AN ABSTRACT OF THE THESIS OF

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Title: Two Studies Addressing Practical Needs of Wheat Farmers, Processors, and Breeders: Changes in Falling Number and Alpha-amylase During Grain Storage, and Improved Predictions of Wheat-flour Dough Properties.

Abstract approved: _____

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Wheat (*Triticum aestivum*) is a globally traded staple food crop. The diverse and pleasing nature of wheat-derived products is a result of the complex interactions of the polymeric components from the wheat endosperm. Changes in the functionality of these polymeric components, as a result of changes in growing conditions or different genetics, impacts market price and end-product quality and directly affects farmers and processors. Wheat is of particular economic importance to the U.S. Pacific Northwest and, specifically, to the state of Oregon. Providing quality wheat for export is paramount to the survival of the Oregon wheat industry. This dissertation focuses on wheat quality from the perspective of serving the practical needs of farmers, processors, and wheat breeders.

The first study, split into two portions, concerns pre-harvest sprouting (PHS) and grain storage. PHS increases alpha amylase (αA) activity in wheat, which, in excess, reduces wheat end-product quality. Falling Number (FN) is the primary test used by industry to gauge PHS damage in wheats. Direct measurement of αA activity is the fundamental frame of reference. The

objective of these studies was to determine if FN and αA activity of wheat samples changed during storage and if changes were a function of storage time, storage temperature, and degree of PHS damage. Samples from three Idaho locations were used. These captured a wide range of PHS degree, and therefore, wide ranges of FN values and αA activities. Samples were subdivided and stored at -20°C, +20°C, and +40°C. Low FN values and high αA activities were observed in soft wheats from locations that had rain events prior to harvest. Overall, FN and αA activity had the curvilinear relationship expected from the literature, indicating the validity of the sample set with regard to the FN/αA relationship.

Changes in FN and αA activity were observed over a 90 day period of grain storage. FN differed between growing environments, wheat varieties, and storage temperatures. αA activity also differed between growing environments and wheat varieties, but not between storage temperatures. Highest rates of increase in FN were observed in hard wheats with high initial (day 0) FN values. Lowest rates of increase in FN were observed in soft wheats with low day 0 FN values. This contrasted with the changes that occurred in αA activity. Decreases in αA activity over storage time were most prevalent in *soft* wheats, particularly sprouted soft wheats (i.e. those with day 0 αA activities > 0.1 Ceralpha Units: CU). There were small decreases in αA activity in hard wheats but the distinction between high and low αA activity samples was not as evident as in the soft wheats because the vast majority of hard wheat samples tested had αA activities < 0.1 CU. Decreases in αA activity were in general not associated with corresponding increases in FN values over grain storage time.

Increases in FN values occurred at a higher rates as storage temperature increased, particularly in hard wheats with high day 0 FN. Grain storage was successful as a way to raise FN values to > 300 s in very few cases. Storage was not effective in decreasing αA activity from > 0.1 CU to < 0.1 CU. Increases in FN over storage time for the hard wheats significantly differed between locations. However, decreases in αA activities over storage time for the hard wheats were not significantly different between locations. This again highlights a lack of correspondence between increased FN and decreased αA activity, suggesting that these two factors are somewhat decoupled when looking at changes in stored grain.

Temperature-induced gluten crosslinking was explored as possible explanation for drastic increases in unsprouted hard wheat FN observed in samples from one location. Total polymeric protein (TPP) content was assessed at the end of the study for unsprouted hard wheats stored at +40°C and -20°C as well as sprouted hard wheats from stored at -20°C. TPP content was assessed as % large unextractable polymeric proteins (%LUPP) and % total unextractable polymeric proteins (%TUPP) using size exclusion high performance liquid chromatography (HPSEC). TPP content was not significantly different between storage temperatures for wheat varieties from the same location. %TUPP, but not % LUPP, was significantly lower in wheat varieties affected by PHS. Changes in FN at high storage temperature were not likely due to increased protein crosslinking.

The second study aimed to validate the use of a rapid method for predicting dough strength at early generations in hard wheat breeding programs. Early generation quality screening improves breeding program efficiency. Hard wheats are used to make leavened bread products. The gluten

proteins, particularly high molecular weight glutenin subunits (HMW-GS), form large, ramifying networks called the glutenin macropolymer (GMP). High GMP content is associated with increased dough strength and bread quality. Genetic differences in HMW-GS, and by inference, GMP, are responsible for differing dough properties between varieties. The Mixograph is used to measure dough mixing properties and predict end-product quality in breeding programs. GMP can also be measured as total polymeric protein (TPP) via HPSEC. The Solvent Retention Capacity (SRC) test has been proposed to predict hard wheat quality, specifically lactic acid SRC (LASRC). The objectives of this research were to provide preliminary information on the usefulness of using LASRC, on its own, to predict dough mixing properties, specifically as applied to early generation screening in a wheat breeding program, and to assess the relationship between LASRC and TPP. Wheat samples were categorized by flour protein concentration (FPC). Mixograph analysis was used as the baseline for dough properties and was analyzed both by eye and by the proprietary Mixsmart software. TPP content was assessed as %LUPP and %%TUPP. As a result of redundancy between the two TPP measures, only %LUPP was used for statistical analysis. Dough mixing parameters were slightly better correlated with LASRC than %LUPP. Correlations between LASRC, %LUPP, and dough mixing parameters were different between FPC categories, particularly in low FPC samples. A strict cutoff of 115% LASRC effectively screened out the bottom 10% of low quality hard wheats but retained a nearly equal amount of low quality hard wheats that would have been screened out by mixograph analysis. LASRC and %LUPP are not likely to be effective *predictors* of dough properties, but may have some value to *screen* for hard wheat quality in the early generations of a wheat breeding cycle.

Two Studies Addressing Practical Needs of Wheat Farmers, Processors, and Breeders: Changes in Falling Number and Alpha-amylase During Grain Storage, and Improved Predictions of Wheat-flour Dough Properties.

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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.

Mike R. Adams, Author

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CONTRIBUTION OF AUTHORS

Dr. Andrew Ross initiated the project. Dr. Andrew Ross guided the project and contributed to the manuscripts. Dr. Teepakorn Kongraksawech assisted with laboratory procedures.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

DEDICATION

This thesis is dedicated to Abigail, for always being happy to see me.

Chapter 1: General Introduction

1.1 Background

Wheat is a food crop of global importance. Modern wheats are primarily cultivars of the hexaploid species *Triticum aestivum* (Wrigley et al 2009). The wheat plant produces an edible seed called a caryopsis that can be milled into flour. Food products derived from wheat flour are diverse and pleasing. The interactions of the polymeric components found in wheat flour create unique foods like noodles, cookies, cakes, and leavened breads. Over 600 million metric tons of wheat are produced annually worldwide, 60 million metric tons of which is produced in the U.S.A. (McFall and Fowler 2009, Wrigley et al 2009). Wheat exportation is of economic importance to the U.S.A. and particularly in the Pacific Northwest (PNW). In Oregon alone, wheat accounts for around $1.3 billion in exports (Agri-Business Council of Oregon 2010). Providing quality wheat for export is paramount to the survival of the Oregon wheat market. This dissertation is focused on wheat quality from the perspective of serving the practical needs of farmers, processors, and wheat breeders.

Pre-harvest sprouting

Pre-harvest sprouting (PHS) is a problem that occurs when mature seeds in the wheat spike germinate prior to harvest. This phenomenon can be triggered by rain events or high humidity (Jones et al 2009). Wheat germination leads to increased alpha amylase (αA) activity as well as the de novo synthesis of other hydrolases: e.g. proteases (Simsek et al 2014). There is an optimum level of αA activity for bread products but millers prefer to start with ungerminated grain and dose αA to a prescribed level. However, in excess, αA activity in flour can yield bread

products with poor volume, sticky brown colored crumb (interior), and an excessively dark crust (Delcour and Hoseney 2010).

Wheat is graded as unacceptable for human food if more than 4% of the seeds are visibly sprouted but PHS can be present in the absence of visible cues (Ross and Bettge 2009). The Perten Falling Number (FN) test is used at grain elevators upon delivery to determine if wheat is damaged by PHS. A FN of < 300 s is presumptive evidence of αA activity in the grain sufficient to degrade both processing performance and end-product quality. αA activity can also be measured directly. αA activity > 0.1 Ceralpha units (CU) is also presumptive evidence of αA activity in the grain sufficient to degrade both processing performance and end-product quality. However, low FN can occur in the absence of high αA activity, most often in wheats with low protein content (Ross et al 2012).

Grain storage

PNW wheat farmers have asked repeatedly, over the most recent few years, if storage of marginally sprouted grain will increase FN sufficiently so that it will test above 300 s and will no longer be subjected to discounts at the first point of sale. There is some evidence that grain storage is be related to increased FN. However, the literature is equivocal about the effects of grain storage (as opposed to flour storage) on FN (Lukow et al 1995, Hruskova et al 2004, Karaoglu et al 2010). Few if any studies have tracked αA activity across grain storage time. This belies the question: even if FN increases from say 275 to 310 s over a practical duration of storage, are any potential processing faults still evident. The scope of this study did not address this perspective, partly through simple lack of sufficiently large grain samples.

Hard wheat quality

Hard wheat is used to make bread products. Characteristics associated with improved bread quality have been extensively studied, wheat protein in particular. The gluten forming proteins in wheat are glutenin and gliadin. These proteins give dough its viscoelastic qualities: glutenin providing elasticity and gliadin providing viscosity. Viscoelasticity gives dough the unique quality of being able to trap gasses produced during microbial fermentation, permanently deform during shaping and rising, and yield a leavened product. The glutenins are the most studied proteins with regard to dough properties and have the most leverage over genotypic differences in dough behavior. Glutenin subunits can be classified as high molecular weight (HMW-GS) and low molecular weight (LMW-GS). Glutenins, primarily the HMW-GS, form large, ramifying networks called glutenin macropolymer (GMP). High levels of HMW-GS, and by inference GMP, are associated with increased dough strength and superior bread quality. Dough strength can be measured using the recording dough mixers (RDMs). In breeding programs the Mixograph method is the most common RDM as a result of smaller sample size (10 g) and high speed, relative to other RDMs. However, compared to other, non-RDM screening methods the Mixograph method requires a relatively large amount of refined flour as compared to other screening methods (mg quantities). GMP can be measured directly as total polymeric protein (TPP) content via high performance size exclusion chromatography (HPSEC). TPP measurement is reported as a percentage of large unextractable polymeric protein (LUPP) and total unextractable polymeric proteins (TUPP). High LUPP content has been associated with increased dough strength (e.g. Tsilo et al. 2010) and bread quality.

Solvent retention capacity

The Solvent Retention Capacity (SRC) test uses four different solvents that selectively swell the polymeric components of wheat flour. The mass of the resulting gel is used to predict flour performance in a variety of end products (Slade and Levine 1994, Kweon et al 2011). The SRC method was developed to predict cookie and cracker performance of soft wheat flours and to replace existing dough rheological tests like the Alveograph, Extensograph, Mixograph, and Farinograph. The SRC test examines the individual contributions of the major polymers and predicts their influence on processing-intermediate functionality and end-product quality (Kweon et al 2011).

In SRC methodology, the four solvents used are deionized water (WSRC), 5% w/w aqueous lactic acid (LASRC), 5% w/w aqueous sodium carbonate (NaSRC), and 50% w/w aqueous sucrose (SucSRC). Lactic acid preferentially swells glutenin subunits, sodium carbonate swells damaged starch, and sucrose swells arabinoxylan (AX) and gliadins. Water is used as the reference solvent as it hydrates all of the polymeric components of wheat flour, but to a lesser degree than the other solvents (Ross and Bettge 2009). A 5:1 solvent-flour ratio keeps the polymers accessible to the solvent (but not extractable) in order to form a swelled and solvent-holding network that will hold up against the force of centrifugation. SRC is useful as a method of predicting baking quality for breeders, millers, bakers, and researchers. End-product testing is sample, time, and labor intensive. Small-scale, high-throughput tests are important for efficiency in processing, research, and breeding programs. SRC profiles are highly correlated with cookie and cracker quality (Slade and Levine 1994).

While initially designed to be a tool for soft wheat quality, SRC has been applied to predicting hard wheat quality as well (Ram and Singh 2004, Ram et al 2005, Xiao et al 2006, Kwan et al 2011, Jayaram et al 2014). The solvent of interest for hard wheat quality is lactic acid, as LASRC favors the swelling of glutenin subunits. Ram et al (2005) showed that grain protein content and LASRC of refined flour explained a large amount of variability in Farinograph water absorption and peak dough development time.

1.2 Objectives and hypotheses

Chapter 2

Chapter 2 is a review of the literature relevant to wheat quality. Wheat classification, kernel anatomy, kernel composition, kernel texture, flour milling, and flour component functionality are described. Perspectives on PHS and grain storage are also explored. It also aims to briefly discuss wheat breeding programs and their techniques and objectives as it pertains to efficiently assessing wheat quality.

Chapter 3

This study is concerned with grain storage time and temperature as a way to mitigate the negative effects of PHS. FN was used to quantify changes in wheat quality over time as it pertains to PHS damage. The objectives of this study are to observe wheat FN changes over storage time and to determine the rate change in FN as a function of storage temperature. We also aim to determine if changes in FN over time manifest into a practical, categorical, change from poor to acceptable quality and if postulated changes are influenced by degree of PHS damage.

Chapter 4

This study is also concerned with grain storage time and temperature as a way to mitigate the negative effects of PHS. αA activity was used to quantify changes in wheat quality over time as it pertains to PHS damage. The objectives of this study are to observe wheat αA activity changes over storage time and to determine the rate change in αA activity as a function of storage temperature. We also aim to determine if changes in in αA activity over time manifest into a practical, categorical, change from poor to acceptable quality and if postulated changes are influenced by degree of PHS damage.

Chapter 5

In this preliminary study we investigate the use of a method that is less sample and labor intensive than measurements of dough characteristics as a screening tool for early generation selections hard wheat genotypes in breeding programs. Mixograph was used as the reference method for hard wheat quality. The usefulness of the LASRC test was explored. Although TPP determination via HPSEC is also labor intensive it was also explored as a predictor of dough characteristics and as an additional reference method against which the utility of LASRC could be gauged. The objective of this study is to determine if either or both LASRC and TPP can be used to predict hard wheat dough characteristics at early generations in a breeding program. We hypothesize that hard wheat quality will be associated with high LASRC and high LUPP values.

Chapter 2: Literature Review

2.1 Wheat

From cookies to bread, and noodles to crackers, people like wheat-based foods. Common wheat, *Triticum aestivum*, is a grass in the *Graminae* family and is grown on more land area than any other food crop. Wheat was domesticated in the Fertile Crescent between 10,000 and 12,000 years ago making it one of the earliest crops domesticated by humans (Gustafson et al 2009, Shewry 2009). Cereal domestication allowed humans to transition from hunting and gathering to farming. Early farmers selected cereals based on their reliability, yield, and storability to give them a constant food source throughout the year. Wheat was an obvious choice because it is a hardy plant that grows in a wide variety of conditions. It can be grown on every continent, excluding Antarctica. Wheat was also an obvious candidate as a food crop because foods derived from wheat are diverse and pleasing.

In the modern era, wheat accounts for roughly one third of global cereal consumption. In 1996 it was estimated that 55% of carbohydrates consumed in the world were from wheat. Global wheat production has tripled since the 1960's. However, with continued population growth world demand is likely to continue to increase (Gustafson et al 2009). It has been estimated that by 2040 the global agriculture demand will increase by 66% (McFall and Fowler 2009). It will be important in the future to supply the world with high-yield and high-quality wheat. Wheat is also a good source of protein. Besides their nutritional properties, wheat (gluten) proteins lend unique functional properties, like gas-holding and cohesive viscoelasticity, to wheat-based foods and their processing intermediates.

Today world wheat production is more than 600 million metric tons per year (McFall and Fowler 2009, Wrigley et al 2009). Average US production over the last decade has remained at around 60 million metric tons, half of which is exported. Because of efficient transport infrastructure from field to ports the US can offer any type of wheat to any buyer across the globe. Of the wheat grown globally about 110 million metric tons are traded internationally (McFall and Fowler 2009). Most countries grow just as much wheat as they consume and export little to none. In contrast, the US grows more than it needs domestically and is consequently responsible for almost one third of wheat traded internationally (Gustafson et al 2009). This equates to an over $6 billion industry in the US. In the Pacific Northwest (PNW) wheat is a major crop. Common wheat is planted on 1.7 million hectares each year in the PNW (Smiley et al 2012). The PNW primarily grows soft white winter wheat. In Oregon alone, wheat accounts for around $1.3 billion in exports (Agri-Business Council of Oregon 2010).

