotypes varied in the amount of soluble brown pigments. They found that genotypes with reddish brown semolina produced brown macaroni, while genotypes with yellow semolina produced bright yellow macaroni. Brownness can be a consequence of enzymatic or non-enzymatic reactions. Non-enzymatic brownness has been proposed to be related to a brown-cupric soluble protein (Matsuo & Irvine, 1967) and to Maillard reaction products (Feillet et al., 2000). Enzymatic brownness is thought to be related to oxidative enzymes such as PPO (Kobrehel et al., 1974; Fuerst et al., 2006) and POD (Kobrehel et al., 1974). Milling (Borrelli et al., 2008) and pasta processing

(Dexter & Marchylo, 2001) can affect the color of the pasta due to a reduction in total yellow pigment content and an increased in brown color (Irvine & Anderson, 1952). During durum milling, the kernel components bran, germ, and endosperm are separated and the endosperm is coarsely ground into semolina. Since the concentration of carotenoid pigments is greater in the outer than inner endosperm, removal of the aleurone layer and some endosperm during milling results in a reduction of carotenoid pigments in semolina. Borrelli et al. (1999) determined that about 8% of carotenes are lost during milling.

Semolina extraction levels above 65% have been reported to cause the deterioration of semolina purity and pasta color (Dexter et al., 2004). Decrease in semolina purity is related to a higher concentration of contaminant particles such as bran and germ into the semolina which results in increased ash content and specks. Peroxidase and PPO activities are higher in the bran layers than semolina. The germ has high POD activity (Fraignier et al., 2000) but very low PPO activity (Marsh & Galliard, 1986). During the milling process, PPO activity is almost totally removed. Although, when a portion of PPOs remain, they can cause serious problems in the color and quality of semolina pasta products (Rani et al., 2001; Demeke et al., 2001; Verlotta et al., 2010). Baik et al. (1994) reported that about 3% of the total PPO activity of the grain was found in the wheat flour. Peroxidases are also removed during milling. However, a small portion of this enzyme has been found in the starchy endosperm (Fraignier et al., 2000). Kobrehel et al. (1974b) reported that pasta made from cultivars with high POD activity had a brownish color with a positive correlation with the brown index of pasta. Contamination of bran and germ can also increase non reducing sugars and free amino acids that can lead to Maillard reaction and the production of non-enzymatic brown components (Resmini et al., 1996).

Borrelli et al. (1999) and DeSimone et al. (2010) determined that the percentage of loss of yellow pigment during pasta processing varied from 4 to 20%. Pasta processing involves hydrating semolina, kneading the dough, extruding the dough to form the pasta, and drying the pasta. Processing conditions such as hydrating and kneading promote oxidation by lipoxygenase (Borrelli et al., 2003). Lipoxygenase oxidizes free unsaturated fatty acids forming free radicals. These free radicals are reduced by the antioxidant activity of yellow pigment, primarily lutein in wheat. The oxidized lutein is colorless. Thus, lipid oxidation contributes to the decline in the yellow appearance of semolina and pasta made from semolina (Sissons, 2008). Drying conditions such as ultra-high temperature promotes Maillard reaction which can mask the yellow color of pasta (Marchylo & Dexter, 1989). There is some uncertainty about the effect of drying conditions on brownness since some authors such as De Stefanis & Sgrulletta (1990) and D'Egidio & Pagani (1997) did not find any relationship between drying temperature and pasta brownness.

Despite the efforts of durum wheat breeding programs to increase the yellow pigment content in newly released cultivars, pasta color score has been decreasing over time for pasta made from durum wheat grown in North Dakota. The reason for this phenomenon is uncertain. This research was conducted to determine the effect of milling and pasta processing on PPO activity, POD activity, soluble brown pigment content, and yellow pigment content and to relate these results to final color of dry pasta.

Materials and Methods

Durum Wheat Samples and Growing Environments

Grain was obtained from 24 genotypes grown at 6 environments in North Dakota, USA. The 24 genotypes included 11 released cultivars ['Alkabo' (Elias & Manthey, 2007a), 'Alzada',

'Carpio' (Elias et al., 2014), 'ND Grano', 'ND Riveland' (Elias & Manthey, 2019), 'Divide' (Elias & Manthey, 2007b), 'Joppa' (Elias & Manthey, 2016), 'Maier' (Elias & Miller, 2000a), 'Mountrail' (Elias & Miller 2000b), 'Strongfield' (Clarke et al., 2006b), and 'Tioga' (Elias & Manthey, 2013)] and 13 experimental breeding lines ('D09555', 'D111068', 'D111156', 'D12846', 'D12863', 'D13344', 'D134003', 'D13500', 'D13526', 'D13541', 'D13720', 'D13750', and 'D13899'). Environments in 2017 were Casselton, Hettinger, and Williston while in 2018 were Dickinson, Hettinger, and Langdon. 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. Subsets of five genotypes with high pasta color scores (Joppa, D13344, D13500, D12846, D12863) and five genotypes with low pasta color scores (Alzada, Tioga, Divide, Strongfield, Mountrail) were selected from these samples.

Grain Milling Procedure

Grain samples (2 kg) were tempered to 14.5% moisture 24 h before milling and further conditioned from 14.5 to 17.5% moisture 45 min before milling. The tempered grain was milled into semolina with a Bühler MLU-202 experimental mill fitted with two Miag laboratory-scale purifiers (Bühler-Miag, Minneapolis, MN, U.S.A.). Additional grain samples (75 g) were ground into whole wheat meal using a UDY cyclone sample mill (UDY Corporation, Ft Collins, CO, USA).

