AN ABSTRACT OF THE THESIS OF

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Title: Oxidative Gelation And Functionality Of Wheat Flour: Effects Of Grain Storage, Flour Aging, And Grain Type (Hard Or Soft).

Abstract approved: _____

Andrew S. Ross

This study examined changes in flour functionality during storage of grain and subsequent aging of flour milled from the grain. Freshly harvested grain was stored for 24 weeks and flour milled from the grain at specified time intervals after harvest (0, 3, 6, 12, and 24 weeks). For each milling date flour functionality was measured on the day of milling (day 0) and at specified intervals after milling (1, 3, 6, 13, 27, and 62 days). Storage and aging were conducted at 23 ± 1°C. The functional properties examined were flour absorption characteristics, quantified using the solvent retention capacity (SRC) test, and oxidative gelation capacity (OGC), which was measured viscometrically, using a Rapid Visco Analyzer (RVA). SRC measures absorption capacity of flour emphasizing different flour polymers in each of the four solvents (water, all polymers: sucrose, arabinoxylans (AX) and gliadins: sodium carbonate, damaged starch: lactic acid, glutenins). Oxidative gelation is a process whereupon a weak gel is formed in a hydrated flour system under oxidative conditions and is thought to be largely a function of the reactivity of ferulic acid residues esterified to AX, although proteins are also involved. To determine if oxidation of flour lipids might accompany changes in OGC, the concentration of malondialdehyde (MDA), a lipid oxidation byproduct, was measured. Four soft-wheat varieties with divergent functionalities were selected to examine the effects of storage and aging. Additionally, a survey was conducted to examine the range of OGC in a selection of hard-grained wheat varieties from the Oregon State University wheat-breeding program. From this survey, one variety with high and one variety with

low OGC were selected for a pilot study to determine the effect of hydrogen peroxide and azodicarbonamide (ADA) concentrations on OGC in straight grade flour.

Variety was the strongest factor in determining flour functionality expressed as SRC and OGC. This is unsurprising, because varieties were chosen based on differences in absorption characteristics and OGC as indicated by preliminary testing. In contrast, variety was the weakest factor in determining changes in MDA concentration.

As a function of grain storage time, water, sucrose and sodium carbonate SRC values increased. In contrast, lactic acid SRC values declined. Although many of these changes were statistically significant, their functional significance remains unclear. As a function of grain storage time, OGC initially increased to week 3 then declined to week 24. Not only was this change statistically significant, but the magnitude of the change could be considered functionally significant. Because OGC is a trait that currently only has theoretical value in food processing (i.e. OGC is not a trait currently taken into consideration during food processing), it is difficult to definitively conclude what constitutes functional significance. Grain storage time had the strongest influence on changes in MDA concentration. The trend of changes in MDA concentration was similar to that observed for OGC.

Flour age was the weakest contributor to changes in SRC. Looking at individual SRC solvents, flour aging time did not significantly influence changes in water SRC values. However, as flour aged, sucrose SRC values significantly increased and sodium carbonate and lactic acid SRC values decreased. Although changes in sucrose, sodium carbonate, and lactic acid SRCs were statistically significant, their functional significance was again unclear. As a function of flour age, OGC increased. As a function of flour age, MDA concentration initially increased, but subsequently declined and remained constant from day 6 to day 62.

Each variety appeared to show a different relationship between peroxide peak viscosity (PPV) and peroxide peak breakdown viscosity (PPBV). Proportional PPBV also appeared

to differ between varieties, and the relationships (PPBV% vs PPV) were nonlinear. This suggested that there was a maximum PPV for each variety at which PPBV no longer increased. Data suggest qualitative differences in the gels formed in each variety that require further investigation.

Speculation allows the idea that the RVA method used here could provide a way of expressing functional differences in OGC that might relate to structure differences in AX.

Oxidative Gelation And Functionality Of Wheat Flour: Effects Of Grain Storage, Flour Aging, And Grain Type (Hard Or Soft).

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Jacob E. Mattson, Author

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CONTRIBUTION OF AUTHORS

Dr. Andrew S. Ross initiated the project, guided it along the way and contributed to the manuscript. Dr. Teepakorn Kongraksawech assisted with statistics and laboratory procedures. Omar Miranda-Garcia assisted with laboratory procedures and data analysis.

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CHAPTER 1 - INTRODUCTION

Background

Cultivated for at least 10,000 to 12,000 years, wheat is grown on more land area than any other commercial food crop (Colledge and Conolly 2007; Curtis 2002a). In the current era over 600 million metric tons of wheat is produced worldwide annually. In 2012, the USA produced around 65 million metric tons, approximately 9% of global production. Within the United States, just over 24 million metric tons of wheat were milled to flour for food use (USDA 2013). This flour is used to make a wide variety of products, such as breads, noodles, coating batters, cookies, and cakes.

The composition of wheat flour determines its processing characteristics and plays a key role in end-product quality. The major components of wheat flour are starch, protein, and non-starch polysaccharides (NSP). In wheat, the most prevalent NSP are arabinoxylans (AX). While much lower in abundance than the major components lipids play an important role in wheat-flour quality. Wheat flour is not an inert system. It undergoes chemical reactions as a result of exposure to light and air (Halton and Fisher 1937; Kozmin 1935). This aging process changes the end-product performance of wheat flour (Chen and Schofield 1996). How soft-wheat flour functionality changes upon hydration at different stages of grain and flour age is the main focus of this dissertation.

The overall hypothesis was that storage of grain and subsequent aging of flour will systematically change the functionality of soft-wheat flour as expressed through the absorption characteristics and oxidative gelation capacity of the resultant flour.

Objectives of the Thesis

1. Determine the effect of grain storage and flour aging on absorption characteristics of break flour milled from soft white winter wheat (Chapter 4).
2. Determine the effect of grain storage and flour aging on OGC and lipid peroxidation of break flour milled from soft white winter wheat (Chapter 5).

3. Survey hard-wheat for OGC, observe changes in OGC after 14 days of flour storage, and determine the effect of varying levels of hydrogen peroxide and ADA on the oxidative gelation of two wheat varieties with differing OGCs (Chapter 6).

CHAPTER 2 - LITERATURE REVIEW

Wheat

Wheat (*Triticum spp.*) is a crop that has both dietary and cultural significance. It was one of the first food crops domesticated: 10,000 - 12,000 years ago in the Fertile Crescent (Gustafson et al. 2009). In some cultures, offering bread is a gesture that welcomes a guest. The motto of the United Nations' Food and Agriculture Organization, "*Fiat panis*" is Latin for "*let there be bread*." According to Wrigley (2009), wheat and bread are symbols of the presence of food, and absence of bread signifies hunger. Many ancient civilizations relied on wheat as a principle food source. Wheat was so important, in fact, that these civilizations attributed the provision of wheat to deities. Osiris and Demeter were deities from Ancient Egypt and Ancient Greece, respectively. Demeter was adopted into Ancient Roman mythology as Ceres (Wrigley 2009).

It is likely that wheat became significant due its ability to grow abundantly in a wide variety of climates, being grown on every continent except Antarctica. Like other desiccated cereal seeds, wheat grain can be stored for extended periods if kept dry, functioning as a secure food source. In the intact kernel cell walls or other such barriers compartmentalize the constituents, minimizing unwanted chemical reactions. These factors are responsible for the stability during storage (Delcour and Hoseney 2010). After wheat is milled to flour, the doughs that result from the addition of water and from the mechanical energy of mixing have the unique ability to retain gas. This phenomenon allows us to make a range of leavened (aerated) products that would otherwise be unavailable to us (Wrigley 2009).

The first types of wheat cultivated were diploids, and included einkorn (*T. monococcum*), *Aegilops speltoides*, and *T. tauschii* (Wrigley 2009). Each of these diploids contains only one genome (A, B, or D). Each diploid genome consists of two groups of seven chromosomes (2n = 2*7 = 14 total chromosomes, e.g. AA). Tetraploid wheats were cultivated next, primarily emmer (*T. dicoccum*). The tetraploid species also include modern durum (*T. durum*), and contain four groups of seven chromosomes (4n = 4*7 =

28 total chromosomes, e.g. AABB). The emmer and durum A and B genomes were derived from the hybridization of *T. monococcum* and *A. speltoides* (Wrigley 2009). Modern common wheat, *T. aestivum*, is hexaploid (6n = 6*7 = 42 total chromosomes, AABBDD) and originated from the hybridization of wild emmer (*T. dicoccoides*, AABB) and *T. tauschii* (DD) (Kimber and Sears 1987; Wrigley 2009). While over 90% of the wheat grown worldwide is modern common wheat, end-product performance can vary greatly between cultivars. Because of this, plant breeding is necessary to develop wheat varieties which best suit the needs of food processors (Wrigley 2009).

Wheat classification

For commercial purposes, wheat is classified using three major factors: kernel hardness, growth habit, and grain color. Based on kernel hardness, wheat can be classified as hard or soft (Feiz et al. 2008; Jolly et al. 1996). Kernel hardness has a profound effect on the spectrum of end-products that a wheat variety can make. As a result of higher starch damage (and therefore higher water absorption in the flour), hard wheats are better suited for high-moisture dough-based products such as breads. Lower starch damage levels (and therefore lower water absorption in the flour) in soft-wheats makes them better suited for low-moisture dough-based products such as cookies and for batter-based products such as cakes, pancakes, and coating batters (reviewed by (Ross and Bettge 2009). However, overly low absorption may be detrimental as this facilitates increased flow at standard solids concentrations with a concomitant need to increase solids concentrations to achieve acceptable flow characteristics (Ross et al. 2014). However, optimum flour absorption characteristics for batters for the plethora of potential uses (cakes, pancakes, coatings for fried and baked foods, etc.) are commonly proprietary knowledge and little if any information is available in the literature (Ross pers. comm.).

Based on growth habit, wheat can also be classified winter or spring. Winter wheats are planted in the Fall and require a period of cold temperature (0 to 5 °C for at least six weeks - a process called vernalization) before they can resume growth and form the heads containing wheat kernels (Atwell 2001; Curtis 2002b; Worland and Snape 2001). Winter wheat is harvested during the summer. Spring wheat, in contrast, does not require

vernalization to form heads (Ross et al. 2014). It is planted in the spring and harvested in late summer or early fall. In North America, spring wheat is planted in regions with harsh winters (e.g. the Dakotas) and winter wheat is planted in regions with milder winters (e.g. the Great Southern Plains and the Pacific North West). Spring wheat may also be planted during the fall in regions with more gentle winters (e.g. southern Oregon and South Asia) (Curtis 2002b)

Three genes control wheat vernalization (*VRN1*). These genes are located in the middle of the long arm on chromosomes 5A, 5B, and 5D (Gooding 2009; Law et al. 1976; Maystrenko 1980). The presence of a dominant allele in any one of the three *VRN1* loci will result in a spring wheat. Winter wheat contains recessive alleles in all three *VRN1* loci (Gooding 2009).

Based on the presence of red pigments in the true seed coat (within the pericarp), wheat is divided into two groups: red and white (Atwell 2001). The presence and intensity of this pigment are controlled by three red (*R*) genes located on the end region of the long arms of chromosomes 3A, 3B, and 3D (McIntosh et al. 1998). Red wheats contain at least one dominant red allele (*R).* The intensity of the pigment generally increases as the number of *R* alleles increases. White wheats contain only recessive (*r*) alleles (Flintham 1993). Red wheat pigments are the polyphenol compounds phlobaphene and proanthocyanidin (Himi and Noda 2005).

Based on the above criteria, most *common* (*T. aestivum*) wheat in the U.S.A. is divided into five market classes: hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), hard white (HW), and soft white (SW). HW and SW include both winter and spring types.

Durum wheat (*T. durum,* tetraploid*)* is another important class of commercial wheat (Stoddard 2004). Durum wheat is harder than hard common wheat. Its endosperm also contains a higher concentration of yellow carotenoid pigments than common wheat (Morris 2002). Durum wheat is primarily used for production of European-style pasta.

Kernel anatomy

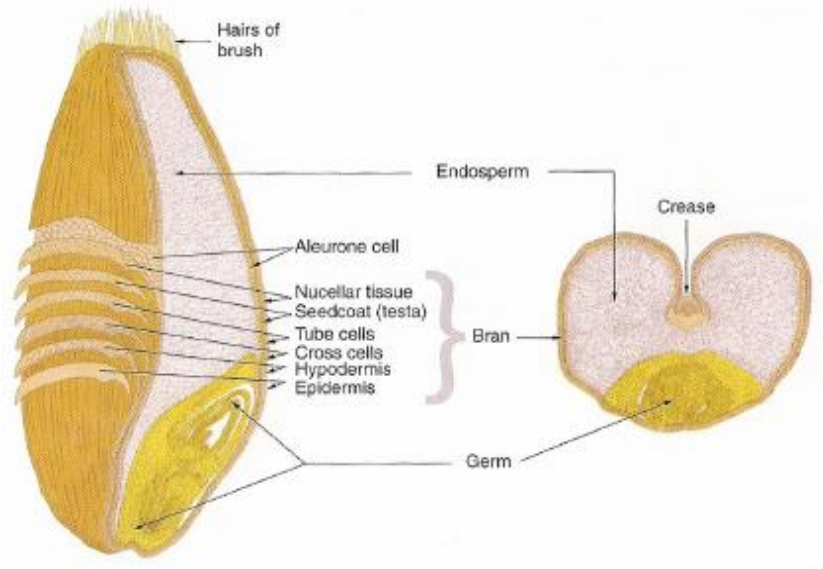

Figure 2.1: The structure of a wheat kernel (Dexter and Sarkar 2004).

Wheat plants produce a one-seeded fruit (caryopsis), which consists of germ (embryo) and endosperm enclosed by a tough outer layer known as bran. A general chemical composition of whole-wheat grain is shown in (Table 2.1). Specific composition can vary greatly depending on genotype and environment.

The endosperm is the largest anatomical structure of the kernel and accounts for about 80% of the total mass. Finely ground endosperm is the major component of white (refined) flour. There are four cell types in wheat endosperm. Aleurone cells are located between the endosperm and the pericarp. However, the aleurone layer is considered part of the bran by operational millers. Peripheral cells are located just inside the aleurone layer. Prismatic cells are located inside the peripheral cells, and central cells are located inside the prismatic cells (Evers and Bechtel 1988). The cells in the non-aleurone endosperm contain starch granules embedded in a protein matrix. Both starch and protein provide nutrition for the germinating embryo prior to photosynthesis. The endosperm is sometimes referred to as the "starchy endosperm," as it is upwards of 80% starch by weight. The endosperm also contains in the order of 2% AX, of which about 75% is

water-unextractable arabinoxylan (WUAX) and 25% is water-unextractable arabinoxylan (WEAX) (Atwell 2001; Ramseyer et al. 2011a). A minor constituent of wheat endosperm is lipid. Endosperm lipid is around 1% by weight of the kernel (Gwirtz et al. 2006).

Bran as defined by flour millers includes the aleurone although botanically it is the outer most layer of the endosperm. Under the millers' definition, bran is in the order of 17% of the mass of the kernel (Delcour and Hoseney 2010). Bran has three major layers: pericarp (fruit coat), testa (seed coat), and nucellar epidermis (hyaline layer). The pericarp surrounds the entire seed and acts as a protective covering. The pericarp contains several layers: epidermis, hypodermis, intermediate cells, cross-cells and tube cells. The seed coat is joined either to cross cells or tube cells on its distal (outer) side, and to the nucellar epidermis on its proximal (inner) side (Delcour and Hoseney 2010). The testa contains three layers: a thick outer cuticle, a colored layer containing pigment, and a thin inner cuticle. The nucellar epidermis lies between and is closely bound to the seed coat and the aleurone layer. It is comprised of mainly NSP (about 75% (w/w) of the total NSP in the wheat kernel), ash (9%), protein (11%), and lipids (5%) (Delcour and Hoseney 2010; Gwirtz et al. 2006; Pomeranz 1987).

The germ is 2 to 4% of the mass of the kernel. It has two major structures: scutellum and embryo. The scutellum is responsible for the transport of nutrients from the endosperm to the embryo during germination (Posner and Hibbs 1997). The germ is commonly removed during the milling process, due to the elevated lipid content (10%). This lipid is susceptible to oxidative rancidity, which reduces flour shelf life and quality (Gwirtz et al. 2006).

Chemical Component	Whole Grain	Bran	Endosperm	Germ
Protein	16.0	16.0	13.0	22.0
Fats	2.0	5.0	1.5	7.0
Carbohydrates	68.0	16.0	82.0	40.0
Dietary Fiber	11.0	53.0	1.5	25.0
Minerals (Ash)	1.8	7.2	0.5	4.5
Other Components	1.2	2.8	1.5	1.5
Total	100	100	100	100

Table 2.1: General whole wheat grain composition and that of its constituents (Anonymous 2002).

Kernel texture

Relative kernel texture (hardness) results from adhesion between starch granules and the surrounding protein matrix. This adhesion is weaker in soft-wheats and stronger in hard wheats. The degree of adhesion is controlled by friabilin proteins (Mikulikova 2007). In soft-wheat friabilins are found in high concentrations on the surface of water-washed starch granules. In hard wheat, friabilins are found in low concentrations. They are absent in durum wheat (Greenwell and Schofield 1986). Friabilin proteins are consist of three polypeptides: puroindolines a and b (pin-a and pin-b) and grain softness protein-1 (Gsp-1). Pin-a and pin-b are the major polypeptides in friabilins (Gautier et al. 1994; Giroux and Morris 1997; Mikulikova 2007; Rahman et al. 1994). Pin-a and pin-b are coded by two *puroindoline* genes (*Pin-a* and *Pin-b*) on the *Hardness* (*Ha*) locus located on the short arm of chromosome 5D (Gautier et al. 1994; Jolly et al. 1993; Morris et al. 1994). Although homologues of *Ha* are present on chromosomes 5A and 5B, they are not expressed (Giroux and Morris 1998).

The presence of wild type *Pin-a* and *Pin-b* genes result in soft kernel texture. An absence or mutation in one or both genes leads to strong protein-starch adhesion, resulting in hard kernel texture (Nadolska-Orczyk et al. 2009). A study observing variation of puroindoline content and heritability of puroindolines in 40 wheat cultivars grown in different locations suggested that *Pin-b* is responsible for kernel hardness (Igrejas et al. 2001). Absence of the D genome in tetraploid wheat species results in a lack of *Pin* genes and therefore a lack of puroindolines. Therefore, the texture of tetraploid wheats (e.g.

durum) is very hard (Bhave and Morris 2008). As this study is focused on soft-wheat, it is of interest to understand the factors that affect kernel texture *within* soft-wheats alone. Within the soft-wheats gradations in kernel texture are not controlled by the *Ha* locus, which is fixed as the wild type. However, the precise genetic control of kernel texture in soft-wheats is not known. Multiple quantitative trait loci (QTL) are implicated with little agreement on QTL between studies (Arbelbide and Bernardo 2006; Bordes et al. 2011; Campbell et al. 1999; G. et al. 2014; Groos et al. 2004; Kongraksawech 2012; Wang et al. 2012). There is also environmental modulation of kernel hardness in soft-wheat. For example grain protein concentration (GPC) is primarily controlled by environment. Ross et al (2012) showed that kernel hardness was significantly positively correlated with GPC across 45 soft-winter wheat genotypes grown at 6 locations in Oregon in 2011, highlighting the environmental effect on kernel hardness within soft-wheats.

Wheat flour milling

Modern wheat flour milling is a dry-milling process performed with roller mills (Bass 1988). It involves breaking wheat kernels open, removing bran, aleurone, and germ from the endosperm, and gradually reducing endosperm particles into "flour" (Bass 1988; Posner 2009). "Flour," defined in the Code of Federal Regulations, is the result of the milling process whereupon greater than 98% of the wheat material passes through a sieve with a mesh size of 212 µm (FDA 2013).

Prior to milling, wheat undergoes a cleaning step to remove any hazardous material that might lower flour quality or damage the mill. Once wheat is cleaned, it is tempered. Tempering is a process whereupon water is added to wheat in order to create a moisture gradient within the grain. This process toughens the bran, reducing the tendency to break into small pieces during milling. Tempering also softens the endosperm, promoting a smaller average particle size within the resulting flour. The amount of water used in the tempering process is a function of grain moisture content, and kernel texture. Soft-wheats are tempered to lower final moisture content than hard wheats. Tempering occurs about 8 and 24 hours prior to milling for soft and hard wheats, respectively.

Once wheat is fully tempered, it can be milled. The roller milling process generally consists of two operations, in series, involving a break system and a reduction system. The objective of the break system is to separate the bran and germ from the endosperm. The break system contains several pairs of steel rollers rotating in opposite directions. Within each pair of rollers, the rolls rotate at different speeds to impart shear as well as crushing force to the milled material. As mill stocks (ground material) passes from one pair of rollers to the next, the gap between the rolls in each set is gradually and progressively decreased (the gradual reduction system). The final products of the break system are bran, germ, coarse endosperm (middlings), and break flour. Middlings are then passed thought the reduction system, which works on the same principle as the break system. The reduction system also contains several pairs of steel rollers rotating in opposite directions and the gap between rollers also decreases gradually and progressively. The final products of the reduction system are shorts (a mill by-product containing bran and endosperm) and reduction flour. Break flour and reduction flour are combined, resulting in "straight grade" flour.

During the milling process, starch granules are physically damaged. Milling soft-wheat generally produces less damaged starch than hard wheat due to the weaker starch-protein adhesion discussed in the **Kernel Texture** section. Soft and hard wheats contain 2 - 4% and 6 - 12% damaged starch, respectively (Stauffer 2007). The level of damaged starch has a profound impact on flour end-use quality (See *Starch*).

Chemical composition of wheat: *Carbohydrates*

Starch

Starch is the most abundant carbohydrate in wheat, accounting for 60 - 75% of the dry weight of the grain (Lineback and Rasper 2009; Stone and Morell 2009). In wheat endosperm starch is synthesized within amyloplasts into either large or small granules (Maningat et al. 2009). The larger granules are lenticular and range from 15 - 30 μm in diameter. The smaller granules are spherical and have a diameter that is typically less than 10 μm (Delcour and Hoseney 2010; Stone and Morell 2009). The large lenticular

granules constitute an average of 7% of the total granules by number, but an average of 73% by weight. Small spherical granules constitute an average of 93% of the total granules by number, but an average of 27% by weight (BeMiller 2007).

Starch granules are semicrystalline, having alternating crystalline and amorphous regions (Stoddard 2004). The granules are complex aggregated structures built from polymers of D-glucopyranose ($C_6H_{12}O_6$). Glucose units within the polymers are connected via α-1,4- and α-1,6- bonds. Alpha-1,4- linkages result in a linear chain, while α-1,6- linkages in starch result in branching (Figure 2.2). The exact frequency and arrangement of these bonds determines whether amylose or amylopectin are formed (Stone and Morell 2009). The typical wheat starch granule contains 25% amylose, and 75% amylopectin (Atwell 2001; Shelton and Lee 2000). Amylose is a linear polymer with infrequent branching (0.2-0.8% of linkages) (Maningat et al. 2009). It contains approximately 500-200,000 glucose units (Edwards 2007; Maningat et al. 2009). Amylopectin is highly branched (4-6% of linkages) and contains approximately 300,000-3,000,000 glucose units (Edwards 2007). Each amylose and amylopectin molecule has only one reducing end, where the first (C1) carbon on the terminal glucose unit is not bound to another glucose molecule, which makes it possible for this glucose unit to exist in the reactive open-chain (carbonyl) form (Atwell 2001).

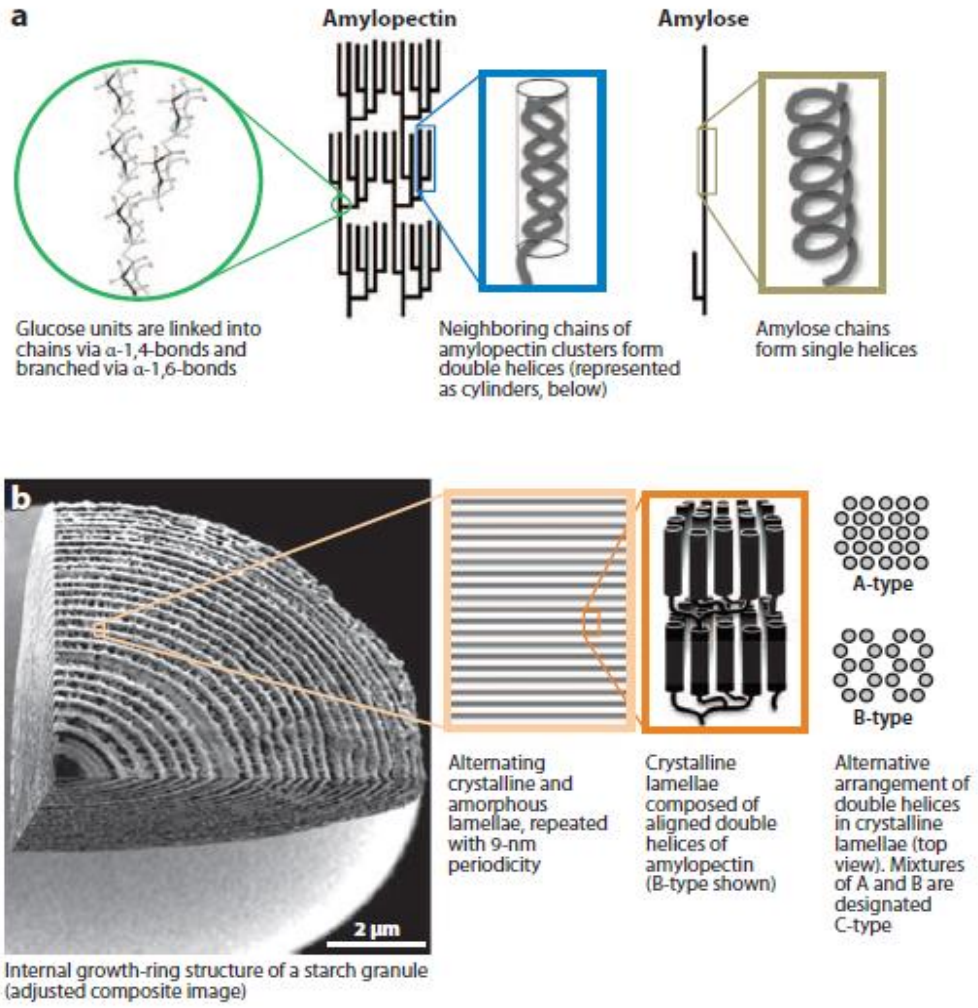

Figure 2.2: The composition and structure of starch granules. (a) A schematic representation of amylose and amylopectin, and the structures adopted by the constituent chains. (b) The relationship between the starch granule (composite image of a potato granule, *left*) and amylopectin structure. Crystalline arrangements of amylopectin (*right*) (Zeeman et al. 2010).

Amylose exists primarily in an unorganized form within amorphous regions of the starch granule (Zeeman et al. 2010). In solution, amylose can be present in a helical conformation. The hydroxyl groups in the amylose helix are located on the exterior, leaving hydrogen atoms inside the coil. This makes the inside (lumen) of an amylose helix hydrophobic (BeMiller 2007). For this reason, amylose can form complexes with free fatty acids, which hinder both their own leaching out of the granule, and entry of water into the granule (Stoddard 2004). Amylopectin terminal chains can form crystalline double helices. The lumen of the double helix is smaller than that of a single helix, so it is

less likely to complex with other molecules, such as iodine. Iodine has commonly been used to analyze the relative proportions of amylose and amylopectin. Amylose can bind 20% of its mass in iodine, while amylopectin can only bind 1% (Stoddard 2004). The color resulting from iodine addition to starch is highly sensitive to chain length. Short chains of glucose (primarily in amylopectin) confer a tan, reddish color. Longer chains of glucose (primarily in amylose) confer a blue color (BeMiller and Huber 2007; Stoddard 2004).

Within the semicrystalline regions, clusters of amylopectin branches are crystalline, while amylose and branched regions of amylopectin are present primarily in the amorphous region of the starch granule (D'Appolonia and Rayas-Duarte 1994). Normal wheat starch has a crystallinity ranging from 23 - 30%. Waxy (little to no amylose) starch has a crystallinity ranging from 37 - 45% (Fujita et al. 1998; Stone and Morell 2009). Because starch granules have a high degree of molecular order, they are able to bend plane polarized light, showing birefringence in the form of an extinction cross (Delcour and Hoseney 2010).

Starch granules are insoluble, but are able to absorb, water (BeMiller and Huber 2007). Upon hydration at ambient temperature, intact starch granules can absorb ~ 30% of their weight in water, increasing the granule volume ~ 5% (Delcour and Hoseney 2010). This process is reversible. However, starch granules that are damaged by the dry milling process can swell up to ~7 times the original volume of the dry granule (Tester and Morrison 1994) with a concomitant increase in water absorption ~170% compared to undamaged starch in commercial bread flour (Ferrand 1964). When starch granules are heated in excess water [water:starch ratio > 1.5 (Buleon and Colonna 2007)], they undergo an irreversible endothermic process called gelatinization. During gelatinization, several changes occur: melting of crystallites, loss of birefringence, a significant increase in swelling, and leaching of soluble components (mainly amylose) (D'Appolonia and Rayas-Duarte 1994; Stoddard 2004). The temperature at which birefringence is lost is defined to be the gelatinization temperature (Delcour and Hoseney 2010). The gelatinization temperature and the range at which the gelatinization process occurs is

dependent on the starch:water ratio, granule type, heterogeneity of the granules, and presence of other components such as lipids and sucrose The presence of low molecular weight cosolutes, such as sucrose, increases gelatinization temperature (BeMiller and Huber 2007). The average gelatinization temperature in wheat starch is 53°C (Delcour and Hoseney 2010).

Upon heating beyond their gelatinization temperatures, starch granules leach amylose, increase water absorption, swell to the point of instability, and possibly collapse. This process is known as pasting, and can occur with or without collapse of starch granules. Collapse of swollen granules usually occurs in the presence of shear (Atwell et al. 1988). Upon pasting, a viscous mass is formed from the increased swelling and eventual granular disruption. Pasting temperatures are always higher than gelatinization temperatures, as pasting is a consequence of gelatinization (Batey et al. 2007). In native wheat starch, pasting temperature varies depending on amylose:amylopectin ratio, degree of crystallinity, presence and concentration of other polymers, e.g. arabinoxylan, and lipid (BeMiller and Huber 2007; Stoddard 2004; Tester and Morrison 1990). Pasting of waxy (low amylose) starch occurs immediately after gelatinization, while pasting in normal starch occurs after the temperature has increased several degrees (Batey et al. 2007).

After pasting and upon cooling, amylose and amylopectin are able to partially re-associate through formation of junction zones (unsubstituted regions of two or more polymer chains associated via noncovalent bonding) (Atwell 2001; BeMiller 2007). This process is called retrogradation, and is more likely to occur in high amylose starch as opposed to waxy starch. If cross-linking through junction zones is extensive, a three-dimensional structure is formed. This leads to either gelation or precipitation depending on factors such as the concentration of starch, rate of cooling, and presence of other components (e.g. protein, lipid, sugars, acids, etc.) (BeMiller and Huber 2007). As junction zones continue to form and grow, water entrapped in the system is released. This process is called syneresis (Stoddard 2004).