Modern wheat breeders seek to produce cultivars that are homogenous and meet the demands of the processors and consumers. Ancient wheat farmers had to deal with wild wheats that had grains with tightly adhering hulls and brittle ears that shattered and scattered seed to the ground after maturity. From those wild wheats early farmers selected types that combined these traits: tough ears, and hull-lessness (Simons et al 2006). The latter allowed the seed to be threshed free of the hull (free-threshing). These traits eased harvesting and cleaning respectively. The *Q* gene controls wheat free-threshing attribute and is associated with rachis strength (Simons et al 2006). This trait was important to early man for convenience and is important now to modern agricultural processing.

Modern common wheat is a hexaploid species with diploid and tetraploid ancestors. Diploid wheats were the first to evolve. This group includes *T. monococcum* (einkorn), *Aegilops speltoides*, and *T. tauschii* (Shewry 2009, Wrigley 2009). These species have one genome of A, B, or D type with two groups of seven chromosomes ($2n = 2x = 14$ chromosomes: AA, BB, or DD). Tetraploid wheats include the older hulled *T. dicoccum* (emmer) and the newer free-threshing *T. turgidum* L. (durum). Tetraploid wheats and have two genomes of A and B types with two sets of seven chromosomes each ($2n = 4x = 28$: AABB). Hexaploid wheats have three genomes of A, B, and D types with two sets of seven chromosomes each ($2n = 4x = 28$: AABBDD) (Shewry 2009, Wrigely 2009).

Prior to use wheat grain is generally milled to flour. Wheat flour has the unique ability, upon its hydration, to create viscoelastic doughs. These doughs are able to retain gasses and form leavened products as a consequence of the presence of the gluten-forming proteins. Formation of a viscoelastic dough requires a solvent (water) and mechanical energy (mixing). The unique ability of gluten to create leavened products arises from its ability to form relatively gas-impermeable films that trap the gases produced by either microbial fermentation or chemical leavening. The properties of wheat grain and its resulting flour are directly attributed to market class, genotype, and growing environment.

2.2 Wheat Classification

In the US wheat is divided into eight market classes. Six of these classes are of economic importance. The market classes are based on kernel texture, seed coat color, and growth habit.

The six major classes are hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), hard white (HW), soft white (SW), and durum (GIPSA 2014). Wheat can also be sold as mixed or unclassed (McFall and Fowler 2009). Kernel texture differentiates common hexaploid hard and soft wheats. Durum wheat kernels are even harder, and are substantially harder than kernels from almost all hard common wheats. Kernel texture interacts with the milling process and has a profound influence on processing and end-use suitability (see section 2.4). Growth habit determines when the wheat is planted and harvested. Winter wheat is planted in fall and harvested in summer of the subsequent year (McFall and Fowler 2009). Winter wheat has a vernalization requirement, the extent of which is determined by genotype. Vernalization is a variable time period at 2 to 7°C that triggers reproductive (seed) development in the planted wheat (McMaster 2009). Spring wheat is planted in spring and harvested in the late summer or fall of the same year. Facultative wheat varieties are bred to be planted in either winter or spring. Seed coat color is a genetic difference analogous to hair or eye color in humans. Expression of seed coat pigment is controlled by the *R-A1*, *R-B1*, and *R-D1* loci on the long arms of chromosomes 3A, 3B, and 3D respectively (Groos et al 2002, Wang et al 1999). As there are three genetic loci, red pigment exists as a gradient across cultivars (Wang et al 1999). Red or white pigmentation is desirable for different processing applications.

HRW wheat is the largest class produced each year and is used to make bread and rolls. HRS wheat is generally higher in protein than HRW and best suited for bread flour. There is a generally held opinion, not necessarily borne out in fact, that HRS is, as a class, of superior quality to HRW (Maghirang et al 2006). However, quality is relative to the intended end-use and not an absolute characteristic. SRW is generally low protein and used for flatbreads, cakes, and

crackers. HW wheat has a similar spectrum of end-uses as hard red wheats, primarily deviating only in seed coat pigmentation and therefore favored for products (e.g. boiled noodles) where low chromatic contribution from the bran is valued. SW wheat is low protein and suitable for cookies, crackers, and cakes. SW wheat is mostly grown in the Pacific Northwest and much of it is exported. Durum wheat is used to make semolina flour for pasta and some bread products (Montana Wheat and Barley Committee 2006).

2.3 Wheat Kernel Anatomy and Composition

The wheat kernel (or caryopsis) is composed of three main anatomical structures known as the endosperm, bran, and germ (Bechtel et al 2009). The endosperm is the largest structure and is primarily composed of carbohydrates (in the form of starch) and proteins. The bran is the outermost layer and is mostly composed of non-starch polysaccharides (NSPs). The germ, or embryo, is the smallest portion and is responsible for growing a new wheat plant upon germination. The germ is high in lipids and minerals. Consideration of these three structures is important for millers and food processers. A caryopsis is typically 8 mm long and 35 mg in mass (Delcour and Hoseney 2010). The seed is round on the dorsal side and has a longitudinal ventral crease that runs the length of the caryopsis; this crease extends almost into the center of the grain (Delcour and Hoseney 2010). The crease is a problem for millers as it makes separation of the bran more difficult and may harbor microbes and dirt.

2.3.1 Endosperm

The endosperm, sometimes called the starchy endosperm, is about 80% of the total kernel weight and is of primary concern when producing refined flour. From an anatomical perspective the

outermost layer of the endosperm is the aleurone (Bechtel et al 2009). It is, mostly, a one-cell thick layer that covers the entirety of the starchy endosperm and the germ. The aleurone layer is removed with the bran in the milling process and is considered to be part of the bran by millers (Delcour and Hoseney 2010). The non-aleurone endosperm is composed of three types of cells: peripheral, prismatic, and central. The peripheral cells are the first layer in from the aleurone and are the smallest. The prismatic cells are the next cells in and are oblong in shape and extend inwards toward the center of the kernel. The central cells are the innermost and are irregular in shape and size (Delcour and Hoseney 2010). Endosperm cell walls are primarily composed of arabinoxylans (AX), with some beta-glucans (βG) and arabinogalactans (Bechtel et al 2009). Cell walls are thickest in the peripheral cells. All non-aleurone endosperm cells are packed with starch granules suspended in a protein matrix. This matrix is primarily made up of the gluten-forming storage proteins (Delcour and Hoseney 2010). There are two main types of starch granules, A and B (Zeng et al 2011). A-types are typically large and lenticular and B-types are typically small and spherical (Delcour and Hoseney 2010, Zeng et al 2011, Simsek et al 2014).

The endosperm of a wheat kernel may appear vitreous or opaque, colloquially differentiated as “steely vs. mealy” or “vitreous vs. floury”. The endosperm of hard wheats is more likely to appear vitreous in contrast to soft wheats where the endosperm generally appears rather opaque (floury). This visual difference is driven by how tightly the starch granules are packed. Loose packing leaves air pockets that scatter light resulting in a white, opaque appearance. Packing occurs while the kernel is drying, if the protein bursts during drying the endosperm is mealy and if it remains intact during drying the endosperm is steely (Delcour and Hoseney 2010). Protein

content will also affect endosperm vitreous appearance. Growing conditions, for example N content of soil, can influence kernel opacity (Morris 2002).

2.3.2 Bran

The bran is a layered, protective structure that surrounds the caryopsis. It is composed of the pericarp, the seed coat, the nucellar epidermis, and (by millers' definition) the aleurone layer. The pericarp is a layered structure and is 5% of the total kernel weight. It is mostly composed of non-starch polysaccharides (NSPs: ~ 70%) but also contains protein (~16%) and minerals (Nandini et al 2001). The outermost portion is typically lost before milling and is referred to as "beeswing" because of its appearance. The seed coat is a three-layered structure. The seed coat is may contain pigment thus determining red or white kernel color. Aleurone cells are cubic and have thick cell walls that are high in cellulose. With the inclusion of the aleurone layer the bran is also high in enzyme activity (Delcour and Hoseney 2010). In the process of milling to create refined flour, bran is seen as a byproduct. Because it is high in dietary fiber and minerals bran is considered nutritious (Nandini et al 2001).

2.3.3 Germ

The germ, or embryo, is 2.5 to 3% of the kernel weight. It is the portion of the seed that is responsible for growing the new plant upon germination. The two main parts of the germ are the embryonic axis and the scutellum (Bechtel et al 2009). The embryonic axis is an undeveloped root and shoot that will grow if the seed is germinated. The scutellum acts as a storage organ for the undeveloped plant. In human consumption the germ is nutritious as it is high in protein, sugars, oil, minerals, B vitamins, tocopherol, and enzymes. The germ is also removed in the

production of refined flour because the high lipid content (25%) can cause flour to go rancid quickly at room temperature (Delcour and Hoseney 2010).

2.4 Kernel Hardness

Wheat kernel hardness has been identified as arguably the single most important factor for determining end-use quality (Morris 2002). Common wheat is either soft or hard. Durum is considered very hard. Kernel hardness, or texture, is both qualitative and quantitative. The division between hard and soft is clear but variation in hardness exists within each classification (Morris 2002). Texture classification is typically done using the Single Kernel Characterization System (SKCS) machine by Perten (AACC International Method 55-31.01) but near-infra red spectroscopy (NIR) and particle size index (PSI) are also used (Morris 2002). Using the SKCS, kernel hardness is determined by the seed's resistance to crushing force between two metal plates. A representative number of seeds, often 200+, are used for a given sample and the crushing force for each seed is used to calculate the Hardness Index (HI).

The difference in hardness between hard and soft hexaploid wheats is genetically driven. It is caused by a difference in the expression of proteins puroindoline-a and puroindoline-b at the hardness (*Ha*) locus on the short arm of chromosome 5D (Wrigley et al 2009, Morris 2002). Soft wheats have both puroindolines because they possess and express both alleles, *Pina-D1a* and *Pinb-D1a* (Wrigley et al 2009, Morris 2002). In hard wheats one or both of the puroindolines are absent or altered and in durum wheat they are entirely absent (no D genome). The biochemical foundation of kernel texture is complex and not definitive. It is theorized that puroindoline changes the affinity that starch granules have for the protein matrix (Pauly et al 2012). In hard

wheats the puroindolines act to bind the storage proteins tightly to the starch granule. The proteins are more tightly bound to the starch granule than the starch molecules are to each other. This determines how the wheat behaves upon milling and qualities of the resulting flour. When milled, the endosperm of hard kernels fractures into larger particles and is more likely to fracture through starch granules. These phenomena yield flour that is both coarser and higher in damaged starch than flour milled from soft wheat. In soft wheat the wild-type puroindolines act as a "non-stick" coating and the storage proteins are less tightly bound to the starch granule. Upon milling soft wheat the endosperm fractures at the protein-starch granule interface yielding flour with smaller particle size and with less starch damage compared to hard wheat flour (Morris 2002).

Starch damage is mechanical damage incurred by the starch granules in the milling process. The granules have been physically scuffed, cracked, or broken. The structural integrity of the granule is compromised and this has processing implications. Different levels of starch damage are desirable/optimal for different applications. Flours with high starch damage hold more water (Hatcher et al 2002). Damaged starch granules are more susceptible to enzyme attack in dough (Mao and Flores 2001). The maltose produced from amylase digestion of damaged starch increases fermentation vigor. If starch damage is excessive amylase can generate an overabundance of sugar, thus reducing water-holding capacity. High levels of starch damage yield sticky doughs that, when baked, set into sticky and inferior crumb structures (Bettge et al 2000).

2.5 Wheat Flour Milling

Wheat kernels are rarely eaten whole and intact, therefore grain is milled into flour before it is processed and eaten. Before milling grain is cleaned. Cleaning starts by removing foreign material such as stones, metal, and other grains from the wheat. One way this is accomplished is by using a series of vibrating conveyer belt screens. Wheat of desired size is retained while large materials are sieved out to the top and smaller materials drop out below. Air is then blown over the grain to remove the chaff (Posner 2009, Delcour and Hoseney 2010).

After cleaning, but before milling, wheat is tempered. Tempering is adding water to dry grain before milling and then letting it rest for some time. The goal of tempering is to strengthen the bran and soften the endosperm. Toughening the bran helps to prevent it from shattering into small pieces, allowing it to be sieved away easily. Tempering also softens the endosperm and makes it easier to decrease flour particle size without creating excessive starch damage. After adding water, the grain rests for a period of time before milling. Rest times are in the order of 6-24 hours, depending on kernel hardness and desired use. Hard wheats are tempered to higher moisture content than soft wheats and are rested longer before milling (Posner 2009, Delcour and Hoseney 2010).

Roller mills are employed to mill wheat to refined flour. The goal of conventional roller milling is to isolate as much of the endosperm as possible, removing bran and germ. This is accomplished using several pairs of rollers. The concept behind roller milling is employing both crushing and shearing forces to both reduce particle size and isolate the anatomical components of wheat. Rollers rotate in opposite directions, seemingly moving towards each other. Rollers

typically rotate at different speeds, and this applies the shearing forces. The crushing forces reduce particle size while shearing forces help isolate bran (initially). The milling process can be categorized into break milling and reduction milling (Posner 2009, Delcour and Hoseney 2010).

In break milling, mill rollers are typically corrugated. Variable numbers of corrugations are employed, with density of corrugation increasing as particle size is reduced. I.e. rollers at the beginning of the process have lower numbers of corrugations per unit length. Multiple sets of rollers are used to gradually reduce particle size and ensure bran is not shattered into pieces that would lower flour quality. Bran contamination in refined flour is associated with darker flour color and decreases end-product quality (Posner 2009, Delcour and Hoseney 2010).

Sieving of flour streams occurs concurrently with break milling in modern milling facilities. Mill streams from the different rollers are diverted to other rollers or sieves based on particle size. Separating mill streams increases efficiency. In the production of refined flour, bran components are sieved away and discarded. Bran and germ particles with large amounts of endosperm still attached after break milling are called middlings. Middlings are diverted to reduction mill rollers for further separation and particle size reduction (Posner 2009, Delcour and Hoseney 2010).

Reduction rollers are typically smooth. Reduction milling takes middlings and reduces them in size. Sieving after reduction milling removes last traces of bran and germ contamination from endosperm components. After sieving, a portion of middlings that was unable to be reduced to reduction flour is called shorts. Shorts are often germ material and are discarded in refined flour production. Allowing a large portion of germ to remain in refined flour limits shelf life potential

as the high fatty acid content of wheat germ speeds the onset of flour rancidity (Posner 2009, Delcour and Hoseney 2010).

The endosperm of wheat is of interest to modern millers for increased profitability. The desirable endosperm components are the starch and the gluten-forming proteins. Extraction is a term used by millers that means percent recovery. Higher extraction flour may increase profitability but may have a high rate of germ or bran contamination. Low ash content is also desirable in refined flour (Posner 2009, Delcour and Hoseney 2010).

Starch damage is a result of the interaction of kernel hardness and the milling process. Softer kernels typically have lower levels of starch damage. Two types of starch damage can be described. If granules are completely broken but retain their semi-crystallinity and birefringence then they are not soluble in water and not susceptible to amylase attack. Granules that lose their crystallinity and birefringence swell in cold water and are susceptible to amylase. Flours with higher damaged starch content tend to have higher water holding capacities. Hard wheats for bread need a certain, desired, level of damaged starch to improve dough handling properties. As damaged starch is susceptible to amylase attack, damaged starch also allows slow release of sugars that can be utilized by microbes during dough fermentation. Excessive starch damage can be problematic causing issues such as excessive crust darkening as a result of production of excessive levels of brownable maltose and detachment of the crumb from the upper crust (keyholing) (Posner 2009, Delcour and Hoseney 2010).

Flour has better bread baking properties if allowed to briefly age after milling. The addition of oxidizers seeks to mimic this process and increase turnaround in flour supply. Chlorine is also used to bleach flour. Chlorine addition is common in cake flours, as flour chlorination is necessary for certain cake formulations. Leavening agents are added to self-rising flour to aid the consumer in making biscuits and quick-breads in the home. Amylase is often added to bread flour in prescribed amounts to help fermentation vigor. This is particularly in lean dough formulations that do not have added sugar (Posner 2009, Delcour and Hoseney 2010).

2.6 Wheat Kernel Proteins

Proteins drive wheat flour functionality. There is no other substance on earth that can match wheat flour's ability to form a cohesive, gas-trapping network that allows the production of leavened bread products. Gluten, specifically, is considered to be the earliest protein extracted from a plant source when Jacobo Beccari rinsed the starch from a dough in 1728 (Egidi et al 2014, Shewry et al 2009). Wheat is made up of 7-20% protein depending on genotype and growing conditions (Shewry et al 2009). Refined-flour protein content is often 1% less than grain protein content as the bran and germ are relatively higher in protein than the endosperm but contribute much less to total kernel weight (Delcour and Hoseney 2010).