Semolina Proximate Analysis

Semolina protein was determined using NIR technology from Foss Infratec 1241 Grain Analyzer. Ash content was determined using AACCI approved method 08-01.01. Visible specks in semolina were counted on a flat surface under a constant light source with three readings on different 6.5 cm^2 areas then converting the average to the number of specks/dm^2.

Pasta Processing

Semolina (1200 g) hydrated to 32% moisture was extruded through an 84-hole spaghetti die using a semi-commercial pasta extruder (DEMACO, Melburne, FL, U.S.A.). Extrusion conditions were: extrusion temperature, 45°C; mixing chamber vacuum, 46 cm of Hg; an auger length to diameter ratio of 8.1:1; and extrusion speed, 25 rpm. Spaghetti was dried in a laboratory dryer (Standard Industries, Fargo, ND, U.S.A.) using a low temperature drying cycle (length, 18 h; peak temperature, 40°C) as describe by Yue et al. 1998. Dry spaghetti (70 g) was ground into flour using a UDY cyclone sample mill.

Pasta Color

Pasta color score was determined using AACCI approved method 14-22.01. Hunter L-value, *a*-value and *b*-value of dried spaghetti were measured with a Minolta CR410 colorimeter (Konica Minolta, Ramsey, NJ). Spaghetti was placed on a black template that was 1.3 cm deep. Three measurements were taken at three different locations on the spaghetti. The data recorded was the average of the three readings. Color difference among L, *a*, and *b* values (ΔE) was calculated to determine if people can differentiate the color of spaghetti using the scale reported by Mokrzycki & Tatol (2012).

$$\Delta E \text{ color change} = \sqrt{((\Delta L)\text{^}2 + (\Delta a)\text{^}2 + (\Delta b)\text{^}2)} \quad (4.1)$$

$0 < \Delta E < 1$ - person does not notice the difference

$1 < \Delta E < 2$ - only experienced observer can notice the difference

$2 < \Delta E < 3.5$ - unexperienced observer also notices the difference

$3.5 < \Delta E < 5$ - clear difference in color is noticed

$5 < \Delta E$ - observer notices two different colors

Pasta color score was determined using the color map developed for model CR310 described for AACCI and Debbouz (1994). Pasta color scores were classified as low with values <8, medium with values between 8-9, and high with values >9.

Yellow Pigment Content

Yellow pigment content was determined by using a modified AACCI approved method 14-50.01. The sample size was 4 g of ground material instead of 8 g. The solvent was prepared in 5:1 ratio, which corresponds to 20 mL of water saturated n-butanol reagent (WSB) added to 4 g of ground sample. Absorbance of the supernatant was measured in a spectrophotometer (Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer) at 436 nm. Measurements per extracted sample were converted to yellow pigment concentration (μg/g or ppm) using β-carotene extinction coefficient 1.6632.

Soluble Brown Pigment Content

Soluble brown pigment content was determined using a modified procedure described by Matuso & Irvine (1967). Ground material (2 g) was added to 4 mL deionized distilled water. The mixture was shaken three times on a vortex mixer for 5 min followed by a 5 min rest; after which the samples were centrifuged (Eppendorf 5810R centrifuge, Rotor: F-34-6-38, Radius: 11.5cm) for 4 min at 18,514 x *g* relative centrifuge force (RCF). Supernatant (1.5 mL) was placed into an Eppendorf tube and centrifuged for 6 min. Absorbance of the supernatant was measured in a spectrophotometer (Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer) at 400 nm. Measurements per extracted were in OD_{400}.

Peroxidase (POD) Activity

Peroxidase activity was determined by using the method described by Honold and Stahmann (1968) and Fraignier et al. (2000) with some modifications. Ground material (150 mg)

was suspended in 1.5 mL of 50 mM phosphate citrate buffer pH 4.6 (1:10 w/v) and rotated for 2 h at room temperature in an orbital shaker (Glas-Col, Terre Haute, IN, USA). After rotation, samples were centrifuged for 10 min at 14,000 × *g*. Then, 150 µL of the supernatant was mixed with 1.5 mL of solution contained 5 mM guaiacol, 10 mM H_2O_2, and 50 mM citrate phosphate buffer (pH 5). POD activity was determined spectrophotometrically (Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer) at 470 nm by measuring the oxidation of guaiacol to tetrahydroguaiacol in the presence of H_2O_2 for a min measuring every 15 sec. The total activity was expressed in units per gram (U/g) of dry weight. One unit of enzyme activity was defined as a change in absorbance unit per min.

Polyphenol Oxidase (PPO) Activity

Polyphenol oxidase activity was determined using AACCI approved method 25-85.01. Solution (1.5 mL) composed of 5 mM of L-3, 4 dihydroxyphenylalanine (L-DOPA) in 50 mM of 3-(N-morpholino) propanesulfonic acid (MOPS) buffer pH 6.5, 0.02% Tween-20 was added to micro-centrifuge tube containing 200 mg of ground material. The tubes were placed on an orbital shaker (Glas-Col, Terre Haute, IN, USA) and were rotated for 1 h at room temperature to allow the reaction. Then samples were centrifuged for 2 min at 14,000 x *g*. Supernatant absorbance was measured at 475 nm (Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer). A control sample was prepared for each sample but treated with a solution without L-DOPA. The absorbance of the control was subtracted from each corresponded sample. The enzyme activity was expressed ΔA_{475}/ min g of sample. One unit of enzyme activity was defined as a change in absorbance unit per min.