Nonstarch polysaccharides

NSP are a group of carbohydrate polymers in the cell well primarily related to cellular structure as opposed to energy storage. Cereal NSP include cellulose, AX, β-glucan, and arabinogalactan. Noncellulosic cereal NSP are commonly referred to as hemicellulose. NSP a water extractable or water-unextractable (Delcour and Hoseney 2010). NSP acts as dietary fiber, as they are indigestible by the human gastrointestinal tract (Lineback and Rasper 2009).

Arabinoxylan

Found in the cell wall, AX are the major NSP (~85%) in wheat grain (Mares and Stone 1973). Because AX is comprised mainly of pentose sugars (arabinose and xylose), they is often referred to as pentosans (Saulnier et al. 2007). AX consist of a linear backbone of (1,4) linked β-D-xylopyranosyl units. These xylopyranosyl units may be unsubstituted, mono-substituted at O-3 or di-substituted at O-3 and O-2 with α-L-arabinofuranosyl units (Ordaz-Ortiz and Saulnier 2005) (Figure 2.3). AX may also be substituted with other moieties, such as acetic acid, ferulic acid, galactose, p-coumaric acid, and uronic acid depending on the tissue of origin (Atwell 2001; Maes and Delcour 2002; Saulnier et al. 2007; Stone and Morell 2009).

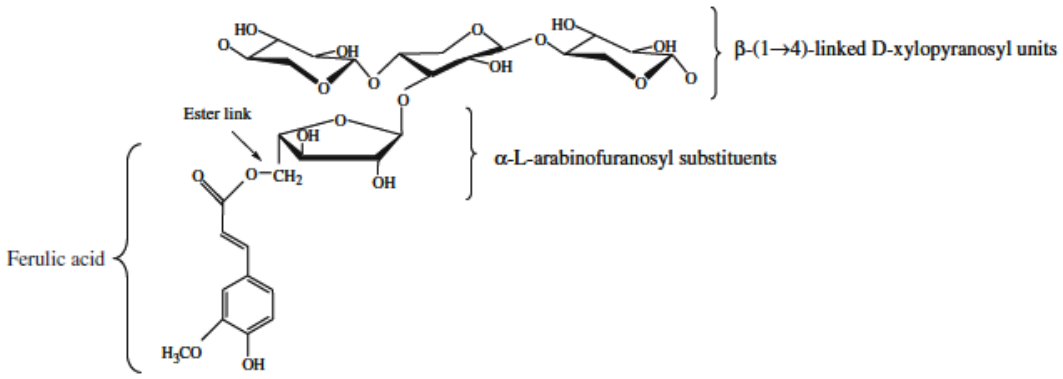

Figure 2.3: Feruloylated arabinoxylan (Nino-Medina et al. 2010).

Arabinose/xylose (A/X) ratio is important in determining the solubility and conformation of AX (Saulnier et al. 2007; Wang et al. 2006). The A/X ratio exhibits a large natural

variation. Typically, average A/X ratio is 0.5 - 0.6. However, extreme low and extreme high values of 0.31 and 1.06 have been reported (Courtin and Delcour 2002a; Dervilly et al. 2000; Dervilly-Pinel et al. 2001). Unsubstituted, mono-substituted, and di-substituted xylose. account for 60 - 65%, 12 - 20%, and 15 - 30%, respectively, of WEAX (see below) (Ordaz-Ortiz and Saulnier 2005). Other factors such as molecular weight and interactions with other cell wall components (protein, cellulose, lignin) also influence AX solubility (Maes and Delcour 2002; Saulnier et al. 2007). Bran contains a significantly higher level of AX than endosperm. AX are 23 - 32% of the bran, and up to 4% of the endosperm (Pomeranz 1988).

Although AX is found in both bran and endosperm, it is more useful to divide AX into two categories based on extractability: WEAX and WUAX. WEAX is lower in abundance, accounting for 0.31 - 0.69% of refined four (Autio 2006). Therefore WUAX accounts for the majority of total AX (Maes and Delcour 2002). WEAX is soluble in cold water while WUAX is soluble in alkali and may be referred to as alkaline-extracted AX (Liukkonen et al. 2007; Ordaz-Ortiz and Saulnier 2005). As a general principal in polysaccharides, as substitution of the backbone polymer increases, so should solubility. Therefore, as A/X ratio increases, the relative water solubility of the AX molecule should increase (Shelton and Lee 2000). However, studies have shown that native wheat WUAX have a higher A/X ratio (higher arabinose substitution) than WEAX (Gruppen et al. 1993; Maes and Delcour 2002), despite the lower solubility of WUAX. This phenomenon occurs as a result of the formation of diferulic bridges between ferulic acid residues esterified to the O-5 position of arabinose on two AX chains. The diferulic cross-linking lowers WUAX solubility (Saulnier et al. 2007; Shelton and Lee 2000). Regardless of extractability, AX are hydrophilic and compete with other constituents for water (Finnie et al. 2006). WEAX and WUAX are able to hold 4 - 6 and 7 - 10 times their mass in water, respectively (Courtin and Delcour 2002a; Finnie et al. 2006).

Upon introduction of WEAX to oxidizing agents (i.e. air, light, hydrogen peroxide), the ferulic acid residues can form covalent cross links with each other. This forms a 3-dimensional network that is able to sequester up to 100 times its weight in water (Bettge

and Morris 2007; Courtin and Delcour 2002a; Finnie et al. 2006; Izydorczyk et al. 1990). The formation of a gel from this process is known as oxidative gelation and will be discussed in detail in a later section (Durham 1925).

AX have significant impact on wheat-based foods and process intermediates. In cookies, WUAX reduce spread more than any other wheat flour fraction (Yamazaki 1955). AX content greatly affects the viscosity of batter products through its large water holding capacity. The viscosity of batter products is important in determining end-product quality as it affects gas retention and texture (Kweon et al. 2010). In bread, WUAX are negatively correlated with bread quality (WUAX compete with gluten for water) while WEAX have either neutral or positive benefits, as they compete for water to a lesser extent, and can chemically interact with gluten (Autio 2006; Courtin et al. 1999; Courtin and Delcour 2002b; Courtin et al. 2001; Goesaert et al. 2005). Development time and viscosity of bread dough has been shown to increase as TAX content increases (Biliaderis et al. 1995; Jelaca and Hlynka 1971; Wang et al. 2003).

Beta-glucan

β-glucan is an unbranched, linear polymer found in the cell walls of many cereal grains such as barley, oats, and wheat (Chawla and Patil 2010). While β-glucan is present in large quantities in barley (5 - 11% w/w) and oats (2 - 9% w/w), wheat contains <1% w/w (Beresford and Stone 1983; Lineback and Rasper 2009; Tiwari and Cummins 2009; Welch et al. 2000). In wheat, β-glucan is found primarily in the aleurone layer (~29%) and the starchy endosperm (~20%) (Bacic and Stone 1981a; b).

β-glucan is comprised solely of D-glucose. These glucose units linked together with both β-(1→3) and β-(1→4) linkages. Each β-(1→3) is followed by either cellotriose or cellotetraose (BeMiller and Huber 2007). β-glucan has in the order of 250,000 glucose units per molecule and an average molecular weight of 487,000 (Chawla and Patil 2010; Li et al. 2006). β-glucan is considered soluble dietary fiber due to the presence of β-(1→3) linkages. These linkages prevent β-glucan molecules from closely associating with each other, making them soluble (Chawla and Patil 2010; Grimm et al. 1995).

Cellulose

Cellulose is a long, linear polymer found in plant cell walls. It is present in all kernel tissues, but primarily found in wheat pericarp (~30% w/w) (Ring and Selvendran 1980; Stone and Morell 2009). Total cellulose content in wheat grain is ~2% (dry), while <0.3% is found in the starchy endosperm (Fraser and Holmes 1959; Stone and Morell 2009). The concentration of cellulose in wheat flour is directly related to the degree of flour extraction from the wheat kernel. Thusly, patent flour (flour comprised of nearly pure endosperm) contains almost no cellulose (Lineback and Rasper 2009).

Like β-glucan, cellulose is comprised solely of D-glucose units. Glucose units in cellulose, however, are only linked together via β-(1→4) bonds (BeMiller and Huber 2007; Shelton and Lee 2000). Due to the lack of β-(1→3) linkages, cellulose can self-associate via hydrogen bonding and van der Waals interactions, forming a rigid, ribbon-like structure (Stone and Morell 2009). Cellulose is partially crystalline, as it contains both highly ordered and non-ordered regions (Shelton and Lee 2000). Subsequently, cellulose is considered insoluble dietary fiber.

Arabinogalactan-peptides

Arabinogalactan-peptides (AGP) are plant cell wall water-extractable polysaccharides. AGP are comprised of carbohydrate material (92 - 94%) covalently linked to a peptide backbone (6-8%) (Van Der Borght et al. 2005). Arabinogalactans contain a linear β-(1→3) linked backbone of D-galactose with random, short D-galactose branches connected via β-(1→6) bonds. These branches can also have L-arabinose units attached via α-(1→3) linkages (Van den Bulck et al. 2002). The peptide backbone of AGP contains 15 - 20 amino acids, notably hydroxyproline, alanine, and glutamine (Van den Bulck et al. 2002). While the general structure of AGP is defined, the exact structure of AGP varies between and within wheat varieties (Loosveld and Delcour 2000). AGP accounts for 0.27 - 0.38% (dry) of wheat flour (Loosveld et al. 1998).

Chemical composition of wheat: Protein

Wheat flour generally contains from 6 - 20% protein depending on the cultivar and growth environment (Dobraszczyk 2001; Finney and Barmore 1948). Wheat protein can be categorized into two groups: non-gluten and gluten-forming.

Non-gluten proteins are soluble in water (albumins) and dilute NaCl solutions (globulins) (Delcour and Hoseney 2010; Osborne 1907). Non-gluten proteins account for 10 - 15% of total wheat flour protein (Swanson 2004). Albumin and globulin are "monomeric" (i.e. non-aggregating) proteins (MacRitchie and Lafiandra 1997).

Gluten proteins are soluble in aqueous 70% ethanol (prolamins) and dilute acids or bases (glutelins) (Delcour and Hoseney 2010; Osborne 1907; Thewissen et al. 2011; Weegels et al. 1996). Gluten proteins account for 80 - 85% of total wheat-flour protein (Swanson 2004; Veraverbeke and Delcour 2002). The prolamins in wheat are the gliadins, and the glutelins are the glute**n**ins (Delcour and Hoseney 2010). When these two classes of protein are hydrated and mechanically worked, a three-dimensional viscoelastic network is formed. This network is known as gluten. Gliadins contribute to gluten viscosity (flow). Glutenins contribute to gluten elasticity (Ciaffi et al. 1996). Gluten can form gas-trapping films that allow leavening gas to be retained within dough.

Gliadins

Gliadins account for 40 - 50% of the total protein content of wheat (Qi et al. 2006). Wheat gliadins have a molecular weight from 30 - 80,000 (MacRitchie and Lafiandra 1997). Gliadins can be classified into four groups based on gel electrophoresis: α-, β-, γ-, and ω- gliadins (MacRitchie and Lafiandra 1997; Woychik et al. 1961). Alpha-, β-, and γ-gliadins overlap with each other in gel electrophoresis due to their relatively similar molecular weights (36 - 40,000) (Bietz and Wall 1980). Alpha- and β-gliadins are sometimes grouped together due to their similarity (Kuktaite 2004). Alpha-, β-, and γ-gliadins are comparable in amino acid composition (MacRitchie and Lafiandra 1997). They are categorized as sulfur-rich prolamins because they have a high number of cysteine residues (2 - 3% by number) (MacRitchie and Lafiandra 1997; Shewry et al.

2009). Although α-, β-, γ-gliadins contain sulfhydryl groups, resulting disulfide bonds are primarily intramolecular, making gliadins monomeric (Delcour and Hoseney 2010). Omega-gliadins are distinguished due to their higher molecular weights (70 - 80,000) (Bietz and Wall 1980; MacRitchie and Lafiandra 1997). ω-gliadins are considered sulfur-poor prolamins, as they contain no cysteine residues (Shewry et al. 2009; Tatham and Shewry 1995). Consequently, ω-gliadins are unable to form disulfide bonds (MacRitchie and Lafiandra 1997; Shewry et al. 1986) and have a relatively expanded conformation compared to other gliadins.

Synthesis of ω-gliadins and most γ-gliadins are regulated by *Gli-A1*, *Gli-B1*, and *Gli-D1* genes located on the short arm of group 1 chromosomes. Synthesis of α-, β-, and some γ-gliadins are regulated by *Gli-A2*, *Gli-B2*, and *Gli-D2*, respectively. These are located on the short arm of group 6 chromosomes (Branlard et al. 2001).

Glutenins

Glutenins account for 30 - 45% of the total protein content of wheat (Cornell 2003). Glutenins are termed "polymeric" proteins. Unlike the monomeric gliadins, glutenins have the ability to aggregate, primarily through intermolecular disulfide bonding (MacRitchie and Lafiandra 1997). Individual glutenin subunits can be isolated via a reduction of disulfide bonds. The resulting glutenin subunits can be separated into two groups: low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS) (Branlard et al. 2001). LMW-GS have molecular weights from 30 - 55,000. HMW-GS have molecular weights from 80 - 160,000 (MacRitchie and Lafiandra 1997; Payne et al. 1980). Both LMW and HMW-GS can be linked via inter- and intramolecular disulfide bridges. The resulting polymeric proteins can have a molecular weight up to several million (MacRitchie and Lafiandra 1997).

Glutenin polymeric proteins are extensively studied because they are important in determining wheat flour quality, baking quality, and dough properties (Shewry 2009). However, glutenin properties can vary between cultivars (Gupta et al. 1996; Johansson et al. 2001; Lindsay and Skerritt 2000; Singh and MacRitchie 2001). Synthesis of HMW-

GS are regulated by *Glu-A1*, *Glu-B1*, and *Glu-D1* genes located on the long arms of the group 1 chromosomes (Bekes et al. 2004). These protein subunits are designated with numbers related to their electrophoretic mobility (e.g. 1, 2, and 2*) and regularly are present in pairs (e.g. 2+12, 5+10, and 17+18) (Payne et al. 1981; Waines and Payne 1987; Yan et al. 2003). Differences within HMW- and LMW-GS are associated with differences in dough strength. For example, HMW-GS 5+10 are associated with stronger dough, while HMW-GS 2+12 are associated with weaker dough (Radovanovic et al. 2002). Synthesis of LMW-GS are regulated by *Glu-A3*, *Glu-B3*, and *Glu-D3* genes located on the short arms of the group 1 chromosomes (Singh and Shepherd 1985). LMW-GS have an impact on dough performance. LMW-GS have been shown to be negatively correlated with Mixograph mixing time and peak resistance (Lee et al. 1999). However, Martinez-Cruz et al (2011) showed that certain LMW-GS (*Glu-A3*) correspond to medium strong and extensible gluten. The *Glu-B3j* allele conferred to low levels of gluten strength and limited extensibility. The *Glu-D3c* allele was associated with strong and extensible gluten (Martinez-Cruz et al. 2011).

Glutenin polymeric proteins can be isolated as unextractable polymeric proteins (UPP) or in a semi-solid form as gluten macropolymer (GMP). UPP are polymeric glutenins which are insoluble in 0.5% SDS, and are solubilized by subsequent sonication (Gupta et al. 1993). GMP is a complex of aggregated glutenins which are insoluble in 1.5% (w/v) sodium dodecyl sulfate (SDS) and form a gel layer after ultracentrifugation of wheat flour in a buffered SDS solution (Don et al. 2003a).

Elevated wheat flour GMP and UPP contents are associated with increased dough firmness, mixing time, and loaf volume (Weegels et al. 1996). It has been established that gluten is formed as the result of mixing/kneading dough. However, GMP decreases in abundance during mixing due to the loss of HMW-GS from the aggregate (Don et al. 2003b; Skerritt et al. 1999; Weegels et al. 1997). This decrease in GMP is slower in dough made from strong-gluten flour compared to dough made from weak-gluten flour (Jood et al. 2001). On subsequent dough resting GMP abundance begins to increase as a result of reaggregation of the glutenins (Don et al. 2003b). This phenomenon also occurs

in noodle processing in doughs with much lower water additions, although the reaggregation on resting is curtailed in alkaline noodle doughs (Ong et al. 2010). Wheat flour UPP can be used to predict dough properties by considering the percentage of unextractable polymeric protein (%UPP) as a function of the total polymeric protein (Field et al. 1983; Gupta et al. 1992). High %UPP indicates a large proportion of high-molecular-weight insoluble glutenin polymer in SDS (MacRitchie 2004). Dough with a high %UPP is associated with more elasticity and a longer mixing time than a dough with low %UPP (Gupta et al. 1993). UPP content and composition is dependent on the genetic composition of flour protein, therefore varietal differences in UPP exist (Kuktaite et al. 2004).

Non-disulfide protein cross-linking

It has been established that protein can cross-link via disulfide bridges. However, linkages between two tyrosine residues may also occur. Tyrosine is a phenolic amino acid, and accounts for 1 - 4% of wheat protein (MacRitchie and Lafiandra 1997). In cell walls, protein plays an important structural role through tyrosine linkages. Protein-bound tyrosine is able to cross-link with other phenolic moieties in cell wall constituents (e.g. ferulic acid moieties on WEAX) (Cooper and Varner 1984; Fry 1986; Miller and Hoseney 1999). The type of linkage, however, depends on the conditions within the system. Dityrosine (Figure 2.4) is formed in the presence of peroxidase and hydrogen peroxide, and isodityrosine (Figure 2.4) is formed through an oxidative, non-free-radical producing mechanism (Cooper and Varner 1984; Fry 1982) and may affect dough properties (Tilley et al. 2001). Tyrosine forms linkages with WEAX via free-radical mechanism similar to that in the formation of dityrosine (See **Oxidative gelation**) (Moore et al. 1990; Neukom and Markwalder 1978).

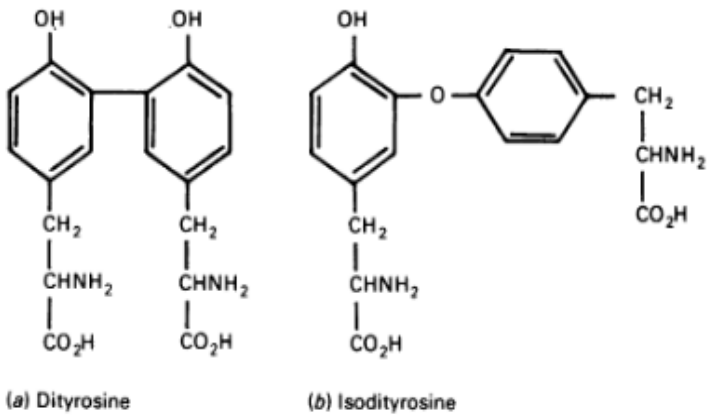

Figure 2.4. The structures of dityrosine and isodityrosine (Fry 1982).

Chemical composition of wheat: Lipids

Lipids are a minor constituent of wheat grain (~2 - 4% w/w, dry: Table 2.2) (Delcour and Hoseney 2010; Morrison 1978). Wheat lipids are present in the form of oil droplets or spherosomes (membrane-bound oil droplets) (Cornell 2003).

Wheat grain lipids are composed of ~70% nonpolar lipids, 20% glycolipids, and 10% phospholipids (Delcour and Hoseney 2010). Approximately 30% of total wheat grain lipids are found in the germ (Chung and Ohm 2000; Morrison 1978; Wrigley et al. 2009). Germ lipids contain 77 - 85% nonpolar and 13 - 17% polar lipids, triglycerides phospholipids being the major components, respectively (Delcour and Hoseney 2010; Morrison 1978). In contrast, wheat endosperm (the largest section of the wheat kernel) contains ~1 - 2.2% (w/w, dry) lipid (Morrison 1978; Wrigley et al. 2009).

Based on extraction methods, lipids can be classified as free, bound, and starch-internal lipids (Pareyt et al. 2011). Non-starch lipids (free and bound lipids) are the major class of lipids in the endosperm (65%), and contain ~60% nonpolar lipids, 25% glycolipids, and 15% phospholipids (Delcour and Hoseney 2010). Starch-internal lipids (35%) consist of 9% nonpolar lipids, 5% glycolipids, and 86% phospholipids (Delcour and Hoseney 2010; Wrigley et al. 2009).

Tissue	Proportion of Whole Kernel (%)	Crude Fat (%)
Whole Grain	100	2.1 - 3.8
Bran	-	5.1 - 5.8
Pericarp	5.0 - 8.9	0.7 - 1.0
Testa, hyaline	0.2 - 1.1	0.2 - 0.5
Aleurone	4.6 - 8.9	6.0 - 9.9
Endosperm	74.9 - 86.5	0.8 - 2.2
Scutellum	1.1 - 2.0	12.6 - 32.1
Embryonic Axis	1.0 - 1.6	10.0 - 16.3

Table 2.2: Crude fat content of whole wheat grain (Morrison 1978).

Nonpolar wheat lipids mainly consist of acylglycerols, free fatty acids, and sterol esters (Wrigley et al. 2009). Acylglycerols are glycerol esters with one, two, or three fatty acids and mono-, di-, and triacylglycerides, respectively. Fatty acids, the simplest form of lipids, are comprised of a long hydrocarbon with a carboxylic acid moiety attached at the end. Palmitic (16:0), stearic (18:0), oleic (18:1, n-9), linoleic (18:2, n-6), and α-linolenic (18:3, n-3) are the most common wheat fatty acids. Sterol esters are unsaturated solid alcohols of the steroid group (Chung et al. 2009; Morrison 1994).

Polar wheat lipids mainly consist of glycolipids (lipids containing sugar groups, usually galactose and glucose in wheat) and phospholipids (lipids with attached phosphate groups) (Delcour and Hoseney 2010; Ito 1983; McDonnell et al. 1982; Wrigley et al. 2009). The majority of wheat glycolipids are galactolipids, namely mono- and digalactosyl diglycerides (Chung et al. 2009). Phospholipids are fat derivatives, containing a phosphate group and one or more fatty acid. Phosphatidycholine and phosphatidylethanolamine are prevalent components of wheat phospholipids (Wrigley et al. 2009).

Although lipids are present in small quantities in wheat, variation of lipid content is extremely important in wheat quality. Variation in wheat flour free lipid content, for example, affects bread loaf volume and texture more than variation in any other flour component (Macritchie 1981; Pareyt et al. 2011). Lipids can interact with protein and starch to form inclusion complexes. Lipids associate with gluten proteins and help

stabilize gas cell structure in dough, resulting in better loaf quality (Goesaert et al. 2005; Macritchie 1981; Pareyt et al. 2011).

Lipids can undergo a radical chain reaction (called lipid oxidation) in the presence of oxidizing agents. Lipid peroxidation has 3 phases: initiation with formation of free-radicals (formation of peroxidized lipids), propagation, and termination (via formation of non-radical products) (Maire et al. 2013). Oxidation of lipids can occur enzymatically via lipoxygenase (Maire et al. 2013). Lipids can also undergo auto-oxidation during flour storage. The level of peroxidized lipids within flour changes as a function of time (Reichenauer and Goodman 2003). Lipid peroxidation leads to the production of rancid flavors in wheat flour, reducing flour shelf-life. Peroxidized lipids may also be a trigger for oxidative gelation of AX (see **Oxidative gelation**).

During the lipid peroxidation process, malondialdehyde (MDA) is formed (Figure 2.5) (Singh et al. 2001). MDA can form an adduct with thiobarbituric acid (TBA) (Figure 2.6), producing a stable chromogen that can be quantified colorimetrically at $\lambda = 532$ nm. (Sochor et al. 2012).

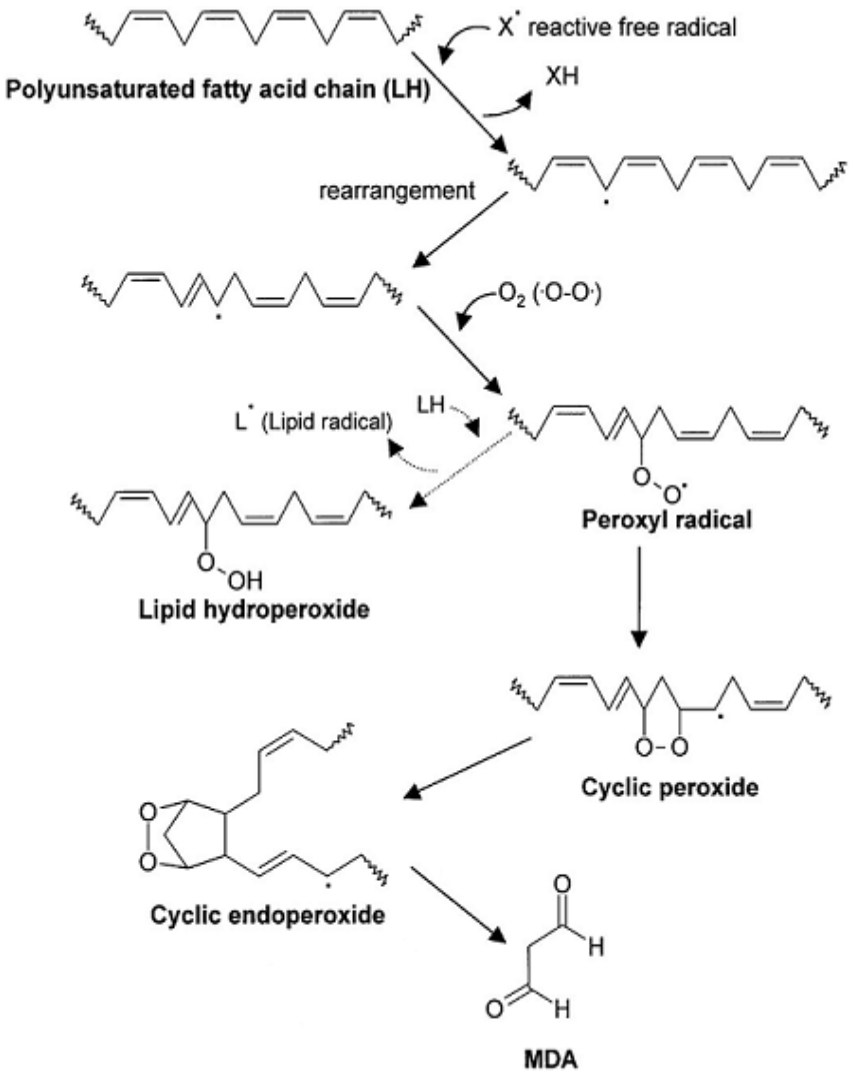

Figure 2.5: Formation of malondialdehyde (MDA) from lipids and reactive free radicals (Singh et al. 2001).

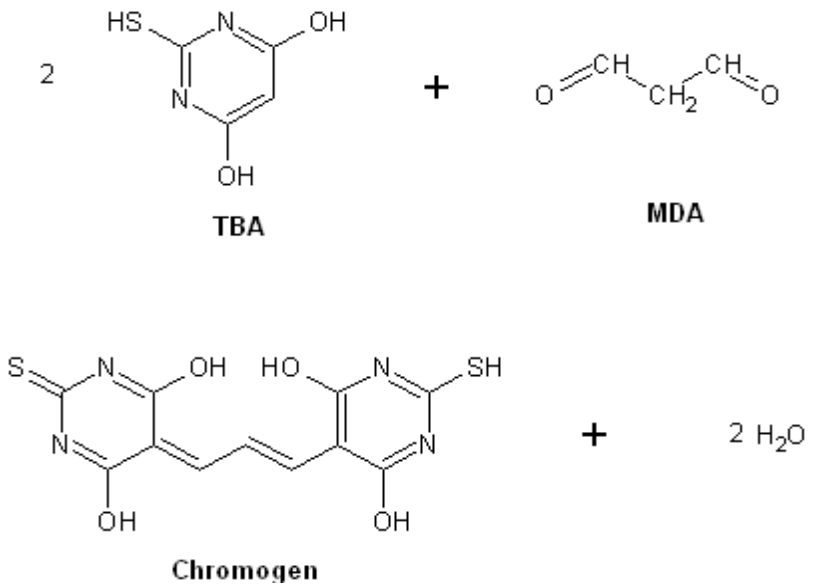

Figure 2.6: Formation of chromogen via condensation of thiobarbituric acid (TBA) with malondialdehyde (MDA) (Sochor et al. 2012).

Viscosity in soft-wheat products

Wheat flour product formulations encompass a large range of water content, resulting in process intermediates ranging from stiff doughs to thin, low viscosity, batters. In bread doughs, sufficient water and mechanical work are used to develop gluten and form a three-dimensional gluten network capable of entrapping fermentation gases. In soft-wheat products (cookies, cakes, donuts, etc.), desired product performance and physical consistency is attained through control of viscosity through appropriate solids concentrations and the use of chemical leavening, which also increases batter viscosity during heating as a result of the small bubbles emanating from the leavening system (Heidolph 1996; Ross and Bettge 2009). Extensive gluten development is generally undesirable due to negative effects on processing and textural quality. Consequently, gluten development is minimized in soft-wheat products. For products with high water contents (cakes, waffles, donuts, coatings, pancakes, etc), viscosity has important influence on end-product quality. Coatings must be viscous enough to adhere to the product without clumping or shearing off. Pancake and donut batters must be viscous enough to retain leavening gases and prevent settling without inhibiting flow and spread. Arabinoxylans, proteins, and their oxidative gels (see **Oxidative gelation**) are likely to directly affect batter properties (Bettge and Morris 2007).

Solvent retention capacity

Solvent retention capacity (SRC) has become widely used in predicting end-use performance for soft-wheat products. The original test that measured absorption in an excess of solvent was the alkaline water retention capacity (AWRC) test. This test used a weakly alkaline solution of 0.84% (w/v) sodium bicarbonate (AACC Approved Method 56-10) (AACC-International 2000). High AWRC absorption was associated with poor cookie spread. The concept of this test was modified to the "sugar water retention capacity" test (Slade and Levine 1994). This test was modified and is now the SRC method (AACC-I Approved Method 56-11) (AACC-International 2000). In contrast to empirical rheological methods, SRC is based on the fundamental swelling behavior of polymer networks in compatible solvents (Kweon et al. 2011). SRC emphasizes the functionality of individual major components by exploiting the capacity of large polymeric molecules to solvate and entangle, rather than dissolve. These entangled polymeric networks can swell more in suitably selected solvents (Kweon et al. 2011). SRC measures overall absorption as well as enhanced absorption related to specific macromolecular components of flour. Water SRC is associated with the overall water holding capacity of all flour polymeric components. Three additional solutions are used to emphasize the functionality of specific flour polymers. Sucrose emphasizes swelling of AX and gliadin. Sodium carbonate emphasizes swelling of damaged starch. Lactic acid is associated with glutenin and dough strength (Duyvejonck et al. 2011).