Proteins are made up of amino acids joined together via peptide bonds. Many amino acids polymerized this way yields a polypeptide. The sequence of amino acids determines protein solubility, folding, and reactivity in a given environment. Wheat kernel proteins have been classified based on their solubility since at least 1907. Thomas Osborne developed an extraction method using four solvents to differentiate proteins from wheat meal (Delcour 2012, Shewry et

al 2009). This method has been refined over the years but has remained a popular and consistent technique of classifying cereal proteins. Albumins are water soluble. Globulins are soluble in dilute salt solutions. Prolamins are soluble in 70% aqueous ethanol. Glutelins are soluble in dilute acid or base (Delcour 2012). The latter two classes contain the wheat proteins gliadin and glutenin respectively. Gliadin and glutenin are the endosperm storage proteins and are responsible for forming the gluten matrix in a hydrated dough system. A driving force behind the gluten proteins being insoluble in water is due to their low abundance of amino acids with ionizable sidechains (Delcour and Hoseney 2010). There is a strict dichotomy between the gluten-forming (gliadin and glutenin) and non-gluten-forming (albumin and globulin) proteins. While glutenins and gliadins are important for gluten formation, wheat enzymes tend to be albumins and globulins (Delcour and Hoseney 2010). Enzymes, such as amylases and proteases, are of particular importance in sprouted wheat (Simsek et al 2014). Albumins and globulins are concentrated in the aleurone layer, the bran, and the germ while glutenins and gliadins are only found in the endosperm (Bechtel et al 2009, Delcour and Hoseney 2010, Delcour 2012). Expression of specific proteins is genetically driven while grain protein content is a function of growing conditions (Pasha et al 2007, Shewry 2007, Shewry et al 2009).

Wheat proteins are also of nutritive importance (Shewry 2006). Cereals are a staple protein source in many countries (Pasha et al 2007, Shewry 2007). Extracted gluten is also used as a meat substitute in Asia called seitan. The protein from whole meal wheat is considered more nutritious than from refined flour due to a nutritionally better balanced amino acid composition. Whole wheat meal is higher in essential amino acids than refined flour, specifically lysine

(Shewry 2006, Shewry 2007, Delcour and Hoseney 2010,). Nutritional quality of cereal protein can be increased through genetic modification (Shewry 2007).

2.6.1 Gliadins

Gliadins are the monomeric component of gluten. Gliadin molecules favor intramolecular bonding. Due to this behavior gliadins are said to contribute to the viscosity and extensibility of dough (Delcour 2012, Wrigley et al 2009, Maghirang et al 2006). Gliadin proteins account for around 40% of grain protein content (Daniel and Triboi 2000). Gliadin relative molecular weight may be as high as 100,000 (Wrigley et al 2009).

As a class, gliadin proteins are numerous and heterogeneous. Three main groups can be distinguished, α-, γ-, and ω-gliadins. These groupings are primarily based on their migration distance in gel electrophoresis and the number of cysteine residues present in the protein (Jackson et al 1983, Rashed et al 2007, Delcour 2012). Another group, β-gliadins, can also be differentiated but they are structurally and functionally similar to α-gliadin (Shewry et al 2009, Delcour 2012). α-Gliadins contain 6 cysteine residues in conserved positions, γ-gliadins contain 8 cysteine residues in conserved positions, and ω-gliadins lack cysteine residues entirely (Delcour 2012). With the absence of cysteines, ω-gliadins are termed sulfur-poor prolamins. Gliadin primary structure consists of three distinct domains. The N-terminal domain contains 5-14 amino acid residues. The central domain is up to 100 residues long and contains one or two repetitive motifs. The C-terminal domain has two unique sequences, one rich in glutamine and the other rich in arginine and lysine (Delcour 2012, Delcour and Hoseney 2009). The C-terminal domain is also the location of all of the cysteine residues in the gliadin molecules. All of the

cysteine residues participate in intramolecular disulfide bonds lending a folded shape. Secondary structure of α- and γ-gliadin consists of β-reverse turns in the repetitive domain and α-helices in the non-repetitive domain lending a compact and globular arrangement (Delcour 2012). ω-Gliadins also have β-turns and low levels of α-helices. It has been postulated that ω-gliadins exist in a stiff coil in solution (Shewry et al 2009). Tertiary structures of α-gliadins tend to be more compact and irregular while γ-gliadins may form extended spirals (Delcour 2012). With all of the cysteine residues involved in intrachain disulfide bridges gliadin is prevented from forming quaternary structures with glutenin subunits.

Gliadin expression is mainly determined by two blocks of genes. One block of genes at the *Gli-1* loci (*Gli-A2*, *Gli-B2*, *Gli-D2*) controls the γ- and ω-gliadins and is found on the short arm of group 1 chromosomes. Expression of α-gliadins (and β-gliadins) is controlled by the *Gli-2* loci (*Gli-A2*, *Gli-B2*, *Gli-D2*) on the short arm of the group 6 chromosomes (Shewry et al 2009, Wrigley et al 2009). There is evidence that the expression of certain gliadin proteins are correlated with superior bread loaf volume but the majority of research is focused on glutenins (Khatkar et al 2001, Rashed et al 2007).

2.6.2 Glutenins

Glutenins are the polymeric component of gluten. While glutenin subunits (GS) themselves are polypeptides and hence polymers, glutenin has ability to interact and covalently crosslink with other glutenin molecules creating a ramifying network. Dough elasticity is due to glutenin (Razmi-Rad et al 2007, Delcour 2012). Glutenin molecules are large and effectively insoluble in normal processing conditions and their structure and amino acid sequences favor intermolecular

disulfide bonding. The difficulty of dissolving glutenin confounds their study (Kuktaite 2004, Lemelin et al 2005).

Glutenin subunits (GS) are distinguished by their molecular weight. There two categories: high molecular weight and low molecular weight GS (HMW-GS and LMW-GS resp.). The molecular weight range of LMW-GS is between 30 and 60,000. LMW-GS are subdivided into B, C, and D-types based on their mobility in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). LMW-GS are further classified by whether the first amino acid residue is serine, methionine, or isoleucine (LMW-GS-s, LMW-GS-m, and LMW-GS-i). LMW-GS are similar to gliadins in sequence and solubility (Lindsay and Skerritt 1999, Delcour and Hoseney 2009, Delcour 2012).

The relative molecular weight of HMW-GS is in the range of 65-150,000. HMW-GS are subdivided into x- and y-types. X-type HMW-GS are more massive and contain 3 cysteine residues in the N-terminal domain while y-types are smaller and contain 5 cysteine residues in the N-terminal domain (Lindsay and Skerritt 1999, Delcour 2012). The HMW-GS are low in abundance but are the key determinants of dough elasticity. HMW-GS have a three domain structure with relatively small N- and C- terminal ends and a large repetitive central domain. The C-terminal domain is a well-defined sequence of 42 amino acid residues. The N-terminal domain is 80-100 residues long. The central domain is 600-850 residues long with several repetitive motifs. The N- and C-terminal domains have secondary α-helical structure (Lindsay and Skerritt 1999, Delcour 2012). The repetitive domain is high in glutamine, proline, and glycine which lead to a series of overlapping β-reverse turns. This series of overlapping turns form the super-

secondary structure known as a β-spiral (Delcour 2012). Because of this large β-spiral HMW-GS is thought to behave as a stiff, extended rod in solution. The large size of HMW-GS limit its extractability, thus the molecular weight distribution and quaternary structure of its native state is difficult to study and not well documented (Lemelin et al 2005).

LMW- and HMW-GS polymerize via intermolecular disulfide bridges. The resulting glutenin macropolymer (GMP) is an aggregate gel. GMP quantity is associated with dough elasticity and bread loaf volume (Weegels et al 1996b, Don et al 2003). Rheological testing is useful for predicting gluten strength and dough quality. While protein content does describe some variability in rheological testing, glutenin and GMP quality are better correlated with dough handling properties and end-product quality (Weegels et al 1996a; 1996b, Harszi et al 2004).

GMP can be measured as total polymeric protein (TPP) content via high performance size exclusion chromatography (HPSEC) (Gupta et al 1993, Johansson et al 2001, Kuktaite et al 2004). TPP analysis via HPSEC distinguishes protein fractions by retention time. Large polymeric proteins (LPP) elute first, small polymeric proteins (SPP) elute second, large monomeric proteins (LMP, i.e. gliadins) elute third, and small monomeric proteins (SMP, i.e. albumins and globulins) elute last. TPP is all polymeric glutenins: i.e. both HMW- and LMW-GS (LPP and SPP, respectively) (Zhang 2015). Unextractable polymeric protein (UPP) are those HMW-GS that cannot be extracted from the flour by the solvent/buffer alone but require sonication for extraction. Total unextractable polymeric protein (TUPP) is the proportion of UPP:TPP. Large unextractable polymeric protein (LUPP) is the proportion of large unextractable polymeric protein to all large polymeric protein. These are normally reported as percentages.

High LUPP content has been associated with increased dough strength and bread quality (Johansson et al 2001).

Genetic expression of GS has been studied extensively and has been mainly concerned with the HMW subunits. Expression of HMW-GS is coded by three complex loci that each code for a pair of subunits unless one gene is silenced: *Glu-A1*, *Glu-B1*, and *Glu-D1* located on the long arms of chromosomes A, B, and D respectively (Wrigley et al 2009). Variations in the *Glu-1* alleles between cultivars are correlated with different potentials for dough strength. Of the identified subunits, subunit pair (5 + 10: *GluD1d*) is considered to be one of the best in terms of quality while subunit pair (2 + 12: *GluD1a*) is considered relatively weak (Weegels et al 1996a, Harszi et al 2004, Wrigley et al 2009).

2.6.3 Gluten

Dough is created when a solvent (typically water) and mechanical energy are added to wheat flour. This cohesive dough contains a network capable of retaining gasses and is called gluten. There are several models that theorize how glutenin and gliadin come together to form this viscoelastic matrix (Weegels et al 1996a, Delcour 2012). Theories include a network model, the "loop and train" model, and a colloidal particle system (Don et al 2003, Delcour 2012)

Rheological testing procedures, e.g. the Mixograph, exist to characterize the quality of hard wheat flours. These tests measure dough strength in varying ways. The mixograph is a small-scale mixer that sits on a free-spinning base. As the dough is developed the base oscillates and a sensor (or pen and paper) read the movements over time. The resulting mixogram provides the

time it took to reach peak dough strength and how quickly the gluten matrix broke down. The extensograph measures resistance to extension (Delcour and Hoseney 2010, Delcour 2012). The farinograph provides similar results as the mixograph but also calculates optimum water absorption for the flour (Ingelin and Lukow 1999). Other tests have been developed to monitor gluten development and strength (Harszi et al 2004)

During intense dough mixing, GMP depolymerizes. GMP eventually re-polymerizes after the dough has been allowed to rest. After resting dough, GMP content appears to increase but this is due to re-polymerization limiting GMP solubility (Weegels et al 1996).

Glutenins undergo disulfide-sulfhydryl (SS-SH) interchanges during mixing. Gliadins are unable to participate because of their stable intramolecular SS bridges. During mixing or dough shaping SS bonds between glutenins are broken and reformed. Resting the dough allows the polymer network to relax and orient itself in the direction of extension (Shewry et al 2009). This polymer alignment is visible macroscopically in the shaping of dough. The gluten network continues to strengthen with increased mechanical energy until the point of failure. Continued energy will destroy the gluten network by encouraging SH-SS interchanges with compounds of lower molecular weight (Delcour 2012). Thixotropy may play a part in this as well.

Protein heat setting is crucial in the final texture of bread products. Initially, in the oven the heat my break hydrogen bonds and begin to degrade the gluten matrix. When the interior of the dough has reached 90°C the intramolecular SS bonds on α- and γ-gliadin undergo SS-SH interchange

and the molecules open up. This ring opening allows α- and γ-gliadin to form aggregates with the glutenins via SS-linkages and increase the size and strength of the gluten matrix (Delcour 2012).

Low MW proteins (albumins and globulins) are also important in dough functionality, but to a lesser degree than the gluten-forming proteins. Higher content of low MW proteins is associated with retarding re-polymerization of GMP. This occurs at such a low rate of addition (0.2% w/w) that it is unlikely to be due to simple dilution (Weegels et al 1996).

2.7 Wheat Kernel Carbohydrates

Carbohydrates account for around 85% of the wheat kernel (Chilkunda et al 2001). The majority of these carbohydrates exist as polymers. The concentration of simple sugars in a wheat kernel is <1% (Delcour and Hoseney 2010). Carbohydrates can be differentiated based on their digestibility. Starch is digestible by humans and their subsequent breakdown provides necessary calories for body and brain function. Non-starch polysaccharides (fiber) are indigestible by human enzymes but are important for gut health (Francois et al 2012).

2.7.1 Starch

Starch is the largest constituent of the human diet. It is important in providing both nutritional and functional properties to many different food products. The starch found in cereals is stored in amyloplasts with one granule in each (Delcour and Hoseney 2010. Zeeman et al 2010). This is called granular starch. Granular starch is composed of 98% polymerized α-D-glucopyranosyl subunits joined together via either α-1,4 or α-1,6 linkages for linear or branched conformations

respectively. Starch molecules contain one reducing end marked by the presence of a hemiacetal group. The two distinct types of starch found in wheat are amylose and amylopectin.

Amylose is the linear component of starch. Depending on variety and grain maturity amylose has molecular weight (MW) of 80,000 to 1,000,000. When solubilized amylose behaves like an elongated linear molecule but 25-55% of the molecules have a side chain attached via an α-1,6 linkage. In solution amylose can be either a random coil or helical. The helical shape can form clathrates with fatty acids, alcohols, and iodine (Delcour and Hoseney 2010. Zeeman et al 2010). In the starch granule amylose exists in a glassy, amorphous state. The amylose content of wheat is typically around 25% of the starch but is variable between varieties.

Amylopectin is the highly branched component of starch found in wheat. The appearance of amylopectin has been described as dendritic: 4-5% of the bonds in amylopectin are α-1,6 branch points. Amylopectin consists of three chain types. Type A is linear and is composed of α-1,4 linkages like amylose. Type B is branched and contains a linear α-1,4 portion culminating in an α-1,6 branch point. Type C also contains both α-1,4 and α-1,6 linkages with the addition of the single reducing end. Each of these chains are typically in the order of 20-30 glucoses long and the MW of amylopectin molecules can be as high as 10^8 (Delcour and Hoseney 2010, Zeeman et al 2010).

Amylose and amylopectin exist in starch granules inside the wheat endosperm (Zeng et al 2011). The granule is composed of alternating amorphous and semi-crystalline lamellae. Amylopectin is the likely contributor to granule crystallinity as crystallinity increases when amylose is leached

from the granule or as amylose content decreases (Cheetham and Tao 1998). The semi-crystalline shells have crystalline and amorphous regions of amylopectin. Short amylopectin chains intertwine into double helices forming crystallites. These crystallites align together in the crystalline portion of the lamellae. The amorphous region of the semi-crystalline lamellae is formed by the branching portions of amylopectin and possibly some amylose (Delcour and Hoseney 2010. Zeeman et al 2010). The amorphous shells are relatively less dense and are mostly made up of glassy amylose and possibly some amylopectin (Delcour and Hoseney 2010).

Starch is typically hydrated and cooked prior to consumption. Changes to starch during cooking and cooling drive starch's functional properties in foods. In heated systems with excess water starch granules undergo an irreversible process called gelatinization. At room temperature water penetrates the starch granule. The granule can hold up to 30% of its weight in water and only swell 5% at room temperature. Microscopy under polarized light conditions can observe the birefringence (indicative of periodic or ordered structure) of the starch granules; as heat is added to the system the granules eventually lose their birefringence. When birefringence is lost the granule is said to be gelatinized; this temperature is the gelatinization temperature of that granule. In a starch system granules exist over a range of sizes and shapes that gelatinize over a 7-10°C range; 50% of wheat starch granules lose their birefringence by 53°C (Delcour and Hoseney 2010, Simsek et al 2014).

An Amylograph or Rapid Viscoanalyser (RVA) can be used to monitor the change in relative system viscosity as temperature increases. Both instruments monitor relative viscosity under shearing conditions with viscosity as a function of temperature, shear force, and concentration of

the starch. After gelatinization occurs and with further heating the starch forms a paste (Simsek et al 2014, Delcour and Hoseney 2010, Abdel-Aal et al 2002). Pasting further increases the viscosity of the system. Starch granules continue to take up water and swell during heating. The granules deform and amylose leaches into the solvent. Both of these phenomena contribute to increased viscosity. When monitoring pasting with the Amylograph or RVA the heating stops at 95°C to prevent water boiling. The granules will continue to swell and release amylose as long as they are intact thus increasing system viscosity (Delcour and Hoseney 2010, Simsek et al 2014). At some point the viscosity of the system will begin to drop as granules begin to disintegrate and molecules begin to orient themselves in the direction of shear. This phenomenon is called thixotropy.