Milling and Processing Effect

Milling and processing effects were determined as the percentage based on whole wheat concentration/activity using the following equations:

Percent after Milling = ((whole wheat value- semolina value) ×100)/ whole wheat value (4.2)

Percent after Processing = ((semolina value-pasta value) ×100)/ semolina value (4.3)

Total Percent = ((whole wheat value – pasta value) ×100)/whole wheat value (4.4)

Experimental Design and Statistical Analysis

Descriptive statistics were computed using all data (n=143). Then ten genotypes were selected as a subset of the 24 genotypes and were subjected to an analysis of variance where the experimental design was a randomized complete block with. Environments (6) were considered as replications. Genotypes and environments were considered fixed effects. Analysis of variance (ANOVA) at 95% level of confidence (F tests: $P \leq 0.05$) was performed on 10 selected genotypes based on pasta color score. PROC GLM procedure and Fisher's-Protected LSD at $P=0.05$ were used. Pearson's correlation was used to evaluate the relationship among all 10 genotypes means (n=60) and averaged genotypes (n=10) across environments for parameters tested. Stepwise multiple linear regression was performed to identify quality parameters that can predict pasta color score, pasta brightness, and pasta yellowness. A significance level of $P \leq 0.05$ was used for forward inclusion of quality traits in the regression model. All analyses were performed by Statistical Analysis System (SAS) version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Descriptive Statistics and Pasta Color Parameters

The median, mean, standard deviation, and range for the overall data (n=143) is summarized in Table 13. Median and mean values were similar for each studied parameters

except POD activity in semolina and pasta where the median and mean values were 9.49 and 15.74 U/g and 0.16 and 10.55 U/g, respectively. These results indicate that more than 50% of these values were smaller than their mean values. Quality traits ranged from poor to excellent quality. For example, pasta color scores ranged from 5.5 which would not be acceptable commercially to 10 which would be considered highly commercially acceptable. Pasta color score had a mean value of 8.3 and median value of 8.5 which ranked most of the genotypes commercially acceptable.

Pasta color parameters and ΔE color change are summarized in Table 14. Genotypes were grouped based on good and poor pasta color scores. Within each group, Hunter L, *a,* and *b* values differed. Interestingly, both groups had similar range and means for L and *a*-values. For example, the L-value means were 53.36 and 53.00 and *a*-value means were 3.06 and 3.04 for good and poor pasta color, respectively. Thus, there does not seem to be a clear relationship between L-value or *a*-values and pasta color score. Pasta yellowness (Hunter *b*-value) showed significant differences between genotypes groups. In this case, genotypes with a good pasta color score had greater *b*-values than did genotypes with poor pasta color scores. An exception was Alzada, which had a moderately high *b*-value but low L-value which resulted in poor overall pasta color score.

Hunter L, *a, b* values for Mountrail, which had the lowest pasta color score, were used as the control comparison to calculate delta E. Mokrzycki & Tatol (2012) developed a scale involving the delta color change that can be used to predict if a person could recognize the differences in color between pasta samples. Part of the scale includes $0 < \Delta E < 1$ **(**person does not notice the difference); $1 < \Delta E < 2$ (only experienced observer can notice the difference); and $2 < \Delta E < 3.5$ **(**unexperienced observer notices the color difference). Based on this scale, only an

experienced observer would notice color differences between the good pasta color group and Mountrail. Interestingly, within the poor pasta color group, an unexperienced observer would be able to notice color difference between Alzada and Mountrail. These results indicate that more research is needed to better understand these results.

Table 13. Descriptive statistics for quality traits based on overall data, n=143.

Quality trait	Median	Mean	SD†	Range
Grain protein (%)	14.40	14.51	1.51	10.8-18.3
Semolina protein (%)	13.20	13.20	1.38	9.7-16.1
Ash content (%)	0.76	0.77	0.09	0.56-0.94
Semolina extraction (%)	66.43	67.15	2.64	60.76-73.17
Specks count (specks/dm^2)	30	32.61	8.61	17-57
Pasta color				
Pasta Hunter L	53.08	53.17	1.73	48.17-56.46
Pasta Hunter *a*	2.83	3.01	0.69	1.92-5.08
Pasta Hunter *b*	25.79	25.46	1.99	20.75-29.12
Pasta color score	8.5	8.3	0.97	5.5-10.0
Yellow pigment content (ppm)				
Whole wheat	8.7	9.2	1.9	5.6-14.5
Semolina	7.0	7.2	1.3	4.0-10.8
Pasta	5.5	5.7	1.2	2.9-9.4
Soluble brown pigment content (OD_{400})				
Whole wheat	0.50	0.52	0.11	0.33-0.96
Semolina	0.16	0.18	0.07	0.04-0.64
Pasta	0.13	0.17	0.08	0.07-0.71
PPO activity (ΔA_{475}/min g)				
Whole wheat	0.33	0.36	0.18	0.04-1.08
Semolina	0.10	0.10	0.06	0.00-0.29
Pasta	0.05	0.05	0.02	0.01-0.19
POD activity (U/g)				
Whole wheat	39.4	40.7	20.2	11.3-104.0
Semolina	9.5	15.7	7.4	3.3-36.8
Pasta	0.2	10.6	6.3	0.9-28.8

† SD= Standard deviation.