Oxidative gelation

Oxidative Gelation is a process whereupon a weak polymeric gel is formed under oxidizing conditions within a hydrated flour system (commonly initiated in research studies using hydrogen peroxide and peroxidase). The extent of gelation is known as oxidative gelation capacity (OGC). This phenomenon has long been known. Durham (1925) indicated that an unidentified water-extractable portion of wheat flour formed a gel with the addition of hydrogen peroxide. It was later discovered that WEAX undergo oxidative gelation through the formation of diferulic acid bridges (Ciacco and D'Appolonia 1982; Morita et al. 1974). The mechanism by which oxidative gelation

occurs has since been expanded to include protein cross-linking with other protein molecules (via dityrosine bridges) and with ferulic acid moieties on WEAX (see below) (Bettge and Morris 2007; Carvajal-Millan et al. 2006; Hoseney and Faubion 1981; Vansteenkiste et al. 2004). It has been shown that break flour reacts more strongly to hydrogen peroxide than straight grade flour (Ramseyer et al. 2011b).

Oxidizing agents are used to treat flour in order to change dough/batter handling characteristics and end-product quality. Oxidative gelation of AX is a free-radical mediated event, and therefore is sensitive to the type of oxidizing agent used. Common oxidizing agents used are hydrogen peroxide, ammonium persulfate, formamidine disulfide, potassium bromate, potassium iodate, ascorbic acid, and azodicarbonamide (ADA). All of these oxidizing agents have been analyzed for their effect on OGC except ADA. It is generally agreed that hydrogen peroxide (in the presence of peroxidase) creates the free radicals necessary for oxidative gelation (Bettge and Morris 2007; Durham 1925; Hoseney and Faubion 1981; Nino-Medina et al. 2010; Schooneveld-Bergmans et al. 1999). Ammonium persulfate and formamidine disulfide are also known to create free radicals. Potassium bromate and ascorbic acid are not considered strong generators of radicals (Hoseney and Faubion 1981; Yeh et al. 1980). Potassium iodate has been described as non-radical forming, thus not influencing OGC (Hoseney and Faubion 1981). Potassium iodate, however, has been shown to decrease the amount of FA in dough systems (Yeh et al. 1980). Yeh et al described this as oxidative gelation, although a change in *cross-linked* WEAX was speculated, not directly measured. ADA has been shown to produce carbamoyl radicals in non-wheat related systems, therefore it is speculated that ADA would influence oxidative gelation.

The process of oxidative gelation results in numerous unique dimers and trimers. As mentioned above, FA on WEAX and protein are both involved in the oxidative gelation process. Ferulic acid may cross-link with other FA moieties either with the phenolic ring or with the activated double bond (Figure 2.7) (Carvajal-Millan et al. 2006; Ralph et al. 1994; Schooneveld-Bergmans et al. 1999). The 5-5’ dimer was previously thought to be the only diferulic acid dimer formed during oxidative gelation. However, studies have

shown that this dimer is present in small amounts compared to dimers formed by coupling at the 8-position of one or both ferulate moieties (Grabber et al. 1995; Ralph et al. 1994). Differing oxidants create of different proportions of these dimers (Schooneveld-Bergmans et al. 1999). Ferulic acid may cross-link with protein tyrosine via phenolic ring, similar to the 5-5' FA-FA' dimer. In a similar fashion, protein tyrosine may cross-link with other protein tyrosine (shown in ***Non-Disulfide Protein Cross-Linking***) (Bettge and Morris 2007; Haynes et al. 2009; Miller and Hoseney 1999; Moore et al. 1990; Nino-Medina et al. 2010). Ferulic acid may also cross link with protein cysteine via thiyl radical addition to the activated double bond on FA (Figure 2.8) (Hoseney and Faubion 1981; Moore et al. 1990).

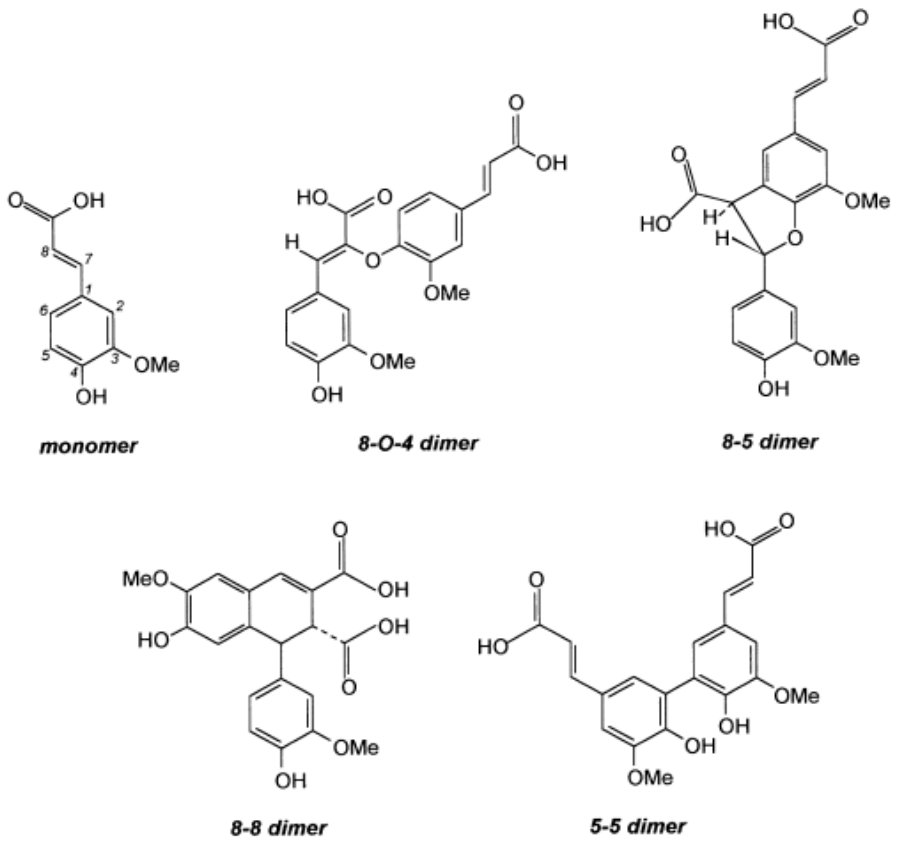

Figure 2.7: Ferulic acid carbon numbering system and common diferulic acid dimers found in plants (Schooneveld-Bergmans et al. 1999).

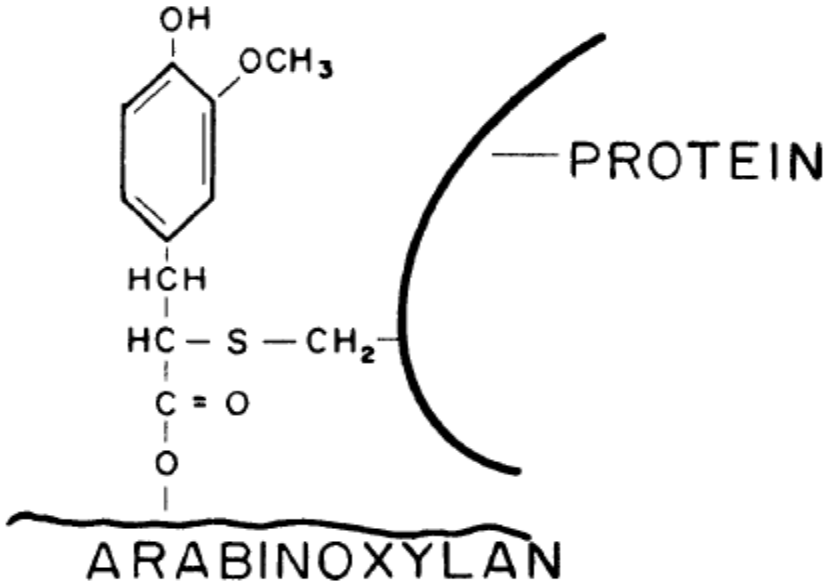

Figure 2.8: Protein cysteine cross-linked with arabinoxylan via thiyl addition to the activated double bond on ferulic acid (Hoseney and Faubion 1981).

Previous methodologies for determining the extent of oxidative gelation include capillary viscometers, spindle-and-plate, or spindle-and-cylinder type instruments, which provide variable amounts of shear. Baker et al (1943) showed that polymeric networks resulting from oxidative gelation were susceptible to shear breakdown. Trough-style flow meters, such as the Bostwick Consistometer, measure viscosity as flow distance under low shear conditions (Bettge and Morris 2007). All of these methods were able to detect differences in oxidative gelation. However, because wheat flour oxidative gels exhibit shear thinning, using a trough-style consistometer proved to be advantageous (Bettge and Morris 2007). Due to inherent limitations of the Bostwick Consistometer (uneven flow down the trough, zero flow if the gel forms before the test starts - making it impossible to gain information about the quality of gelation), a method for measuring oxidative gelation using the Rapid Visco Analyzer (RVA) has been proposed, and is detailed in **Materials and Methods** section (Ross et al. 2014).

WEAX and protein polymers create viscous suspensions at low concentrations due to their large size (Izydorczyk and Biliaderis 1992). However, the oxidative gels they can

form exhibit shear-thinning (Izydorczyk et al. 1991). This rheological trait indicates that their impact could be reduced in mechanically worked doughs (e.g. breads) compared to their impact in batter formulations (e.g. cakes) (Bettge and Morris 2007). Additionally, the effect of disulfide cross links formed during gluten development likely overshadows the effects of di-tyrosine and FA-tyrosine bonds in bread dough (Bettge and Morris 2007; Hoseney and Faubion 1981).

Flour aging

The aim of the milling industry is to provide flours of consistent quality, which are optimally matched to the requirements of the end product (Gras et al. 2001). Historically it has been thought that there is a progressive increase in the dough mixing requirement as the storage period of grain increases before it is milled into flour. Blame for the variations in mixing properties has been attributed to the miller, breeder, grain supplier, etc. It was only later that storage was investigated as a possible factor (Gras et al. 2001). It has been shown that over a period of grain storage, free fatty acids increase, flour extraction decreases (Rose et al. 2011), alpha amylase decreases, falling number increases, fat acidity increases, glutenin subunit solubilization increases (Gonzalez-Torralba et al. 2013), wet gluten content increases, and alveograph *W* parameter (deformation energy) decreases (Mezei et al. 2007). Grain stored at temperatures over 30 °C have been shown to increase maximum dough resistance and decrease dough extensibility (Gonzalez-Torralba et al. 2013).

Changes in flour components during flour aging have also been reported: peroxidized lipids fluctuate (Reichenauer and Goodman 2003), enzymatic activity fluctuates, non-esterified fatty acids increase (Doblado-Maldonado et al. 2012), free fatty acids increase (temperature dependent), sulfhydryl contents decrease (due to oxidation), and a glutathione contents decrease (Nishio et al. 2004). Changes in these components depend on storage temperature, moisture content, and oxygen concentration (Gras et al. 2001).

While chemical changes within wheat grain and flour related to aging have been reported, the effects on baking quality have been variable, and ranged from negative to positive.

For grain storage, reduced bread volume and longer dough mixing times were observed (Gras et al. 2001). For flour aging, there have been observations of no effects (Lin et al.) or positive effects on baking quality (Chen and Schofield 1996; Nishio et al. 2004). Additionally, Nishio et al. (2004) showed that Farinograph stability increased on flour aging. The disagreement in the literature could be due to differences in whether grain or flour was stored, for how long grain was stored prior to milling, differences in storage conditions (Gras et al. 2001), and differences in cultivars used (Nishio et al. 2004; Schofield and Chen 1995).

Gras et al. (2001) showed that grain stored for 12 months at temperatures at and below 23° C showed negligible changes in Farinograph stability, dough development time, Extensograph properties, and loaf volume. The amount of oxygen present in the storage atmosphere alone does not affect the rate at which changes within grain properties change (e.g. low atmospheric oxygen will not slow the rate of change in quality unless the storage temperature is also reduced) (Gras et al. 2001) suggesting that modified atmosphere storage may not be effective unless O_2 is completely purged.

CHAPTER 3 - MATERIALS AND METHODS

Plant materials

Soft-wheat aging study

Grain samples were obtained from the Oregon State University Wheat Breeding Program. The samples were harvested in 2012 from Pendleton, Oregon. Wheat varieties were chosen based on preliminary testing of their OGC (Ross et al. 2014) and known varietal differences in absorption capacities (via SRC). The chosen SW winter-wheat varieties were Tubbs, Goetze, Skiles, and Bobtail. Grain and flour were stored at ambient temperature and monitored using a ThermoWorks (Lindon, UWAH) TW-USB-2LCD+ recording thermometer. A portion of grain for each variety was archived at -20 °C in order to preserve the grain's characteristics, and provide a negative aging control.

Hard wheat survey

23 varieties of hard winter wheat were obtained from the Oregon State University Wheat Breeding Program. Samples were harvested in 2013 from LaGrande, Oregon. All samples were milled to straight grade flour and tested for their reactivity to hydrogen peroxide on two separate days. Samples were tested 1 and 14 days after milling to observe any effect of flour aging on OGC. 2 samples (OR2080227H and OR2090107H with the highest and lowest reactivity to hydrogen peroxide, respectively) from this material were chosen to conduct a dose-response study (detailed in Chapter 6) of hydrogen peroxide and ADA on OGC.

Other Materials

All chemicals were analytical grade or better.

Methods

Determination of wheat quality

Kernel characteristics

Two hundred kernels from each sample were prepared by removing broken and non-uniform kernels and foreign material. Each individual kernel was tested for hardness index (HI), moisture content, weight, and diameter using a Perten 4100 Single Kernel Characterization System (Perten Instruments, Inc., Springfield, IL) according to AACC-I Approved Method 55-31 (AACC-International 2000). Protein was measured according to AACC-I Approved Method 39.11 (AACC International 2000).

Milling

Grain was milled to flour using a modified Brabender Quadrumat Senior (Quad Senior: Brabender Instruments Inc. GmbH & Co. KG, Germany) milling method established by the USA-ARS-WWQL (Jeffers and Rubenthaler 1977). 12 to 18 hours prior to milling, wheat grain samples (250g) were tempered with deionized water. Soft and hard wheats were tempered to 14% and 15% moisture, respectively. Tempered wheat was milled using Quadrumat Senior experimental mills. Total mass of tempered grain was recorded. Grain was fed into the break roll unit at a rate of 150 g/min. Once all of the grain passed through the rolls, the rolls were manually brushed to remove retained flour. The interior of the mill was vacuumed between samples to eliminate cross contamination of samples. Milled wheat was then sifted through 500 µm and a 150 µm sieves (Fisher Scientific, Pittsburgh, PA) for 1 minute using a mechanical sieve shaker (Great Western Manufacturing, Leavenworth, KS). Bran retained on the 500 µm sieve was weighed and discarded. The remaining mixture was sifted for a further 2 min. Break flour was weighed and stored in closed zip top bags. Middlings retained on the 150 µm sieve were re-milled through the reduction milling unit at a rate of 50 g/min. As before, the reduction mill was brushed out and vacuumed between samples. The output from the reduction mill was sifted through a 150 µm sieve for 3 min using the sieve shaker. "Shorts," (fine bran) retained on the 150 µm sieve was weighed and discarded. Reduction flour was weighed.

Reduction flour can be combined with break flour to form straight grade flour as in the hard wheat survey. However, in the soft-wheat study, break flour was used to measure solvent retention capacity and OGC. Break flour rather than straight-grade flour was chosen because Ramseyer et al (2011b) showed that break flour reacted more strongly to the addition of hydrogen peroxide. Break flour was not available for the hard-wheat study.

Flour yields were calculated as follows:

$$\text{Break Flour Yield (\%)} = \left(\frac{\text{Break flour}}{\text{Total weight of tempered wheat}} \right) * 100$$

$$\text{Total Flour Yield (\%)} = \left(\frac{\text{Break flour} + \text{Reduction flour}}{\text{Total weight of tempered wheat}} \right) * 100$$

Milling and testing schedule

Wheat grain was transported from Pendleton to Corvallis, Oregon, cleaned and brought to the lab. Grain arrived within 2 weeks of harvest and would be considered "freshly harvested" in the grain trade. Two weeks was the quickest that the transport, cleaning, and other preparations could be achieved.

Week 0 day 0 is defined to be the day that the grain was delivered to the lab and the aging study began. To observe the effect of grain aging, grain was milled into flour 0, 3, 6, 13, and 24 weeks after harvest. To observe the effects of flour aging, flour testing was performed 0, 1, 3, 6, 13, 27, and 62 days after each milling. Grain sub-samples (1.5 kg) for each variety were frozen at week 0 day 0 as the control for the study. Grain was kept at ambient temperature throughout the aging period.

Approximately 9 grams of break flour was subsampled from the OGC and SRC material on each testing day and archived at -20 °C. Subsampled material was used for analysis of total peroxidized lipids.

Flour protein determination

Protein contents of flour used were determined using near infrared spectroscopy (Infratec 1241, FOSS NIT Systems Inc., Denmark) according to the AACC approved method 39-11 (AACC-International 2000).

Solvent retention capacity

SRC tests were conducted on wheat break flours according to a modification of AACC-I Approved Method 56-11.02 (AACC-International 2000). Solvents used were deionized water, 5% (w/v) sodium carbonate in water, 50% (w/v) sucrose in water, and 5% (v/v) lactic acid in water. For each solvent separately, 50 mL screw cap tubes were weighed and the weight recorded. Flour, 5.00 ± 0.05 g, was weighed into each tube and 25 mL of the appropriate solvent was added. The mixture was shaken vigorously by hand for 5 s to suspend the flour. The mixture was then allowed to hydrate for 20 min. At 5-min intervals during hydration the mixture was shaken by hand for ~5 s. Tubes were immediately transferred to a Beckman GS-15R centrifuge (Beckman Coulter, Inc., Brea, CA) and centrifuged at 1,000g for 15 min. The supernatant was decanted and the tubes drained at a 90° angle for 10 min on a paper towel. Total weight of tube, cap, and pellet was measured. Weight of pellet was calculated by subtracting total weight of tube and cap from total weight of tube, cap, and gel. SRC (%) value was calculated as follows (Haynes et al. 2009):

$$\%\ SRC = \left\{\left[\left(\frac{Tube, Cap, Gel\ weight - Tube, Cap\ weight}{Flour\ weight}\right) - 1\right] * \left(\frac{86}{100 - Flour\ Moisture}\right) * 100\right\}$$

Oxidative gelation capacity

OGC testing of flour was conducted using the method of Ross et al. (2014). This is method uses the Rapid Visco Analyzer (RVA 4500, Perten Instruments, Warriewood, Australia) rather than the Bostwick Consistometer (Bettge and Morris, 2007; Ross et al. 2014). Break flour (soft-wheat aging study, Chapter 6) or straight-grade flour (hard-wheat survey and oxidant dose response study, Chapter 7) (12.5 ± 0.05 g) was weighed into a 50 mL screw-capped centrifuge tube. Deionized water (25.0 ± 0.05 mL) was added,

and the resulting mixture was shaken vigorously by hand for ~5 seconds. The flour-water suspension was placed in a Labquake tube shaker (Barnstead/Thermolyne, Dubuque, Iowa) and hydrated for 20 minutes. As quickly as possible (within 20 sec), and without allowing the suspension to settle by continued gentle hand shaking, 30.0 ± 0.05 g was transferred quantitatively to an RVA canister. The can, with paddle, was placed into the RVA. Test settings were: temperature 30°C; speed 960 rpm for 10 seconds, 160 rpm for 60 seconds. Viscosity at 120 seconds was recorded for the water-only baseline. The RVA was stopped for 20 seconds to allow the manual addition of 65 μL of 3% hydrogen peroxide. The RVA restarted at 960 rpm for 15 seconds and 160 rpm for a further 300 seconds for measurement.

Measured parameters were:
-Final viscosity in water, prior to addition of hydrogen peroxide (reported as "baseline viscosity").
-Peak viscosity after addition of hydrogen peroxide (reported as peroxide peak viscosity: "PPV"). For purposes of this research, OGC is equivalent to PPV.
-Final viscosity in hydrogen peroxide after mixing for 300 sec (not reported).

Calculated parameters were:
-“Reactivity to peroxide”; calculated as the difference between baseline viscosity and PPV.
-“Peroxide peak breakdown viscosity” was calculated as a comparison of final viscosity in hydrogen peroxide to PPV, reported as an absolute value (PPBV: final viscosity - PPV) or as a proportion (PPBV% : final viscosity/PPV, expressed as a percent).

Determination of total peroxidized lipids

MDA analysis was carried out using a method modified from that described in the included manual. Modifications made were to increase MDA extraction, and are described below. Each flour sample was extracted twice and each extract was measured in duplicate.

10 (±0.5) mg of flour was weighed into 2 mL microcentrifuge tubes in duplicate. 297 µL of MDA Lysis Buffer and 3 µL of BHT-100x (butylated hydroxytoluene) were added to each tube. Each sample was mixed for 5 seconds in a VWR Analog Vortex Mixer (VWR International, LLC) to suspend the flour. Samples were then sonicated using a Model 100 Sonic Dismembrator (Fischer Scientific, Pittsburgh, PA) for 20 seconds. Samples were again vortexed for 5 seconds to suspend any flour that might be attached to the tube wall above the buffer/BHT solution. The suspension was allowed to stand at room temperature for 10 minutes. Samples were centrifuged at 13,000 x g for 10 minutes to remove insoluble material. 200 µL of supernatant from each sample was transferred to a new microcentrifuge tube. 600 µL of the TBA solution was then added to the 200 µL of supernatant. Samples were incubated at 95° C in a VWR Analog Dry Block Heater (VWR International, LLC) for 60 minutes to form the TBA-MDA adduct. Samples were removed from heat and placed in an ice bath for 10 minutes and allowed to cool to room temperature. To determine the total amount of MDA in each sample, 200 µL of each TBA-MDA adduct was placed into a 96-well plate in duplicate (for a total of 4 wells per sample). The plate was then placed into a VERSAmax Tunable microplate reader (Molecular Devices, Inc., Sunnyvale, CA) and optical density determined at $\lambda = 532$ nm.

MDA colorimetric standards were made by diluting 10 µL of MDA standard, included in the kit, with 407 µL of deionized water. This resulted in a 0.1 M MDA standard solution. 20 µL of this solution was further diluted with 980 µL of deionized water to prepare the 2 mM MDA standard. 0, 2, 4, 6, 8, and 10 µL of the 2mM MDA standard was added to separate microcentrifuge tubes and deionized water was added to each tube to bring the respective volumes to 200 µL. Doing so generated a 0 (blank), 4, 8, 12, 16, and 20 nmole standards to make a standard curve. 200 µL of each standard was placed in duplicate in a 96 well plate. The absorbance from the blank standard was subtracted from all other data obtained from the colorimeter.

A standard curve was produced using the absorbance values obtained from the standards (after subtracting the blank). MDA content was calculated from the standard curve.

TBA solution was prepared by adding the contents of the stock TBA bottle to 7.5 (±0.05) mL of Glacial Acetic Acid, and adding water to a final volume of 25 mL (±0.05).

To determine the amount of MDA from an individual colorimeter reading, the absorbance value was substituted into the standard curve regression equation for "y" [absorbance (unitless)]. The equation was then solved for "x," giving MDA amount (nmol).

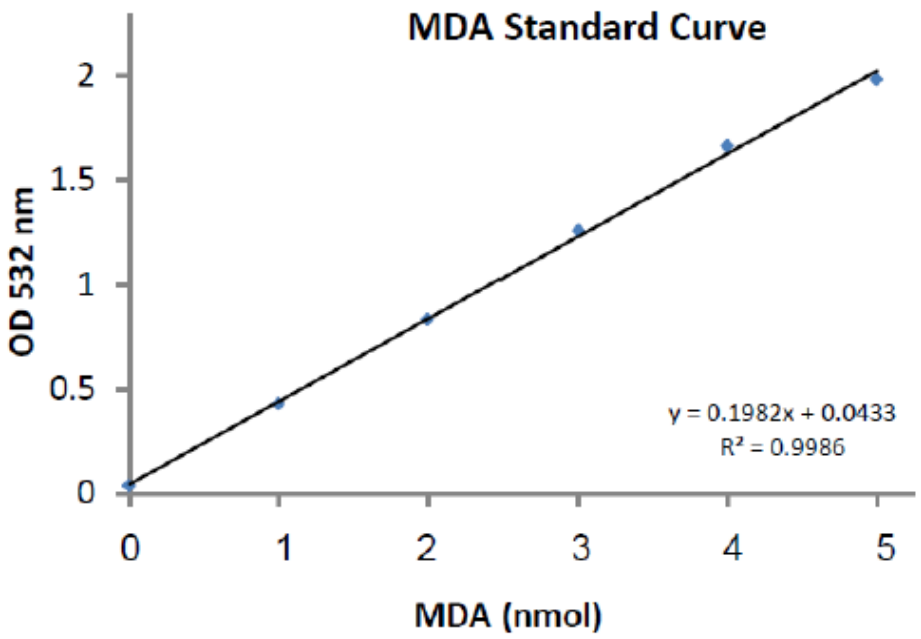

Figure 3.1: Example standard curve included in the Lipid Peroxidation (MDA) Assay Kit

Determination of MDA Concentration

$$MDA\,Concentration = \left(\frac{\left(\frac{x_1 + x_2}{2} \right)}{S} \right) * 4 * D$$

x_1 and x_2 are the MDA amounts (nmol) from the standard curve for the duplicate readings within each sample.

S is the amount of sample weighed into each 2 mL microcentrifuge tube (in mg)
4 is the correction for using 200 μL of the 800 μL reaction mix
D is the dilution factor (D in this case was 1 because no further dilution was conducted)

Response of oxidative gelation to dosage of hydrogen peroxide and ADA

In order to observe the response of OGC to the dose of hydrogen peroxide and ADA, a modification of the OGC method described above was employed. Oxidant levels of 10, 20, 40, 60, 80, and 100 ppm were used. 10, 20, 40, 60, 80 and 100 ppm corresponds to 8, 17, 33, 50, 67, and 83 μL of 3% hydrogen peroxide, respectively. 10, 20, 40, 60, 80 and 100 ppm corresponds to.5, 0.4, 0.3, 0.2, 0.1, and 0.05 g ADA-spiked flour.

In order to conduct the dose response with ADA, spiked flour samples (2500 ppm ADA) were used instead of addition of an ADA solution (ADA has low solubility, ~50 ppm, in water at ambient temperature). Therefore, a modification of the OGC test was used. A small amount of ADA-spiked flour was added into the RVA (at the same time that hydrogen peroxide would be added) in order to give flour-water system the desired oxidant concentration. 10, 20, 40, 60, 80 and 100 ppm corresponds to addition of 0.5, 0.4, 0.3, 0.2, 0.1, and 0.05 g ADA-spiked flour.

To keep the flour:water ratio consistent with the standard OGC test, the addition of ADA-spiked flour was taken into account (e.g. to have an ADA concentration of 100 ppm, 0.5 g of 2500 ppm ADA-spiked flour was added to the RVA canister, which contained 12 g ADA free flour and 25 g water).

A negative control test (0 ppm oxidant addition) was not explicitly conducted because each OGC test contains a control, measured as final water viscosity (viscosity before the addition of oxidant).

Measured parameters in this study are the same as those declared in the *Oxidative Gelation Capacity section* above. Reported values are final viscosity in water (reported as

0 ppm oxidant addition) and peak viscosities after addition of hydrogen peroxide or ADA (reported as "peak viscosity" for all oxidant addition levels above 0 ppm).

Statistical analyses

All analyses were performed in duplicate. One-way and multifactor analysis of variance were performed to determine the significance of each main effect and possible interaction at a probability level of P = 0.01. Multiple comparisons were calculated using Tukey's HSD at a probability level of P = 0.01 unless otherwise specified. Linear correlation coefficients were calculated and significance for r was given at a probability level of P = 0.01 unless otherwise specified. Multiple-variable analysis (correlation) was used to compare reactivity to peroxide and PPV in the soft-wheat aging study. Statistical analyses were performed using Statgraphics Centurion XVI.I (Statpoint Technologies Inc., Warrenton, VA).

CHAPTER 4: RESULTS AND DISCUSSION: ABSORPTION CHARACTERISTICS OF SOFT-WHEAT FLOUR

Soft-wheat kernel characteristics

Table 4.1 shows hardness, moisture, and protein data for the four wheat samples. Goetze and Tubbs grouped with the highest kernel hardness. Skiles and Bobtail grouped with the lowest kernel hardness. Skiles had the highest wheat protein content and Bobtail the lowest.

Variety	Hardness (Hardness Index)		Moisture Content (%)		Protein Content (%)**
	Mean	SD*	Mean	SD*	
Goetze	33.3	17.0	10.3	0.5	9.9
Tubbs	34.8	15.3	10.4	0.5	9.9
Skiles	17.1	12.4	10.4	0.5	10.6
Bobtail	18.9	16.3	10.6	0.6	8.3

Table 4.1: Hardness index, moisture content, and protein content of Goetze, Tubbs, Skiles, and Bobtail. *Standard deviation of 200 individual kernels. **Measured in singlet: repeatability of NIR protein ± 0.5% (Osborne and Fearn 1983).

Soft-wheat break-flour yield

ANOVA (Table 4.2) shows that variety and weeks after harvest (WAH) each had a significant effect on break flour yield (BFY). There interaction term was not significant.

Main Effects	***F*-Ratio**	**P-Value**
Variety	60.1	< .01
Weeks After Harvest	23.7	< .01
Interactions		
Variety x Weeks After Harvest	1.12	0.3934

Table 4.2: 3 way ANOVA results for break flour yield showing main effects and 2-way interaction terms.

Varieties

Figure 4.1 shows that among varieties, summed across WAH, Goetze, Tubbs, and Skiles grouped together with the lowest BFY. Bobtail had the highest BFY. Variety was the strongest contributor to differences in BFY in the ANOVA model as determined by the *F*-ratios. Based on experience, it is surprising that Skiles, with the softest kernel texture

of the four (Table 4.1), did not group with Bobtail for high BFY. The commonly observed relationship between soft kernel texture and high BFY was confirmed by reanalysis of data presented by Bettge et al. (2002) that showed a significant correlation ($r = -0.96$) between SKCS kernel hardness and BFY, and data of Bettge and Morris (2000) that showed similar with $r = -0.89$.