In approved starch pasting methods, after a holding period at 95°C the starch/water paste is cooled to 50°C (Crosbie et al 1999). During cooling viscosity increases quickly; this is called setback. Setback is due to the decrease in energy that allows increased hydrogen-bonding and entanglements between amylose chains to occur (Delcour and Hoseney 2010, Simsek et al 2014). Setback magnitude varies between wheat varieties.

During cooling in a static system the starch can form a gel. A gel is a liquid system with solid properties. The water is retained in the system and behaves as liquid water but it is trapped in-between junction zones. Junction zones are formed by hydrogen-bonding between amylose chains. In storage starch retrogrades. Retrogradation is the return to crystalline order. In one sense only amylopectin can retrograde, as amylose was not crystalline to begin with (Delcour and Hoseney 2010). Amylose crystallization is simply referred to as amylose crystallization. The

degree of heating and shearing during cook-up determines the properties of the cooled starch mixture. If granules are still intact and densely packed then a paste that flows instead of a gel will form. Loosely packed starch granules suspended in a predominantly amylose gel network is another potential result. If enough shear was present during cook-up then a gelled dispersion of amylose and amylopectin molecules will be left after cooling (Delcour and Hoseney 2010).

When amylose crystallizes it forms a double helix with another amylose molecule, these double helices determine the stiffness of a starch gel upon cool down. Amylose crystallization occurs above its glass transition temperature (Tg, below 0°C) and below its melt temperature (Tm, 120°C). Amylose crystallization occurs quickly during cooling from 95 to 50°C because there is a broad temperature range where crystal nucleation and crystal growth overlap (Abdel-aal et al 2002). Amylopectin has a T_g of below 0°C and a Tm of 50-60°C. This temperature range allows for little to no crystal nucleation or growth during cooling from 95 to 50°C (Delcour and Hoseney 2010, Simsek et al 2014). As the starch system continues to stand over a few days at ambient temperature, amylopectin crystals will form. Amylopectin is responsible for the long-term stiffening of cooked-up starch products in storage. As the gel ages the starch chains continue to interact and junction zones grow larger. This forces water, an incompressible fluid, out of the system in a process called syneresis (Delcour and Hoseney 2010, Simsek et al 2014).

2.7.2 Arabinoxylan

Arabinoxylan (AX) is the primary wheat non-starch polysaccharide at 6-7%, by weight in the seed. AX is made up of β-1,4-linked D-xylopyranosal subunits with α-L-arabinofuranose subunits substituted at either the second or third oxygen position (Delcour and Hoseney 2010).

The amount and location of these arabinose substitutions determine the solubility of AX. The ratio of arabinose to xylose of AX is useful in predicting its behavior. A typical arabinose to xylose ratio is around 0.5 for wheat endosperm. Wheat seed endosperm cell walls are primarily composed of AX. The endosperm is 1-3% AX by weight. Differences in AX composition are found between cultivars (Finnie et al 2006). Ferulic acid moieties are esterified to the substituted arabinoses (Ramseyer et al 2011).The differences in AX composition drive solubility and functionality. Soluble AX is called water-extractable arabinoxylan (WEAX) and insoluble AX is called water-unextractable arabinoxylan (WUAX). WUAX may also be insoluble due to crosslinking of ferulic acid residues in cell walls and under certain other conditions such as oxidation.

Under oxidative conditions WEAX can form weak, ramifying gels via diferulic acid bridges. This is called oxidative gelation (OG) and it may have quality implications in wheat quality. This may be specifically useful for soft wheats as these are destined for products that do not rely on the development of an extensive gluten network. (Bettge and Morris 2007, Delcour and Hoseney 2010). OG capacity (OGC) is a genetic trait that occurs strongly in some varieties, mildly in some varieties, and not at all in others (Finnie et al 2006, Mattson 2014). Oxidative gelation occurs in the presence of free radicals (Ramseyer et al 2011, Ross et al 2014). Measuring OGC can be done in an RVA. Initial flour viscosity is measured and then hydrogen peroxide is added to encourage the reaction to occur. A sharp increase in viscosity is indicative of high OGC (Ross et al 2014).

OGC has been postulated as a useful metric for flour quality. It has been used to describe the variation in quality between different mill streams (Ramseyer et al 2011). It has been estimated to be of use in batter-based product quality as well. Batter viscosity is important in soft wheat products, as gluten matrix is not developed. OGC may play a major role in batter product quality by thickening batters and trapping gases produced in leavening. This could lead to higher cake volume. OGC has been theorized to reduce cookie diameter and postulated to reduce spread in pancakes. (Bettge and Morris 2007). However, recently OGC was found to be not correlated with several metrics for pancake quality including diameter and spreading (Fajardo and Ross 2015).

2.8 Wheat Lipids

Wheat has lipids (Ross 2015, personal correspondence). The wheat grain is roughly 3% crude fat by weight. The relative abundance of lipids in wheat meal is 70% nonpolar lipids, 20% glycolipids, and 10% phospholipids (Delcour and Hoseney 2010). The anatomical structure with the highest lipid content is the germ (28% by weight). The aleurone layer is roughly 8% lipids and the bran is roughly 5% lipids (Delcour and Hoseney). The germ, aleurone, and bran are removed when milling refined flour. This is helpful in preventing rancidity in refined flour, contributing to its storability (Doblado-Maldonado et al 2012). The endosperm and pericarp are least abundant in lipids at around 1% by weight (Delcour and Hoseney 2010). Whole meal flour rancidity is caused by hydrolytic lipases, oxidative lipoxygenases, and autoxidative activity in presence of atmospheric oxygen (Doblado-Maldonado et al 2012). Hydrolytic rancidity occurs first and then is followed by oxidative rancidity. Enzymatic and non-enzymatic oxidative rancidity operate via different mechanisms but create hydroperoxides via the addition of oxygen

to a polyunsaturated fatty acid (Doblado-Maldonado et al 2012). This produces several classes of volatile compounds with negative sensory and end-use quality consequences (Sjovall et al 2000, Doblado-Maldonado et al 2012). Vitamin E, or tocopherol, is a lipid of primary nutritional concern in wheat meal. Tocopherols serve as antioxidants and are found at levels of 3-10 µg/g in wheat meal, primarily in the germ (Moore et al 2005, Zhou et al 2005).

2.9 Wheat Enzymes

Wheat enzymes tend to be from the albumin and globulin wheat protein classes. Enzymes are important for a variety of functions in the caryopsis. Wheat enzyme utility in food processing may be positive or negative depending on the level of enzyme activity present or processing situation. Enzymes are primarily concentrated in the aleurone layer of the endosperm.

2.9.1 Starch-degrading Enzymes

Wheat has two main starch degrading enzymes, α-amylase (αA) and β-amylase (βA). These enzymes are responsible for starch liquefaction (αA) and saccharification (βA).

Liquefaction decreases starch viscosity (making it more liquid) via enzymatic hydrolysis at α-1,4 linkages randomly along starch backbone (Delcour and Hoseney 2010). Because αA acts upon the middle of the starch chain it is called an endoamylase. Liquefaction is achieved by reducing degree of polymerization (MW) of starch chains thus lowering system viscosity. The hydrolysis products from starch by αA are called dextrins. Optimum pH for αA activity is around 5.3 (Delcour and Hoseney 2010). Wheat seeds are deficient in αA until they are sprouted unless they are of a variety that expresses late maturity αA (LMA) production (Mares and Mrva 2008,

Wrigley et al 2009). Excessive αA activity is a defect in wheat as it degrades end use products (Ross and Bettge 2009, Wrigley et al 2009) but a small and measured amount of αA activity is necessary for the production of fermentable sugars in lean (no added sugar) doughs. To achieve this wheat processors prefer to start with effectively no αA and then add a prescribed amount to achieve desired processing outcomes. Each αA molecule requires a calcium ion to function. If replaced with a silver ion it deactivates αA functionality (Crosbie et al 1999). Starch is most susceptible to αA attack after gelatinization and pasting. However, αA can hydrolyze granular starch given enough time. This is primarily seen in sprouted grains. Sprouted grains produce more than enough αA to hydrolyze all starch contained in the endosperm, but the slow diffusion of αA into intact starch granules is rate-limiting. αA can act upon starch from damaged granules post-milling but the majority of fermentable disaccharides are produced by the combined action of αA and βA.

Saccharification, by βA, is the production of maltose (a disaccharide) via enzymatic attack from the non-reducing end of starch chains. Maltose sugar is important as an energy source during microbial fermentation. βA is found in sound grain but does not act upon intact starch granules (Delcour and Hoseney 2010). The pH optimum of βA is 5.5 and βA is less heat-stable than αA (Delcour and Hoseney 2010). βA relies on αA to produce non-reducing ends for βA to hydrolyze.

2.9.2 Protein-degrading Enzymes

Protein degrading enzymes are called proteases. Proteases break down storage proteins upon germination. Typically these proteins are endoproteases that break large proteins into smaller

polypeptides. These smaller polypeptides are then susceptible to degradation by exoproteases and peptidases. Protease content is low in sound grain but proteases are synthesized at high levels upon germination (Wrigley et al 2009). Fungal infection may also serve as a source of protease in wheat (Carson and Edwards 2009). Proteases may be used as a dough improver, though excess protease content in wheat flour has been associated with decreased processing-intermediate functionality and end-product quality in both bread and noodles (Carson and Edwards 2009, Wrigley et al 2009).

2.10 Pre-harvest Sprouting

Pre-harvest sprouting (PHS) can be a problem. PHS occurs when mature seeds in the wheat spike germinate prior to harvest because of high humidity or rainfall. Physical indicators of PHS include kernel swelling, germ discoloration, seed coat splitting, and visible root or shoot protrusion in extreme cases. Wheat is graded as unacceptable for milling into flour if more than 4% of the seeds are visibly sprouted. However, PHS can be present in the absence of visible cues (Ross and Bettge 2009, Simsek et al 2014). Grain that has been affected by PHS is sold at a significant discount (20-50% price reduction) and negatively impacts farmer's incomes and the prosperity of rural communities. It is not only a farmer problem; sprout damaged wheat reduces yield and quality at the mill and decreases product quality in processing facilities. The primary culprit that leads to the poor processing outcomes of PHS damaged wheat is excessive alpha-amylase (αA) activity. PHS is a genetic trait that is linked to seed coat color via "coat-imposed embryonic pathways regulated by separate genetic systems" (Simsek et al 2014). In general, red seed coat color is correlated with a higher degree of sprout tolerance. Falling Number can be used to determine genetic predisposition to PHS tolerance. Genotypes have been identified as

tolerant to PHS (Nornberg et al 2015). While PHS can be bred for, even PHS-tolerant cultivars will sprout in right conditions (Van Eeden and Labuschagne 2012).

Sprout damage is detected in industry using the Perten Falling Number (FN) instrument (AACC International Method 56-81.03). This test uses the viscosity of a hydrated wheat meal sample as a proxy for determining αA activity. A 300 gram, representative sample of wheat kernels is collected and milled to a meal of determined particle size. Seven grams of this meal is hydrated in 25 mL of water and shaken mechanically. A metal plunger is inserted into the tube and immersed into a boiling water bath. Within 5 seconds the machine begins to mix the sample by moving the plunger up and down for 60 seconds. After 60 seconds the plunger is drawn to the top of the sample and is allowed to free-fall through the flour suspension while the timer continues to count up. When the plunger reaches the bottom of the tube the timer stops and reports FN in seconds, this reading includes the initial 60 seconds of mixing. The time between the insertion of the tube into the boiling water bath and the time it takes to reach the temperature at which αA is denatured is purported to be sufficient time for αA to degrade enough starch to significantly decrease the viscosity of the suspension. Given the rapid rate of temperature increase the FN test is remarkably insensitive to the low levels of αA activity present at the critical cut-off between what buyers consider acceptable and unacceptable. There is a curvilinear relationship between FN and αA activity. (Perten 1964). Low FN has been observed in soft white winter wheats in the absence of high αA activity, primarily in wheats with low protein content (Ross et al 2012). Direct measurement of αA activity is a more reliable metric of PHS but is relatively more time and labor intensive than FN.

Simsek et al (2014) described the effect of PHS on the physiochemical properties of wheat starch and found that PHS causes several changes. Starch from PHS damaged wheat had reduced water binding capacity and paste stability likely due to degradation of amylose and amylopectin chains. Starch granules from sprouted wheat also lost their resistance to swelling in presence of high αA activity. Comparing sound and sprouted samples of the same variety showed a decrease in high molecular weight amylopectin (HMW-AP) and corresponding increase in low molecular weight amylopectin (LMW-AP) and amylose (AM). This increase was not due to new synthesis of LMW-AP and AM but as a likely outcome of HMW-AP hydrolysis via αA. Although AM concentration went up slightly the average MW of AM was reduced by one order of magnitude. This MW decrease is a likely cause for reduced pasting viscosity in PHS damaged wheat starch. Scanning electron microscopy (SEM) was used to visually compare the starch granules of sound and sprouted wheat. The sound samples showed intact granules suspended in a dense protein matrix as expected but sprouted samples exhibited pitting on the surface of the granules. Not only starch was affected; SEM showed that the storage protein matrix was significantly degraded in sprouted samples as well. Huang and Varriano-Marston (1980) reported that proteolytic enzymes degraded the protein matrix surrounding the granules, making the granules more susceptible to αA attack. Physiologically, the degradation of protein and starch provides the only source of amino acids and carbohydrates to the developing embryo prior to photosynthesis.

It has been postulated that post-harvest grain storage may alter FN over time. Abid et al. (2009) tracked FN in six commercial wheat varieties over 12 months; all six varieties showed a decrease in FN over the course of the study. Another study compared FN over time on the incoming wheat stream from five producers feeding a production mill in two harvest years. Wheat storage

temperature was uncontrolled but was generally observed to be between 20-25°C. In the 2001 harvest year they observed a slight increase in FN over 11 months but in 2002 they observed inconclusive fluctuations (Hruskova et al 2004). They cited the inhomogeneity of the incoming wheat stream as a potential source of error.

2.11 Grain Storage

Wheat is stored for a variable period of time after harvest. The amount of time wheat is stored is, mostly, dependent on the supply remaining from previous harvests. Wheat quality has been shown to change over storage time. It is a common practice for millers to blend 5-15% newer wheat into older wheat and gradually increase as stock changes to current crop year (Posner and Deyoe 1986).

It is commonly accepted by industry that wheat milling properties change over grain storage time. The preference is for wheat that has been stored for at least 2-3 months post-harvest prior to milling (Posner and Deyoe 1986, Gonzalez-Torralba et al 2013). Flour milled from freshly harvested wheat has poorer baking qualities when compared to wheat that is stored for several weeks (Posner and Deyoe 1986, Gonzalez-Torralba et al 2013). After a brief storage period, baking qualities increase and then degrade only slowly. Specifically, loaf volume has been shown to peak in flour from grain stored up to 120 days (Posner and Deyoe 1986). This may be due to gluten polymers stiffening and losing rotational freedom of peptide bonds over grain storage, (Pinzino et al 1999). Changes in protein solubility occur during storage (Gonzalez-Torralba et al 2013). Increased protein extractability may influence the functional properties of dough.

Protein content has been observed to increase over grain storage time. High temperature and low humidity storage discourage H-bonding and allow proteins to be more easily solubilized. This may manifest as an artificial increase in protein content (Gonzalez-Torralba et al 2013). Enzymatic reactions occur in wheat kernels during storage. For example, lutein esterifies to linoleic or palmitic fatty acids. Carotenoids, like lutein, act as antioxidants during grain storage, scavenging radicals (Fleurat-Lessard 2002). Sharp decline in carotenoids after six years of storage has been observed. Free radicals are simultaneously produced and scavenged *in situ*. Decline in enzymatic activity occurs slightly but insignificantly over storage time as well (Fleurat-Lessard 2002).

As grain is stored post-harvest, flour quality parameters change. Increases in extraction, particle size, patent flour extraction, and water absorption are observed in flours from stored grain (Posner and Deyoe 1986). Break flour percentage increases until 21 weeks of storage. Flour extraction and other qualities that increase over storage time are associated with higher profits for millers (Posner and Deyoe 1986).

It is accepted by industry that post-milling flour aging is important for bread quality as well (Posner and Deyoe 1986). Aging flour is different than storing grain. Higher surface area and moisture content can encourage changes to occur more quickly than would happen in grain. Whole meal and refined flour quality changes over time. Whole meal quality degrades at a higher rate than refined flour. This is a primarily a function of the fatty acid content of the germ.). Aging of whole meal flour at 4°C and at 20°C failed to produce significant changes in

FN and gruel viscosity (Zarzycki and Sobota 2015). OGC and Solvent retention capacity (see Section 2.12) have been shown to change as soft wheat is stored and flour is aged (Mattson 2014).

Changes in grain quality can be difficult to monitor and predict. Studies on grain aging have been conducted since at least the 1970's (Delouche and Baskin 1970). Studies using different temperature and humidity levels have shown different changes in the same quality parameters. Posner and Deyoe (1986) proposed a model to predict cost-benefit analysis of storing grain to maximize increases in profit and minimize cost of storage. Suggest that after 14 weeks of storage (98 days) the cost of storage begins to outweigh revenue increases from quality parameters.