Table 14. Means for pasta color parameters and ΔE change color averaged across genotype.

Genotype	Pasta HL[†]		Pasta H*a*		Pasta H*b*		Pasta color score		ΔE color change
Good color score									
Joppa	53.73	a	2.88	de	26.09	a	8.6	a	1.9
D13344	53.78	a	2.97	cde	26.08	a	8.6	a	1.9
D13500	53.07	bc	3.12	bc	25.69	ab	8.6	a	1.8
D12846	52.88	c	3.32	b	26.06	a	8.7	a	0.7
D12863	53.36	abc	3.01	cd	25.89	a	8.7	a	1.9
Mean	53.36		3.06		25.96		8.6		1.6
Bad color score									
Alzada	51.72	d	3.76	a	25.15	bc	8.0	b	2.8
Tioga	52.98	bc	3.05	cd	24.99	cd	8.0	b	1.4
Divide	53.58	ab	2.78	e	24.48	cde	7.9	b	0.6
Strongfield	52.74	c	3.09	bcd	24.82	de	7.9	b	1.5
Mountrail	53.98	a	2.50	f	24.22	e	7.7	b	0.0
Mean	53.00		3.04		24.73		7.9		1.26

[†] Pasta HL= pasta Hunter L-value; Pasta H*a*= pasta Hunter *a*-value; Pasta H*b*= pasta Hunter *b*-value. Values followed by different letters in the columns are significantly different at $P \leq 0.05$.

Yellow Pigment Content

Means for yellow pigment content in whole wheat, semolina, and pasta, and the loss of yellow pigment content caused by milling and processing are summarized in Table 15. Overall mean for yellow pigment content was greatest in whole wheat fraction (9.0 ppm), intermediate in semolina (7.1 ppm) and least in pasta (5.6 ppm). Similar responses to milling and processing were obtained by Matsuo et al. (1982) who reported yellow pigment content in whole wheat of 7.6 ppm, in semolina of 5.2 ppm, and of 3.6 ppm in pasta. Clarke et al. (2006a) obtained similar results for yellow pigment content in the endosperm (semolina) (~ 5 to 8 ppm). On average, milling reduced yellow pigment content by 21.6% and pasta processing reduced yellow pigment content 21.1% for a total decline in yellow pigment content of 38.1%. Based on results presented

in their paper, Matsuo et al. (1982) reported reduction in yellow pigment by milling and processing of 31.6 and 30.8% with a total reduction from whole wheat to pasta of 52.6%.

Table 15. Means for yellow pigment content and its milling, processing and total effect averaged across genotype.

Genotype	Yellow pigments, ppm						Loss of yellow pigment, %		
	Whole wheat		Semolina		Pasta		Milling effect	Processing effect	Total change
Good color score									
Joppa	9.3	cd	7.7	abc	6.1	b	17.1	21.1	34.6
D13344	9.8	bc	7.5	a-d	5.8	bc	23.2	22.9	40.7
D13500	10.6	a	7.8	ab	6.3	ab	25.8	19.7	40.4
D12846	10.2	ab	8.0	a	6.8	a	21.4	15.5	33.6
D12863	8.7	de	7.6	a-d	6.0	b	13.4	21.2	31.7
Mean	9.7		7.7		6.2		20.2	20.1	36.2
Bad color score									
Alzada	9.3	cd	7.1	bcd	6.1	b	23.8	13.3	34.0
Tioga	8.6	e	6.8	de	5.3	c	21.1	21.7	38.2
Divide	7.7	f	6.0	e	4.3	d	21.4	29.4	44.5
Strongfield	8.9	de	6.9	cd	5.4	c	22.6	22.3	39.9
Mountrail	6.9	g	5.1	f	3.9	d	25.8	23.5	43.2
Mean	8.3		6.4		5.0		22.9	22.0	40.0
Overall mean	9.0		7.1		5.6		21.6	21.1	38.1

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

For genotype main effect, yellow pigment content in whole wheat, semolina, and pasta was greater for good vs poor pasta color score genotypes (Mean= 9.7 vs 8.3, 7.7 vs 7.1, and 6.2 vs 5.6 ppm, respectively). In all the cases Mountrail and Divide had the lowest yellow pigment contents while in most of the cases D13500 and D12846 had the highest values.

Milling resulted in greater yellow pigment loss for genotypes with poor (22.9%) than with good (20.2%) pasta color scores. Joppa and D12863 had lowest losses due to milling.

Milling losses for D13344, D13500 and D12846 were similar to those for genotypes with poor pasta color scores. In fact, D13500, good score and Mountrail, poorest score, had the same percentage loss of yellow pigment content caused by milling. Differences in percent loss due to milling could indicate that the genotypes differed in yellow pigment distribution in the grain. Pasta processing generally caused greater percentage loss in poor pasta color genotypes than good pasta color genotypes which ranged from 15.5 to 22.9% for good pasta color and 13.3 to 29.4% for poor pasta color. Alzada which had poor pasta color, had noticeably the lowest loss of yellow pigment by processing of any other genotypes. Divide lost the most (29.4%) while Alzada lost the least amount (13.3%) of yellow pigments.