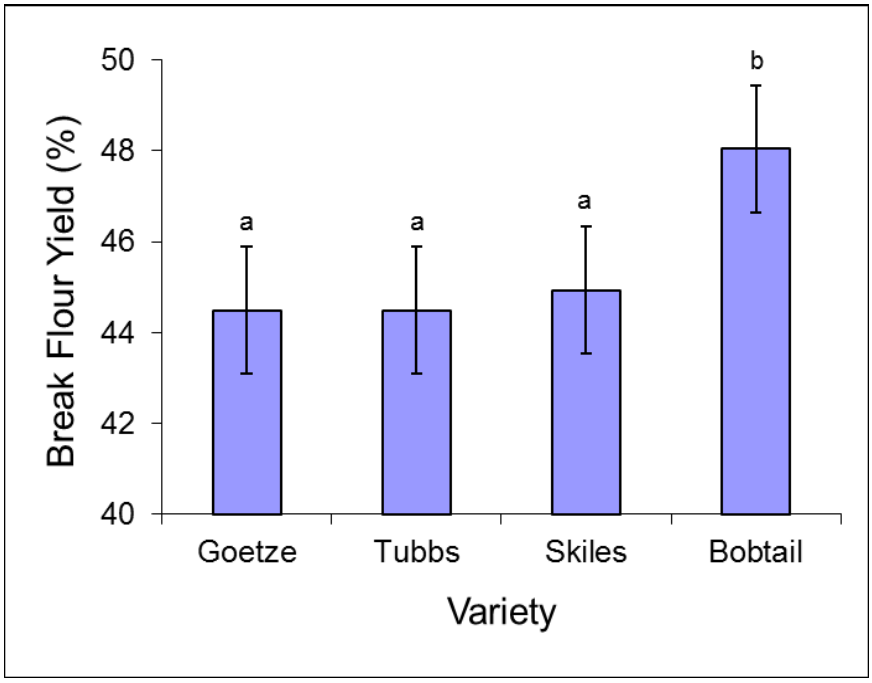

Figure 4.1: Break flour yield for all varieties tested, summed across all weeks after harvest. Different letters indicate significantly different mean values for BFY. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 4.2 shows that, when summed across varieties BFY increased with grain storage time for the first 6 weeks. After the maximum BFY at 6 WAH, BFY then decreased steadily until 24 weeks of grain storage. Grain stored for 24 weeks had BFY not significantly different to BFY of freshly harvested grain (stored for 0 weeks). There appear to be no reports in the peer-reviewed literature regarding changes in soft-wheat BFY across storage times of this magnitude. However Lin et al. (2010) at the 57th Soft-Wheat Research Review reported very small but monotonic decreases in BFY across 15 weeks of storage, in contrast to the initial increase and subsequent decrease observed here.

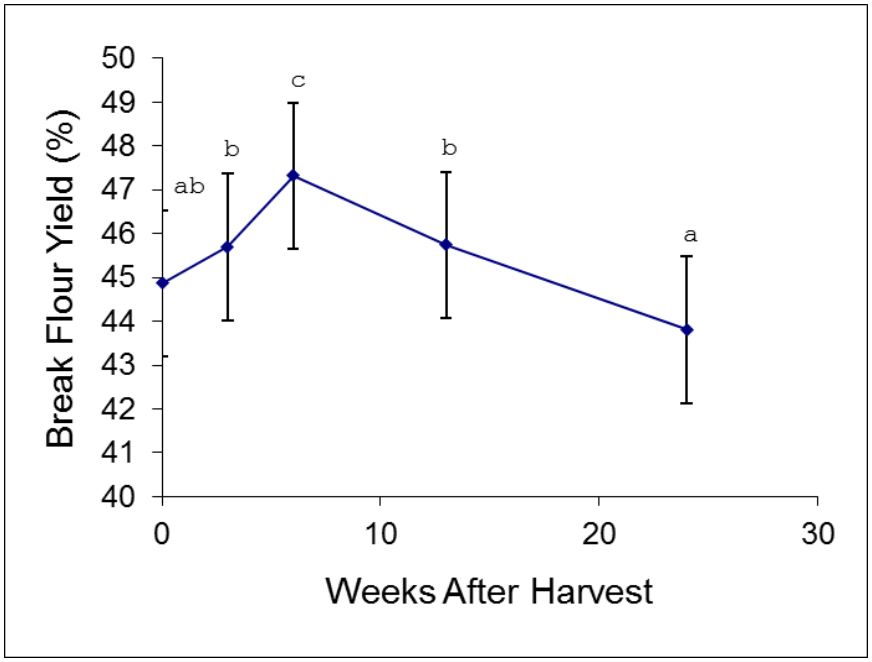

Figure 4.2: Break flour yield for the weeks after harvest, summed across all varieties tested. Different letters indicate significantly different mean values for BFY. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Water solvent retention capacity

ANOVA (Table 4.3) shows that only variety and WAH had a significant effect on the water SRC. At $p \leq 0.01$ there were no significant two- or three-way interactions.

Main Effects	***F*-Ratio**	**P-Value**
Variety	445.45	< .01
Weeks After Harvest	12.54	< .01
Days After Milling	2.28	0.0393
Interactions		
Variety x Weeks After Harvest	0.96	0.4897
Variety x Days After Milling	1.1	0.3613
Weeks After Harvest x Days After Milling	1.69	0.0321
Variety x Weeks After Harvest x Days After Milling	1.11	0.2918

Table 4.3: 3 way ANOVA results for water solvent retention capacity showing main effects, 2-way, and 3-way interaction terms.

Varieties

Figure 4.3 shows that among varieties, summed across WAH and days after milling (DAM), Skiles and Bobtail grouped with the lowest water SRC values and Goetze and

Tubbs grouped with the highest. This effect was the strongest contributor to the overall ANOVA model as determined by the *F*-ratios. This observation also conformed to multi-year multi-location SRC data showing the high (Tubbs, Goetze) and low (Skiles, Bobtail) absorption capacities of these varieties (Ross pers. Comm.; USDA Western Wheat Quality Laboratory G&E study http://public.wsu.edu/~wwql/php/vqs.php accessed 2014-03-28). Water SRC also corresponded to kernel hardness (Table 4.1) with the expected result that the two softer textured wheats would have the lowest absorption characteristics (Xiao et al. 2006). In practice the differences between the two low and two high water SRC groups (approximately 5%) would be considered a practically relevant difference (Ross pers Comm.). There are no reports in the literature that define what "practical" differences in SRC values are, and this holds true for all 4 solvents.

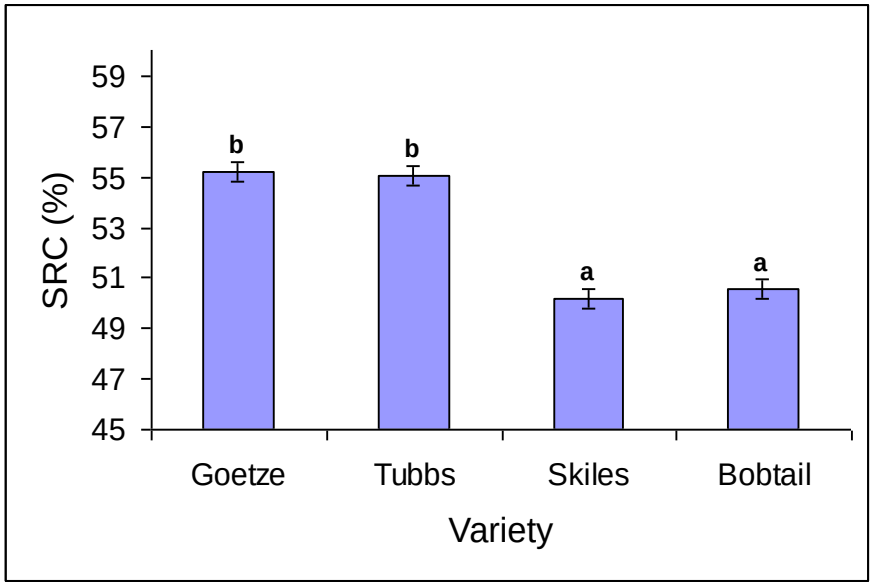

Figure 4.3: Water solvent retention capacity (SRC) for the varieties tested, summed across all weeks after harvest and days after milling. Different letters indicate significantly different mean values for water SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 4.4 shows that, when summed across varieties and DAM, there was a small but significant decrease in water SRC (approximately 0.6%) from week 0 to week 3 of grain storage, followed by a small but significant increase (approximately 1.4%) in water SRC until week 24. There were no significant differences between water SRC values in flour

tested from grain stored at 6, 13, and 24 weeks. The week 24 mean value across varieties and DAM was significantly higher than the week 0 value. The reported changes over time were statistically significant but their practical significance is not clear. Kweon et al (2014) outlined the expected repeatability of water SRC at ± 0.5%. Based on this criterion, the changes in water SRC from week 0 to week 3 (52.2% and 51.9%, respectively) and between week 0 and week 24 (52.2% and 53.3%, respectively) appear to border on practical significance. However, the changes across grain storage time (WAH) were smaller in magnitude than those between the high and low varieties (Figure 4.3).

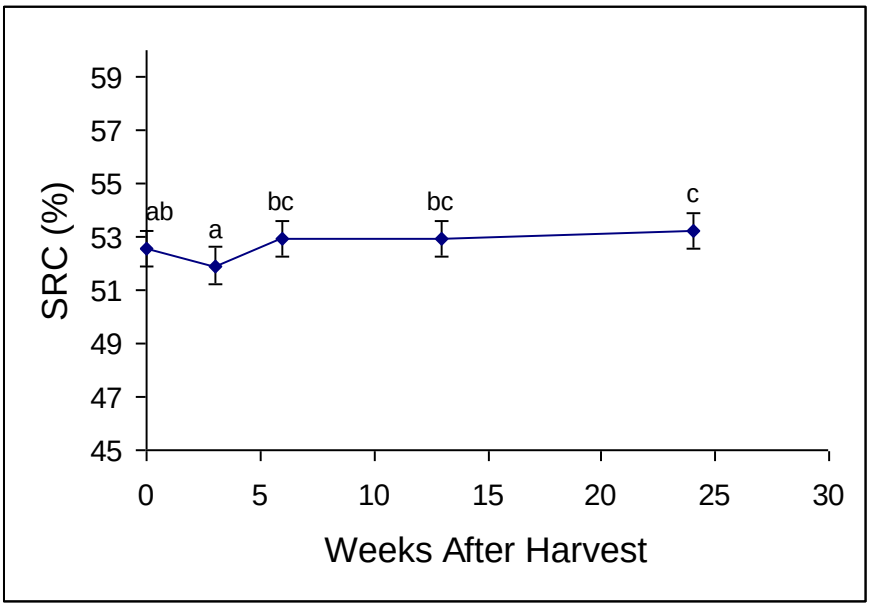

Figure 4.4: Water solvent retention capacity (SRC) for the weeks after harvest, summed across all varieties tested and days after milling. Different letters indicate significantly different mean values for water SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Sucrose solvent retention capacity

ANOVA (Table 4.4) shows that variety, WAH, and DAM each had a significant effect on sucrose SRC. At $p \leq 0.01$, there was a significant two-way interaction for WAH x DAM.

Main Effects	***F*-Ratio**	**P-Value**
Variety	2080.65	< .01
Weeks After Harvest	49.57	< .01
Days After Milling	12.2	< .01
Interactions		
Variety x Weeks After Harvest	1.77	0.0587
Variety x Days After Milling	1.23	0.246
Weeks After Harvest x Days After Milling	5.41	< .01
Variety x Weeks After Harvest x Days After Milling	1.18	0.2059

Table 4.4: 3 way ANOVA results for sucrose solvent retention capacity showing main effects, 2-way, and 3-way interaction terms.

Varieties

Figure 4.5 shows that among varieties, summed across WAH and DAM, Skiles had the lowest sucrose SRC value and Goetze had the highest. Variety was the strongest contributor to the overall ANOVA model as determined by The *F*-ratios. Congruent with the water SRC results (Figure 4.3), Tubbs and Goetze, although significantly different from each other, grouped as the high absorption group and Bobtail and Skiles again grouped as low absorption types. The difference in sucrose SRC between Bobtail and Tubbs (approximately 5%) would be considered of practical significance (Ross pers Comm).

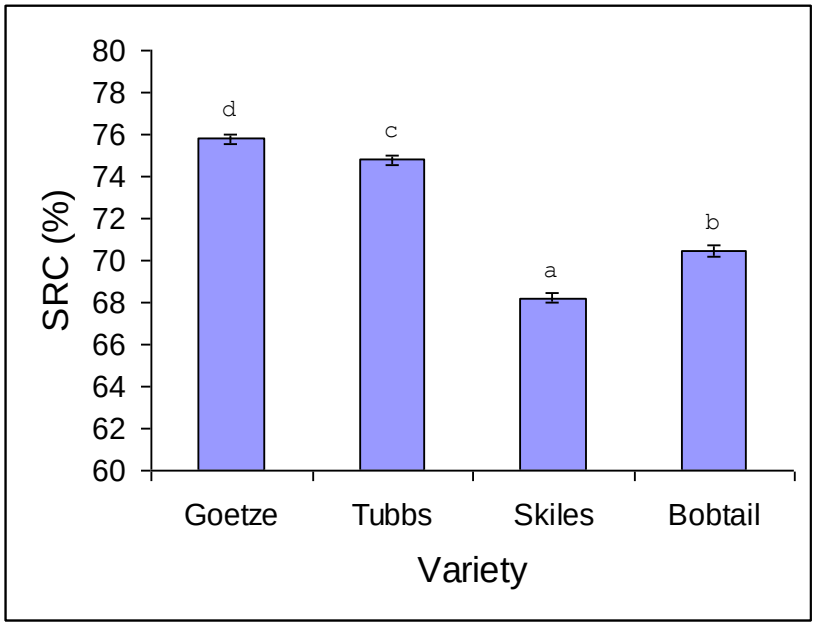

Figure 4.5: Sucrose solvent retention capacity (SRC) for the varieties tested, summed across all weeks after harvest and days after milling. Different letters indicate significantly different mean values for sucrose SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 4.6 shows that, when summed across variety and DAM, the general trend in sucrose SRC resembled that of water SRC. There was a small but significant decrease (approximately 1.2%) in sucrose SRC from week 0 to week 3 of grain storage, followed by a small but significant increase (approximately 1.5%) in sucrose SRC until week 24. Although the reported changes over time are statistically significant their practical significance is again not clear. Kweon et al (2014) also outlined the expected repeatability of sucrose SRC at ± 1%. Based on this criterion, the changes in sucrose SRC from week 0 to week 3 (72.6% and 71.4%, respectively) and between week 0 and week 24 (72.6% and 73.1%, respectively) appear to border on practical significance. However, these changes across time are again smaller in magnitude than the varietal differences.

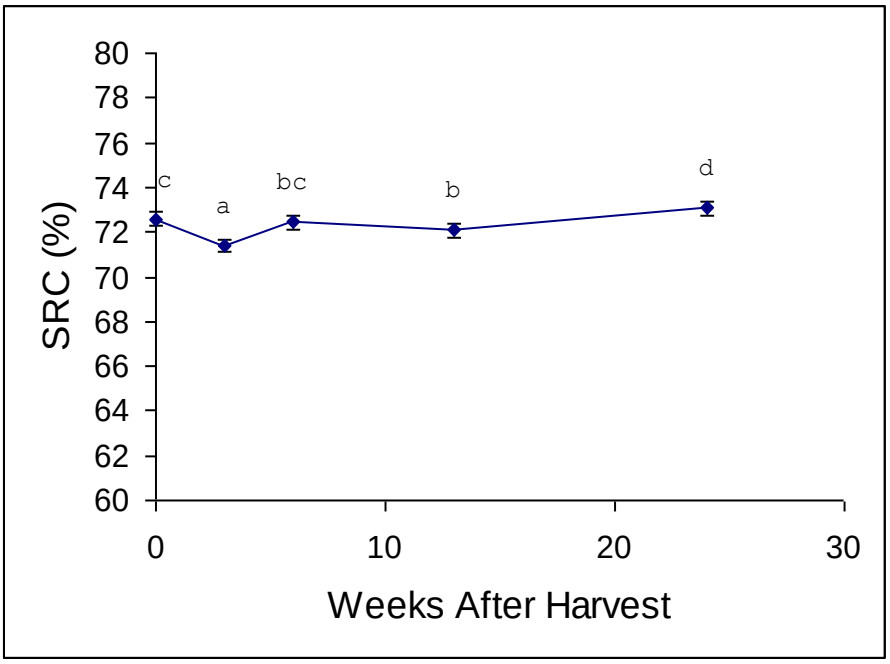

Figure 4.6: Sucrose solvent retention capacity (SRC) for the weeks after harvest, summed across all varieties tested and days after milling. Different letters indicate significantly different mean values for sucrose SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Days After Milling

Figure 4.7 shows that, when summed across variety and WAH, there was an overall upward trend in sucrose SRC. Although differences in sucrose SRC across DAM were statistically significant, their practical significance compared to the other main effects variety and WAH were smaller as determined by the *F* -ratios.

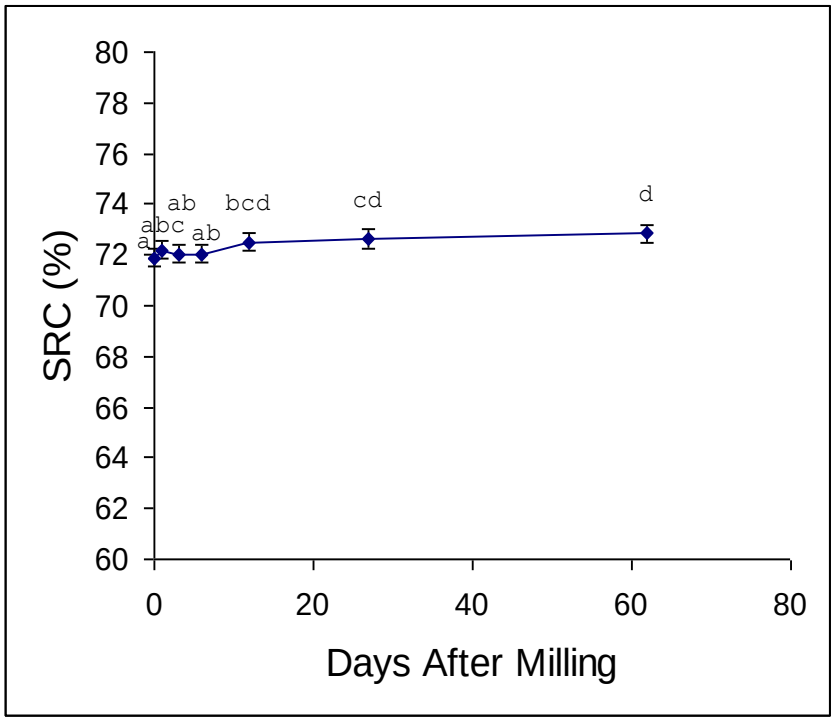

Figure 4.7: Sucrose solvent retention capacity (SRC) for the days after milling, summed across all varieties tested and weeks after harvest. Different letters indicate significantly different mean values for sucrose SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Interactions

Table 4.4 shows that the WAH x DAM interaction was statistically significant. The contribution of the interaction to the overall ANOVA model was comparable to the main effect DAM as determined by the *F*-ratios. Therefore, it was concluded that the interaction could be of some practical significance. The interaction plots (Figure 4.8) show, for example, there was a decrease in sucrose SRC value for all DAM between 0 and 3 weeks of grain storage except for days 6 and 27 (Figure 4.8 D and F, respectively). There were also variations in slope that helped account for the significant variety x WAH interaction term. Although these interactions were statistically significant, their practical significance compared to the main effects variety and WAH was smaller as determined by the *F*-ratios.

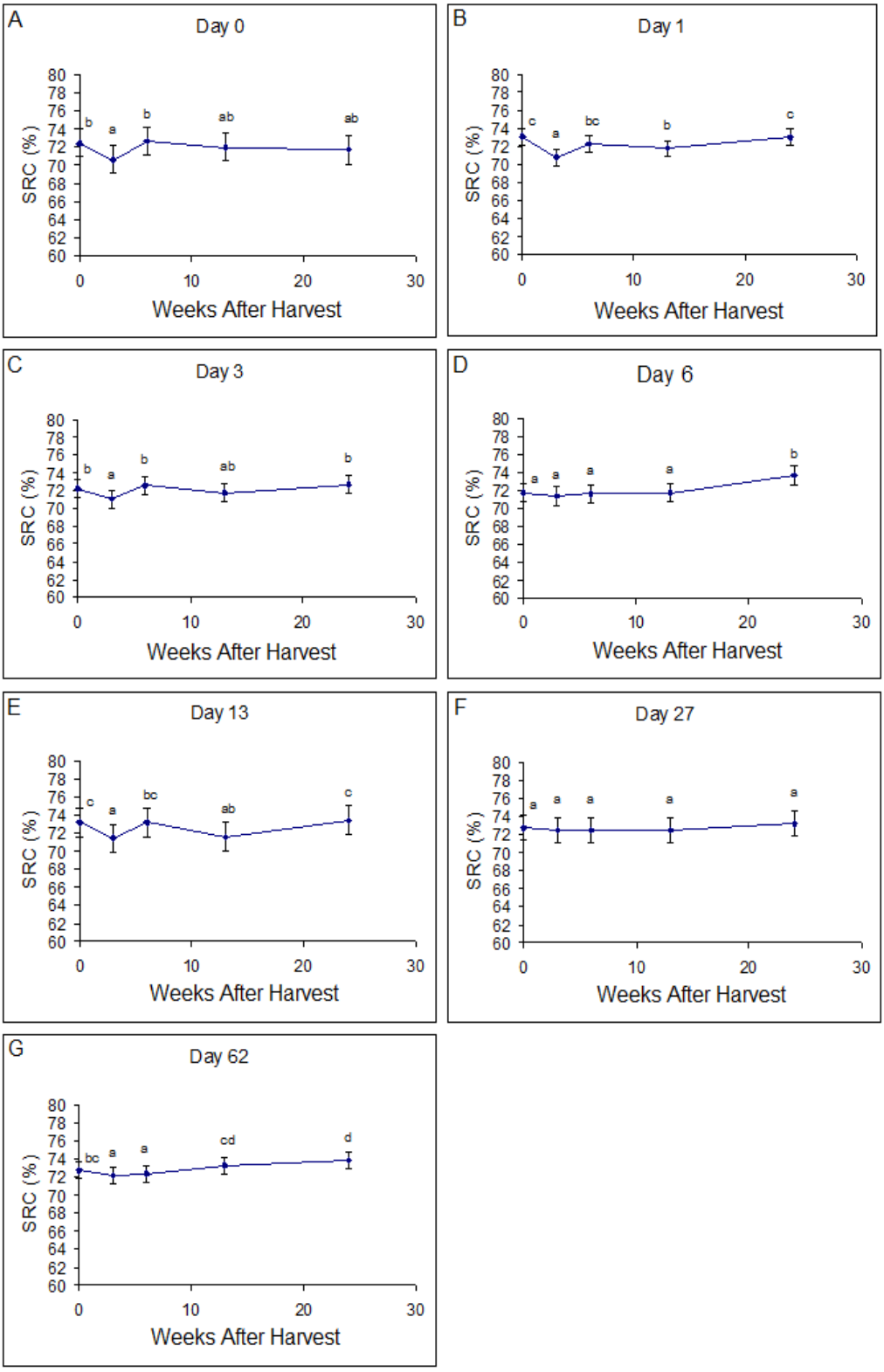

Figure 4.8: Sucrose solvent retention capacity (SRC) for all days after milling within each milling week, summed across all varieties tested. Different letters indicate significantly different mean values for sucrose SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Sodium carbonate solvent retention capacity (day 62 removed)

Due to laboratory error, data for flour aged 62 days was corrupted and irretrievable, and was omitted from the statistical analyses.

ANOVA (Table 4.5) shows that variety, WAH, and DAM each had a significant effect on the sodium carbonate SRC. At $p \leq 0.01$, there was also significant a two-way interaction for WAH x DAM.

Main Effects	***F*-Ratio**	**P-Value**
Variety	1217.99	< .01
Weeks After Harvest	18.29	< .01
Days After Milling	3.63	< .01
Interactions		
Variety x Weeks After Harvest	1.8	0.0561
Variety x Days After Milling	1.43	0.1441
Weeks After Harvest x Days After Milling	3.77	< .01
Variety x Weeks After Harvest x Days After Milling	1.05	0.4003

Table 4.5: 3 way ANOVA results for sodium carbonate solvent retention capacity (day 62 removed) showing main effects, 2-way, and 3-way interaction terms.

Varieties

Figure 4.9 shows that among varieties, summed across WAH and DAM, Bobtail had the lowest sodium carbonate SRC and Tubbs had the highest. Xiao et al (2006) showed a positive relationship ($r = 0.49$, $p \leq 0.0001$) between kernel hardness and sodium carbonate SRC value that is generally congruent with the relationships between kernel hardness and carbonate SRC observed in this study. The effect of variety was the strongest contributor to the overall ANOVA model as determined by the *F*-ratios.

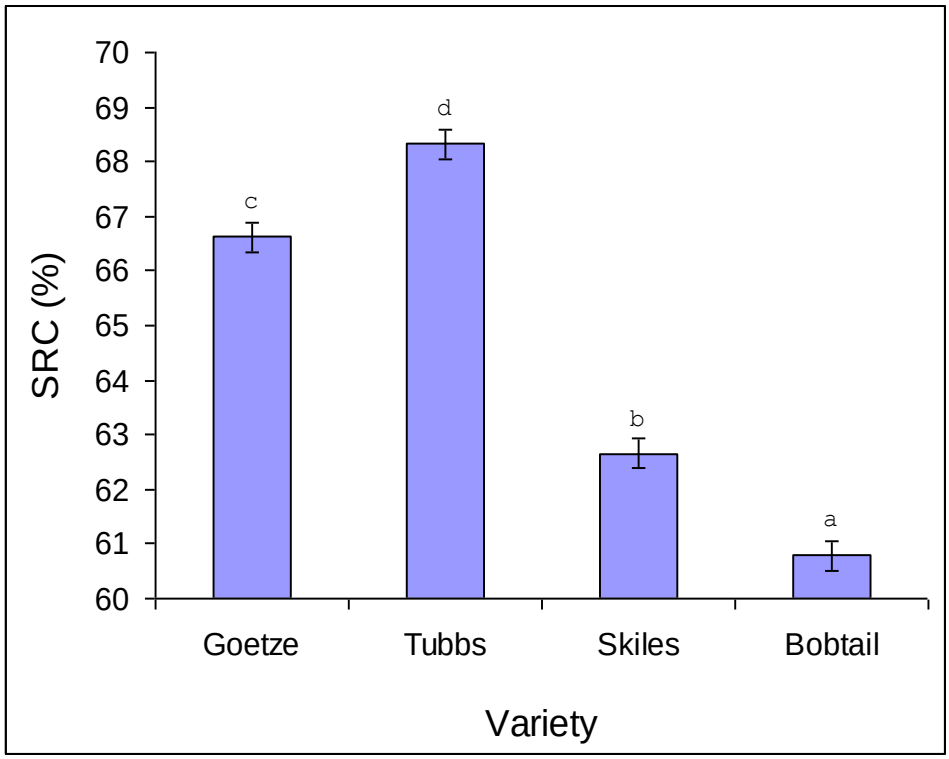

Figure 4.9: Sodium carbonate solvent retention capacity (SRC) (day 62 removed) for the varieties tested, summed across all weeks after harvest and days after milling. Different letters indicate significantly different mean values for sodium carbonate SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 4.10 shows that, summed across variety and DAM, the general trend in sodium carbonate SRC resembled that of both water and sucrose SRC. There was a significant decrease in the sucrose SRC from week 0 to week 3 of grain storage, followed by a general increase in sucrose SRC until week 24. However, unlike the trend in water and sucrose SRC, the sodium carbonate SRC value of flour tested from grain stored 24 weeks did not exceed that of grain stored 0 weeks. Although the reported changes over time are statistically significant their practical significance is once again not clear. Kweon et al (2014) also outlined the expected repeatability of sodium carbonate SRC at ± 0.5%. Based on this criterion, the changes in sodium carbonate SRC from Week 0 to Week 3 (65.2% and 63.9%, respectively) appear to be of practical significance.

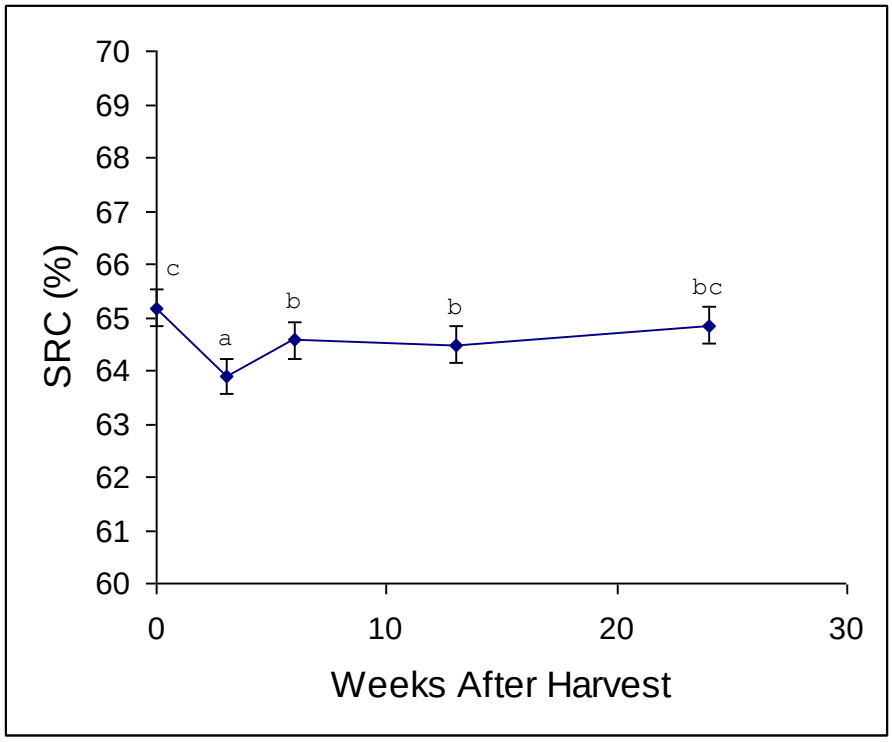

Figure 4.10: Sodium carbonate solvent retention capacity (SRC) (day 62 removed) for the weeks after harvest, summed across all varieties tested and days after milling. Different letters indicate significantly different mean values for sodium carbonate SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Days After Milling

Figure 4.11 shows that, summed across variety and WAH, there was a downward trend in sodium carbonate SRC. This trend did not follow that of either water or sucrose SRC.