Well-designed silos have the ability to adjust temperature, humidity, and aeration. Heat and CO_2 are produced in silos in presence of moist grain, loss of dry material in wheat. Models can predict change in nutritional degradation and losses due to infestations or infections. No models predict enzyme degradation. (Fleurat-Lessard 2002). Wheat storage in commercial-scale grain silos at different temperature and RH conditions are associated with changing grain quality parameters over time. Protein content degraded over time, either as a result of decreased solubility or due to fungal metabolism. There was an increase in aflatoxin content over storage time, specifically in higher humidity samples. GMC changed over time depending on RH. FN increased significantly in wheats stored at higher temperature and lower RH. Wheats at low temperature and high RH showed slight decrease in FN over time. There were also slight decreases in test weight and ash content (Mhiko 2012).

High temperature and RH storage conditions are, in general, associated with detrimental effects on grain quality (Gonzalez-Torralba et al 2013). However, for FN this may not be the case. FN has been shown to increase over grain storage time (Gonzalez-Torralba et al 2013). The rate of increase was accelerated with higher temperature but not humidity. High humidity conditions increased kernel moisture content over storage time. Test weight decreased over time, particularly in low humidity conditions. This likely occurred as a result of moisture loss.

2.12 Solvent Retention Capacity

The Solvent Retention Capacity (SRC) test uses four different solvents that selectively swell the polymeric components of wheat flour. The mass of the resulting gel is used to predict flour performance in a variety of end products (Slade and Levine 1994, Kweon et al 2011). The SRC method was developed in 1980's at Nabisco to predict cookie and cracker performance of soft wheat flours. It was developed to assist and replace existing rheological tests like the alveograph, extensograph, and farinograph. The desire to replace rheological tests came from the assertion that rheological tests only examine the cumulative effect of the polymeric components in wheat flour. Slade and Levine desired to examine the individual contributions of the major polymers to predict their influence on processing-intermediate functionality and end-product quality (Kweon et al 2011). The likely inspiration for the concept of the SRC method is the alkaline water retention capacity test (AWRC). The AWRC test used weakly alkaline solution to predict cookie spreading, high AWRC values are associated with poorly spreading cookies (Ross and Bettge 2009).

Rheological testing is based on kinetics, where a solvent is used in limited quantity to plasticize polymeric flour components and allow them to form a covalently-linked network. The SRC method, in contrast, is based on energetics. In energetics, excess solvent is employed to solvate and swell polymeric flour components which then subsequently entangle. As such, any element that introduces shear to the system violates the principal of the method. Energetics is based on solvent-polymer thermodynamic compatibility. Solvent-polymer compatibility is quantified using the Hildebrand solubility parameter, with higher compatibility indicating a higher goodness of fit of the solvent to the polymer (Kweon et al 2011). Solvent compatibility is necessary when solvating polymers because, unlike small molecules, they do not readily dissolve in excess solvent. Solvated polymers swell and form gels. The extent of polymer swelling can be characterized by measuring changes in length, weight, or volume. The SRC test relies on gel weight as a measure of polymer swelling (Slade and Levine 1994, Kweon et al 2011).

In SRC methodology, the four solvents used are deionized water (WSRC), 5% w/w aqueous lactic acid (LASRC), 5% w/w aqueous sodium carbonate (NaSRC), and 50% w/w aqueous sucrose (SucSRC). Lactic acid preferentially swells glutenin subunits, sodium carbonate swells damaged starch, and sucrose swells arabinoxylan (AX) and gliadins. Water is used as the reference solvent as it hydrates all of the polymeric components of wheat flour, but to a lesser degree than the other solvents (Ross and Bettge 2009). Flour is weighed into centrifuge tubes and a mass of solvent five times the weight of the flour is added, i.e. five g flour and 25 g solvent. The sample is shaken to fully suspend flour into solvent. The tube is then agitated periodically by hand or constantly via a gentle mechanical shaker for exactly 20 min. After 20 min, the tube is centrifuged at 1,000 RCF for 15 min. The tube is then drained up-ended at 90° for exactly 10

min. The resulting gel pellet is weighed and %SRC is reported on a 14% moisture basis (AACCI approved method 56-11.02). The 5:1 solvent-flour ratio is important to keep the polymers accessible (but not extractable) in order to form a swelled and solvent-holding network that will hold up against the force of centrifugation. Excess solvent is also important to avoid influence of kinetic effects during shaking and agitation steps (Kweon et al 2011). The use of smaller scale SRC tests have been explored and found useful (Bettge et al 2002). SRC on whole meal wheat has also been identified as a useful tool in identifying quality wheat cultivars at early generations when sample is limited (Ram and Singh 2004).

SRC is useful as method of predicting baking quality for breeders, millers, bakers, and researchers. End-product testing is sample, time, and labor intensive. Small-scale, high-throughput tests are important for efficiency in processing, research, and breeding programs. SRC profiles are highly correlated with cookie and cracker quality. For example, a desirable SRC profile for a flour destined for cracker processing would ideally have low water holding capacity, high gluten strength, a low amount of damaged starch, and be low AX. Practically, an SRC profile would be <51% WSRC, >87% LASRC, <64% NaSRC, and < 89% SucSRC (Kweon et al 2011). Another use of SRC is confirming flour quality from different mill streams, particularly when blending wheats from other locations or cultivars (Kweon et al 2011).

While initially designed to be a tool for soft wheat quality, SRC has been applied to predicting hard wheat quality as well (Ram and Singh 2004, Ram et al 2005, Xiao et al 2006, Kwan et al 2011, Jayaram et al 2014). The solvent of interest for hard wheat quality is lactic acid, as LASRC favors the swelling of glutenin subunits. Glutenin quality and functionality has been described

above (see section 2.6.2 and 2.6.3). Ram et al (2005) showed that grain protein content and LASRC of refined flour explained a large amount of variability in farinograph water absorption. The gluten performance index (GPI) has been proposed as a variation on SRC interpretation for predicting hard wheat quality (Kweon et al 2011). GPI = LASRC / (NaSRC + SucSRC). The goal of this method is to reduce the confounding effect of water being a major component of all three other solvents.

2.13 Breeding Programs

It is the goal of wheat breeders to produce distinct cultivars that meet the demand of the market and population. Wheat breeding involves a number of different strategies like pedigree selection, bulk selection, doubled haploid production, and backcrossing. The idea behind wheat breeding is to start with numerous crosses from germplasm (either within or from outside one's program) and ultimately release named commercial varieties. This relies on greenhouse and field trials and high throughput quality testing to make the decision on which of the thousands of progeny to keep and which ones to throw away. After each subsequent generation more sample is planted and more sample is produced. This allows for different quality metrics to be applied. Eventually lines reach field trials that are sown across multiple growing environments. Most lines are dropped from the program based on agronomic characteristics like height, lodging, and disease susceptibility. Those that are harvested go to a quality lab where fast decisions have to be made based on limited quality information. These decisions have to be particularly fast for winter wheat breeding programs where the turnaround between planting and harvest is short. Any method that can accurately predict wheat end-product quality, either positive or negative, with a

limited amount of samples (early generations) can help save the breeding program time, money, and field space. (Baenziger and DePauw 2009).

Wheat breeders are always looking for ways to screen wheats for quality at earlier generations However, early generation screening means limited sample is available and later generation research plots are expensive and labor intensive. LASRC has been explored as method for predicting dough strength (Ram et al 2005). These authors used the one gram of flour or whole meal version (Bettge et al. 2002) and thus is appropriate for early generation screening. LASRC was shown to have a strong positive correlation with peak mixing time and storage modulus of dough in dynamic rheometry. Whole meal LASRC was useful, but the correlation not as predictive of loaf volume as the refined flour version (Ram et al 2005). Ram and Singh (2004) also showed whole meal LASRC to have a weak but significant correlation with refined flour LASRC ($r = 0.50$, $p < 0.001$). In the Ram and Singh study LASRC was negatively correlated with cookie diameter and width/thickness ratio. This is of value to know as it has shown that greater amounts of HMW glutenins are also associated with reduced cookie diameter (Ohm et al. 2009).

Chapter 3: The influence of storage time and temperature on Falling Number in sprouted and unsprouted wheats

Mike R. Adams and Andrew S. Ross

Abstract

Pre-harvest sprouting (PHS) is a problem that can reduce wheat end-product quality. Falling Number (FN) is the primary test used by industry to gauge PHS damage in wheats. The objective of this study was to determine if FN of wheat samples changed during storage and if changes were a function of storage time, storage temperature, and degree of PHS damage. Sixteen wheat varieties (ten soft, six hard) from three Idaho locations were used. Samples captured a wide range of FN values. Samples were stored at -20°C, +20°C, and +40°C and assessed for FN after 0, 14, 30, 60, and 90 days of storage. Changes in FN were observed over grain storage time. FN was different between growing environments, wheat varieties, and storage temperatures. Highest rates of increase in FN were observed in hard wheats with high initial (day 0) FN values. Lowest rates of increase in FN were observed in soft wheats with low initial day 0 FN values. Increases in FN values occurred at a higher rates as storage temperature increased, particularly in hard wheats with high initial FN. Grain storage successfully raised FN to values > 300 s in very few cases. Total polymeric protein (TPP) content was also assessed at the end of the study to see if FN increases in unsprouted hard wheats stored at +40°C were a result of changes to gluten proteins. TPP content was assessed as % large unextractable polymeric proteins (%LUPP) and % total unextractable polymeric proteins (%TUPP) using size exclusion high performance liquid chromatography. TPP content was not significantly different between storage temperatures when growing environment was kept constant. %TUPP, but not % LUPP, was significantly lower in

wheat varieties affected by PHS. Changes in FN at high storage temperature were not likely a result of increased protein crosslinking.

3.1 Introduction

Pre-harvest sprouting (PHS) appears to be an increasingly frequent problem in the U.S. Pacific Northwest (PNW). Rain prior to harvest germinates wheat in the field, which leads to increased alpha amylase (αA) activity in the intact caryopsis, as well as the de novo synthesis of other hydrolases: e.g. proteases (Simsek et al 2014). PHS can reduce both the functionality of processing intermediates and the quality of end-products. Wheat is visually inspected for signs of PHS upon delivery and is graded as unacceptable for milling purposes if more than 4% of the seeds are visibly sprouted (Simsek et al 2014). However, PHS can be present in the absence of visible cues (Ross and Bettge 2009). Falling Number (FN: AACCI Approved Method 56-81.03) is the standard analytical method performed at grain elevators when farmers deliver their wheat. A FN of > 300 s is presumptive evidence of low αA activity in the grain. Wheat with a FN < 300 s is presumptive evidence of excessive αA activity in the grain and is considered poor quality by processors. In consequence, grain testing with FN < 300 s leads to discounted prices paid to farmers (Hareland 2003). This is a serious problem for farmers and exporters in the PNW. While FN is a useful tool for grading, it strictly measures the enzyme's effect on the starch endogenous to the grain as opposed αA activity directly.

Direct measurement of αA activity is the reference method for framing all other measures of PHS. At αA activities < 0.1 Ceralpha units (CU: AACCI Approved Method 22-02.01) αA activity in the grain is sufficiently low so that it does not cause processing problems. The main

drawback of using αA activity as an index for PHS is that it is time consuming and both labor and cost intensive. As αA is the primary enzyme related to reduced end-product quality, effort has been spent to either reduce αA activity in PHS-affected wheat or mitigate its effects. For reduction, Hareland (2003) found that removing the bran via pearling wheat increased FN and decreased αA activity, a likely result of removing the enzyme-containing aleurone layer. However, pearling had no effect on dough characteristics (as indexed by Farinograph stability), bread loaf volume, or crumb characteristics as compared to non-pearled controls (Hareland 2003). This suggests pearling may not be a viable option to improve PHS-affected wheat processing characteristics and other methods must be explored.

Post-harvest grain storage has been suggested as another way to reduce the effects of PHS damage. Lunn et al (2000) cited anecdotal evidence that suggested that increased FN during storage was a result of inactivation pericarp-derived αA. The effect of grain storage on FN has been explored before. A number of studies have observed increased FN during grain storage. Harvest year, storage temperature, growing environment, and grain moisture content were reported to have significant effects (Lukow et al 1995, Hruskova et al 2004, Karaoglu et al 2010). Brandolini et al (2010) also observed an increase in FN with increasing storage time and temperature. However this was in stored wheat flour and not intact grain. On the contrary, Abid et al (2009) observed decreases in FN to < 300 s in six wheat varieties over 12 months of storage, but this was also done with flour as opposed to intact grain. Therefore, there is a lack of consensus on the changes in PHS-affected grain during storage.

Some of the deleterious effects of PHS can be observed in the intact grains which result in reduced end-product quality. For example, wheat starch physiochemical properties are modified in presence of PHS (Simsek et al 2014). PHS was shown to decrease high molecular weight amylopectin (HMW-AP) content of wheat starch with a concurrent increase in low molecular weight amylopectin (LMW-AP) and amylose (AM) via enzymatic degradation (Simsek et al 2014). In PHS the enzyme αA is an index of sprout damage but there are also increases in, for example, protease enzymes that can also affect end-product quality. Degradation of storage proteins in PHS-affected wheat has been observed and grain protein content has been identified as a factor modulating FN (Huang and Varriano-Marston1980, Ross et al 2012). Storage protein degradation increases the amount of free amino acids in wheat processing intermediates that participate in Maillard browning with dextrins. This further promotes crumb discoloration and may promote overly dark crust colors as a result of the increase in brownable sugars, primarily maltose.

The common thread missing in many of the studies cited above are observations of changes in intact grains, with varying levels of sprout damage, across different relevant storage temperatures, over practical storage durations. The goals of this study are as follows: to observe if FN and αA activity of intact grain are altered by storage, if changes in FN or αA activity are affected by storage temperature, if changes during grain storage are effective in increasing wheat from poor to acceptable FN values and αA activity levels, and if potential changes in FN and αA activity are affected by the degree of PHS. We hypothesize that the extent of mitigation will be a function of wheat variety, growing conditions, storage time and temperature, and the extent to which the grain has been damaged.. In addition, total polymeric protein (TPP) content was also

investigated to observe any possible changes in gluten proteins over storage time and in the presence or absence of sprout damage.

3.2 Materials and methods

3.2.1 Materials

Wheat samples

Ten soft and 6 hard wheat varieties from were selected three Idaho locations (Aberdeen, Parma, and Kimberly) were screened for FN and αA activity. Samples from Aberdeen and Kimberly were obtained from research plots planted, maintained, and harvested by the University of Idaho Agronomy Program. Samples from Parma were obtained from research plots planted, maintained, and harvested by the Oregon State University Wheat Breeding Program. Varieties and locations were chosen to capture a range of FN values from poor to acceptable. The location Aberdeen was harvested on August 11, 2014 and received on October 6, 2014. Parma was harvested on August 3, 2014 and received on September 25, 2014. Kimberly was harvested on August 20, 2014 and received October 3, 2014. After harvest, samples were stored indoors at ambient temperature and humidity in plastic tubs (Parma) or paper bags (Aberdeen and Kimberly). Upon receiving, samples were screened for FN and αA activity within 5 days. Samples were then subdivided and stored at -20°C, +20°C, and +40°C in zip-closure plastic bags in a temperature controlled cabinet. Temperature and relative humidity were continuously monitored over storage time.

Milling

For each day of testing 25 g of each wheat sample was pulverized in a Perten Laboratory Mill (LM 3100, Perten Instruments, Inc., Springfield, IL) with a 0.8 mm screen. All samples were milled 24 hours before testing. Frozen subsamples were allowed 12 hours to equilibrate to room temperature before milling.

3.2.2 Methods

Falling Number Analysis

FN analyses were performed in duplicate after 0, 14, 30, 60, and 90 days of storage for each of the three storage temperatures. FN was performed according to the AACCI approved method 56-81.03 using a FN apparatus (FN 1700, Perten Instruments, Inc., Springfield, IL) with one modification. Samples of 25 g, rather than the standard 300 g, were milled as a result of a limited supply of sample. Samples of wheat meal (7 g) were added to Perten Falling Number tubes followed by 25 ml deionized water. Tubes were stoppered and placed into a Perten Shakematic (SM 1095, Perten Instruments, Inc., Springfield, IL) to shake (3 s) and to hydrate wheat meal; tubes were inverted once after mixing to ensure all wheat meal was suspended. FN plungers were used to scrape down residual slurry on stoppers and upper part of tubes and then placed into tubes. Tubes were placed into boiling water bath of the FN apparatus. Within 5 s the apparatus engaged the plungers and began mixing the wheat meal suspension using an up and down oscillating motion. After 60 s of mixing, the plungers were automatically lifted to the top of the tubes and released. The plungers were allowed to fall freely through the samples and upon reaching the bottom of the tubes the timers were automatically stopped. The numbers on the

timers are the FN values and include the initial 60 s mixing step. FN values were reported as is (without moisture correction).