Soluble Brown Pigment Content

Means for soluble brown pigment content in whole wheat, semolina, and pasta, and the loss of soluble brown pigment content caused by milling and processing are summarized in Table 16. Whole wheat fraction had the highest overall mean for soluble brown pigment content (0.53 OD_{400}), followed by semolina (0.18 OD_{400}) and pasta (0.16 OD_{400}). Matsuo et al. (1982) reported average OD_{400} value of 0.30 in pasta.

For genotype main effect, the soluble brown pigment content varied with genotype. D13500 had the highest and Mountrail had the lowest soluble brown pigment in the whole wheat, with the overall average greater with good pasta color genotypes than with the poor pasta color genotypes. Milling caused the decline in brown pigments for all genotypes. On average, the reduction in soluble brown pigments was greater for good color score than for genotypes with poor pasta color (Mean=69.5 vs 59%, respectively). Divide (46.1%) and Mountrail (50.6%) were the genotypes with the lowest reduction in soluble brown pigment content. After milling, soluble

brown pigment content tended to be greater in semolina of poor pasta color than good pasta color genotypes.

Table 16. Means for brown pigment content and its milling, processing and total effect averaged across genotype.

Genotype	Brown pigments, OD_{400}						Loss of brown pigments, %		
	Whole wheat		Semolina		Pasta		Milling effect	Processing effect	Total change
Good color score									
Joppa	0.51	bcd	0.14	b	0.16	b	71.9	-7.1	69.9
D13344	0.49	bcd	0.16	b	0.18	b	67.6	-9.5	64.5
D13500	0.62	a	0.18	ab	0.16	b	71.9	7.1	73.9
D12846	0.55	abc	0.15	b	0.15	b	71.8	3.0	72.6
D12863	0.51	bcd	0.18	ab	0.14	b	64.3	20.8	71.7
Mean	0.54		0.16		0.16		69.5	2.9	70.5
Bad color score									
Alzada	0.51	bcd	0.17	ab	0.24	a	66.2	-40.7	52.4
Tioga	0.58	ab	0.18	ab	0.14	b	68.5	21.8	75.4
Divide	0.47	cd	0.25	a	0.14	b	46.1	46.0	70.9
Strongfield	0.50	bcd	0.18	ab	0.14	b	63.8	24.6	72.7
Mountrail	0.45	d	0.22	ab	0.15	b	50.6	33.2	67.0
Mean	0.50		0.20		0.16		59.0	17.0	67.7
Overall mean	0.53		0.18		0.16		64.3	10.0	69.1

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

Pasta processing caused an increase in brown pigment content for some genotypes. Alzada had the highest increase equal to 40.7% while Divide had the greatest decline equal to 46.0% (Table 16). All genotypes had similar amount of soluble brown pigment in the pasta (0.14 to 0.18 OD_{400}) except for Alzada which had the highest brown pigment content (0.24 OD_{400}). Because of the increase in content in some of the genotypes, the effect of processing was greater

for genotypes with poor pasta color score than good color score (mean=17 vs 2.9%, respectively).

PPO Activity

Means for PPO activity in whole wheat, semolina, and pasta, and the loss of PPO activity caused by milling and processing are summarized in Table 17. Whole wheat fraction showed the greatest PPO activity (overall mean = 0.316 ΔA_{475}/min), intermediate in semolina (overall mean = 0.086 ΔA_{475}/ min) and the least in pasta (overall mean = 0.049 ΔA_{475}/min). Different studies have reported that flour and germ have low PPO activity (Marsh & Galliard, 1986; Baik et al., 1994) while bran has high activity (Okot-Kotber et al., 2001).

Table 17. Means for PPO activity and its milling, processing and total effect averaged across genotype.

Genotype	PPO Activity, ΔA_{475}/min						Loss of PPO Activity, %		
	Whole wheat		Semolina		Pasta		Milling effect	Processing effect	Total change
Good color score									
Joppa	0.36	ab	0.08	a	0.05	a	76.5	41.2	86.2
D13344	0.32	abc	0.10	a	0.05	ab	69.5	53.7	85.9
D13500	0.40	a	0.12	a	0.06	a	69.4	52.9	85.6
D12846	0.25	c	0.11	a	0.06	a	56.3	47.7	77.2
D12863	0.29	bc	0.06	a	0.05	ab	78.0	29.4	84.4
Mean	0.32		0.10		0.05		69.9	45.0	83.9
Bad color score									
Alzada	0.32	abc	0.09	a	0.04	ab	72.9	49.0	86.2
Tioga	0.37	ab	0.07	a	0.05	a	80.6	23.5	85.2
Divide	0.27	bc	0.10	a	0.04	b	62.5	64.6	86.7
Strongfield	0.36	ab	0.09	a	0.06	a	76.4	34.6	84.6
Mountrail	0.23	c	0.07	a	0.04	b	70.9	44.1	83.7
Mean	0.31		0.08		0.05		72.7	43.2	85.3
Overall mean	0.32		0.09		0.05		71.3	44.1	84.6

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

For genotype main effect, there were small differences among genotypes for PPO activity in whole wheat and pasta, while PPO activity in semolina was similar for all genotypes. Both good and poor pasta color groups contained genotypes with high and low PPO activity in whole wheat. PPO activity in pasta with good color was similar for genotypes; however, for poor color, PPO activity was significantly lower in Divide and Mountrail. Overall, PPO activity in whole wheat (0.54 and 0.50 ΔA_{475} /min), semolina (0.16 and 0.20 ΔA_{475}/ min) and pasta (0.16 and 0.16 ΔA_{475}/ min) was similar for genotypes with good and poor pasta color, respectively.