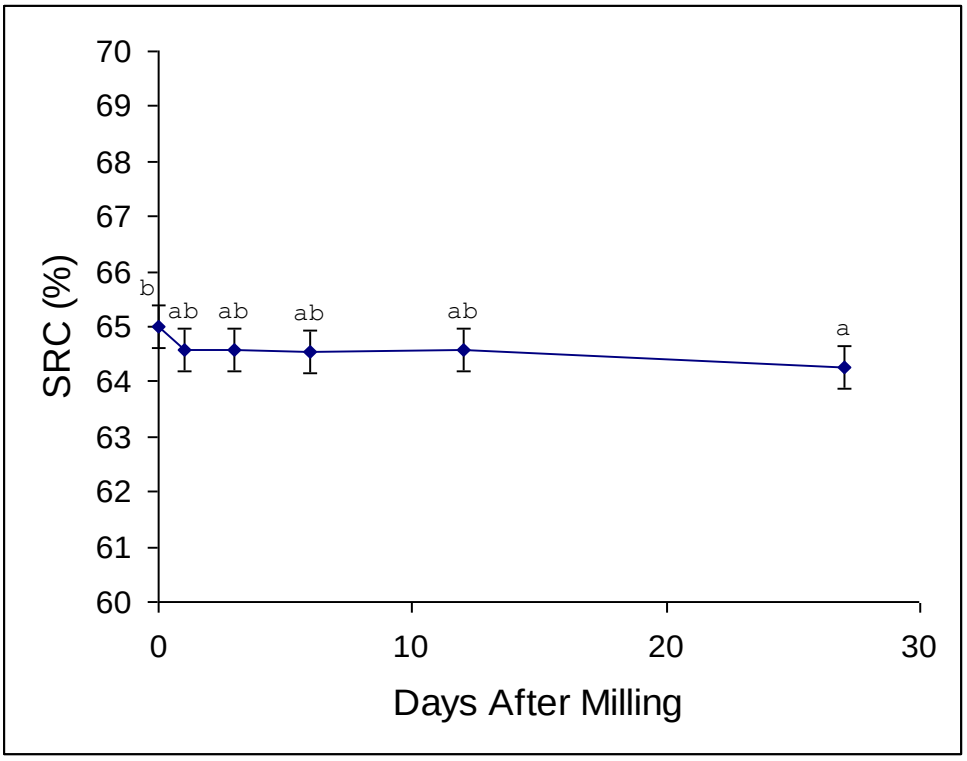

Figure 4.11: Sodium carbonate solvent retention capacity (SRC) (day 62 removed) for the days after milling, summed across all varieties tested and weeks after harvest. Different letters indicate significantly different mean values for sodium carbonate SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Interactions

Table 4.5 shows that the WAH x DAM interaction was statistically significant. The contribution of the interaction to the overall ANOVA model was comparable to one main effect, DAM, as determined by the *F*-ratios. Therefore, it was concluded that the interaction was of some practical significance. The interaction plots (Figure 4.12) shows differences in the slopes for carbonate SRC at each milling date dependent on the age of the flour that account for the significant interaction.

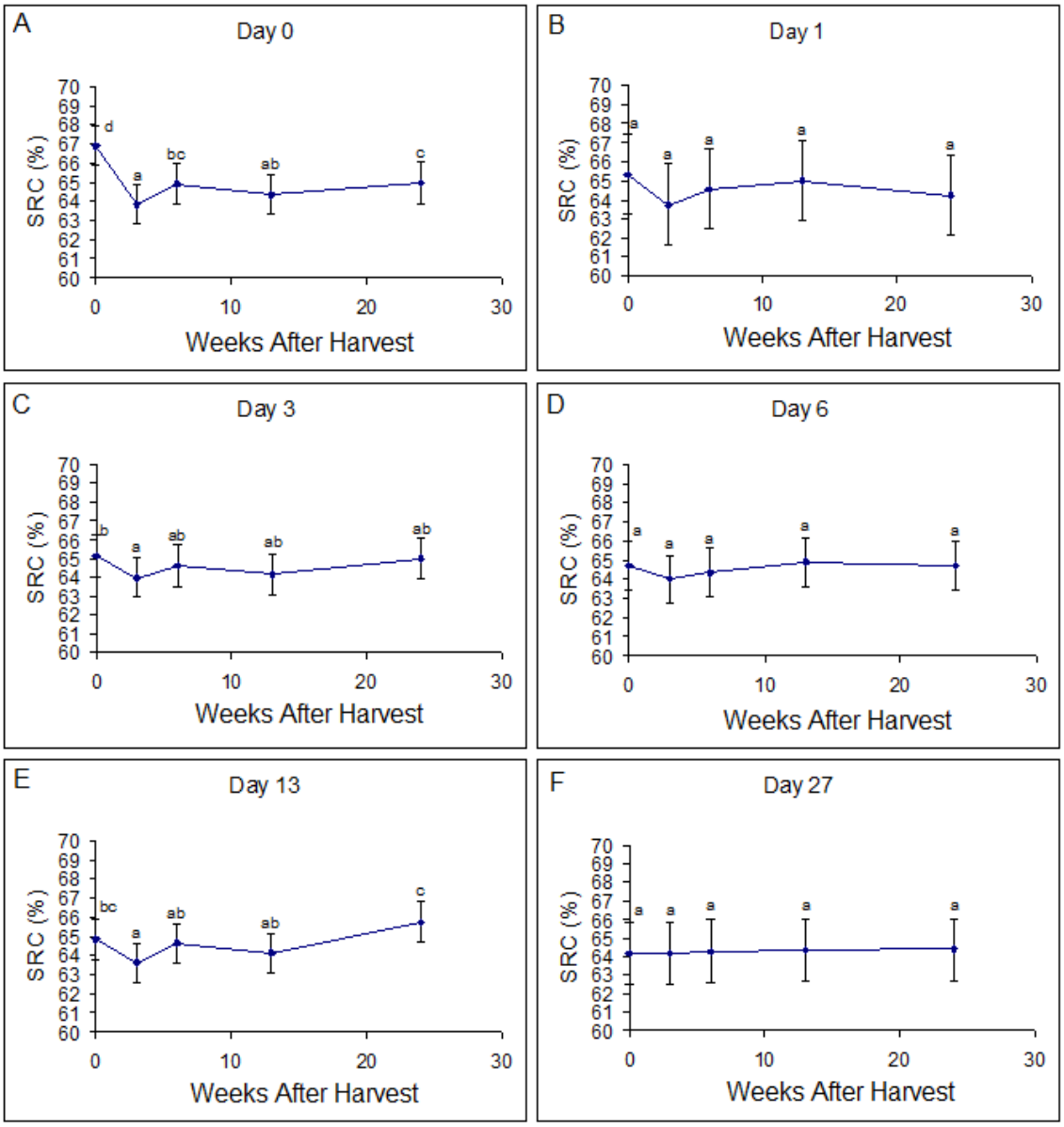

Figure 4.12: Sodium Carbonate solvent retention capacity (SRC) (day 62 removed) for all days after milling within each milling week, summed across all varieties tested. Different letters indicate significantly different mean values for sodium carbonate SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Lactic acid solvent retention capacity (weeks 6 and 24 removed)

Due to laboratory error, data for flour milled from grain stored 6 and 24 weeks was corrupted and irretrievable, and therefore was omitted from the analysis.

ANOVA (Table 4.6) shows that variety, WAH, and DAM each had a significant effect on lactic acid SRC. At $p \leq 0.01$, there were no significant interaction terms.

Main Effects	***F*-Ratio**	**P-Value**
Variety	2221.57	< .01
Weeks After Harvest	43.71	< .01
Days After Milling	10.52	< .01
Interactions		
Variety x Weeks After Harvest	2.08	0.0641
Variety x Days After Milling	1.35	0.178
Weeks After Harvest x Days After Milling	1.28	0.2478
Variety x Weeks After Harvest x Days After Milling	1.08	0.3826

Table 4.6: 3 way ANOVA results for lactic acid solvent retention capacity (weeks 6 and 24 removed) showing main effects, 2-way, and 3-way interaction terms.

Varieties

Figure 4.13 shows that among varieties, summed across WAH and DAM, Tubbs had the lowest lactic acid SRC and Bobtail had the highest. This grouping does not conform with the other SRC tests. Variety was the highest contributor to the overall ANOVA model as determined by the *F*-ratios.

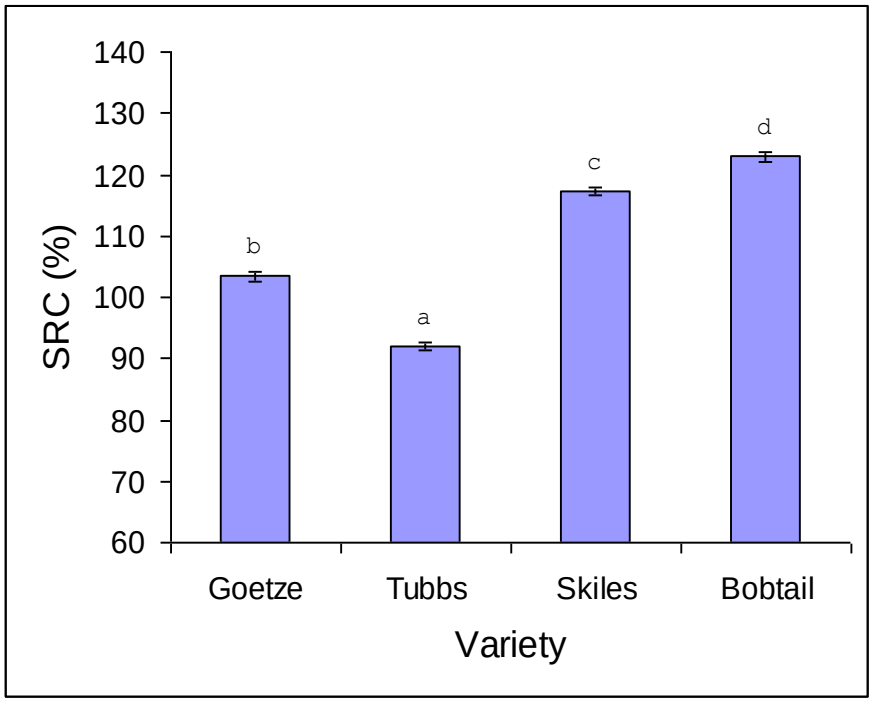

Figure 4.13: Lactic acid solvent retention capacity (SRC) (weeks 6 and 24 removed) for the varieties tested, summed across all weeks after harvest and days after milling. Different letters indicate significantly different mean values for lactic acid SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 4.14 shows that, when summed across variety and DAM, there was an overall downward trend in lactic acid SRC in flour tested from grain stored until week 13. The lactic acid SRC value at weeks 0 and 3 were not significantly different. This trend conforms to data reported in *Milling New Crop Wheat: Myth and Reality* (Lin et al. 2010), showing a downward trend in lactic acid SRC as grain storage time increased. Although the reported changes over time are statistically significant their practical significance is not clear. However, Kweon et al (2014) also outlined the expected repeatability of lactic acid SRC at ± 1%. Based on this criterion, the changes in lactic acid SRC from Week 0 to Week 13 (110.0% and 107.0%, respectively) appear to be of practical significance.

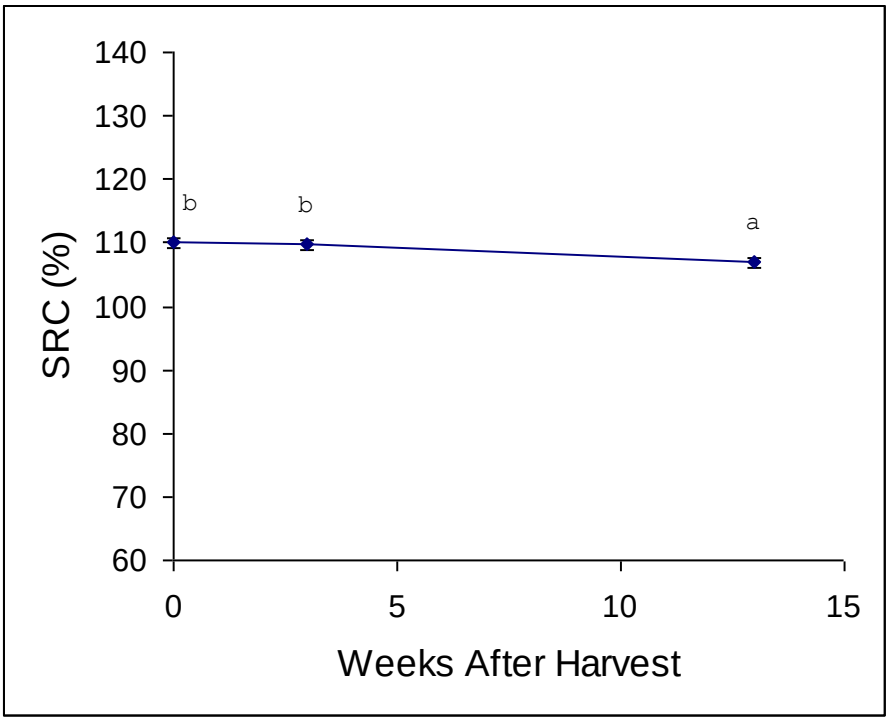

Figure 4.14: Lactic acid solvent retention capacity (SRC) (weeks 6 and 24 removed) for the weeks after harvest, summed across all varieties tested and days after milling. Different letters indicate significantly different mean values for lactic acid SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Days After Milling

Figure 4.15 shows that, when summed across variety and WAH, there were no significant changes in lactic acid SRC values in the first 27 days of flour aging. After 27 days of flour aging, lactic acid SRC decreased significantly.

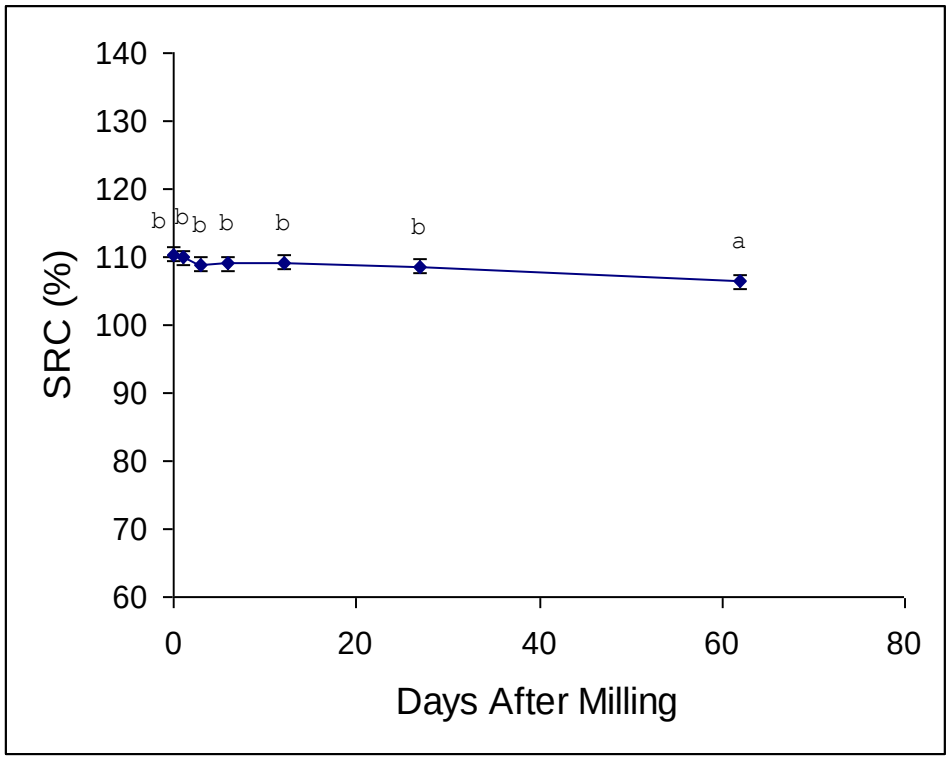

Figure 4.15: Lactic acid solvent retention capacity (SRC) (weeks 6 and 24 removed) for the days after milling, summed across all varieties tested and weeks after harvest. Different letters indicate significantly different mean values for lactic acid SRC. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Despite omission of data from the analyses for lactic acid SRC, the data was in agreement with the data presented by Lin et al. (2010: not peer reviewed) showing an overall downward trend in lactic acid SRC across grain and flour storage time.

Solvent retention capacity in fresh and maximally aged flour: practical considerations

Table 4.7 compares the SRC values for all 4 solvents for "fresh" flour (flour from 0 WAH and 0 DAM) and for maximally aged ("Aged") flour. For water and sucrose SRCs, maximally aged flour is defined to be the flour milled at 24 WAH and then aged for 62 DAM. For carbonate SRC maximally aged flour is defined to be the flour milled at 24 WAH and aged 27 DAM. For lactic acid SRC maximally aged flour is defined to be the flour milled at 13 WAH and aged 62 DAM. What was notable for water and sucrose SRCs was the greater level of change for the group (Skiles, Bobtail) with the lowest

absolute values of the two SRCs. For lactic acid SRC the trend appeared reversed and the higher lactic acid group (Skiles, Bobtail) also had the largest declines in lactic acid SRC across time. When formally published this data will be the first report of these changes in the literature and as such is preliminary in nature and requires considerable confirmatory work.

Variety	SRC							
	Water		Sucrose		Sodium Carbonate		Lactic Acid	
	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged
Goetze	54.6 a	56.2 b	76.1 a	77.2 a	69.2 a	66.3 a	105.1 b	100.5 a
Tubbs	54.4 a	55.9 a	75.2 a	76.3 a	70.9 a	67.8 a	93.9 a	87.9 a
Skiles	48.9 a	51.3 a	68.4 a	69.9 b	64.7 a	62.3 a	119.1 b	111.2 a
Bobtail	49.8 a	52.0 a	70.1 a	72.0 a	62.9 a	61.1 a	125.0 b	116.9 a

	Differences			
Goetze	1.6*	1.1	-2.8	-4.6*
Tubbs	1.5	1.1	-3.1	-6.0*
Skiles	2.4	1.6*	-2.4	-7.8
Bobtail	2.2	1.9	-1.8	-8.1*

Table 4.7: Average solvent retention capacity (SRC) values and differences therein of fresh flour and maximally aged flour within each variety tested. "Fresh" flour is defined to be flour from 0 weeks after harvest and 0 days after milling. Maximally aged ("Aged") flour is defined to be flour from 24 weeks after harvest and 62 days after milling for Water and Sucrose SRC, 13 weeks after harvest and 62 days after milling for Lactic Acid SRC, and 24 weeks after harvest and 27 days after milling for Sodium Carbonate SRC. *indicates significance at $p \leq 0.01$. Different letters also indicate significance at $p \leq 0.01$.

CHAPTER 5: RESULTS AND DISCUSSION: OXIDATIVE GELATION AND LIPID PEROXIDATION IN SOFT-WHEATS

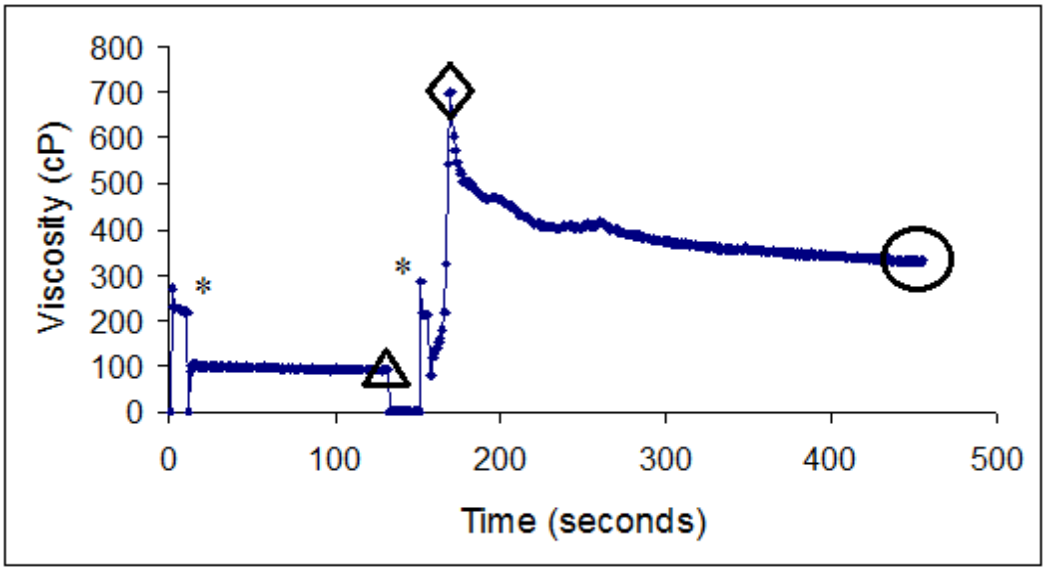

Figure 5.1: A sample plot of viscosity versus time from the Rapid Visco Analyzer based oxidative gelation capacity test. The triangle denotes the value for baseline viscosity. The diamond denotes the value for peroxide peak viscosity. The circle denotes the value for final viscosity. *peaks resulting from high speed mixing, unrelated to oxidative gelation.

Baseline Viscosity

ANOVA (Table 5.1) shows that variety, WAH, and DAM each had a significant effect on baseline viscosity. At $p \leq 0.01$, there were also significant two-way interactions for variety x WAH and WAH x DAM.

Main Effects	***F*-Ratio**	**P-Value**
Variety	714.26	< .01
Weeks After Harvest	50.5	< .01
Days After Milling	28.55	< .01
Interactions		
Variety x Weeks After Harvest	3.5	< .01
Variety x Days After Milling	1.11	0.3506
Weeks After Harvest x Days After Milling	20.04	< .01
Variety x Weeks After Harvest x Days After Milling	1.01	0.4688

Table 5.1: 3 way ANOVA results for baseline viscosity showing main effects, 2-way, and 3-way interaction terms.

Varieties

Figure 5.2 shows that among varieties, summed across WAH and DAM, Bobtail had the lowest baseline viscosity and Goetze the highest. Variety was the highest contributor to the overall ANOVA model as determined by the *F*-ratios. These results reflect the water SRC data (Figure 4.3), essentially grouping Bobtail and Skiles as the low absorption group (low flour/water viscosity) and Tubbs and Goetze (high flour/water viscosity) as the high absorption group. Baseline viscosity and water SRC both appeared to reflect absorption characteristics of flour in a similar fashion. However, Kweon et al. (2011) were adamant that "*SRC technology is based on energetics (related to thermodynamic polymer-solvent compatibility), not kinetics (related to mobility constraints for poor plasticizers)*" and that the "*SRC method deliberately avoids kinetic effects, which would be incorrectly introduced by a rheological method such as RVA…, the deliberate use of shear would violate the principle of the SRC method*". However, we observed alignment between water SRC and RVA baseline viscosities of the varieties that suggest, at least for water SRC, that the energetic and kinetic behaviors might be related.

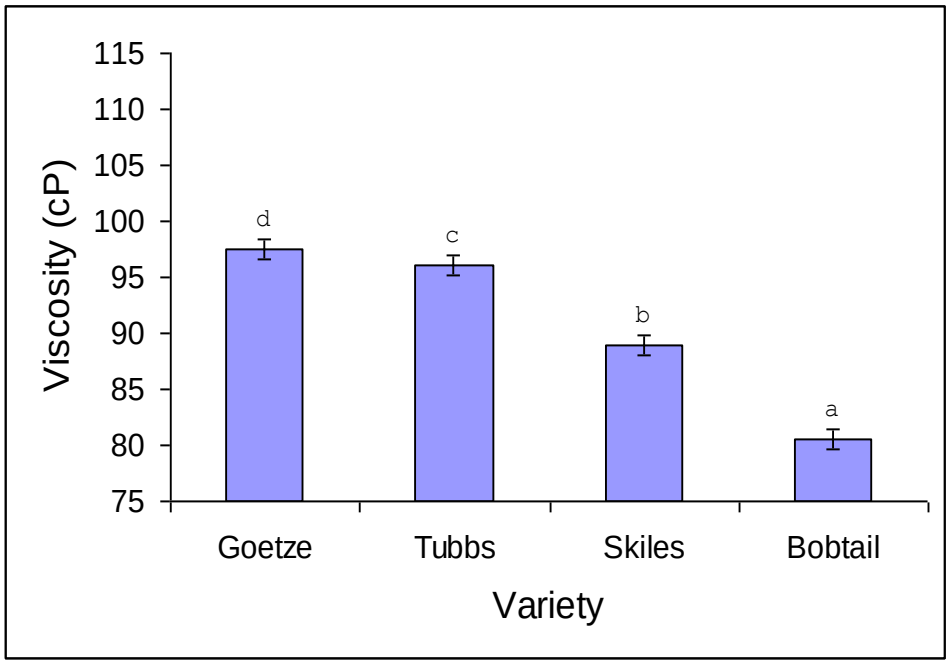

Figure 5.2: Baseline viscosity for the varieties tested, summed across all weeks after harvest and days after milling. Different letters indicate significantly different mean values for baseline viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 5.3 shows that, summed across varieties and DAM, the baseline viscosity did not significantly change after 24 weeks of grain storage. Similar to trends seen in the SRC data above, there was a significant decrease in baseline viscosity in flour tested from grain stored 0 and 3 weeks. Although there were statistically significant differences, the practical significance is negligible (±5 cP was the approximate maximum range of values in this data: Figure 5.2). Therefore the changes in baseline viscosities across WAH, although statistically significant, were considered to be "noise," and not definite trends. It might be expected that both water SRC and water baseline RVA viscosity would follow the same trends over time. However, while water SRC increased as a function of grain storage, baseline viscosity did not change. OGC and SRC tests hydrate flour under different conditions (no shear vs shear in SRC and RVA, respectively), which could have led to this difference and may support the contentions of Kweon et al (2011) detailed above.

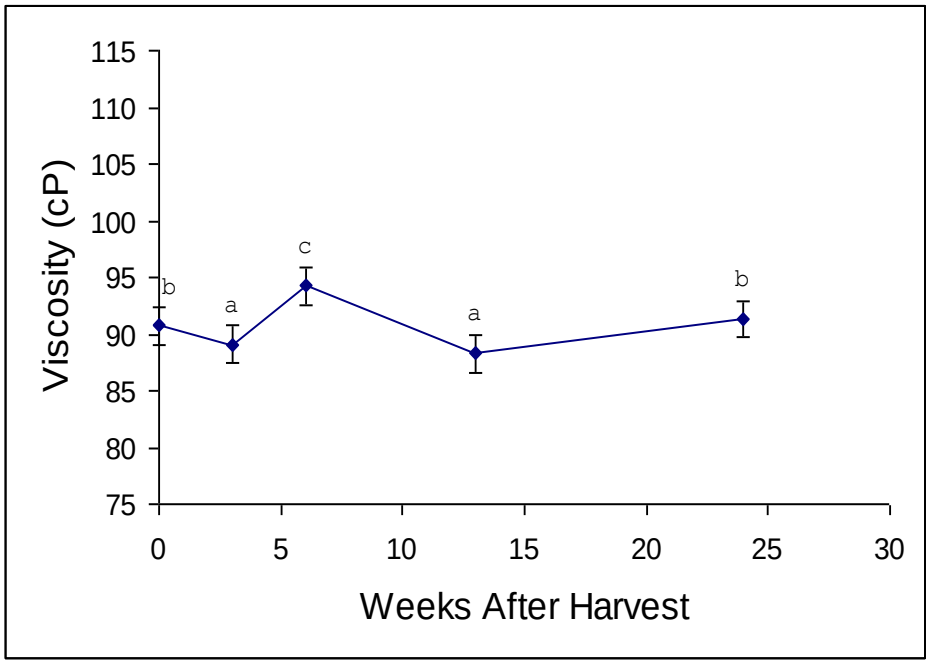

Figure 5.3: Baseline viscosity for the weeks after harvest, summed across all varieties tested and days after milling. Different letters indicate significantly different mean values for baseline viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Days After Milling

Figure 5.4 shows that, summed across variety and WAH, there was an overall upward trend in baseline viscosity. Baseline viscosity increased significantly in flour aged 13 and 27 days and plateaued thereafter.

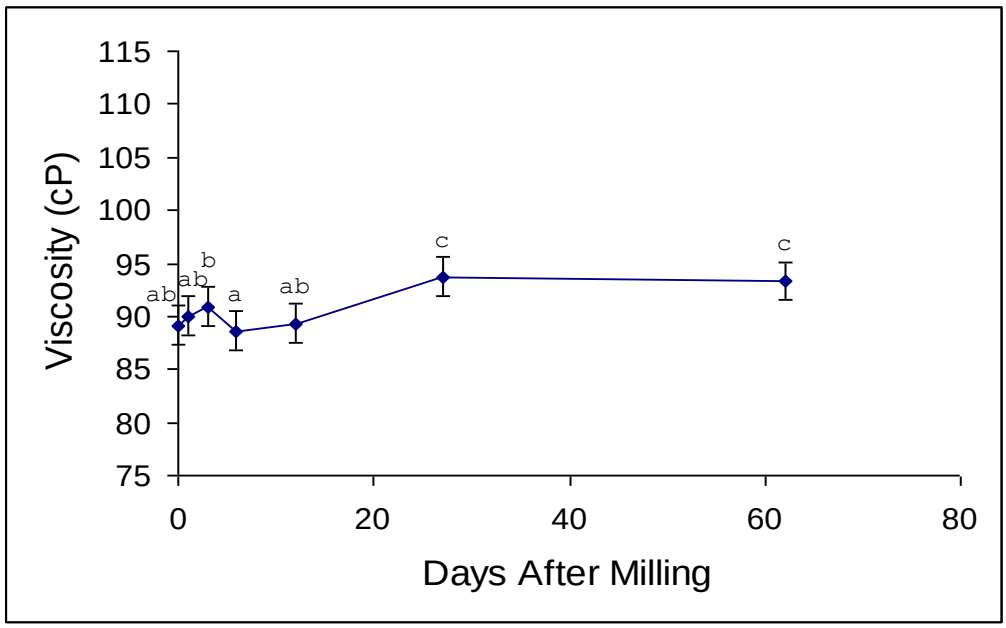

Figure 5.4: Baseline viscosity for the days after milling, summed across all varieties tested and weeks after harvest. Different letters indicate significantly different mean values for baseline viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Interactions

Even though the variety x WAH interaction was statistically significant, the contribution of the interaction to the overall ANOVA model was minor compared to the main effects as determined by the *F*-ratios. Therefore, the interaction was concluded to be of low practical significance.

Table 5.1 shows that the WAH x DAM interaction was also statistically significant. The contribution of the interaction to the overall ANOVA model was comparable to the main effect DAM as determined by the *F*-ratios. Therefore, it was concluded that the interaction was of some practical significance. The interaction plots (Figure 5.5) show a non-systematic change in baseline viscosity within the first 6 days of flour aging for all

WAH. There were also variations in slope that partly contributed to the significant WAH x DAM interaction term.