Total Polymeric Proteins

TPP analysis was performed using a two-stage sodium dodecylsulphate (SDS) extraction adapted from Gupta et al 1993. The first extraction yields SDS-extractable proteins and the second extraction yields the SDS-unextracatable proteins (Kuktaite et al 2004, Batey et al 1990, Singh et al 1990a, Singh et al 1990b). Wheat meal (0.01 g) was suspended in 1 mL 0.5% SDS at pH 6.9, vortexed for 5 s, and then centrifuged (16,000 RCF) for 20 min. The resulting supernatant was filtered (0.45 µm, Samplicity Filtration System, Thomson Instrument Company, Oceanside, CA) into HPLC vials. The remaining pellet received 1 mL of the SDS buffer and was resuspended. The resuspended samples were then sonicated (30 s, amplitude 5) to extract the insoluble polymeric proteins, centrifuged for 20 min at 16,000 RCF, and the resulting supernatant was filtered (0.45 µm, Samplicity Filtration System, Thomson Instrument Company, Oceanside, CA) into HPLC vials (Gupta et al 1993). Wheat protein was fractionated via size-exclusion high performance liquid chromatography (SE-HPLC) in a Waters 2695 Separations Module using a Phenomenex BioSep-SEC-s4000 column (5 µm particle size, 500 Å pore size) with a KJO-4282 guard column and AJ0-4489 cartridge. The detector was a Waters 2996 Photodiode Array detector set at $\lambda = 214$ nm (Kuktaite et al 2004). Separation was achieved in 15 minutes by loading 10 µl of sample in elution conditions of a 1:1 ratio 50% (v/v) acetonitrile and deionized water containing 0.1% (v/v) trifluoroacetic acid (TFA) with a flow rate of 0.45 ml/min (Johansson et al 2001). The chromatograms were integrated at 4.85, 5.55, and 7.80 min (Zhang 2015: Figure 3.1) which divided the chromatogram into 4 main sections of decreasing molecular

size: HMW-glutenin (A), LMW-Glutenin (B), gliadins (C), and albumins + globulins (D) in the first (a) and second (b) extractions. The percentage of large unextractable polymeric protein (%LUPP) was calculated as [A2 / (A2 + A1)] x 100. The percentage of total unextractable polymeric protein (%TUPP) was calculated as [(A2 + B2) / (A1 + B1 + A2+ B2] x 100.

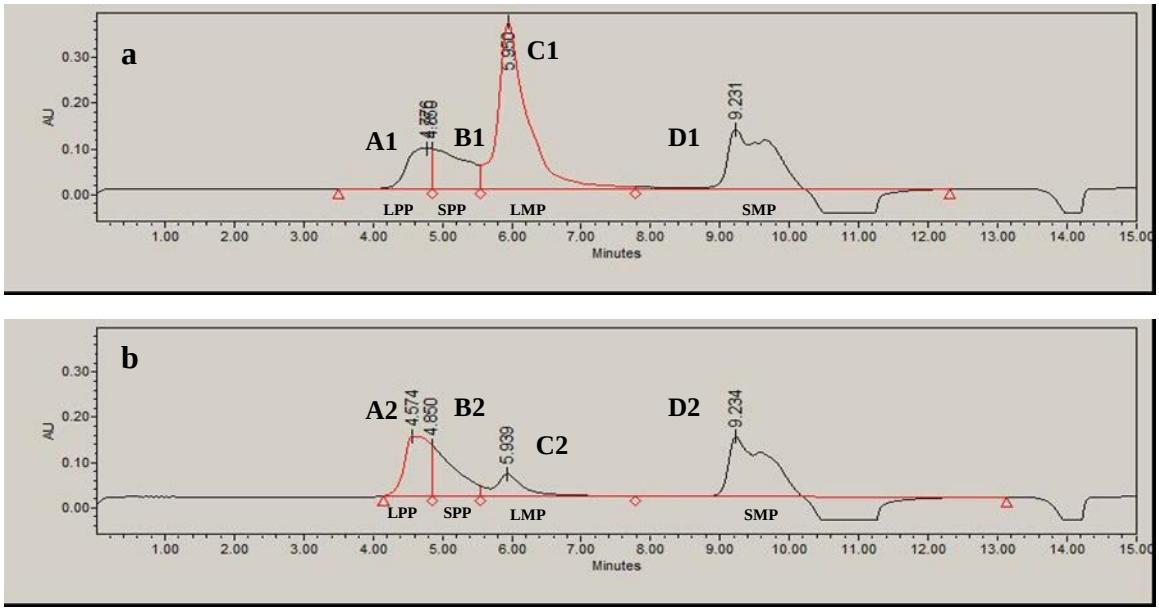

Figure 3.1 - Representative SE-HPLC chromatograms (a) First extraction: SDS-extractable proteins and (b) Second extraction SDS-unextractable proteins. Chromatograms were separated into four parts including large polymeric protein (LPP), small polymeric protein (SPP), large monomeric protein (LMP), and small monomeric protein (SMP). A1 and A2 represent the LPP fractions, B1 and B2 represent the SPP fractions, C1 and C2 represent the LMP fractions, and D1 and D2 represent the SMP fractions (Zhang 2015).

Statistical Analysis

Multifactor analyses of variance and correlation analyses were carried out using JMP 11 (SAS Institute Inc., Cary, NC). Statistical significance was set at $p < 0.01$.

3.3 Results and Discussion: Soft wheats

Summary statistics for soft wheat FN results are shown in Table 3.1. The full-factorial four-way ANOVA showed that FN varied significantly between growing locations, wheat varieties, and days and temperatures of storage (Table 3.2). F-ratios from the ANOVA indicated that environment had the largest influence on FN, followed by variety and storage time. Storage temperatures were less influential. All two-way interaction terms were significant. The largest F-ratios were for location*variety and temperature*storage time interactions.

Table 3.1 shows that the location Kimberly had both the lowest absolute FN value and the lowest mean FN value of the 3 locations. This indicated that Kimberly was likely to have been affected by PHS. This was expected as our collaborators at the University of Idaho indicated a noteworthy rain event after grain reached physiological maturity. Parma had the highest mean FN of the three locations. The lowest FN value observed at Parma was 289 s, which is

suggestive, but not diagnostic, that PHS may have affected this sample (αA activity for this sample (see Section 4.1) suggested otherwise). Aberdeen had the highest maximum FN value, although this location's mean FN was lower than that of Parma. Parma was harvested by our own field team and was harvested prior to the rain event. Aberdeen had a minimum FN value of 221 s indicating some degree of PHS. Aberdeen also had a rain event prior to harvest.

Table 3.1 - Summary Statistics of soft wheat Falling Number values (s).

Location	**Min**	**Max**	**Mean**	**SD**	**SE**
Parma	289	461	353	29.8	3.4
Aberdeen	221	515	336	63.0	3.4
Kimberly	83	399	204	71.9	3.3
Variety					
Skiles	284	476	363	42.9	8.3
OR2090473	241	488	332	55.9	8.4
Mary	202	515	331	80.4	8.3
Bobtail	186	475	311	72.6	8.3
Rosalyn	209	472	309	63.8	8.4
WB 1070 CL	149	461	295	91.2	8.3
SY Ovation	227	388	291	37.9	8.3
Kaseberg	125	423	280	99.5	8.3
LCS Biancor	96	389	236	99.1	8.3
LCS Artdeco	83	394	225	108	8.3
Temperature					
-20°	83	405	290	84.5	5.1
+20°	83	422	291	84.9	5.1
+40°	83	515	310	93.7	5.1
Storage Time (Days)					
0	83	391	279	79.2	6.5
14	83	412	289	82.7	6.5
30	85	412	298	86.0	6.5
60	84	447	307	92.6	6.6
90	85	515	314	96.1	6.5

All wheat varieties had maximum FN values of > 300 s. This indicated either no evidence of PHS at one or more locations, or increased FN over storage time. All wheat varieties had minimum FNs of < 300 s suggesting that they were all affected by PHS at one or more locations. The variety Skiles had the highest mean and minimum FN values among all varieties suggesting either low susceptibility to PHS, or that it may be later maturing variety. LCS Biancor and LCS Artdeco had the lowest mean FN values. This suggests that both varieties either had high susceptibility to PHS, or that they may be earlier maturing varieties. In the cases of LCS Biancor and LCS Artdeco seed dormancy may have already been eroding while the harvest-ripe seeds waited in the field to be harvested. The logistics of harvesting breeding nurseries means that in practice all plots are harvested on the same day, and only after the latest maturing varieties reach harvest maturity.

Table 3.2 - F-statistics from ANOVA of Falling Number (FN) values (s) of all factors and interactions in soft wheats.

Soft Wheats	**F-statistic**
Location	7637.7*
Variety	627.0*
Temperature	187.9*
Storage Time	528.5*
Location*Variety	216.3*
Location*Temperature	12.0*
Location*Storage Time	32.8*
Variety*Temperature	2.9*
Variety*Storage Time	3.7*
Temperature*Storage Time	144.9*
Location*Variety*Temperature	ns
Location *Variety*Storage Time	2.3*
Location*Temperature *Storage Time	11.9*
Variety*Temperature*Storage Time	ns
Location* Variety*Temperature*Storage Time	ns

*significant at P < 0.01, ns not significant at P < 0.01

Mean FN values were significantly different between the 3 storage temperatures when assessed by F-ratio from the four-way ANOVA (Table 3.2). The +40°C storage temperature also had the highest maximum FN value. This was higher than maximum FN values at either -20 or +20°C (Table 3.1). Days of storage were significantly different between the beginning (day 0) and end (day 90) of the study (Tables 3.1, 3.2).

F-statistics from 4-way ANOVA (Table 3.2) indicated significant influences on FN for all main effects. The majority of interaction terms were also significant. Given the number of significant interactions it was necessary to analyze each location*storage temperature treatment separately. Figures 3.2, 3.3 and 3.4 show changes in FN values over time of storage for the 10 soft varieties for each location*storage temperature treatment. It is evident that FN changed over time differentially at each temperature and that there were substantial differences in FN between locations and between varieties. However, all of the interaction terms that were not significant included the main effect “variety” (Table 3.2). This suggests that wheat variety may not be as important as the other main effects.

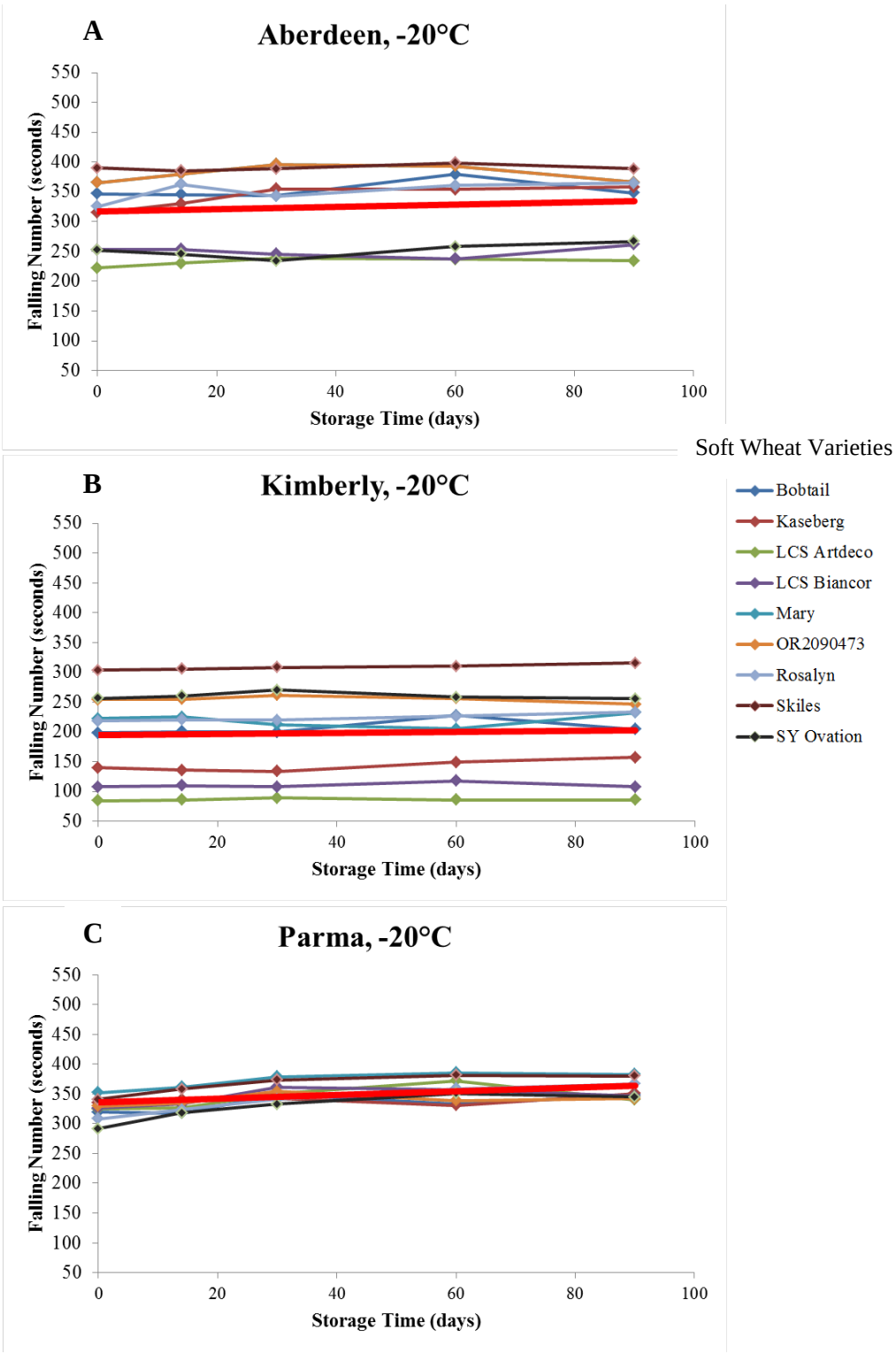

Figure 3.2 - Mean Falling Number (FN) values (s) of soft wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at -20°C. Linear regression of overall FN activities (averaged across varieties) are presented in red.

Table 3.3 - F-statistics (F-stat) from two-way ANOVA of soft wheat Falling Number (FN) values (s) by storage time, variety, and the storage time*variety interaction for each storage location*temperature treatment.

Aberdeen, -20°	**F-stat**	**Kimberly, -20°**	**F-stat**	**Parma, -20°**	**F-stat**
Storage Time	8.6*	Storage Time	ns	Storage Time	15.2*
Variety	253.6*	Variety	860.6*	Variety	11.2*
Storage Time*Variety	ns	Storage Time*Variety	2.3*	Storage Time*Variety	ns

Aberdeen, +20°	**F-stat**	**Kimberly, +20°**	**F-stat**	**Parma, +20°**	**F-stat**
Storage Time	33.9*	Storage Time	21.6*	Storage Time	12.4*
Variety	645.9*	Variety	3668.9*	Variety	13.6*
Storage Time*Variety	5.8*	Storage Time*Variety	15.6*	Storage Time*Variety	ns

Aberdeen,+ 40°	**F-stat**	**Kimberly, +40°**	**F-stat**	**Parma, +40°**	**F-stat**
Storage Time	148.6*	Storage Time	80.3*	Storage Time	48.8*
Variety	196.3*	Variety	1012.1*	Variety	10.1*
Storage Time*Variety	2.6*	Storage Time*Variety	4.5*	Storage Time*Variety	ns

*significant at $P < 0.01$, ns not significant at $P < 0.01$

3.3.1 Storage at -20°C

Two-way ANOVA for the location*temperature treatment, Aberdeen at -20°C, is shown in Table 3.3. There were significant differences between storage times and varieties. The storage time*variety interaction term was not significant. The F-ratios indicated the major influence was variety. Regression analyses (Table 3.4) showed that only one variety, OR2090473, had a significant increase in FN across the 90 days of storage. The rate of increase for OR2090473 was 12.9 s per 30 days. The correlation of the overall FN for each day (across varieties) with storage time was also not significant. The results show that for this location*storage time treatment, storage of grain was not associated with systematic increases in FN.

Two-way ANOVA for the location*temperature treatment, Kimberly at -20°C, is shown in Table 3.3. There were significant differences between wheat varieties but not storage times. The storage time*variety interaction term was also significant, but to a much lower degree than the main effect variety. Regression analyses (Table 3.4) showed that only one variety, Kaseberg, had a significant increase in FN across the 90 days of storage. The rate of increase for Kaseberg was 7.2 s per 30 days. The correlation of overall FN for each day (across varieties) with storage time was also not significant. The results show that for this location*storage time treatment, storage of grain was not associated with systematic increases in FN.