Milling and pasta processing effects caused a reduction in PPO activity for all genotypes. Means between the two groups indicate that reduction in PPO activity due to milling was similar for good and poor color score genotypes (69.9 vs 72.7%, respectively). D12846 (good pasta color) had the lowest reduction of PPO activity due to milling (56.3%) while Tioga (poor pasta color) had the highest decline (80.6%). Pasta processing caused the highest decline in PPO activity (64.6%) with Divide (poor pasta color) and the least (29.4%) with D12863, a genotype with good pasta color.

POD Activity

Means for POD activity in whole wheat, semolina, and pasta, and the loss of POD activity caused by milling and processing are summarized in Table 18. Whole wheat fraction had the greatest overall POD activity (37.7 U/g), intermediate in semolina (14.6 U/g) and the least in pasta (9.4 U/g).

For genotype main effect, in whole wheat, POD activity was greatest with Alzada (50.6 U/g) while the remaining nine genotypes had similar POD activities that ranged from 33.4 to 38.8 U/g. POD activity in semolina and pasta varied with genotype but the range in activity was similar for good and poor pasta color groups for semolina (9.6 to 24.4 U/g and 9.4 to 26.9 U/g,

respectively) and pasta (5.8 to 19.1 U/g and 6.3 to 17.3 U/g, respectively). Fraigner et al. (2000) reported a big range in POD activity (0.5 to 21.8 U/g) between in the semolina of two different genotypes. Milling and pasta processing caused the decline of POD activity in all genotypes.

Milling reduced POD activity, the least with Joppa (34.5%) and Alzada (46.9%) and the most with D13344 (72.3%), D12846 (73.5%), D12863 (72.6%) and Tioga (73.8%). The reduction due to pasta processing was the greatest with Mountrail (53.2%) and the least with Joppa (21.5%).

Table 18. Means for POD activity and its milling, processing and total effect averaged across genotype.

Genotype	POD activity, U/g						Loss of POD activity, %		
	Whole wheat		Semolina		Pasta		Milling effect	Processing effect	Total change
Good color score									
Joppa	37.2	b	24.4	a	19.1	a	34.5	21.5	48.6
D13344	36.8	b	10.2	bc	5.8	c	72.3	43.3	84.3
D13500	33.4	b	12.6	bc	7.8	cb	62.2	37.8	76.5
D12846	36.2	b	9.6	c	6.6	c	73.5	31.3	81.8
D12863	37.3	b	10.2	bc	5.4	c	72.6	47.2	85.5
Mean	36.2		13.4		8.9		63.0	36.2	75.3
Bad color score									
Alzada	50.6	a	26.9	a	17.3	a	46.9	35.7	65.8
Tioga	35.8	b	9.4	c	7.3	c	73.8	22.2	79.6
Divide	37.0	b	14.7	b	7.2	c	60.6	51.2	80.6
Strongfield	38.8	b	14.3	b	11.0	b	63.2	22.7	71.5
Mountrail	33.7	b	13.4	bc	6.3	c	60.3	53.2	81.4
Mean	39.2		15.7		9.8		60.9	37.0	75.8
Overall mean	37.7		14.6		9.4		62.0	36.6	75.6

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

Model for Pasta Color/Score

Stepwise multiple linear regression was performed for pasta color score and its components, pasta Hunter L-value and pasta Hunter *b*-value (n=60) using grain protein content, semolina protein content, semolina ash content, semolina extraction, semolina speck count and whole wheat, semolina and pasta contents of yellow pigment and soluble brown pigment and activities of PPO and POD as potential predictive variables. Stepwise multiple linear regression equations to predict pasta color score and its components (pasta Hunter L-value and pasta Hunter *b*-value) are presented in Table 19.

Stepwise multiple linear regression analysis indicated that speck count (partial R^2= 0.55), semolina yellow pigment content (partial R^2= 0.15), and semolina ash content (partial R^2= 0.15) were major factors and grain protein (partial R^2= 0.03), pasta yellow pigment content (partial R^2= 0.01), and semolina soluble brown pigment content (partial R^2= 0.01) were minor factors in determining pasta color, all of which explained 90% of the variability of the data. For pasta brightness (Hunter L-value), regression analysis indicated that semolina protein content (partial R^2= 0.75) and semolina ash content (partial R^2= 0.09) were major factors and speck count (partial R^2= 0.02) and pasta POD activity (partial R^2= 0.01) were minor factors, all of which explained 87% of the variability of the data. For pasta yellowness (Hunter *b*-value) regression analysis indicated that speck count (partial R^2= 0.59), semolina yellow pigment content (partial R^2= 0.17), and semolina protein content (partial R^2= 0.10) were major factors and pasta yellow pigment content (partial R^2= 0.04) and semolina ash content (partial R^2= 0.02) were minor factors, all of which explained 91% data variability. Overall, regression equations indicate that pasta color score was enhanced by semolina yellow pigment content and pasta yellow pigment

content and diminished by grain protein, semolina ash content, speck count and semolina brown pigment content.

Table 19. Stepwise multiple linear regression for pasta HL and H*b* color values and pasta color score vs quality traits, n=60.