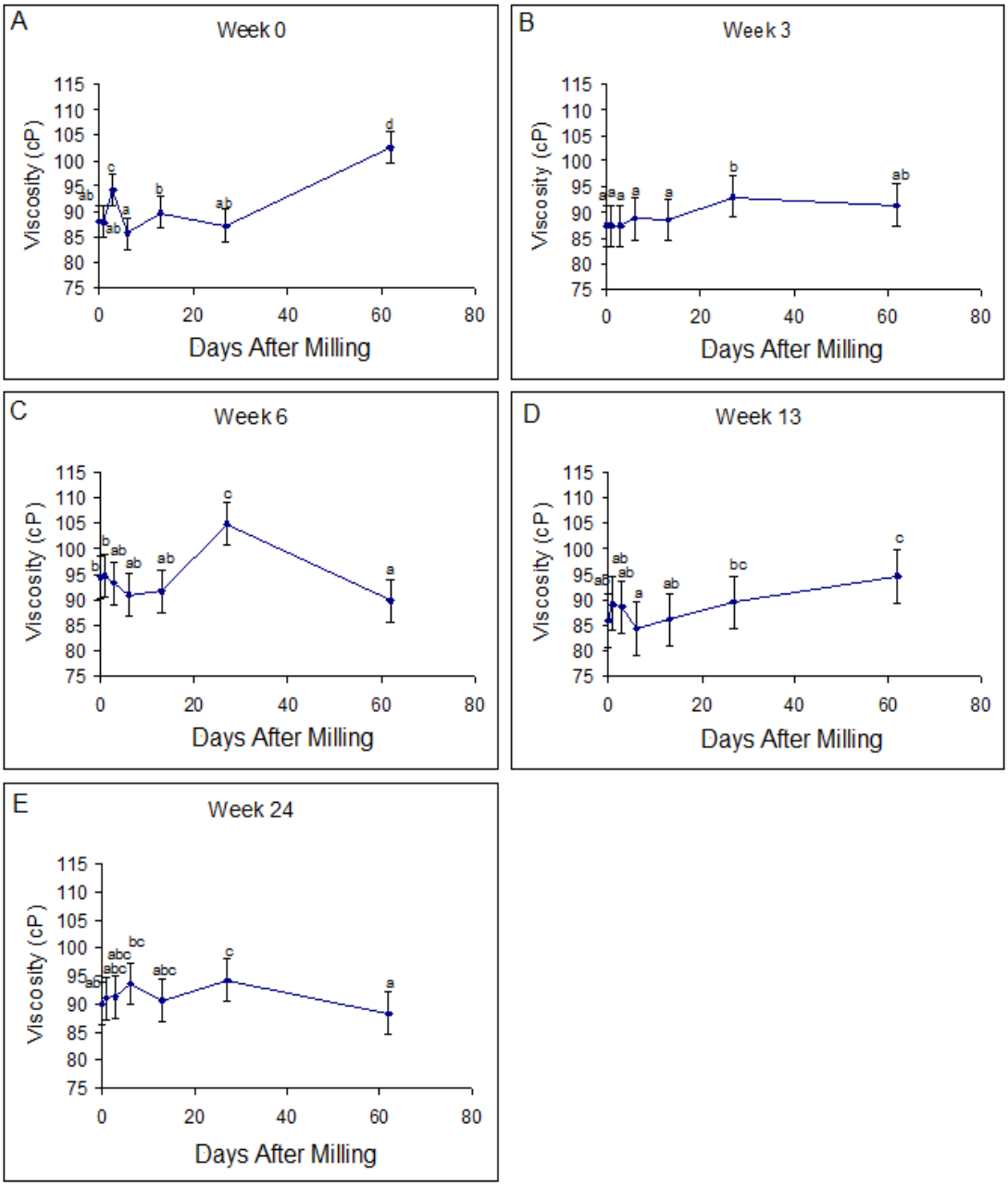

Figure 5.5: Baseline viscosity for the days after milling in all milling weeks, summed across all varieties tested. Different letters indicate significantly different mean values for baseline viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Changes in baseline viscosity appeared to be nonsystematic. For example, the increase in baseline viscosity at week 6 of grain storage (Figure 5.5 C) between days 13 and 27 of

flour aging appears quite large but is small in contrast to the differences in viscosity of up to 100s of cP for PPV when compared across varieties (Figure 5.6).

Peroxide peak viscosity (PPV)

ANOVA (Table 5.2) shows that variety, WAH, and DAM each had a significant effect on the PPV. At $p \leq 0.01$, there were also significant two-way interactions for variety x WAH, variety x DAM, and WAH x DAM, and significant three-way interactions for variety x WAH x DAM.

Main Effects	***F*-Ratio**	**P-Value**
Variety	13251	< .01
Weeks After Harvest	550.6	< .01
Days After Milling	55.24	< .01
Interactions		
Variety x Weeks After Harvest	93.38	< .01
Variety x Days After Milling	6.12	< .01
Weeks After Harvest x Days After Milling	5.59	< .01
Variety x Weeks After Harvest x Days After Milling	1.85	< .01

Table 5.2: 3 way ANOVA results for peroxide peak viscosity showing main effects, 2-way, and 3-way interaction terms.

Varieties

Figure 5.6 shows that among varieties, summed across WAH and DAM, Bobtail had the lowest PPV and Tubbs the highest. These data group the four varieties differently than both water SRC and RVA baseline viscosity (in which Skiles was grouped with Bobtail, and Goetze was grouped with Tubbs). Upon addition of hydrogen peroxide, Tubbs had the highest PPV (PPV). Goetze and Skiles had similar PPV, grouping them together. Bobtail saw little-to-no increase in viscosity upon addition of hydrogen peroxide. Because oxidative gelation involves cross linking of pentosans, it would be expected that PPV would group similarly to sucrose SRC data. This was not the case and therefore might indicate that OGC is a unique property that cannot be predicted by SRC alone. A modification of the SRC protocol to add hydrogen peroxide is a viable additional experiment that could be examined for its utility. Variety was the largest contributor to the overall ANOVA model as determined by the *F*-ratios.

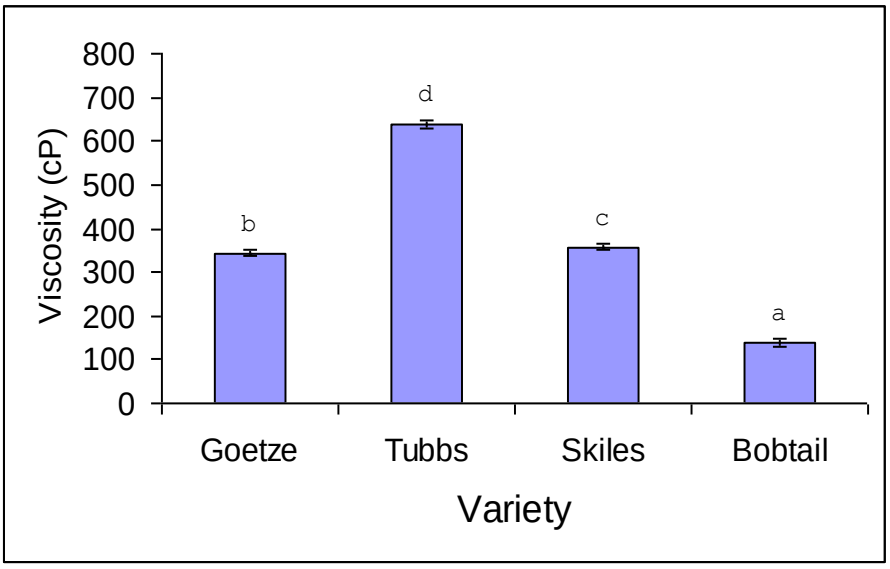

Figure 5.6: Peroxide peak viscosity for the varieties tested, summed across all weeks after harvest and days after milling. Different letters indicate significantly different mean values for peroxide peak viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 5.7 shows that, when summed across variety and DAM, there was an overall downward trend in PPV. Flour tested from grain stored 3 weeks had the highest PPV. This trend is in direct opposition to that found in the SRC data (i.e. there was a consistent decrease in sucrose and sodium carbonate SRC values (Figure 4.6 and 4.10, respectively) after 3 weeks of grain storage, followed by an increase through week 24). Not only that, but the magnitude of the decline from the maximum at 3 WAH, to the minimum at 24 WAH is of the same order as the differences in PPV between varieties (Figure 5.6). This suggests a change across WAH that is of practical significance. Despite decades of research into oxidative gelation, this is the first report of changes in OGC (in this case PPV) across time in the literature. Therefore, there is no frame of reference from which to gauge the practical relevance of the observed changes.

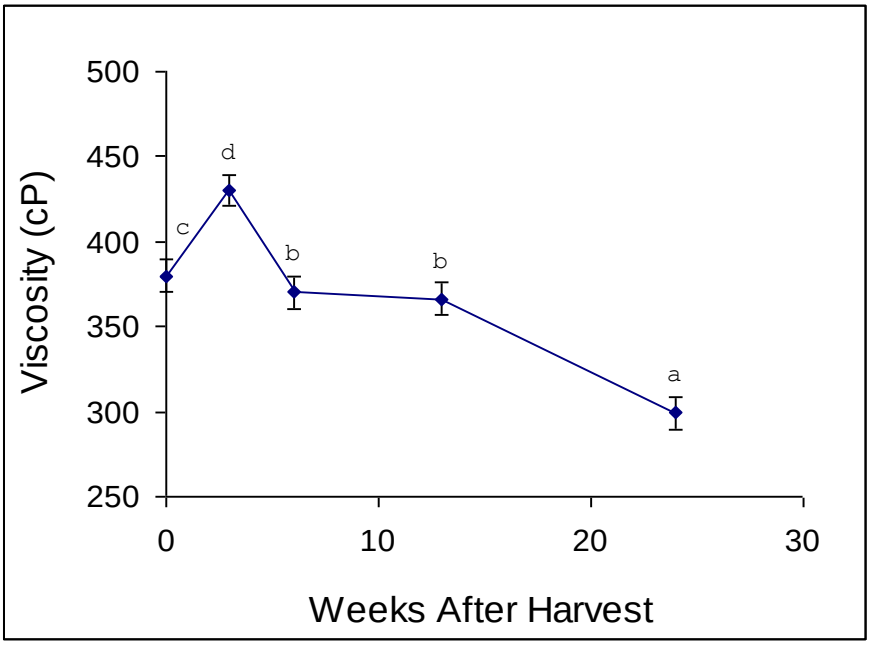

Figure 5.7: Peroxide peak viscosity for the weeks after harvest, summed across all varieties tested and days after milling. Different letters indicate significantly different mean values for peroxide peak viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Days After Milling

Figure 5.8 shows that, summed across variety and WAH, there was an overall upward trend in PPV as flour aged. There were no significant differences in PPV between 0 and 13 DAM. The viscosity significantly increased each testing day thereafter to day 62. This trend was similar to that found in the sucrose SRC data (Figure 4.7).

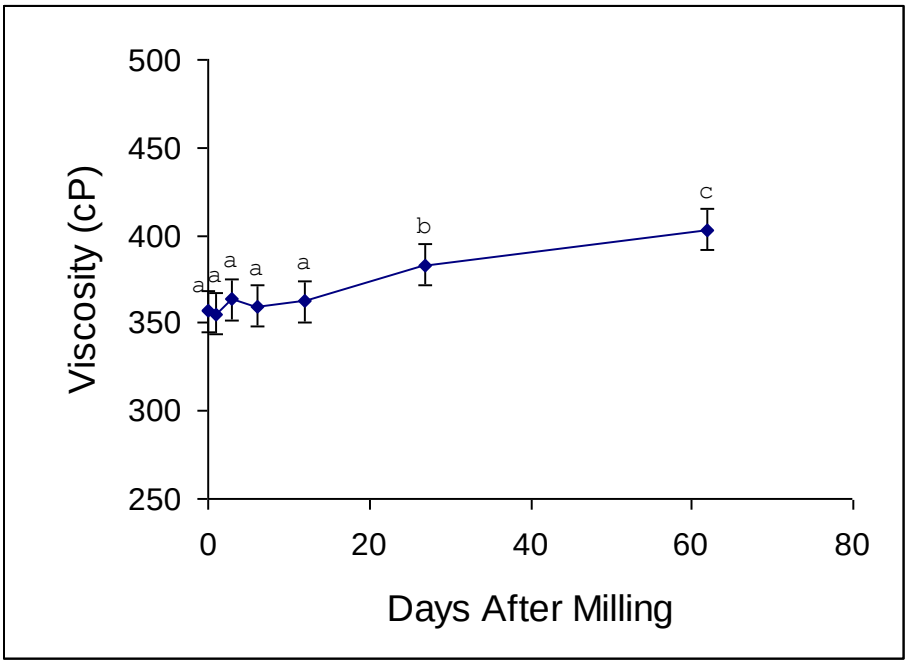

Figure 5.8: Peroxide peak viscosity for the days after milling, summed across all varieties tested and weeks after harvest. Different letters indicate significantly different mean values for peroxide peak viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Interactions

Even though the variety x DAM, WAH x DAM, and variety x WAH x DAM interactions were statistically significant, the contributions of the interactions to the overall ANOVA model were minor compared to the main effects as determined by the *F*-ratios. Therefore, the interactions were concluded to be of low practical significance.

Table 5.2 shows that the variety x WAH interaction was statistically significant. The contribution of the interaction to the overall ANOVA model was greater to the main effect DAM as determined by the *F*-ratios. Therefore, the interaction was concluded to be practically significant. The interaction plots (Figure 5.9) shows an overall downward trend in PPV in flour tested from 0 to 24 weeks of grain storage among all varieties. However, between 6 and 13 WAH, Goetze (Figure 5.9 A) increased in PPV while the other varieties decreased in viscosity. There were also variations in slope that helped account for the significant variety x WAH interaction term.

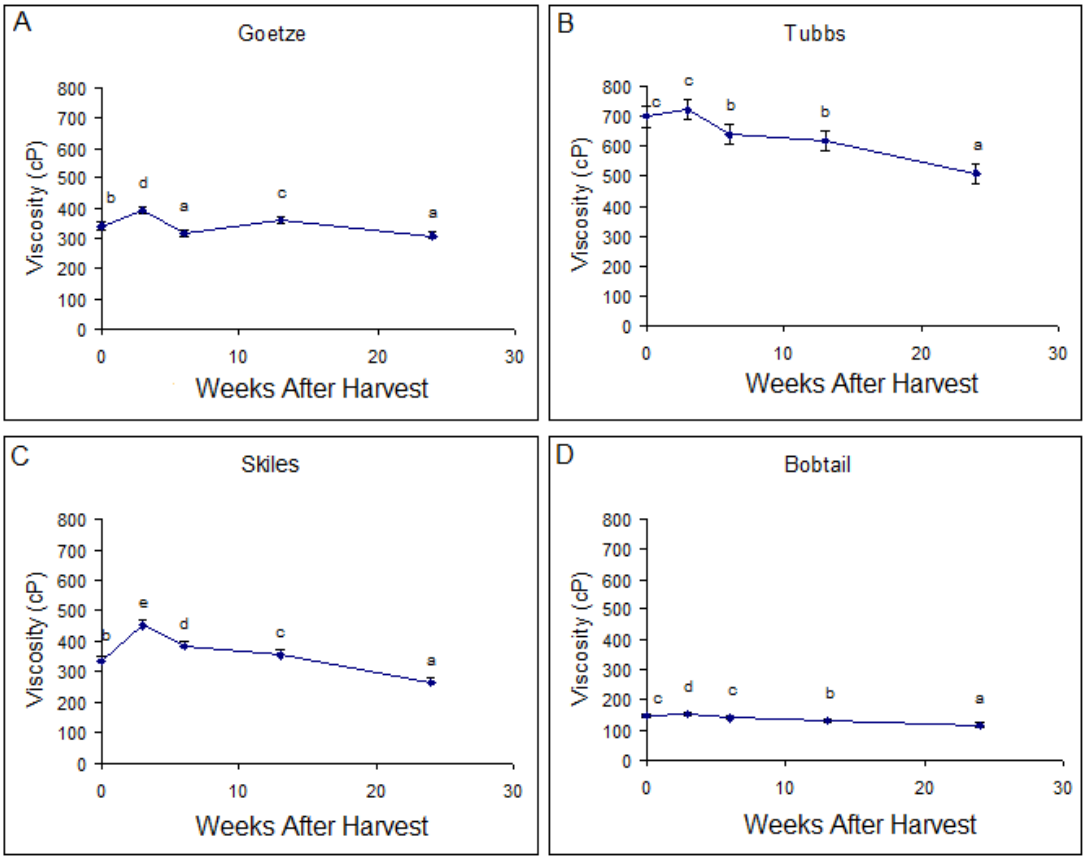

Figure 5.9: Peroxide peak viscosity for all varieties within each milling week, summed across all days after milling. Different letters indicate significantly different mean values for peroxide peak viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Figure 5.10 shows the variety x WAH interaction plot for PPV as normalized data (i.e. Week 0, Day 0 = 100% for each variety). Figure 5.10 shows a similarity in trend between varieties as determined by slopes but there were sufficient slope differences and some cross-overs that account for the statistically significant interaction term. Nonetheless, for example, the proportional changes for Tubbs and Bobtail (the high and low reactive varieties, respectively) were almost identical. Normalizing the data gave insight to the mechanism of oxidative gelation. The contribution of variety to the overall ANOVA model (non-normalized vs normalized) was substantially reduced (F = 13251.65 vs F = 798.37), indicating fewer varietal differences. This suggests that OGC changes *proportionally* as a function of grain storage time in a similar fashion across varieties. The one exception was Skiles. Here the pattern of change across time (WAH) was similar

to the pattern exhibited by the other varieties, but the magnitude of the proportional change was greater. The data do not allow speculation regarding the mechanism.

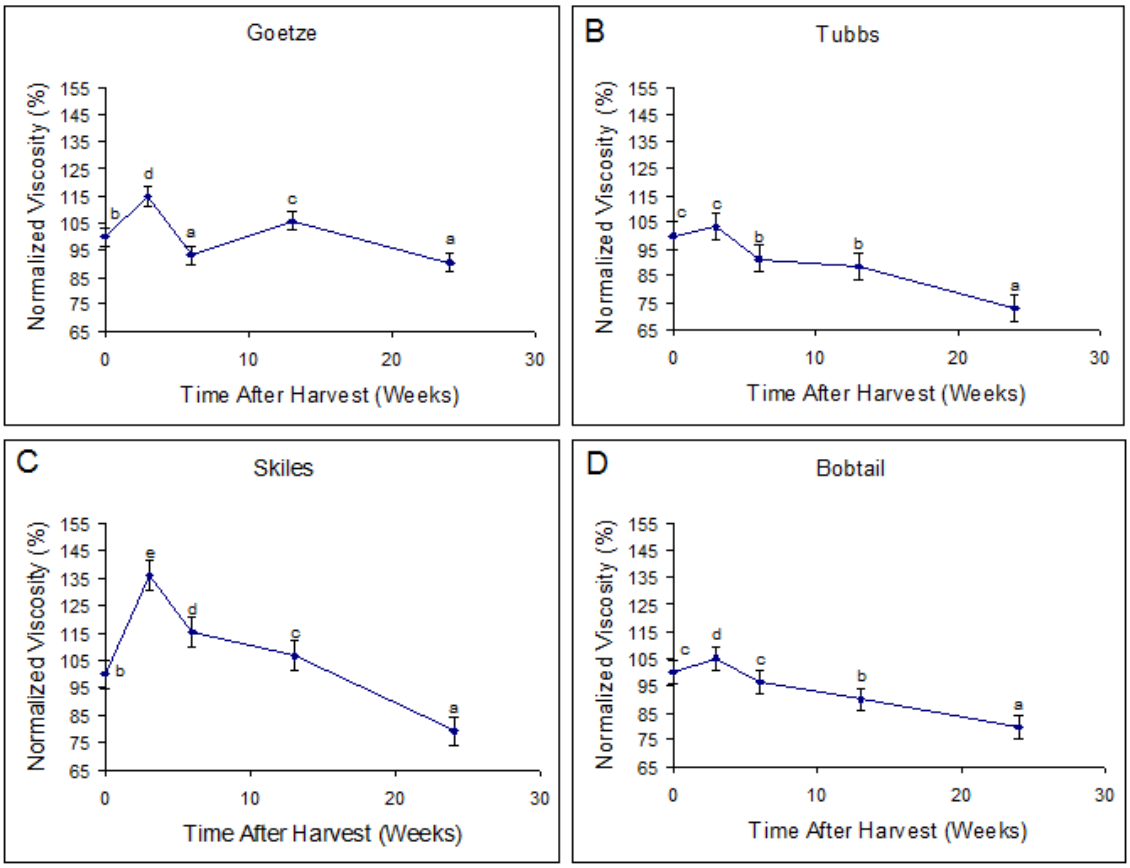

Figure 5.10: Normalized peroxide peak viscosity for all varieties within each milling week, summed across all days after milling. Different letters indicate significantly different mean values for peroxide peak viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Reactivity to peroxide

Figure 5.11 shows the linear regression analysis of PPV vs. reactivity to peroxide (PPV – baseline viscosity). The strong correlation ($R = 0.99$, $p \leq 0.01$) justifies reporting only one of the datasets. PPV was chosen as it is a direct measurement and not derivative of a calculation between to data points and was reported above.

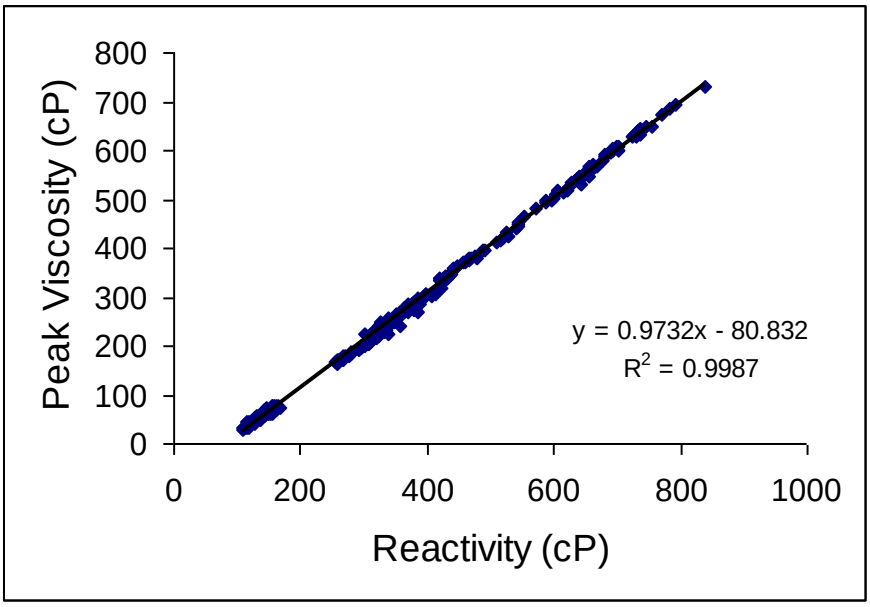

Figure 5.11: Linear regression analysis of peroxide peak viscosity versus reactivity to peroxide.

Viscosity change over time of mixing (PPBV%)

ANOVA (Table 5.3) shows that variety, WAH, and DAM each had a significant effect on the PPBV% (a measure of the thixotropic behavior of the oxidized gel measured as the loss of viscosity over 300 seconds of mixing at 160 rpm expressed as a percentage of PPV: Figure 5.1). At $p \leq 0.01$, there were also significant two-way interactions for variety x WAH, variety x DAM, and WAH x DAM, and a significant three-way interaction for variety x WAH x DAM.

Main Effects	***F*-Ratio**	**P-Value**
Variety	16324.07	< .01
Weeks After Harvest	969.25	< .01
Days After Milling	14.36	< .01
Interactions		
Variety x Weeks After Harvest	232.66	< .01
Variety x Days After Milling	3.48	< .01
Weeks After Harvest x Days After Milling	4.1	< .01
Variety x Weeks After Harvest x Days After Milling	2.06	< .01

Table 5.3: 3 way ANOVA results for proportional peroxide peak breakdown viscosity showing main effects, 2-way, and 3-way interaction terms.

Varieties

Figure 5.12 shows that among varieties, summed across WAH and DAM, Bobtail had the lowest PPBV% and Tubbs had the highest. This grouping was similar to that of PPV. Variety was the strongest contributor to the overall ANOVA model as determined by the *F*-ratios.

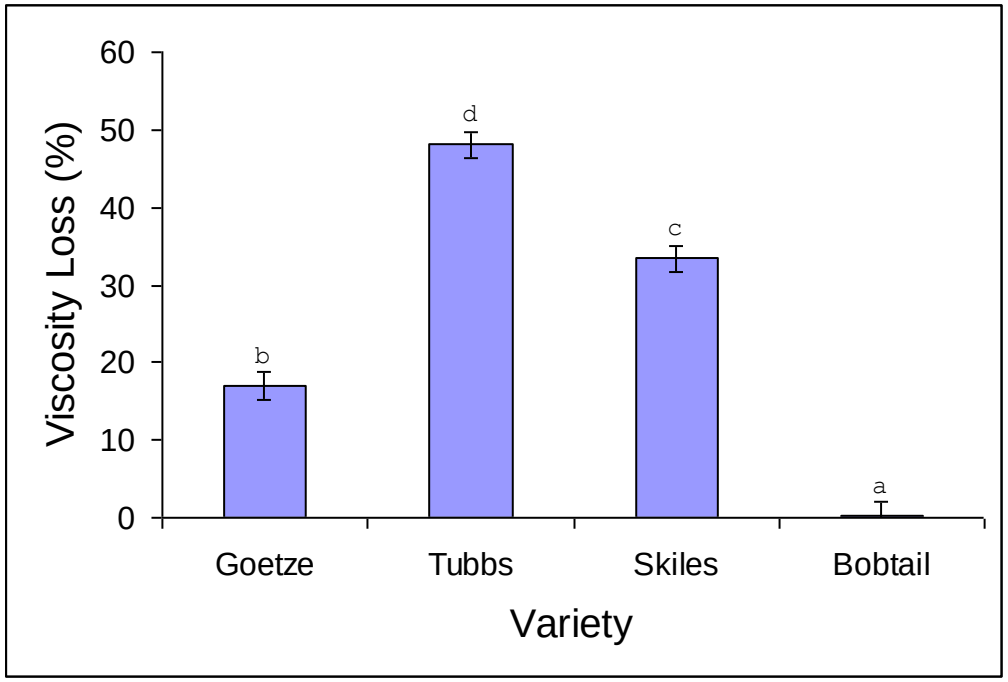

Figure 5.12: Proportional peroxide peak breakdown viscosity for the varieties tested, summed across all weeks after harvest and days after milling. Different letters indicate significantly different mean values for proportional peroxide peak breakdown viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 5.13 shows that, summed across variety and DAM, there was an increase in peroxide peak breakdown from week 0 to week 3, followed by a downward trend to week 24. This trend was similar to that shown by PPV across WAH (Figure 5.7).

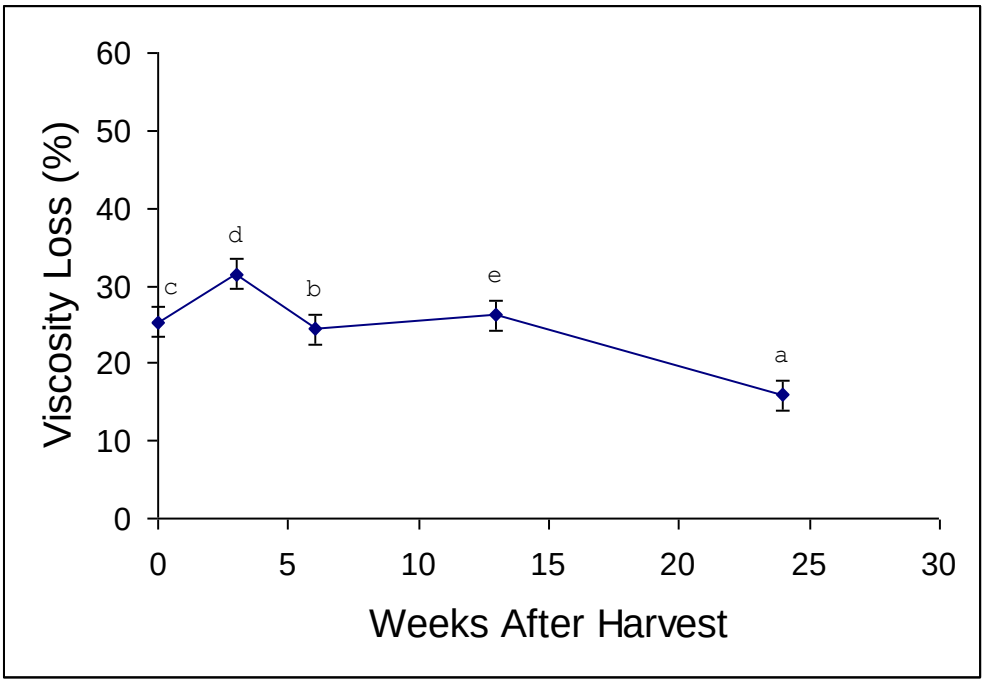

Figure 5.13: Proportional peroxide peak breakdown viscosity for the weeks after harvest, summed across all varieties tested and days after milling. Different letters indicate significantly different mean values for proportional peroxide peak breakdown viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Days After Milling

Figure 5.14 shows that, summed across variety and WAH, peroxide peak breakdown did not significantly change until the flour had been aged for 62 days at which juncture a significant increase was observed. Although this change was statistically significant, the contribution of the effect to the overall ANOVA model was minor compared to the other main effects as determined by the *F*-ratios. This trend was again similar to that shown by PPV across DAM (Figure 5.8).

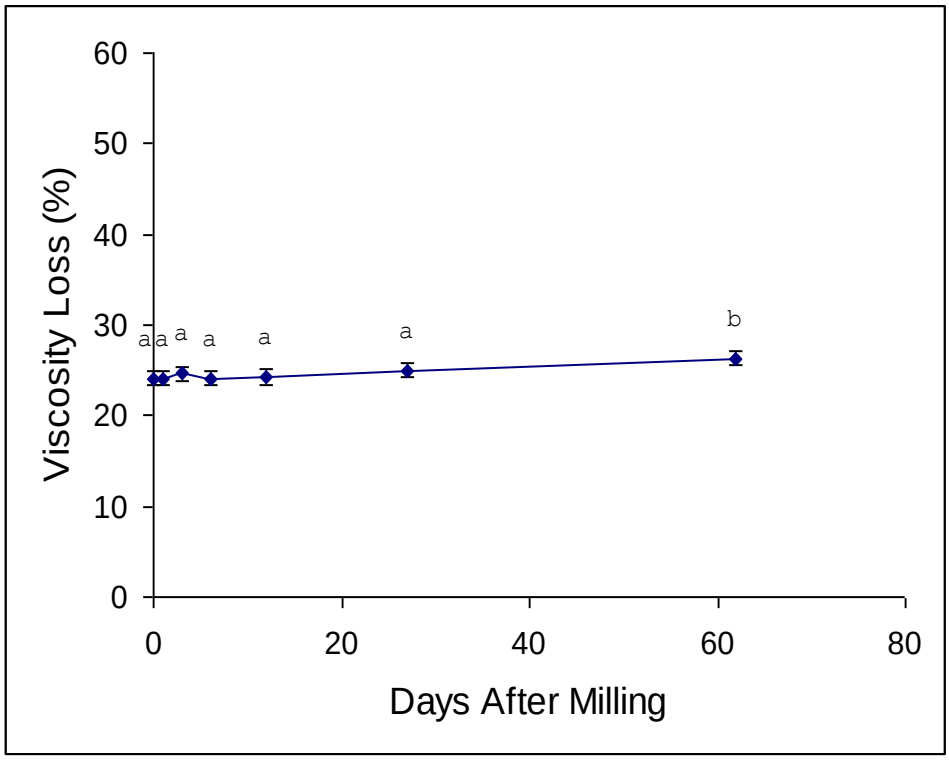

Figure 5.14: Proportional peroxide peak breakdown viscosity for the days after milling, summed across all varieties tested and weeks after harvest. Different letters indicate significantly different mean values for proportional peroxide peak breakdown viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Interactions

Even though the variety x DAM, WAH x DAM, and variety x WAH x DAM interactions were statistically significant, the contribution of the interactions to the overall ANOVA model was minor compared to the main effects as determined by the *F*-ratios. Therefore, the interactions were concluded to be of low practical significance, and are not discussed further.