Two-way ANOVA for the location*temperature treatment, Parma at -20°C, is shown in Table 3.3. There were significant differences between storage times and varieties. The storage time*variety interaction term was not significant. The F-ratios indicated that storage time and wheat variety were each about equally influential. Regression analyses (Table 3.4) showed that two varieties, Rosalyn and SY Ovation, had significant increases in FN across the 90 days of storage. The rate of increase for Rosalyn was 19.5s per 30 days and the rate of increase for SY Ovation was 16.8 s per 30 days. The correlation of overall FN for each day (across varieties) with storage time was weak but significant, although this may simply be due to the significant increases in FN in Rosalyn and SY Ovation. The results show that for this location*storage time treatment, storage of grain was associated with some systematic increases in FN.

In summary, at -20°C there were very few systematic changes in FN values associated with grain storage. The biggest increases were seen in two varieties from Parma that had initial FN values > 275 s. When a linear model was applied to this location*storage temperature treatment across all

varieties, excluding the two significant varieties, the overall change in FN was still significant. In observing Figure 3.2C it is evident that there was general trend towards higher FN values over storage time, in contrast to the flat trends for the other two locations (Figure 3.2A, B). The significant positive correlations seen when looking across all varieties may have been a function of the higher degrees of freedom in the analysis afforded by not dividing the analyses by variety. The variety SY Ovation from Parma increased from an initial FN < 300 s to > 300 s after 14 days of storage. This increase in FN was significant.

Table 3.4 - Linear regression of Falling Number (FN) values (s) of soft wheats from Aberdeen, Kimberly, and Parma stored at -20°C for 90 days.

-20°C	**Aberdeen**				**Kimberly**				**Parma**			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Bobtail	347	0.15	0.13	ns	200	0.16	0.16	ns	322	0.29	0.27	ns
Kaseberg	325	0.44	0.53	ns	134	0.24	0.69	<0.001	337	0.09	0.08	ns
LCS Artdeco	228	0.12	0.20	ns	86	0.00	0.00	ns	332	0.28	0.2	ns
LCS Biancor	249	0.03	0.01	ns	109	0.03	0.05	ns	336	0.23	0.19	ns
Mary	380	0.00	0.00	ns	218	0.03	0.00	ns	359	0.34	0.55	ns
OR2090473	351	0.43	0.59	<0.001	258	-0.09	0.11	ns	336	0.1	0.61	ns
Rosalyn	339	0.32	0.29	ns	217	0.17	0.41	ns	315	0.65	0.75	<0.001
Skiles	388	0.05	0.02	ns	304	0.13	0.28	ns	351	0.41	0.39	ns
SY Ovation	243	0.23	0.30	ns	262	-0.04	0.03	ns	306	0.56	0.62	<0.001
WB 1070 CL	319	0.12	0.13	ns	159	0.25	0.33	ns	370	0.07	0.01	ns
Overall FN	**317**	**0.19**	**0.01**	**ns**	**195**	**0.09**	**0.00**	**ns**	**336**	**0.3**	**0.16**	**<0.0001**

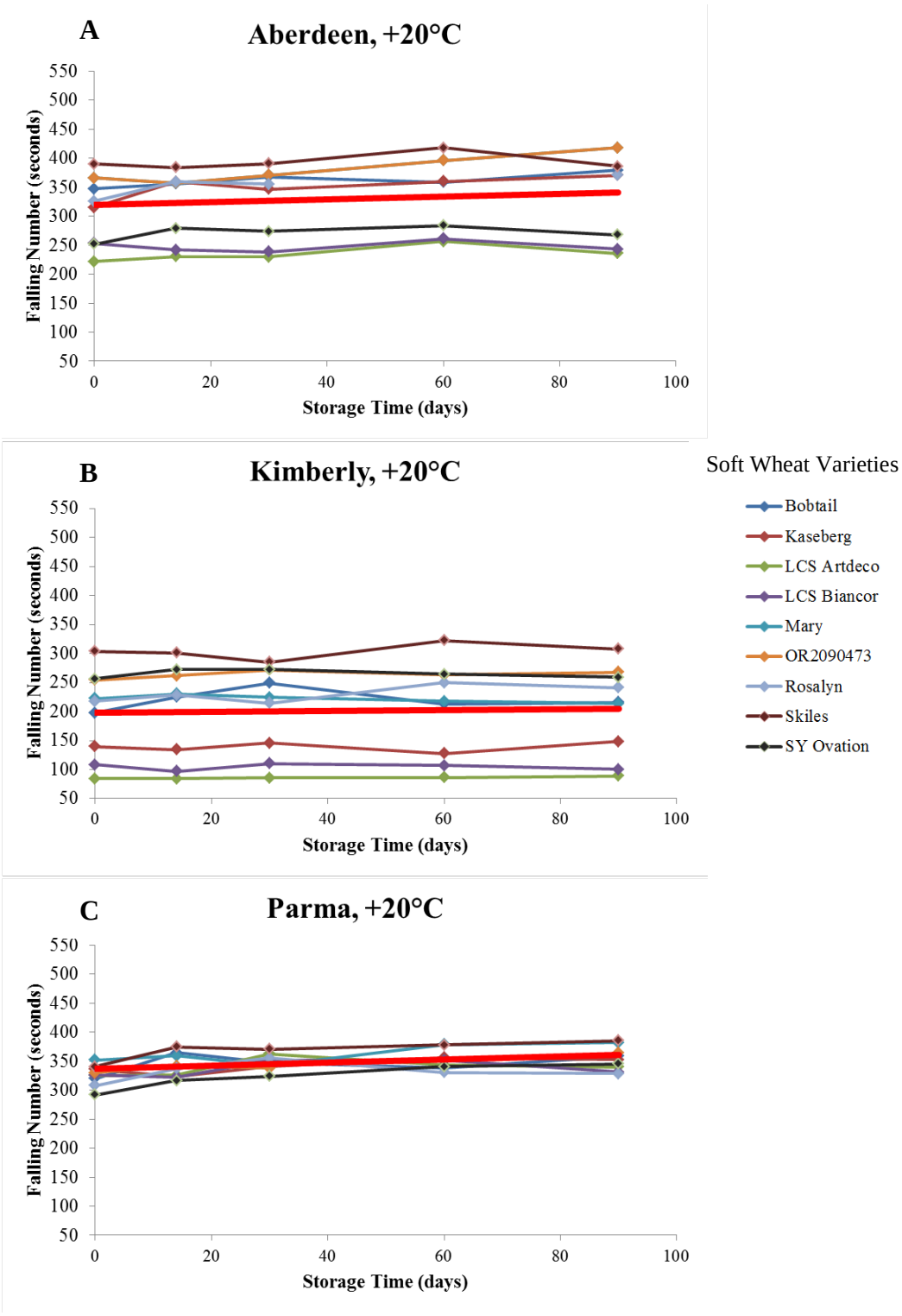

Figure 3.3 - Mean Falling Number (FN) values (s) of soft wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at +20°C. Linear regression of overall FN activities (averaged across varieties) are presented in red.

3.3.2 Storage at +20°C

Two-way ANOVA for the location*temperature treatment, Aberdeen at +20°C, is shown in Table 3.3. There were significant differences between both storage times and varieties. The F-ratios indicated the major influence was variety and then storage time. The storage time*variety interaction term was also significant. Regression analyses (Table 3.5) showed that only one variety (Mary) had a significant increase in FN across the 90 days of storage. The rate of increase for Mary was 19.8 s per 30 days. The correlation of the overall FN for each day (across varieties) with storage time was also not significant. The results show that for this location*temperature, storage of grain was not associated with overall systematic increases in FN.

Two-way ANOVA for the location*temperature treatment, Kimberly at +20°C, is shown in Table 3.3. There were significant differences between storage times and varieties. The F-ratios indicated the major influence was variety. The storage time*variety interaction term was also significant. Regression analyses (Table 3.5) showed that only one variety (LCS Artdeco) had a significant increase in FN across the 90 days of storage. The rate of increase for LCS Artdeco was barely detectable at 1.5 s per 30 days. The correlation of the overall FN for each day (across varieties) with storage time was also not significant. The results show that for this location*temperature treatment, storage of grain was not associated with overall systematic increases in FN.

Two-way ANOVA for the location*temperature treatment, Parma at +20°C, is shown in Table 3.3. There were significant differences between storage times and varieties. The F-ratios indicated that storage time and wheat variety were each about equally influential. The storage

time*variety interaction term was not significant. Regression analyses (Table 3.5) showed that only one variety (SY Ovation) had a significant increase in FN across the 90 days of storage. The rate of increase for SY Ovation was 16.5 s per 30 days. The correlation of the overall FN for each day (across varieties) with storage time was weak but significant, although this may be due primarily to the significant increases in FN in SY Ovation. The results show that for this location*temperature treatment, storage of grain was associated with some overall systematic increases in FN.

In summary, at +20°C there were again only a few systematic changes in FN values associated with grain storage. However, this was, as it was for -20°C, primarily in wheats from Parma. The variety SY Ovation was the only variety that had significant increases in FN values over storage time at Parma. This significant increase in SY Ovation from Parma was sufficient in changing initial FN of < 300 s to > 300 s in 14 days of storage, as was observed in SY Ovation from Parma when stored at 20°C. When a linear model was applied to this treatment, without SY Ovation, the mean change in FN over time was still significant. In observing Figure 3.2C it is evident that there was general trend towards higher FN values over storage time at +20°C. The contrast with the other two locations is not as evident as it was at -20°C. Notably, the wheat variety SY Ovation from Parma had significant increases in FN values over storage time when stored at both -20°C and +20°C, which may suggest that FN of grain from this variety is more responsive to change when stored at cold to moderate these temperatures.

Table 3.5 - Linear regression of Falling Number (FN) values (s) of soft wheats from Aberdeen, Kimberly, and Parma stored at +20°C for 90 days.

+20°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Bobtail	351	0.28	0.53	ns	219	0.02	0.00	ns	338	0.2	0.14	ns
Kaseberg	332	0.46	0.54	ns	137	0.06	0.05	ns	330	0.3	0.28	ns
LCS Artdeco	226	0.22	0.32	ns	84	0.05	0.68	<0.001	333	0.15	0.07	ns
LCS Biancor	247	0.01	0.00	ns	106	-0.03	0.05	ns	332	0.12	0.04	ns
Mary	356	0.66	0.86	<0.001	228	-0.15	0.56	ns	348	0.38	0.52	ns
OR2090473	365	0.29	0.33	ns	260	0.10	0.27	ns	331	0.36	0.63	ns
Rosalyn	340	0.39	0.51	ns	218	0.31	0.53	ns	329	0.08	0.02	ns
Skiles	390	0.09	0.06	ns	298	0.16	0.18	ns	355	0.37	0.34	ns
SY Ovation	267	0.11	0.11	ns	267	-0.04	0.05	ns	302	0.55	0.75	<0.001
WB 1070 CL	320	0.08	0.07	ns	164	0.21	0.34	ns	364	0.26	0.3	ns
Overall FN	**319**	**0.25**	**0.02**	**ns**	**198**	**0.07**	**0.00**	**ns**	**336**	**0.28**	**0.14**	**0.0002**

3.3.3 Storage at +40°C

Two-way ANOVA for the location*temperature treatment, Aberdeen at +40°C, is shown in Table 3.3. There were significant differences between storage times and varieties. The F-ratios indicated that variety and storage time were equally influential. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 3.6) showed that all varieties had significant increases in FN across the 90 days of storage. Increases in FN ranged from a low of 13.2 s per 30 days for LCS Biancor to a high of 48 s per 30 days for Mary. The correlation of the overall FN for each day (across varieties) with storage time was also significant. The overall rate of increase across varieties was 31.2 s per 30 days. The results show that for this location*temperature treatment, storage of grain was associated with overall systematic increases in FN and that the magnitude of the increases were greater than seen at either of the lower temperatures (Tables 3.4 and 3.5).

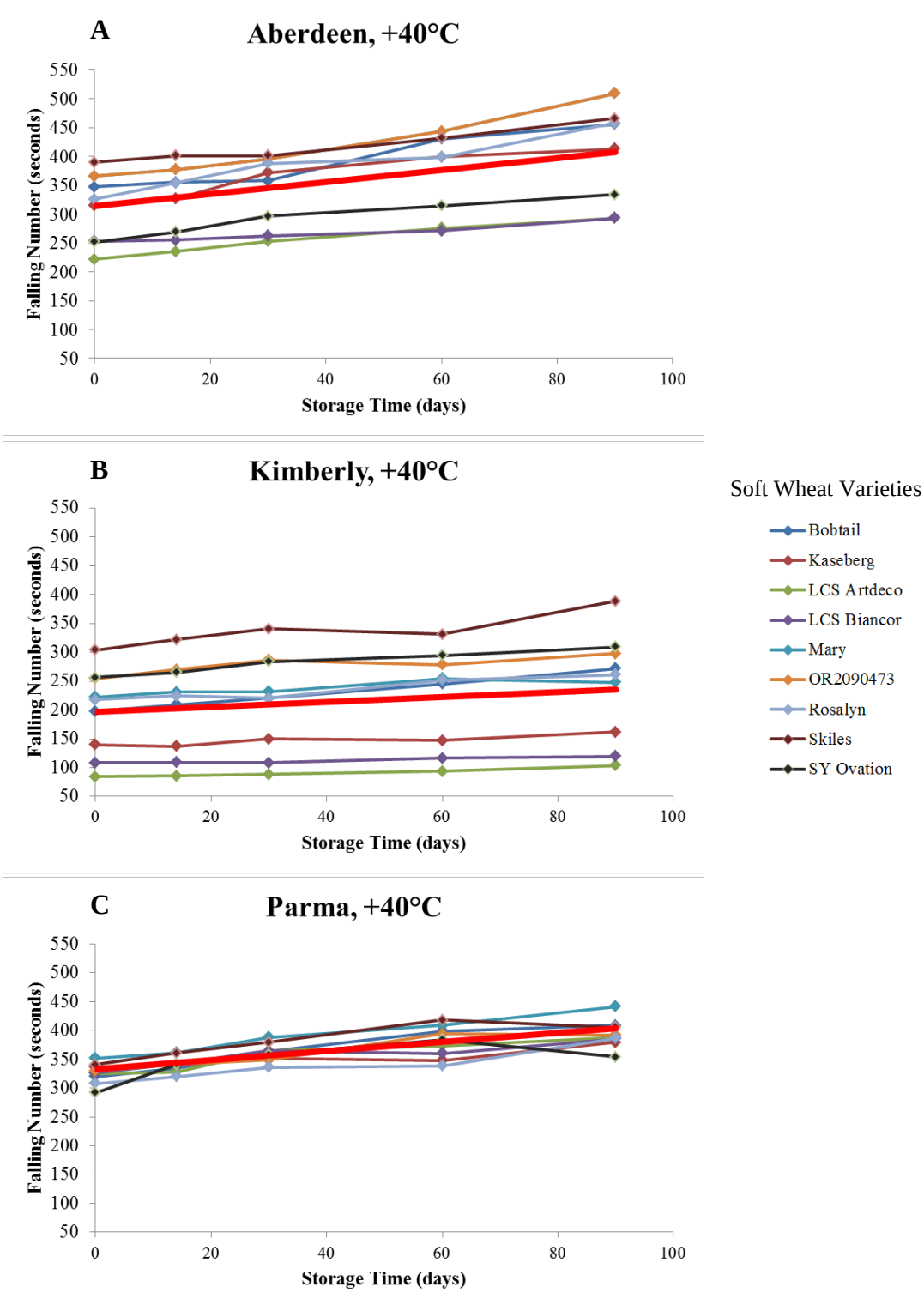

Figure 3.4 - Mean Falling Number (FN) values (s) of soft wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at +40°C. Linear regression of overall FN activities (averaged across varieties) are presented in red.

Two-way ANOVA for the location*temperature treatment, Kimberly at +40°C, is shown in Table 3.3. There were significant differences between storage times and varieties. The F-ratios indicated the major influence was variety. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 3.6) showed that all varieties except WB 1070 CL had significant increases in FN over the 90 days of storage. The correlation of the overall FN for each day (across varieties) with storage time was also not significant. However, observation of Figure 3.4B shows that wheat varieties with initial (Day 0) FN values > 200 s may have behaved differently over storage time than wheat varieties with initial FN values < 200 s. This conjecture is supported by strong influence variety had on FN as assessed by F-ratio (Table 3.3). Separate linear regression models were applied to wheat varieties from Kimberly stored at +40°C with initial FN values > 200 s and < 200 s. There was a significant increase in FN over time in wheat varieties with initial FN > 200 s ($r = 0.40$, $p = 0.004$). The overall rate of increase in FN from wheats with initial FN > 200 s was 15.6 s per day. There was no significant change in FN over time in wheat varieties with initial FN < 200 s ($r = 0.04$, $p = 0.14$). This supports an assertion that change in FN over storage time is influenced by degree of PHS damage. The results show that for this location storage of grain for 90 days at +40°C was not associated with systematic increases in FN when calculated across all varieties. However, there were systematic increases in FN for varieties with initial FN > 200 s.