Color parameter	Quality trait	Effect[†]	Partial R^2	R^2
Pasta brightness	Semolina protein content	(-)	0.75	0.87
	Semolina ash content	(-)	0.09	
	Speck counts	(-)	0.02	
	Pasta POD activity	(-)	0.01	
Equation	$(71.01-0.71_{SP}-7.09_{Ash}-0.05_{SC}-0.04_{PD})$			
Pasta yellowness	Speck count	(-)	0.59	0.91
	Semolina yellow pigment content	(+)	0.17	
	Semolina protein content	(-)	0.09	
	Pasta yellow pigment content	(+)	0.04	
	Semolina ash content	(-)	0.02	
Equation	$(34.59-0.61_{SP+}0.18_{yp}-6.78_{Ash}-0.02_{SC}+0.72_{PYP})$			
Pasta color score	Speck count	(-)	0.56	0.90
	Semolina yellow pigment content	(+)	0.15	
	Semolina ash content	(-)	0.15	
	Grain protein content	(-)	0.03	
	Pasta yellow pigment content	(+)	0.01	
	Semolina soluble brown pigment	(-)	0.01	
Equation	$(12.68-0.18_{GP}-3.87_{Ash}-0.03_{SC}+0.28S_{YP}+0.27_{PYP}-1.03_{SB})$			

[†]SP=semolina protein; SC=specks count; WP=PPO activity in whole wheat; PD=POD activity in pasta; WB= whole wheat brown pigment content; GP=grain protein; SYP=semolina yellow pigments; PYP=pasta yellow pigments; SB=semolina brown pigments; WD=whole wheat POD

Discussion

Grain harvested in the twelve environments ranged from poor to excellent quality (Table 13). Pasta color scores ranged from 5.5 which would not be acceptable commercially to 10 which would be considered highly commercially acceptable. Pasta color is one of the most important quality parameters for consumer's choice (Troccoli et al., 2000). Pasta color is often

measured using a reflectance colorimeter method which determines Hunter L, *a*, *b*-color values, where L-value indicates brightness, *a*-value indicates redness when positive and greenness when negative, and *b*-value indicates yellowness when positive. Hunter L and *b*-values are used to assign a pasta color score on a scale of 1 to 12 where scores over 8 are commercially acceptable (AACCI approved method 14-22.01). Among factors that could affect final pasta color, the implication of yellow pigment content, brown pigment content, PPO activity and POD activity have been reported by Matsuo & Irvine (1967); Kobrehel & Gautier (1974); Kruger (1976); Borrelli et al. (1999); Clarke et al. (2006a). In general, genotypes with good pasta color score had more yellow pigment content, higher L and *b* values, and less soluble brown pigment content than genotypes with poor pasta color scores (Tables 14, 15, and 16).

Whole wheat meal contained more yellow pigment, soluble brown pigment and greater PPO activity and POD activity compared to semolina (Table 15, 16, 17, and 18). Some of the yellow pigment content, soluble brown pigment content, and PPO and POD activities in the semolina are due to the level of milling extraction and bran contamination, as indicated by the presence of bran specks in the semolina of the tested genotypes which ranged from 28 to 39/dm^2 (data not shown). The average speck count for good pasta color score genotypes was lower (31/dm^2) than for poor pasta color score genotypes (34/dm^2). Greater amount of bran and outer endosperm occurs in semolina as milling extraction increases. Dexter et al. (2004) reported that pasta color declined as milling extraction increased above 65%. Results of this research indicate that milling caused a reduction in yellow pigment content, soluble brown pigment content, and PPO and POD activities (Tables 15, 16, 17, and 18). Yellow pigment content declined less during milling than did soluble brown pigment content or POD and PPO activities. The effect of milling reflects the distribution of yellow pigment and soluble brown pigment and POD and PPO

activities in the grain kernel. Yellow pigments have been reported to be more concentrated in the outer than inner endosperm; however, it is genotype dependent, with genotypes varying in their concentration gradient from the outer to inner endosperm (Fu et al., 2017; Borrelli et al., 1999). This could explain differences in the loss of yellow pigment content during milling ranged from 13.4% to 25.8% (Table 15). In addition, PPO activity has been reported to be greater in the bran (Hatcher & Kruger, 1993; Okot-Kotber et al., 2001) while POD activity has been reported to be greater in the bran and germ (Fraignier et al., 2000) than the endosperm. Within the endosperm, PPO and POD activities are greater in the outer layer of the endosperm. Different isozymes have been reported for POD in the milled fractions and could be related to pasta brownness (Fraignier et al., 2000). To our knowledge, there is no information about the localization of brown soluble pigments in durum wheat but based on the results of this research it seems that they are also found in greater concentrations in the bran/germ layer than in the endosperm.