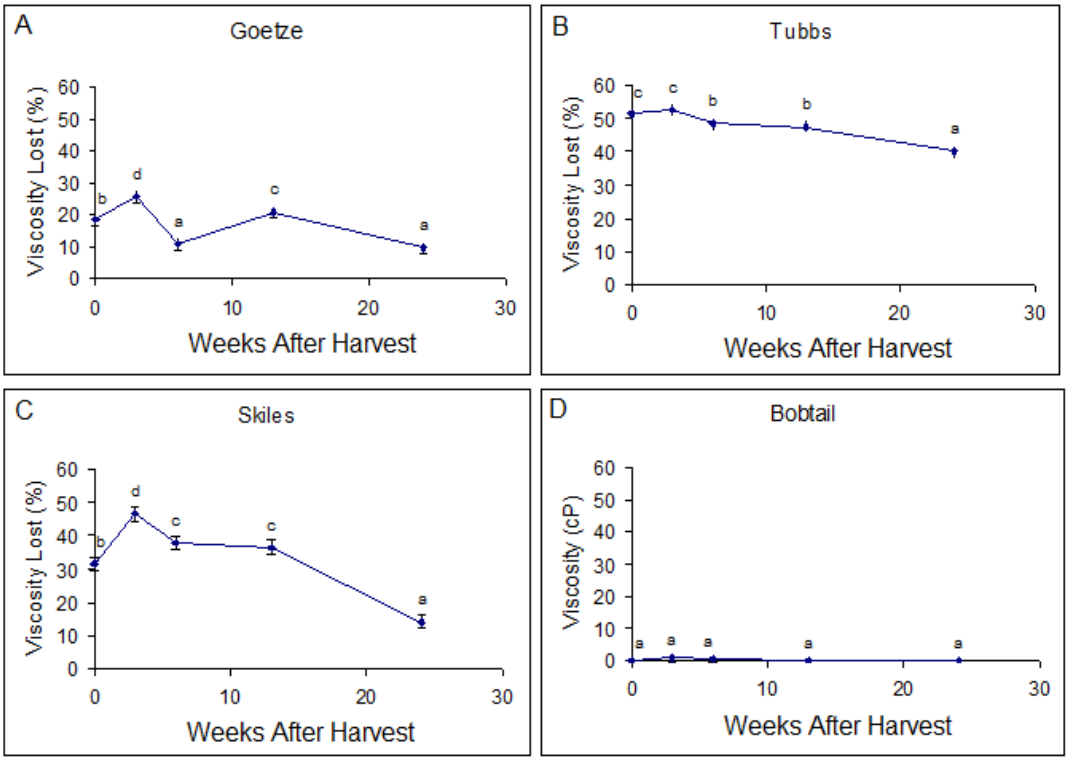

Figure 5.15: Proportional peroxide peak breakdown viscosity for each milling week of all varieties, summed across all days after milling. Different letters indicate significantly different mean values for proportional peroxide peak breakdown viscosity. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Relationships between PPV and PPBV

The close concordance of PPV and PPBV across time (WAM and DAH) suggested that they were highly correlated, in a similar fashion to the correlation observed for the relationship between PPV and reactivity to peroxide (Figure 5.11). Plotting PPV against the PPBV showed this not to be the case. The correlation between PPV and reactivity to peroxide (Figure 5.11) shows the scatter of data points tightly grouped around a single significant regression line. Clearly Bobtail is shown as a cluster at the lower left of the regression but it aligns tightly to a single line of best fit. The PPV and PPBV scatterplot (Figure 5.16) showed obvious clustering of groups of data points away from a common line of best fit. Further examination showed that the clusters were each associated with one of the varieties used in the study (Figure 5.17). Linear correlations between PPV and absolute PPBV were numerically strong and highly significant within each varietal cluster (Figure 5.17). The exception was the non-reactive variety, Bobtail, which showed

no relationship between PPV and PPBV. The slopes and intercepts of the regressions were also significantly different ($p > 0.01$), even though it is not evident visually, indicating a qualitative difference in gelation between varieties.

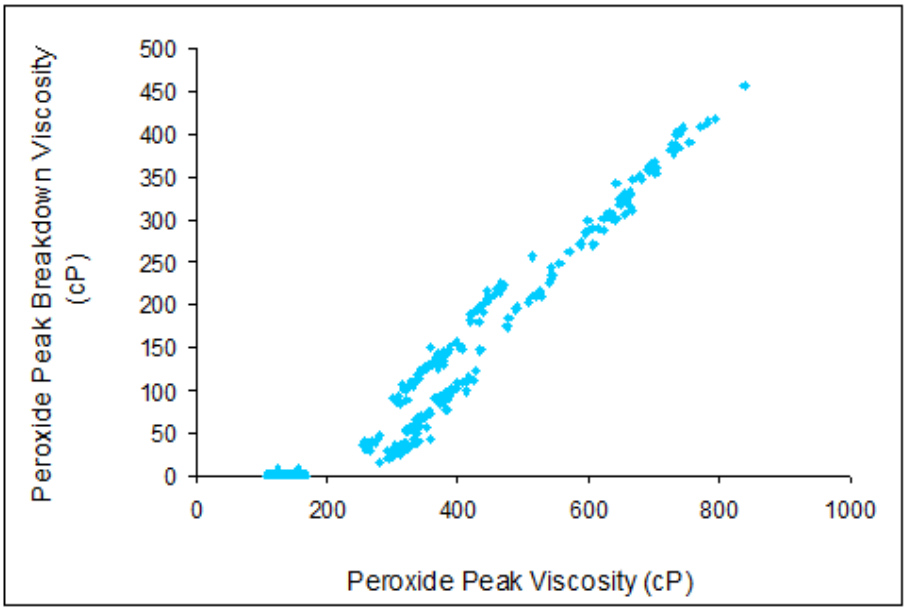

Figure 5.16: Absolute peroxide peak breakdown viscosity (PPBV) versus peroxide peak viscosity (PPV) for all varieties tested.

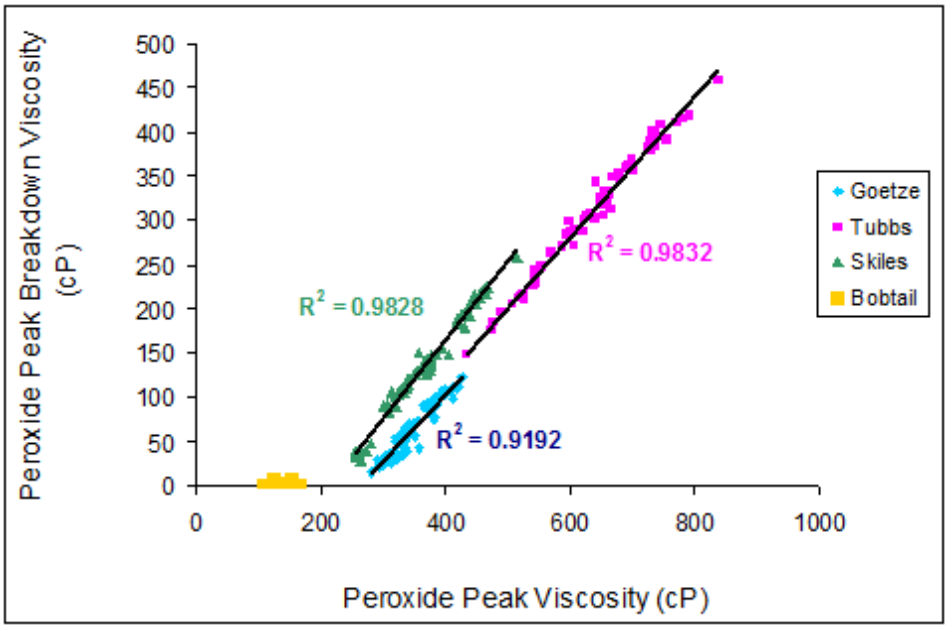

Figure 5.17: Absolute peroxide peak breakdown viscosity (PPBV) versus peroxide peak viscosity (PPV) with varietal clusters identified and linear regressions and coefficients of determination indicated.

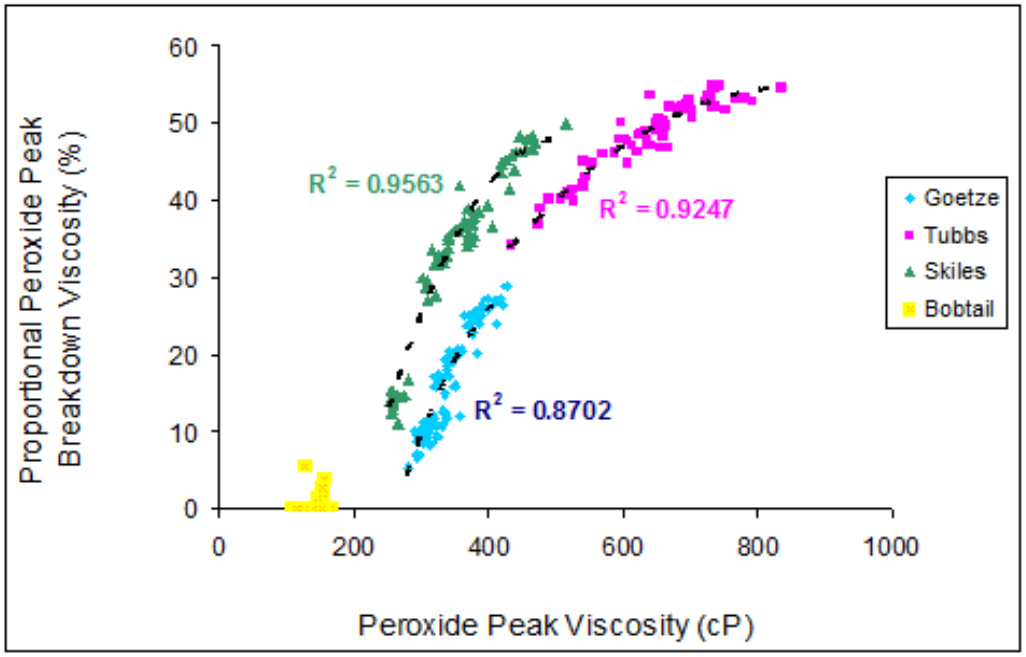

Figure 5.18 Proportional peroxide peak breakdown viscosity (PPBV%) versus peroxide peak viscosity (PPV) with varietal clusters identified and 2nd order polynomial regressions and coefficients of determination indicated.

Examination of Figure 5.18, which plots *relative* PPBV (PPBV%) against PPV shows an even more intriguing picture. Firstly the clustering remains evident. Secondly, within each variety PPBV% increased with increasing PPV. Thirdly, there appears to be a leveling-off (asymptotic response) of the relative increase in PPBV as absolute PPV increased **within each varietal cluster** as it reaches its maximum PPV. Once again the exception was the non-reactive variety Bobtail. Across varieties the relationship "increased PPBV% with increasing PPV" did not hold. For example, at PPV of ~ 450 cP, each variety had a different PPBV%: 48% for Skiles, 28% for Goetze, and 40% for Tubbs. This suggests substantial *qualitative* differences in the nature of the gels between varieties that are consistent across aging of both the grain and the flour. This data does not allow speculation regarding the nature of the potential qualitative differences but does suggest avenues of investigation (AX content, arabinose substitution ratio, or degree of ferulic acid esterification) that might be investigated.

An important observation was that for PPV against PPBV% (Figure 5.18), "r" values for the 2nd order polynomial fits were numerically greater than the "r" values for the equally significant linear fits (polynomial: 0.93, 0.96, 0.98 and linear: 0.92, 0.94, 0.95 for Goetze, Tubbs, and Skiles, respectively). Evidence of an asymptotic relationship between PPBV% and PPV indicates that there is a residual gel structure that survives shearing, and that the persistence of the gel differs between the varieties examined here. Further investigation of this trait may finally shed some light on the early observation of Baker et al. (1943) that oxidative flour-gels were susceptible to shear breakdown. The observation of residual viscosity after peroxide oxidation and shearing, which was larger than the water-only baseline viscosity, also lends credence to the assertions of Ross et al. (2014) that 1) some functional element of the oxidative gel persists after extensive shearing and 2) that the RVA OGC method can provide additional information about the rheological nature of the oxidative gel not available in the Bostwick Consistometer method (Bettge and Morris 2007).

MDA Concentration

MDA concentration was measured in an attempt to determine if there was significant oxidation of flour lipids that accompanied changes in OGC across time. ANOVA (Table 5.4) shows that variety, WAH, and DAM each had a significant effect on MDA concentration. At $p \leq 0.01$, there were also significant two-way interactions for variety x WAH, variety x DAM, WAH x DAM, and significant three-way interactions for variety x WAH x DAM. Notably, compared to most other analyses, a time parameter (WAH) was the largest main effect contributor to the ANOVA, not variety (Tables 4.3 to 4.6 and 5.1 to 5.3).

Main Effects	***F*-Ratio**	**P-Value**
Variety	16.17	< .01
Weeks After Harvest	74.79	< .01
Days After Milling	27.41	< .01
Interactions		
Variety x Weeks After Harvest	12.45	< .01
Variety x Days After Milling	6.84	< .01
Weeks After Harvest x Days After Milling	50.63	< .01
Variety x Weeks After Harvest x Days After Milling	6.78	< .01

Table 5.4: 3 way ANOVA results for total peroxidized lipids (as MDA) showing main effects, 2-way, and 3-way interaction terms.

Varieties

Figure 5.19 shows that among varieties, summed across WAH and DAM, Bobtail had the lowest MDA concentration and Tubbs had the highest. However, MDA concentration was not significantly different between Goetze, Skiles and Bobtail. This grouping was similar to the absolute PPV, Tubbs highest, Bobtail lowest (Figure 5.6) but did not reflect the similarity in proportional changes in OGC (PPV) for Bobtail and Tubbs (Figure 5.10), and also did not reflect the higher proportional change in OGC for Skiles (also Figure 5.9).

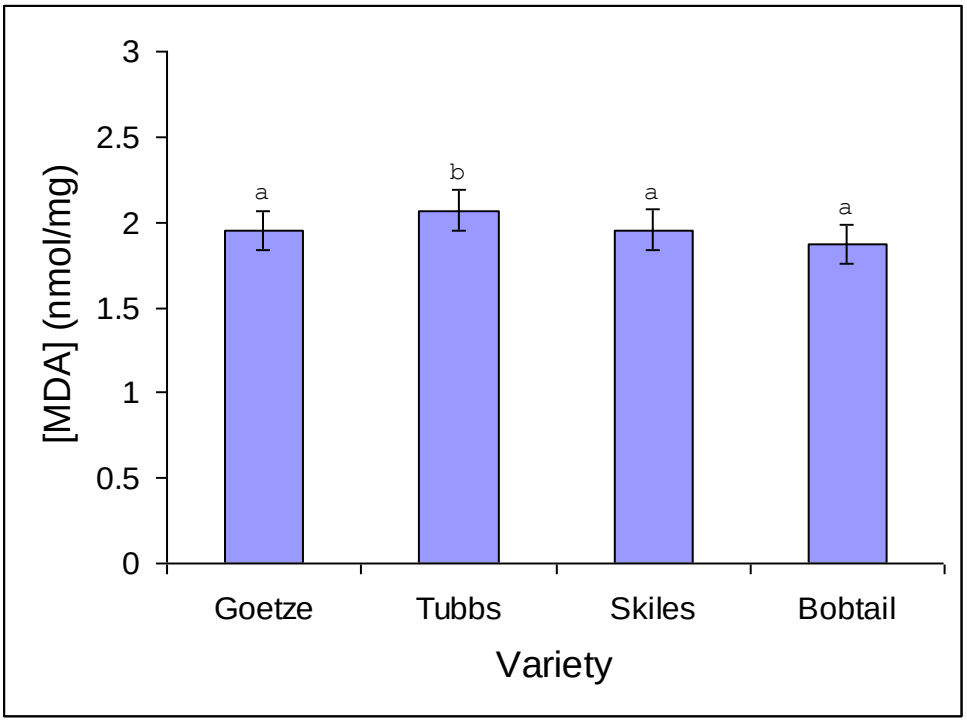

Figure 5.19: Malondialdehyde (MDA) concentration for the varieties tested, summed across all weeks after harvest and days after milling. Different letters indicate significantly different mean values for MDA concentration. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Weeks After Harvest

Figure 5.20 shows that, when summed across variety and DAM, there was a general decline in MDA concentration from 0 to 24 WAH, which resembled the trends seen in PPV and PPBV% (Figures 5.7, and 5.13). This effect was the strongest contributor to the overall ANOVA model as determined by the *F*-ratios. The result is also contrary to the expected trend of increased indicators of oxidative changes across time as reported by Reichenauer and Goodman (2003).

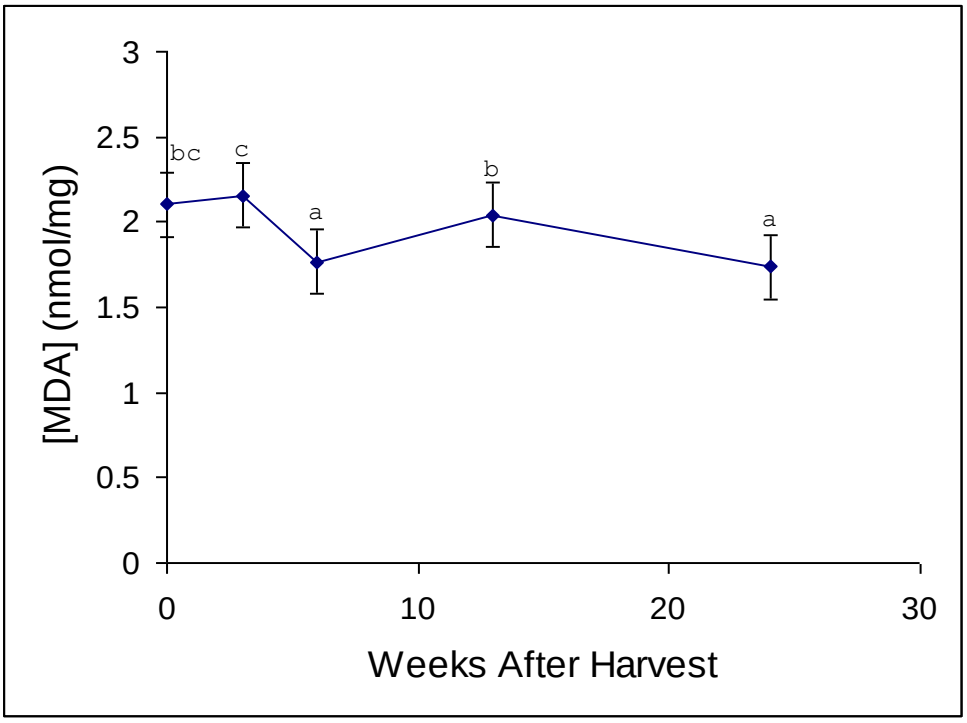

Figure 5.20: Malondialdehyde (MDA) concentration for the weeks after harvest, summed across all varieties tested and days after milling. Different letters indicate significantly different mean values for MDA concentration. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Days After Milling

Figure 5.21 shows that, summed across variety and WAH, there was an initial increase in MDA concentration from day 0 to day 3. MDA concentration then declined significantly to day 6 and remained unchanged until day 62. The day 62 value was significantly higher than the day 0. The trend did not follow the observed increase in PPV across DAM (Figure 5.8). The initial increase in MDA suggests oxidation. However, the subsequent decline may indicate the consumption of oxidize lipids in downstream redox reactions.

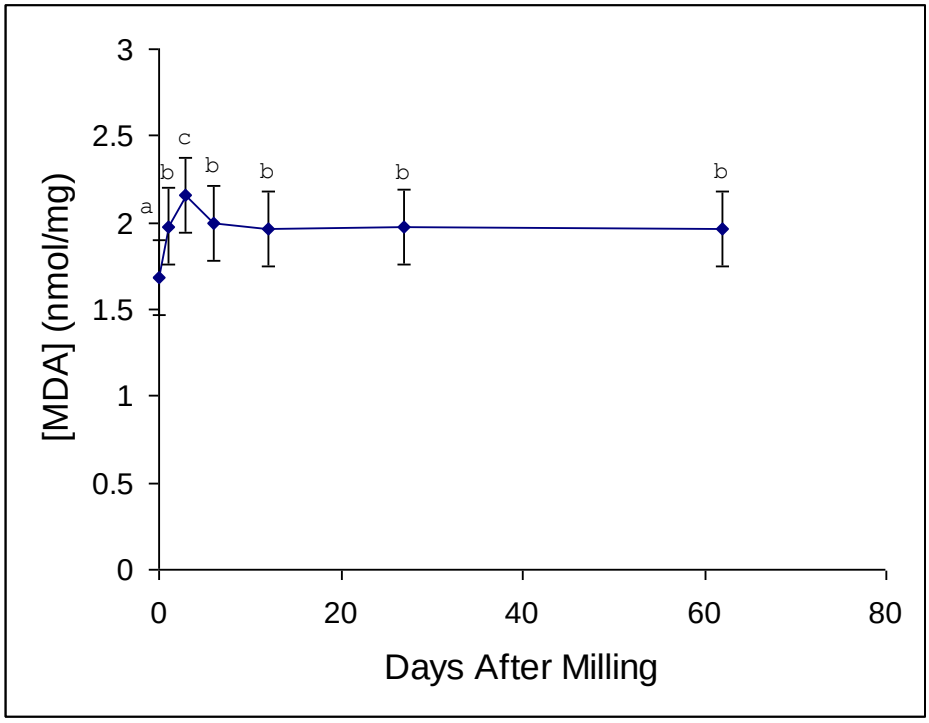

Figure 5.21: Malondialdehyde (MDA) concentration for the days after milling, summed across all varieties tested and weeks after harvest. Different letters indicate significantly different mean values for MDA concentration. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Interactions

Of the interaction terms it is notable that the interaction term with the highest *F*-ratio was the term with both time effects (Table 5.4). The magnitude of this interaction was comparable to the strongest main effect, WAH, also a time parameter and not variety.

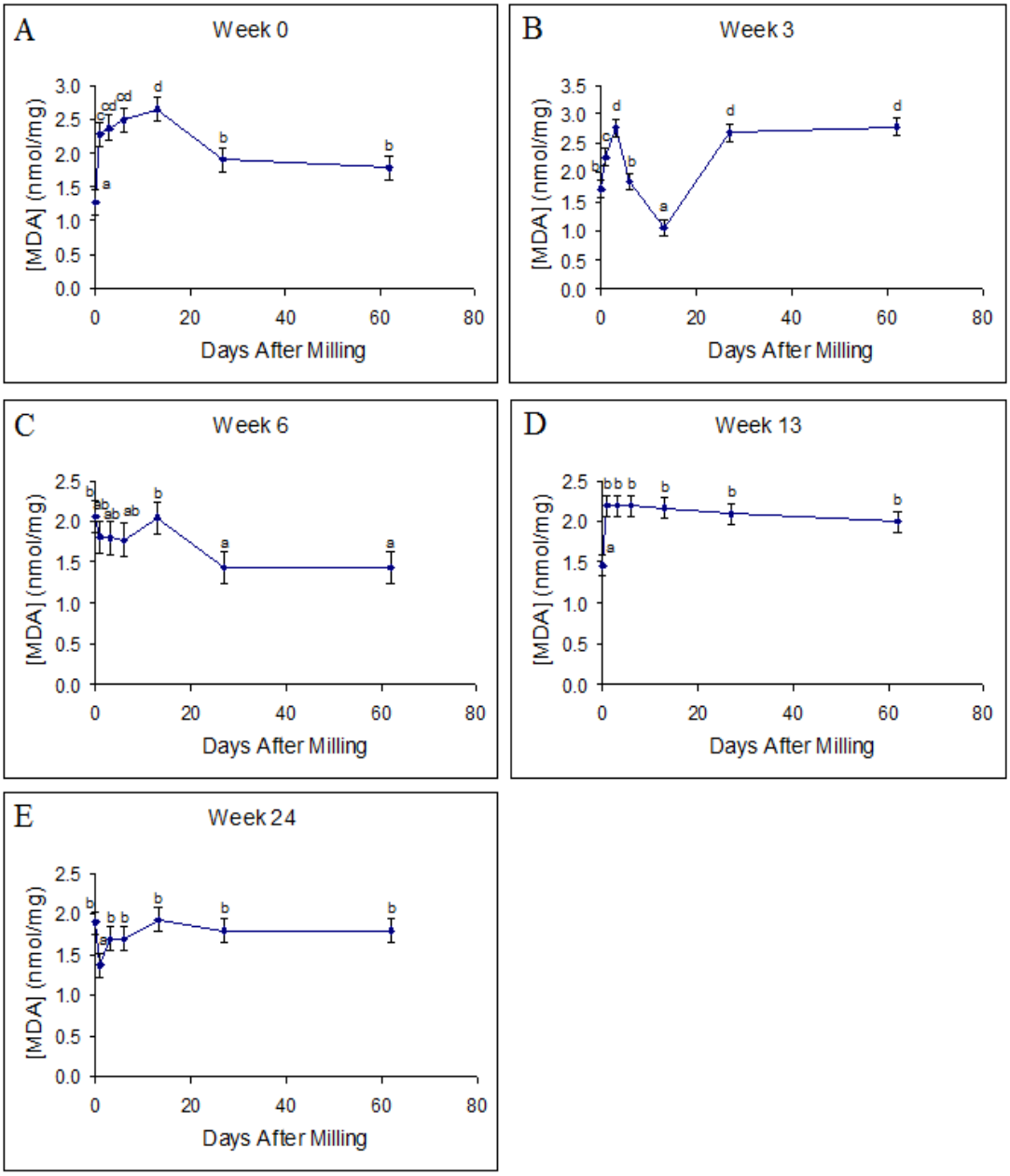

Figure 5.22: Malondialdehyde (MDA) concentration for the days after milling in all milling weeks, summed across all varieties tested. Different letters indicate significantly different mean values for MDA concentration. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Due to time restrictions, the flour samples analyzed for MDA were stored for up to 5 months at -20 °C before peroxidized lipid analysis was completed. It is possible that -20 °C was not cold enough to halt chemical activity in the flour. Ideally, the flour would have been tested on the same day that SRC and OGC tests were conducted.

Further considerations

There are numerous changes that can occur during wheat grain storage that could potentially influence OGC. Reichenauer and Goodman (2003) observed an increase in MDA across flour aging. However, we only observed an increase over the early stages of flour aging followed by a decrease, as discussed above. Elements of MDA reactivity that may be relevant were not studied. For example, MDA can cross-link two lysine residues (Chio and Tappel 1969), which could also affect OGC. There are other lipid peroxidation products (other aldehydes) that were not analyzed in this thesis, such as 4-hydroxynonenal that could have also accumulated as a function of storage and aging time (Sochor et al. 2012).

Apart from lipid oxidation products, it has been shown that early and late Maillard products (e.g. Amadori compounds) can accumulate in wheat grain during storage, although the two might be related. A positive correlation between lipid peroxidation and Maillard product accumulation was observed by (Strelec et al. 2008). Generation of reactive species resulting from the Maillard reaction may influence changes in OGC across time. Therefore Maillard products are viable candidates for further studies of changes in flour functionality over time.

Were changes in OGC over time of functional significance? Although the OGC phenomenon has been known for almost a century, the effects of variety, grain storage time, and flour aging have not been extensively studied with respect to OGC. Likewise, the place of OGC in manufacturing in cereal-based foods is not well understood. It potentially plays a role in all batter-based systems (e.g. cake batters, pancake batters). It may play an even greater role in fried batter systems due to the high degree of lipid oxidation that occurs as the oil degrades over long periods of use. Varieties with high OGC, like Tubbs, might then theoretically be better suited for fried batter applications. The stronger, most extensive gelation could be a clean-label method for reducing oil absorption into the fried product.

CHAPTER 6: RESULTS AND DISCUSSION: SURVEY AND DOSE RESPONSE STUDY OF HARD WHITE WINTER WHEAT

The aim of the study reported in this chapter was to confirm that OGC was a variety-dependent phenomenon in hard wheats, and to observe if OGC in hard wheats changed as a result of flour aging. An additional aim was to observe the dose-response of one high and one low OGC variety to 2 oxidants, hydrogen peroxide and the common bread-making oxidant ADA.

Hard-wheat kernel characteristics and the effect of aging on oxidative gelation capacity: a survey

Figure 6.1 shows the average OGC in fresh and aged flour for all hard-wheat varieties tested. The majority of varieties tested had PPVs above 500 cP. There were also varieties that did not undergo oxidative gelation upon addition of hydrogen peroxide. Similarities were found between this data and that reported by Ross et al (2014). Ross et al. reported that half of the hard wheat varieties tested had PPVs higher than 500 cP. They also showed the presence of non-reactive varieties. However, the range of PPVs found by Ross et al. was smaller than that found in our study (~230 to 725 cP vs 130 to 2804 cP in Ross et al. and our study, respectively. As observed in the soft-wheat aging study, variety heavily influences OGC. Therefore, differences in results in the two studies likely derived from differences in the spectrums of varieties tested.