Pasta processing is the critical final point for pasta color. There was reduction in yellow pigment content and PPO and POD activities associated with pasta processing. Interestingly, pasta processing caused both increase and decrease in soluble brown pigment content, depending on genotype. Overall, milling reduced yellow pigment content, POD activity, PPO activity and soluble brown pigment content 1.3, 4.4, 6.2 and 17.5 X more than did pasta processing. Contrarily to our findings, Borrelli et al. (1999) determined that pasta processing was the most responsible phase for yellow pigment loss with an overall average reduction of beta-carotene equal to 8% during milling and 16% during pasta processing. Most reduction in yellow pigment content by pasta processing is attributed to lipoxygenase activity (Borrelli et al., 1999; Borrelli et al., 2003). For Joppa, D13344, and Alzada, the soluble brown pigment content increased with pasta processing. This increase could be related to absorption at 400 nm by other soluble

compounds such as phenolic compounds co-extracted or to an increase in brown pigments due to Maillard reaction. Although, more research needs to be done to confirm this hypothesis, data suggests that processing had a relatively small effect on PPO and POD activity, particularly when compared with milling effect. On average PPO activity declined 0.230 units with milling and 0.037 units with processing similarly POD activity declined 23.1 U/g with milling and 5.2 U/g with processing. These results make sense, since more of the enzymes are removed during milling resulting in low activity in the semolina. Therefore, it is unlikely that PPO and/or POD have an important role in pasta color (Dexter et al., 1994); besides, POD requires hydrogen peroxide as its substrate which is absent during processing (Feillet et al., 2000).

Pasta color score is based on Hunter L and *b* values. Stepwise multiple linear regression indicated that the most important parameters determining pasta color were: speck counts, semolina protein content, semolina yellow pigment content, and semolina ash content (Table 19). The results suggest that yellow pigment content enhanced pasta color while speck count, protein content, and semolina ash content reduced pasta color. Specks, small particles of bran and germ, in the semolina represent impurities that are associated with elevated ash and protein contents. Several authors have reported that high levels of ash and protein in the semolina can reduce pasta and semolina yellowness and brightness (Kobrehel et al., 1974; Matsuo et al., 1982; Baik et al., 1994; Borrelli et al., 1999). Regression equation for pasta color score support the importance of speck count (partial R^2= 0.55). Specks represent bran and germ particles both of which are high in ash content, protein content and PPO and POD activities. Kobrehel et al. (1974) reported that high the protein content was associated with brownness of pasta. Breeders are looking for high protein content to improve cooking qualities; however, this could be detrimental for pasta color.

Neither enzymatic activity nor soluble brown pigment content indicates that they have an important role in affecting the final color of the pasta.

Conclusions

Overall, results indicate the importance of milling on end-product quality of pasta and identified semolina quality traits that can be used to select semolina that would produce the desired pasta color. There was 1.3X more loss of yellow pigments, 4.4X more loss of POD, 6.2X more loss of PPO, and 17.5X more loss of brown pigments due to milling than processing. The milling effect on yellow pigment and soluble brown pigment contents and PPO and POD activities is attributed to their distribution in the kernel. Although not measured in this research, the reduction of yellow pigments during pasta processing was most likely attributable to lipoxygenase activity. Brown pigment could be changed to other soluble components or formed by Maillard reaction. Data suggests that pasta processing had a relatively small effect on PPO and POD activity. Regression analysis determined that the most important parameters that explain pasta color were yellow pigment content (positive effect) and semolina protein content, semolina ash content, and speck counts (all with negative effect). For durum breeders, these results show the importance of selecting genotypes that have high yellow pigment content and excellent milling qualities.

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OVERALL CONCLUSIONS

The results of this research indicate that lower grain moisture content, higher temperature, and the addition of wet/dry events caused an increase in water gain. A single exposure to moisture (rainfall, heavy dew, or high relative humidity) seems to be enough to cause a declined in grain quality. Exposure to a single bulk water wet/dry cycle was enough to increase the kernel width and cause a rough and wrinkled bran surface which resulted in a decline in test weight and vitreous kernel content while kernel size and brightness increased. A single relative humidity event was enough to reduce vitreous kernel content and increase kernel size. Scanning electron microscope and light microscope show that the germ and ventral surface of grain are important sites for water absorption.

The major role of the environment on the response of evaluated traits can determine the end quality of high inherited traits, being relevant to consider it during selection. In general, the number of days with RH $\geq$ 80% diminished overall pasta color while the number of days with $\leq$ 13°C enhanced pasta yellowness and pasta color score. The negative effect of high relative humidity could be related to speck counts and soluble brown pigments while the positive effect of low temperature was not clear.

In this study, milling had a greater effect than did pasta processing on pasta color. The milling effect on yellow pigment, brown pigment, PPO, and POD activities was attributable to their distribution in the kernel. For the studied parameters, yellow pigment content had a positive effect, while protein content, semolina ash content, and speck counts had a significant negative impact on pasta color.

FUTURE RESEARCH AND INDUSTRIAL APPLICATIONS

Further research is needed to identify genotypes with tolerance to bleaching. Based on the results, the best way to evaluate genotypes would be by performing the wet/dry cycles experiment using bulk water and high relative humidity and to evaluate grain quality traits such as test weight, vitreous kernel content, kernel size, and kernel brightness.

In this research, it was found that pasta yellowness (*b*-value) might not be related to only yellow pigment content and it is unlikely that PPO/or POD had an effect on pasta color. Soluble brown pigment content increased in some genotypes after pasta processing. More research could be done to identify if Maillard products are involved in this increase. For example, determination of furosine, reducing sugars, and lysine which can be correlated to the extent of Maillard reaction. The determination of phenolic compounds which might be helpful to understand the role of brown pigments in pasta color. Besides the identification of soluble brown pigments components by using chromatography. High protein content in semolina was related to lower pasta color, more research could be done to identify if the protein quality or the composition are related to this phenomenon.

This information will be useful for farmers for agronomic decisions and breeders for future selection and crosses.