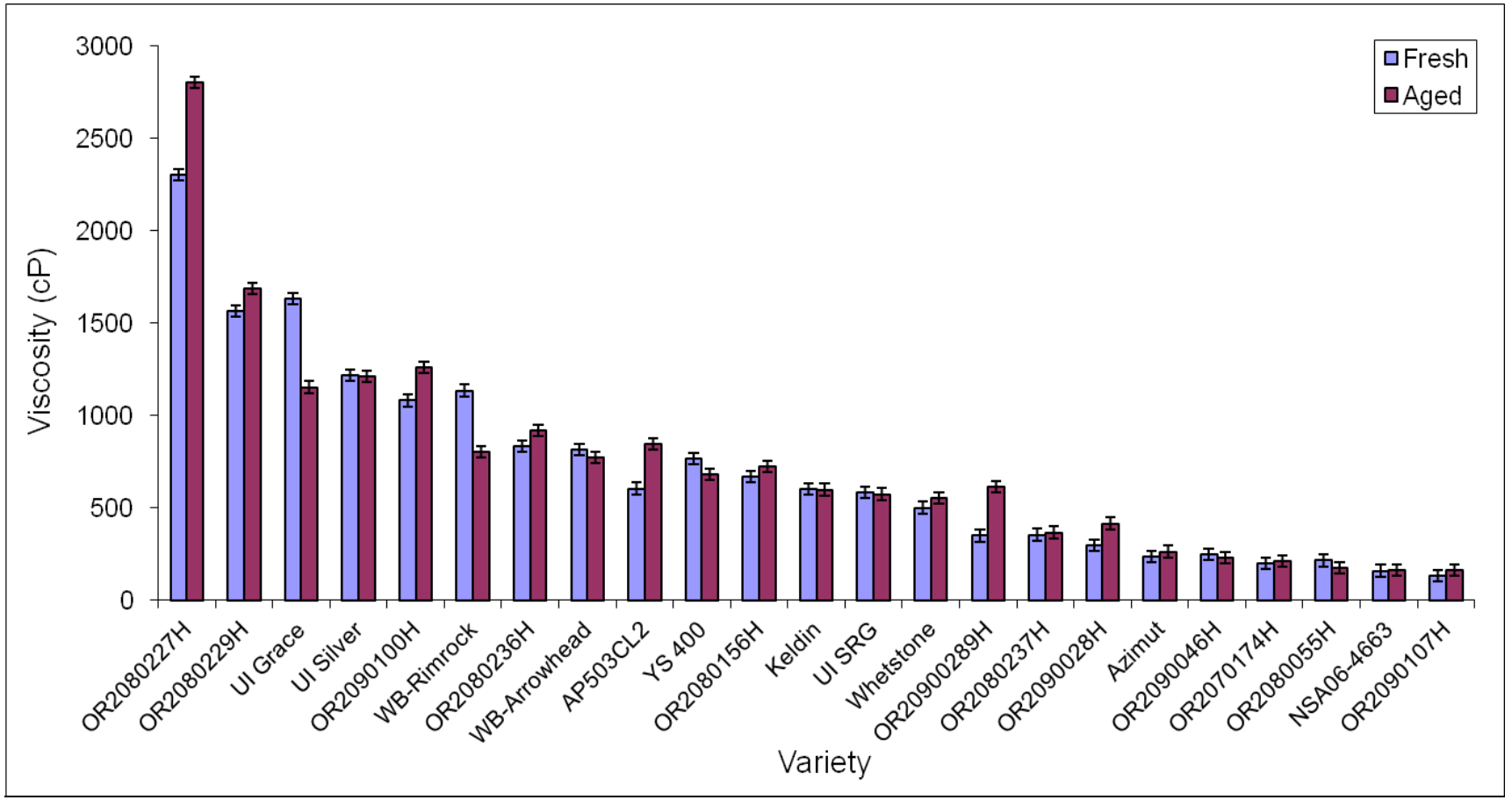

Figure 6.1: Average peroxide peak viscosities for all hard wheats surveyed in fresh and aged flour. "Fresh" flour is defined to be the day after milling. "Aged" flour is defined to be 14 days after milling. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Response of oxidative gelation in OR2080227H and OR2090107H to dosage of Hydrogen Peroxide

The two varieties that were chosen to conduct the dose-response study of hydrogen peroxide and ADA on OGC were OR2080227H and OR2090107H. These varieties had the strongest and weakest responses to peroxide respectively in the hard-wheat survey. Table 6.1 shows hardness, moisture, protein , BFY and total flour yield data for the two wheat samples.

Variety	Hardness (Hardness Index)*		Moisture Content (%)*		Protein Content (%)**	Break Flour Yield (%)	Total Flour Yield (%)
	Mean	SD	Mean	SD	Mean	Mean	Mean
OR2080227H	81.9	16.1	8.4	0.75	15.5	32.9	64.6
OR2090107H	31.2	20.8	8.8	0.71	16.0	50.2	64.2

Table 6.1: SKCS hardness index and moisture content, protein content, break flour and total flour yields of OR2080227H and OR2090107H. *Standard deviation of 200 individual kernels. **Measured in singlet: repeatability of NIR protein ± 0.5% (Osborne and Fearn 1983).

ANOVA (Table 6.2) shows that variety and dose of peroxide each had a significant effect on OGC. Variety, however, had a much larger influence on peak viscosity. This result was expected, as the two varieties were chosen based on their extreme differences in OGC. At $p \leq 0.01$, there were also significant two-way interactions for variety x dose of peroxide.

Main Effects	***F*-Ratio**	**P-Value**
Variety	21534.56	< .01
Dose of Peroxide	778.49	< .01
Interactions		
Variety x Dose of Peroxide	696.01	< .01

Table 6.2: ANOVA results for response of OR2080227H and OR2090107H on dosage of hydrogen peroxide showing main effects and 2-way interaction terms.

Varieties

Figure 6.2 shows that, summed across dose of peroxide, OR2080227H responded more strongly to the addition of hydrogen peroxide than OR2090107H. This effect was the strongest contributor to the overall ANOVA model as determined by the *F*-ratios.

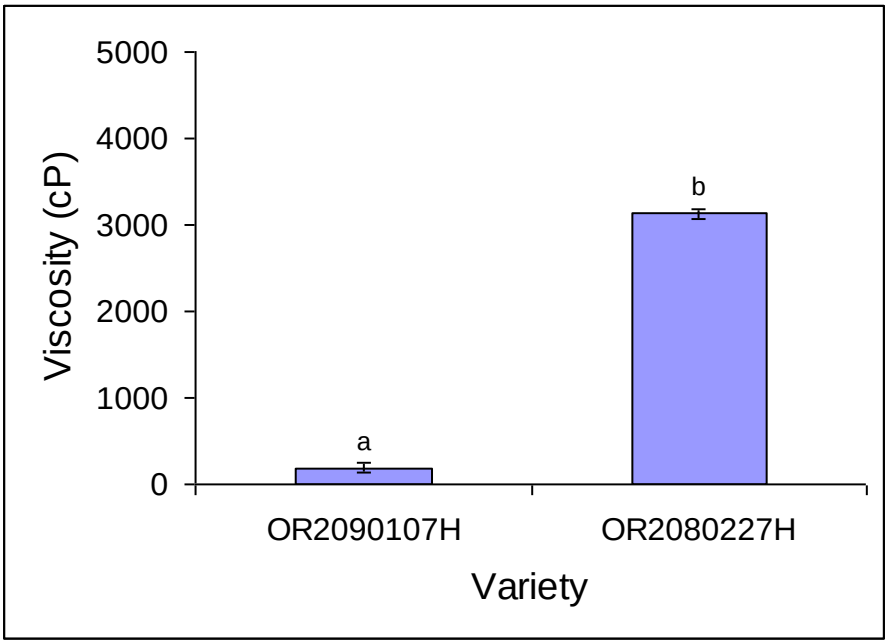

Figure 6.2: Peak viscosity for the varieties tested, summed across dose of peroxide. Different letters indicate significantly different mean values for dose of peroxide. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Dose of peroxide

Figure 6.3 shows that, summed across variety, oxidative gelation occurred upon addition of 40+ ppm hydrogen peroxide. There was a significant increase in peak viscosity from 40 to 60 ppm hydrogen peroxide. Peak viscosity did not change at higher doses.

It is clear that a dose of ≥ 40 ppm hydrogen peroxide is needed to initiate oxidative gelation in an aqueous wheat-flour suspension. It is also clear that the 75 ppm dose used by both Bettge and Morris (2007) and Ross et al. (2014) was sufficient to yield the maximum PPV. Further increase in peroxide concentration above 60 ppm did not increase the extent of the reaction as monitored by viscosity.

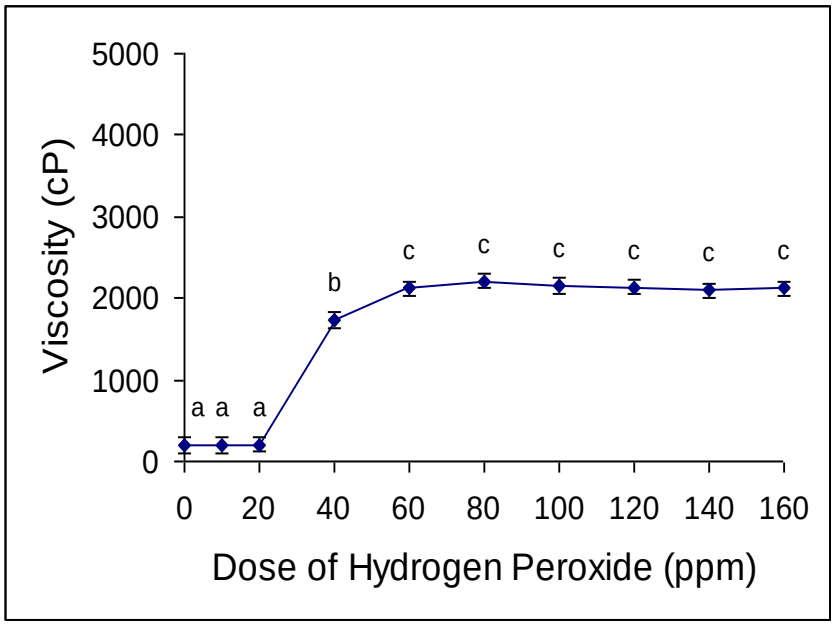

Figure 6.3: The effect of hydrogen peroxide dosage on peak viscosity summed across variety. Different letters indicate significantly different mean values for dose of peroxide. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Interactions

Table 6.2 shows that the variety x dose of peroxide interaction was statistically significant. The contribution of the interaction to the overall ANOVA model was comparable to the main effect dose of peroxide as determined by the *F*-ratios. Therefore, it was concluded that the interaction was of some practical significance. The interaction plots (Figure 6.4) show that both varieties tested undergo oxidative gelation upon addition of 40+ ppm hydrogen peroxide. However, OR2080227H responded much more strongly to hydrogen peroxide than OR2090107H.

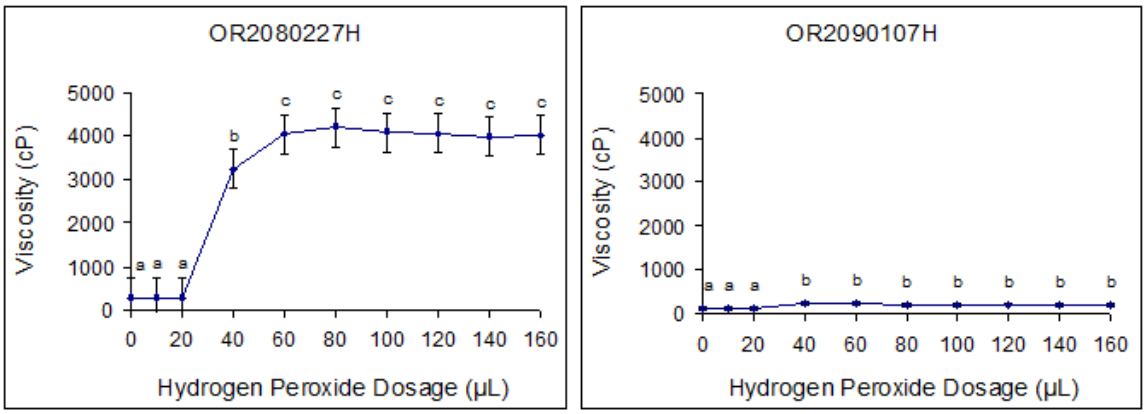

Figure 6.4: The effect of hydrogen peroxide dosage on peak viscosity for each variety tested. Different letters indicate significantly different mean values for dose of peroxide. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Response of oxidative gelation in OR2080227H and OR2090107H to dosage of Azodicarbonamide

ANOVA (Table 6.3) shows that variety and dose of ADA each had a significant effect on oxidative gelation. At $p \leq 0.01$, there were also significant two-way interactions for variety x dose of peroxide.

Main Effects	***F*-Ratio**	**P-Value**
Variety	5333.61	< .01
Dose of ADA	7.07	< .01
Interactions		
Variety x Dose of ADA	9.25	< .01

Table 6.3: ANOVA results for response of OR2080227H and OR2090107H on dosage of azodicarbonamide (ADA) showing main effects and 2-way interaction terms.

Varieties

Figure 6.5 shows that, summed across dose of ADA, OR2080227H had a higher peak viscosity than OR2090107H. This effect was the strongest contributor to the overall ANOVA model as determined by the *F*-ratios. The average peak viscosities for OR2080227H and OR2090107H were substantially lower than those observed when peroxide was used as the oxidant.

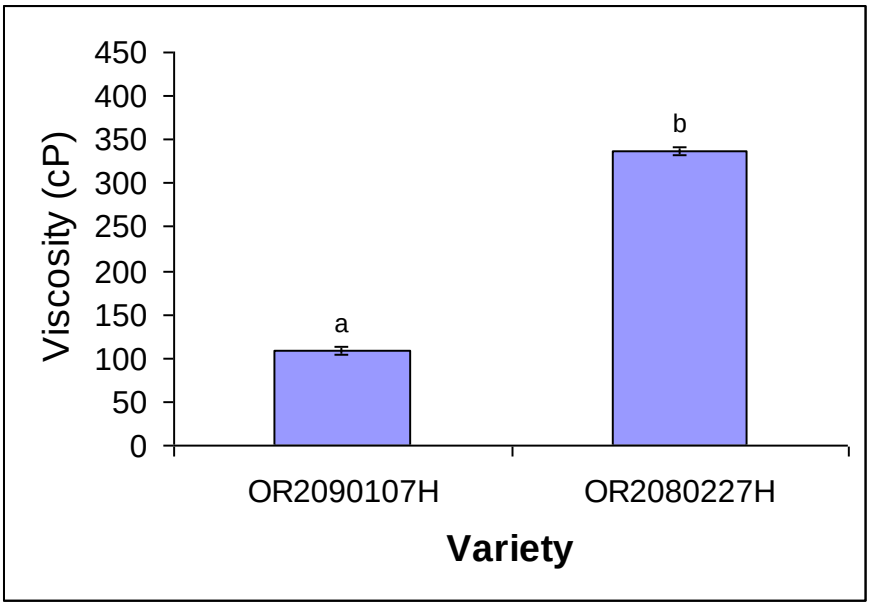

Figure 6.5: Peak viscosity for the varieties tested, summed across dose of azodicarbonamide. Different letters indicate significantly different mean values for dose of peroxide. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Dose of ADA

Figure 6.6 shows, summed across variety, peak viscosity of flour tested did not change significantly between 0 ppm and 160 ppm ADA. Although differences in peak viscosity in ADA across variety were statistically significant, their practical significance compared to the other main effect, variety, were smaller as determined by the *F*-ratios.

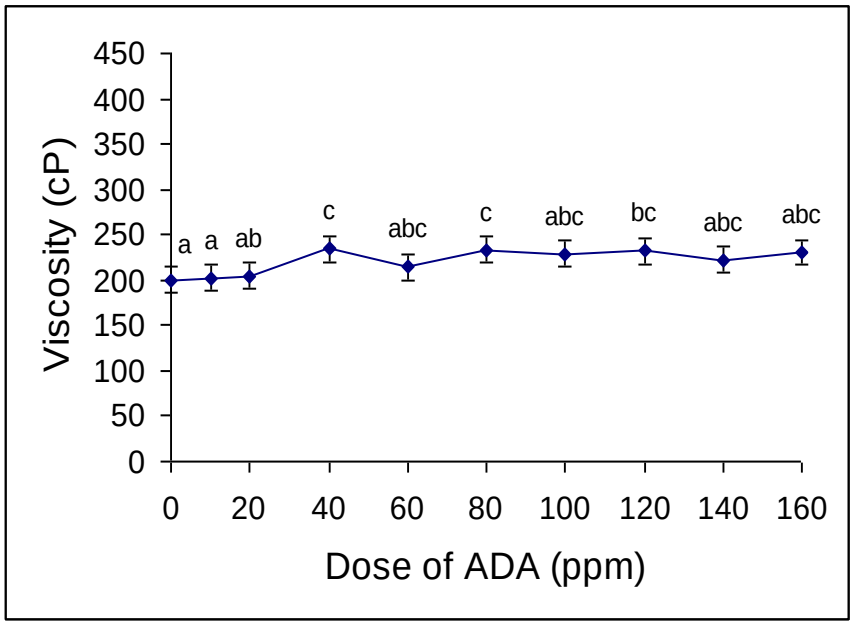

Figure 6.6: The effect of azodicarbonamide (ADA) dosage on peak viscosity summed across variety. Different letters indicate significantly different mean values for dose of peroxide. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

Interactions

Table 6.3 shows that the variety x dose of ADA interaction was statistically significant. The contribution of the interaction to the overall ANOVA model was comparable to the main effect dose of ADA as determined by the *F*-ratios. The interaction plots (Figure 6.7) show that OR2080227H had a higher peak viscosity than OR2090107H. There was no significant difference in peak viscosity in ADA between 0 and 160 ppm ADA for either variety. Because the viscosity did not change, addition of ADA did not start the oxidative gelation process in either flour tested.

ADA does not produce the free radicals necessary to kick start oxidative gelation at room temperature (Figure 6.7). (Noonan et al. 2008) showed when ADA is used in bread formulations, ADA does not produce free radicals until baking. This indicated that ADA needed to be introduced to elevated temperatures in order to kick start oxidative gelation. In order to observe the response of oxidative gelation to dosage of ADA, this test would need to be repeated at elevated temperatures.

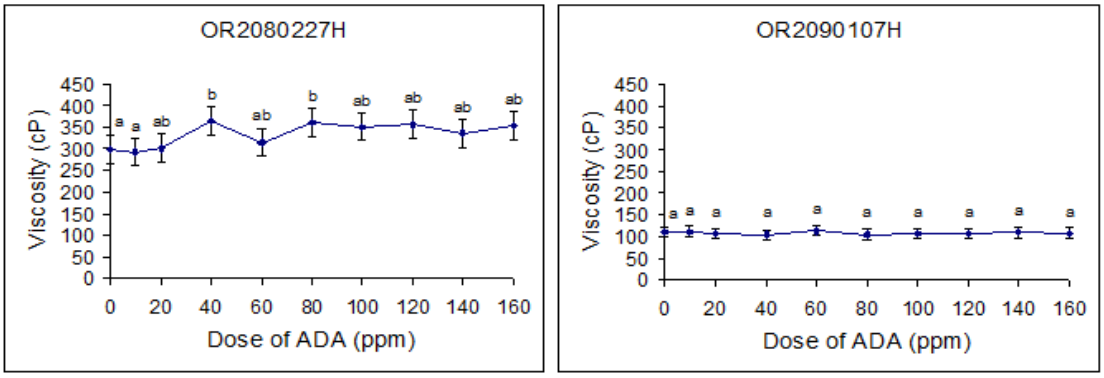

Figure 6.7: The effect of azodicarbonamide (ADA) dosage on peak viscosity for each variety tested. Different letters indicate significantly different mean values for dose of peroxide. Error bars indicate ± Tukey's HSD at $p \leq 0.01$.

CHAPTER 7: CONCLUSIONS

Conclusions: The effect of grain storage and flour aging on absorption characteristics of break flour milled from soft-wheat

Break flour yield

- The strongest contributing factor to variability in BFY was variety. Figure 4.1 showed that Bobtail had significantly higher BFY than the other three varieties. In general, high BFY is associated with lower absorption characteristics and this is shown by the low water SRC of Bobtail (Figure 4.3). The relationship between BFY and absorption characteristics, in part, justifies our selection of the four varieties used in this study.

- BFY also changed across WAH with an initial increase to week 6, then declining to a level equivalent to the freshly milled BFY at week 24 (Figure 4.2).

Water SRC

- The strongest contributing factor to variability in water SRC was variety. Water SRC data grouped Bobtail and Skiles as the low absorption group (low flour/water viscosity) and Tubbs and Goetze (high flour/water viscosity) as the high absorption group (Figure 4.3).

- Water SRC was also influenced by grain storage time. In all varieties tested, water SRC values dipped slightly at week 3 before increasing slightly by week 24 (Figure 4.4). Although water SRC was higher at week 24, the absolute value of the increase may not be of practical significance.

- Flour aging had no significant effect on Water SRC (Table 4.3).

Sucrose SRC

- The strongest contributing factor to variability in sucrose SRC was variety (Table 4.4, Figure 4.5). Sucrose SRC data also grouped Bobtail and Skiles as the low absorption group and Tubbs and Goetze as the high absorption group (Figure 4.5).

However, for sucrose SRC all four varieties were significantly different from each other (e.g. Bobtail had significantly higher sucrose SRC than Skiles). This is partial confirmation that sucrose SRC provides a different perspective of absorption than does water SRC and conforms to the theoretical discussion of Kweon et al. (2011).

- Sucrose SRC was influenced by grain storage time. Summed across WAH and DAM, sucrose SRC values dipped significantly at week 3 before increasing by week 24 (Figure 4.6). Although sucrose SRC was significantly higher at week 24 than at week 0, the absolute value of the increase may not be of practical significance.

- Sucrose SRC was influenced by flour aging time. In general, there was a small but significant increase in sucrose SRC during flour aging (Figure 4.7). Although sucrose SRC was significantly higher at week 24 than at week 0, the absolute value of the increase may not be of practical significance.

Sodium Carbonate SRC

- The strongest contributing factor to variability in sodium carbonate SRC was variety (Table 4.5, Figure 4.9). Sodium carbonate SRC data also grouped Bobtail and Skiles as the low absorption group and Tubbs and Goetze as the high absorption group (Figure 4.9). However, for sodium carbonate SRC all four varieties were significantly different from each other (e.g. Bobtail had significantly lower sodium carbonate SRC than Skiles). This is further confirmation that different SRC solvents each provide a different perspective of absorption characteristics (Kweon et al. 2011).

- Sodium carbonate SRC was influenced by grain storage time. Summed across WAH and DAM, sodium carbonate SRC values dipped significantly at week 3 before increasing by week 24 (Figure 4.10). Sodium carbonate SRC was not significantly different at week 24 than at week 0.

- Sodium carbonate SRC was influenced by flour aging time. In general, there was a small but significant decrease in sodium carbonate SRC during flour aging (Figure 4.11). Although sodium carbonate SRC was significantly lower at week 24 than at week 0, the absolute value of the decrease may not be of practical significance.

Lactic Acid SRC

- The strongest contributing factor to variability in lactic acid SRC was variety (Table 4.6, Figure 4.13). Lactic acid SRC measures the absorption of glutenins, and is more an indicator of gluten strength than what we would commonly call "flour absorption." Not surprisingly, lactic acid SRC data grouped the varieties differently than the other SRC solvents. Bobtail and Skiles were grouped as the high gluten strength group, and conversely Tubbs and Goetze as the low gluten strength group (Figure 4.13). However, all four varieties were significantly different from each (e.g. Bobtail had significantly higher lactic acid SRC than Skiles). This is further confirmation that different SRC solvents each provide a different perspective of absorption characteristics (Kweon et al. 2011).

- Lactic acid SRC was influenced by grain storage time. Despite omission of unreliable data from weeks 6 and 24, summed across WAH and DAM, lactic acid SRC values declines monotonically as time of grain storage increased (Figure 4.14). Although lactic acid SRC was significantly lower at week 13 than at week 0, the absolute value of the decrease, summed across varieties, may not be of practical significance. However, for Goetze, Skiles, and Bobtail individually, the decrease was both significant and of a magnitude to be of practical significance (Table 4.7).

- Lactic acid SRC was influenced by flour aging time. In general, there was a small but significant decrease in lactic acid SRC during flour aging (Figure 4.15). Although lactic acid SRC was significantly lower at day 62 than at day 0, summed across varieties, the absolute value of the decrease may not be of practical significance.

Conclusions: The effect of grain storage and flour aging on oxidative gelation and lipid peroxidation of break flour milled from soft-wheat

Baseline viscosity

- The strongest contributing factor to variability in baseline viscosity was variety (Table 5.1, Figure 5.2). Although the differences in baseline viscosity were statistically significant, the variation between varieties (~15 - 20 cP: Figure 5.2) was essentially inconsequential when compared to, for example, the PPV of Tubbs at ~650 cP (Figure 5.6). Therefore the differences in baseline viscosities between varieties, although statistically significant, were considered to be "noise," and not definitive differences.

- Baseline viscosity data reflected water SRC data, essentially grouping Bobtail and Skiles as the low absorption group (low flour/water viscosity) and Tubbs and Goetze (high flour/water viscosity) as the high absorption group. However, in comparison to water SRC (Figure 4.3), Skiles is much closer to the high absorption group.

- ANOVA indicated that baseline viscosity was influenced by grain storage time (Table 5.1, Figure 5.3). However, the magnitude in differences was small (~5 cP) and there appeared to be no systematic change across WAH. Therefore the differences in baseline viscosities between varieties, although statistically significant, were considered to be "noise," and not definitive differences.

- ANOVA indicated that baseline viscosity was influenced by flour aging time (Table 5.1, Figure 5.4). However, once again, the magnitude in differences was small (~5 cP). However, there appeared to be systematic increase to day 27 which then plateaued to day. This observation may indicate a real trend, but this data does not allow further interpretation.

Peroxide peak viscosity

- The strongest contributing factor to variability in PPV was variety (Table 5.2, Figure 5.6). Grouping for PPV was different than both water SRC and baseline viscosity. They were divided into three categories: low OGC (Bobtail), intermediate (Goetze and Skiles), and high (Tubbs). The different groupings suggests that OGC is a trait that is independent of and cannot be explained fully by absorption characteristics, although clearly they are related because AX are related to both absorption and OGC (Bettge and Morris 2007, (Kiszonas et al. 2013), Ross et al. 2014).

- PPV was also influenced by grain storage time. In all varieties tested, PPV increased though week 3 and subsequently declined to week 24. (Figure 5.7). Week 24 PPV was significantly lower than week 0 PPV. The magnitude of this difference may be of practical significance as the changes observed were a substantial proportion compared to absolute values of PPV.

- PPV was influenced by flour aging time. In general, there was a small but significant increase in PPV during flour aging (Figure 5.8). Day 62 PPV was significantly higher than day 0 PPV. The magnitude of this difference may also be of practical significance as the changes observed were a substantial proportion compared to absolute values of PPV.

- Trends in PPV observed across WAH and DAM were different (Figures 5.7 and 5.8). Over the total period of grain storage, PPV decreased. In contrast, across DAM, PPV increased. The contrasting trends in PPV across grain storage and flour aging times could be a function of the state of the stored material: intact grains vs milled flour. Specifically, reduced particle size, increased surface area to volume ratio, leading to increased exposure to oxygen, and loss of compartmentalization of potential reactants in flour compared to their anatomical locations within the intact seed.

- OGC changed proportionally as a function of grain storage and flour aging time in a similar fashion across all varieties tested, regardless of the absolute values

(Figures 5.6 and 5.10). This suggest an underlying similarity in reactivity independent of magnitude OGC (PPV).

- Reactivity to peroxide was highly correlated to PPV ($r > 0.99$) for all varieties at all stages of grain storage and flour age.

- PPBV was measured in order to confirm or refute earlier reports (Baker et al. 1943) that oxidative gels were shear sensitive. In this case, thixotropy (time dependent decrease in viscosity at constant shear rate) was used to observe the sensitivity of the oxidative gel to shear.

- PPBV followed a similar trends to PPV across varieties, WAH and DAM (Figures 5.12, 5.13, and 5.14). For example, viscosity loss declined across WAH and increased across DAM. The similarities between PPBV% and PPV suggest that the gel formed upon addition of hydrogen peroxide broke down proportionally to the strength of gel formed.

- Within reactive varieties (Goetze, Tubbs and Skiles), PPV and PPBV were highly correlated (Figure 5.16). However, there was obvious clustering that showed each of the reactive varieties to have separate regressions for PPV vs PPBV with significantly different slopes and intercepts (Figure 5.17).

- When observing PPBV as a proportion of PPV (PPBV%), the data showed that there was a non-linear response of PPBV% to increasing PPV. PPBV% increased asymptotically as PPV increased within each varietal cluster.

- Across varieties, however, the relationship between PPV and PPBV% did not hold (i.e. at a given PPV, varieties differed significantly in PPBV%). This suggests qualitative differences in the nature of the gels formed. This finding may be the most intriguing result in the entire study and the RVA technique may provide a method of assessing functionality of reported differences in, for example A/X ratio in WEAX (Souza et al. 2011).

Malondialdehyde

- Although variety was a significant contributor to the variability in MDA concentration, the *F*-ratio was the lowest out of all three main effects (Table 5.4). In contrast to the analyses relating to OGC and SRC, MDA concentration changed primarily as a function of grain storage time.

- As grain was stored, MDA concentration decreased (Figure 5.20). However, as flour aged, MDA concentration initially increased to day 3, decreased at day 6, and remained constant to day 62. The initial increase and subsequent decrease of MDA concentration as a function of flour age may indicate the initial production and subsequent consumption of oxidized lipids, the latter in downstream redox reactions.

Conclusions: The effect of varying levels of hydrogen peroxide and azodicarbonamide on the oxidative gelation of two wheat varieties with differing oxidative gelation reactivities

- There were significant differences in PPV within hard-wheat varieties surveyed.

- Surprisingly, not all varieties increased in PPV as a function of flour age (Figure 6.1). Increased PPV might have been expected based on results from the soft-wheat study reported in Chapter 5.

- The highest PPV observed in the hard-wheat survey was 2804 cP, which is ~2000 cP higher than that observed in both the soft-wheat aging study in the study of Ross et al. (2014) that included hard-wheats. The lowest PPV, however, was similar to that in the soft-wheat aging study.

- The response of PPV to dosage of hydrogen peroxide showed that a threshold level of hydrogen peroxide was necessary to induce oxidative gelation. A dose of $\geq$ 40 ppm hydrogen peroxide was required. Once 60 ppm was added, PPV did not increase further at higher doses. Because of this, the use of 75 ppm hydrogen peroxide used in the literature (Bettge and Morris 2007, Ross et al. 2014) was appropriate.

- ADA did not induce oxidative gelation in either variety tested. Testing conditions (low temperature) were insufficient in creating free radicals necessary to do so.

General Conclusions

Variety

- Variety was the strongest factor in determining flour functionality expressed as SRC and OGC. This is unsurprising, because varieties were chosen based on differences in absorption characteristics and OGC as indicated by preliminary testing. Variety was the weakest factor in determining changes in MDA concentration.

Grain storage time

- As a function of grain storage time, water, sucrose and sodium carbonate SRC values increased. In contrast, lactic acid SRC values declined as a function of grain storage time. Although many of these changes were statistically significant, their practical significance was unclear. As a function of grain storage time, OGC initially increased to week 3 then declined to week 24. Not only was this change statistically significant, but the magnitude of the change could be considered practically significant. Because OGC is a trait that currently only has theoretical value in food processing (i.e. OGC is not a trait currently taken into consideration during food processing), it is difficult to definitively conclude what constitutes practical significance. Grain storage time had the strongest influence on changes in MDA concentration. The trend of change in MDA concentration was similar to that of OGC.

Flour age

- Flour age was the weakest contributor to changes in SRC. Looking at individual SRC solvents, flour aging time did not significantly influence changes in water SRC values. However, as flour aged, sucrose SRC values significantly increased and sodium carbonate and lactic acid SRC values decreased. Although changes in

sucrose, sodium carbonate, and lactic acid SRCs were statistically significant, their practical significance was again unclear. As a function of flour age, OGC increased. As a function of flour age, MDA concentration initially increased, but subsequently declined and remained constant from day 6 to day 62.

Relationship between PPBV and PPV

- Each variety appeared to show a different relationship between PPV and PPBV. The proportional breakdown in viscosity also appeared to differ between varieties, and the relationships were nonlinear. This suggested that there was a maximum PPV for each variety at which PPBV no longer increased.

- Speculation allows the idea that the RVA method used here could provide a way of expressing functional differences in OGC that might relate to structure differences in AX.

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