Two-way ANOVA for the location*temperature treatment, Parma at +40°C, is shown in Table 3.3. There were significant differences between storage times and varieties. The F-ratios indicated that storage time was slightly more influential than variety. The storage time*variety interaction term was not significant. Regression analyses (Table 3.6) showed most varieties had a

significant increase in FN across the 90 days of storage. Changes in FN ranged from a low of 0 s per 30 days for Kaseberg, Skiles, and SY Ovation (ns) to a high of 32 s per 30 days for WB 1070 CL. The correlation of the overall FN for each day (across varieties) with storage time was significant. The overall rate of increase across varieties was 23.4 s per 30 days. The results show that for this location*temperature treatment, storage of grain was associated with systematic increases in FN.

In summary, at +40°C there were systematic increases in FN values associated with grain storage in this collection of soft wheat varieties. There were some exceptions. The correlation of the overall FN across all varieties from Kimberly was not significant. However, when a linear regression model was fit for wheat varieties from Kimberly with initial (day 0) FN > 200 s there were systematic increases in FN over storage time. All wheat varieties from Aberdeen had significant systematic increases in FN values over storage time. Most wheat varieties from Parma had significant systematic increases in FN values over storage time. The wheat variety SY Ovation had significant increases in FN values to over 300 s over storage time at -20°C and +20°C but did not have significant increases in FN values over storage time at +40°C suggesting that its FN was not systematically more responsive to grain storage time than the other varieties studied here. In almost all cases (excluding the location*temperature treatments Parma at -20°C and Parma at +40°C), variety was the most significant effect as assessed by F-ratio (Table 3.9). The significance of the effect “storage time” increased as storage temperature increased, with the exception of these location*temperature treatments, Parma at -20°C, and Parma at +20°C (Table 3.9). This suggests that storing grain at higher temperatures accelerated the increases in FN that were observed in this collection of soft wheats over time.

Table 3.6 - Linear regression of Falling Number (FN) values (s) of soft wheats from Aberdeen, Kimberly, and Parma stored at +40°C for 90 days.

+40°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Bobtail	337	1.34	0.87	<0.0001	197	0.81	0.98	<0.0001	325	1.04	0.76	<0.001
Kaseberg	321	1.14	0.87	<0.0001	138	0.07	0.62	<0.001	334	0.42	0.49	ns
LCS Artdeco	225	0.79	0.94	<0.0001	83	0.21	0.90	<0.0001	328	0.72	0.75	<0.001
LCS Biancor	250	0.44	0.89	<0.0001	107	0.14	0.90	<0.0001	333	0.57	0.82	<0.001
Mary	356	1.60	0.96	<0.0001	225	0.32	0.64	<0.001	352	0.99	0.78	<0.001
OR2090473	352	1.32	0.89	<0.0001	262	0.39	0.61	<0.001	331	0.77	0.69	<0.001
Rosalyn	333	1.34	0.84	<0.0001	215	0.52	0.80	<0.001	307	0.78	0.76	<0.001
Skiles	386	0.84	0.89	<0.0001	306	0.79	0.75	<0.001	350	0.78	0.56	ns
SY Ovation	259	0.89	0.87	<0.0001	259	0.58	0.84	<0.001	321	0.63	0.42	ns
WB 1070 CL	321	0.70	0.78	<0.001	171	0.26	0.24	ns	352	1.08	0.87	<0.0001
Overall FN	**314**	**1.04**	**0.24**	**<0.0001**	**196**	**0.43**	**0.03**	**ns**	**333**	**0.78**	**0.50**	**<0.0001**

3.4 Results and Discussion: Hard wheats

Summary statistics for hard wheat FN results are shown in Table 3.7. The full factorial ANOVA showed that FN varied significantly between growing locations, wheat varieties, and days and temperatures of storage (Table 3.8). F-ratios from ANOVA indicated, as for the soft wheats (Table 3.2), that environment had the largest influence on FN. Storage temperatures and times were less influential. All two-way interaction terms (except variety*storage temperature) were significant and the largest F-ratios were for temperature*storage time and then location*storage time interactions.

Table 3.7 shows that the location Kimberly had both the lowest absolute FN value and the lowest mean FN value of the 3 locations. This indicated that Kimberly was likely to have been affected by PHS. This was expected as our collaborators at the University of Idaho indicated a noteworthy rain event after grain reached physiological maturity. Aberdeen had the highest

absolute FN value and mean FN value of the three locations. Aberdeen also had a rain event prior to harvest, but Aberdeen had a minimum FN value of 375 s. This indicates no PHS damage at this location in these hard-grained varieties. This contrasts with the soft wheat results (Table 3.1). Parma had a minimum FN value of 283 s, which is suggestive, but not diagnostic, that PHS may have affected this sample (αA activity for this sample, see Section 4.2, suggested otherwise).

All wheat varieties had maximum FN values of > 300 s. This indicated either no evidence of PHS at one or more locations, or increased FN over storage time. All wheat varieties had minimum FNs of < 300 s suggesting that they were affected by PHS at one or more locations.

Mean FN values were significantly different between the three storage temperatures when assessed by F-ratio in the 4-way ANOVAs (Table 3.8). The +40°C storage temperature also had the highest maximum FN value. This was higher than maximum FN values at either -20 or +20°C (Table 3.7). Days of storage were also significantly different (Table 3.8).

Table 3.7 - Summary Statistics of hard wheat Falling Number values (s).

Location	**Min**	**Max**	**Mean**	**SD**	**SE**
Aberdeen	375	848	480	92.2	5.2
Parma	283	574	399	45.3	5.2
Kimberly	120	421	260	64.8	5.3
Variety					
WB Arrowhead	280	848	405	115.3	12.1
Whetstone	244	728	393	86.5	12.0
OR2100081H	228	770	388	103.3	12.0
Norwest 553	254	664	381	77.2	12.0
OR2080236H	193	743	361	120.5	12.0
Keldin	120	762	351	160.3	12.0
Temperature					
-20°	120	535	363	95.9	8.4
+20°	142	529	361	90.9	8.4
+40°	147	848	416	142.5	8.4
Storage Time (Days)					
0	152	472	346	81.1	10.8
14	146	566	360	89.0	10.9
30	147	650	374	102.5	10.8
60	120	693	397	120.3	10.8
90	120	848	422	151.5	10.8

Table 3.8 - F-statistics from ANOVA of Falling Number (FN) values (s) of all factors and interactions in hard wheats.

Hard Wheats	**F-statistic**
Location	5583.5*
Variety	86.8*
Temperature	426.7*
Storage Time	1021.2*
Location*Variety	185.0*
Location*Temperature	76.1*
Location*Storage Time	214.1*
Variety*Temperature	ns
Variety*Storage Time	3.5*
Temperature*Storage Time	403.0*
Location*Variety*Temperature	4.7*
Location *Variety*Storage Time	4.9*
Location*Temperature *Storage Time	77.3*
Variety*Temperature*Storage Time	ns
Location* Variety*Temperature*Storage Time	2.4*

*significant at $P < 0.01$, ns not significant at $P < 0.01$

F-statistics from 4-way ANOVA (Table 3.8) indicated significant influences on FN for all main effects. As assessed by F-ratio, location was the most significant main effect impacting FN followed by storage time. The majority of interaction terms were also significant. Given the number of significant interactions it was necessary to analyze each location*temperature treatment separately. Figures 3.5, 3.6, and 3.7 show changes in FN values over time of storage for the six hard varieties for each location*temperature treatment. It is evident that FN changed over time differentially at each temperature and that there were substantial differences in FN between locations, particularly in wheats from Aberdeen stored at +40°C. There were substantial differences between changes in FN over time between varieties as well.

3.4.1 Storage at -20°C

Two-way ANOVA for the location*temperature treatment, Aberdeen at -20°C, is shown in Table 3.9. There were significant differences between storage times and wheat varieties. The F-ratios indicated that storage time and variety were equally significant. The storage time*variety interaction term was not significant. Regression analyses (Table 3.10) showed that two varieties (Norwest 553 and Whetstone) had significant increases in FN across the 90 days of storage. The rate of increase for Norwest 553 was 22.5 s per 30 days and for Whetstone, 18.3 s per 30 days. The correlation of the overall FN for each day (across varieties) with storage time was also significant, though this may be due largely to the significant increases in Norwest 553 and Whetstone. The results show that for this location*temperature treatment, storage of hard-grained wheat was associated with some overall systematic increases in FN.

Table 3.9 - F-statistics (F-stat) from two-way ANOVA of hard wheat Falling Number (FN) values (s) by storage time, variety, and the storage temperature*variety interaction for each storage location*temperature treatment.

Aberdeen, -20°	**F-stat**	**Kimberly, -20°**	**F-stat**	**Parma, -20°**	**F-stat**
Storage Time	24.0*	Storage Time	6.2*	Storage Time	7.7*
Variety	31.8*	Variety	499.9*	Variety	23.2*
Storage Time*Variety	ns	Storage Time*Variety	3.3*	Storage Time*Variety	4.4*
Aberdeen, +20°	**F-stat**	**Kimberly, +20°**	**F-stat**	**Parma, +20°**	**F-stat**
Storage Time	25.1*	Storage Time	4.3*	Storage Time	10.7*
Variety	32.0*	Variety	1093.1*	Variety	10.9*
Storage Time*Variety	ns	Storage Time*Variety	26.6*	Storage Time*Variety	ns
Aberdeen, +40°	**F-stat**	**Kimberly, +40°**	**F-stat**	**Parma, +40°**	**F-stat**
Storage Time	313.5*	Storage Time	177.8*	Storage Time	110.2*
Variety	40.0*	Variety	718.1*	Variety	44.1*
Storage Time*Variety	ns	Storage Time*Variety	11.6*	Storage Time*Variety	3.35*

*significant at $P < 0.01$, ns not significant at $P < 0.01$

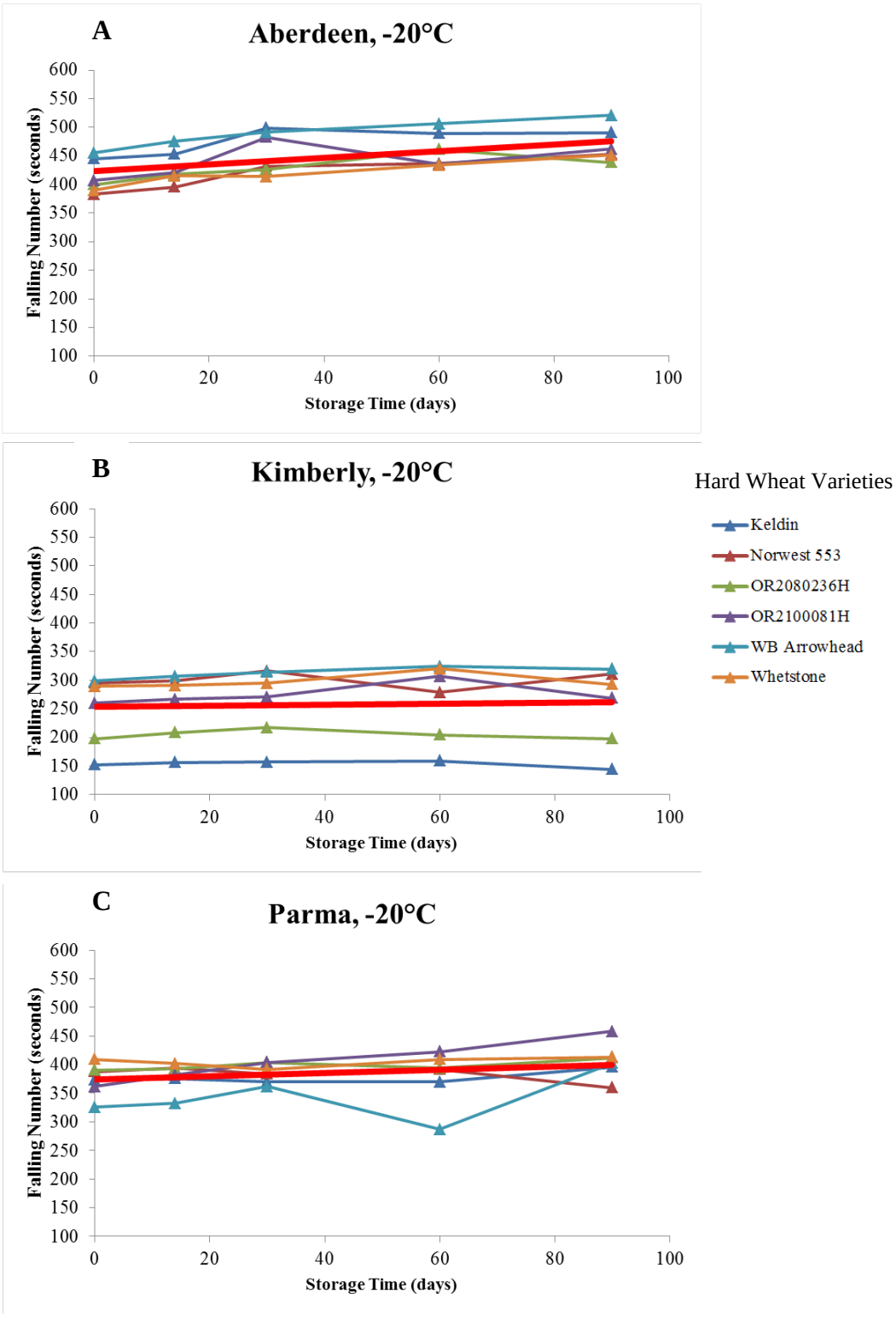

Figure 3.5 - Mean Falling Number (FN) values (s) of hard wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at -20°C. Linear regression of overall FN activities (averaged across varieties) are presented in red.

Two-way ANOVA for the location*temperature time treatment, Kimberly at -20°C, is shown in Table 3.9. There were significant differences between storage times and wheat varieties. The F-ratios indicated that the major influence was wheat variety. The storage time*variety interaction term was also significant. Regression analyses (Table 3.10) showed that no wheat varieties had a significant increase in FN across the 90 days of storage. The correlation of overall FN for each day (across varieties) with storage time was also not significant. The results show that for this location*temperature treatment, storage of hard-grained wheat was not associated with overall systematic increases in FN. It is notable also that the initial FN values were lower for this location than the other 2 locations. The lack of response at Kimberly may be related to the low initial FN values, as similar was observed for the varieties with initial FN above 200 s (FN responsive to storage time) and below 200 s (FN *not* responsive to storage time) for the soft-wheats from Kimberly stored at +40°C (Figure 3.4B).

Two-way ANOVA for the location*temperature treatment, Parma at -20°C, is shown in Table 3.9. There were significant differences between storage times and wheat varieties. The F-ratios indicated that the major influence was wheat variety. The storage time*variety interaction term was also significant. Regression analyses (Table 3.10) showed that only one variety, OR2100081H, had significant increases in FN across the 90 days of storage. The rate of increase for OR2100081H was 30.6 s per 30 days. The correlation of overall FN for each day (across varieties) with storage time was not significant. The results show that for this location*temperature treatment, storage of grain was not associated with overall systematic increases in FN.

In summary, at -20°C there were very few systematic changes in FN values associated with grain storage. The biggest increases were seen in two varieties from Aberdeen that both had initial FN values > 350 s. When a linear model was applied to this location*storage temperature treatment without the two significant varieties, the overall change in FN over time was still significant (r = 0.48, p = 0.002). In observing Figure 3.5A it is evident that there was a general trend towards higher FN values over storage time at Aberdeen, in contrast to the flat trends for the other two locations (Figure 3.5B, C). The significant positive correlations seen when looking at the overall correlation, across varieties, may have been a function of the higher degrees of freedom in the analysis afforded by not subdividing the analyses by variety.

Table 3.10 - Linear regression of Falling Number (FN) values (s) of hard wheats from Aberdeen, Kimberly, and Parma stored at -20°C for 90 days.

-20°C	**Aberdeen**				**Kimberly**				**Parma**			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Keldin	456	0.49	0.69	ns	157	-0.08	-0.42	ns	369	0.20	0.42	ns
Norwest 553	391	0.75	0.89	<0.001	299	0.03	0.07	ns	394	-0.28	-0.46	ns
OR2080236H	410	0.49	0.66	ns	207	-0.06	-0.21	ns	391	0.19	0.44	ns
OR2100081H	424	0.46	0.49	ns	266	0.22	0.39	ns	366	1.02	0.94	<0.0001
WB Arrowhead	463	0.69	0.76	ns	304	0.24	0.70	ns	323	0.50	0.42	ns
Whetstone	397	0.61	0.88	<0.001	292	0.15	0.33	ns	401	0.10	0.27	ns
Overall FN	**424**	**0.58**	**0.50**	**<0.0001**	**254**	**0.08**	**0.04**	**ns**	**374**	**0.29**	**0.28**	**ns**

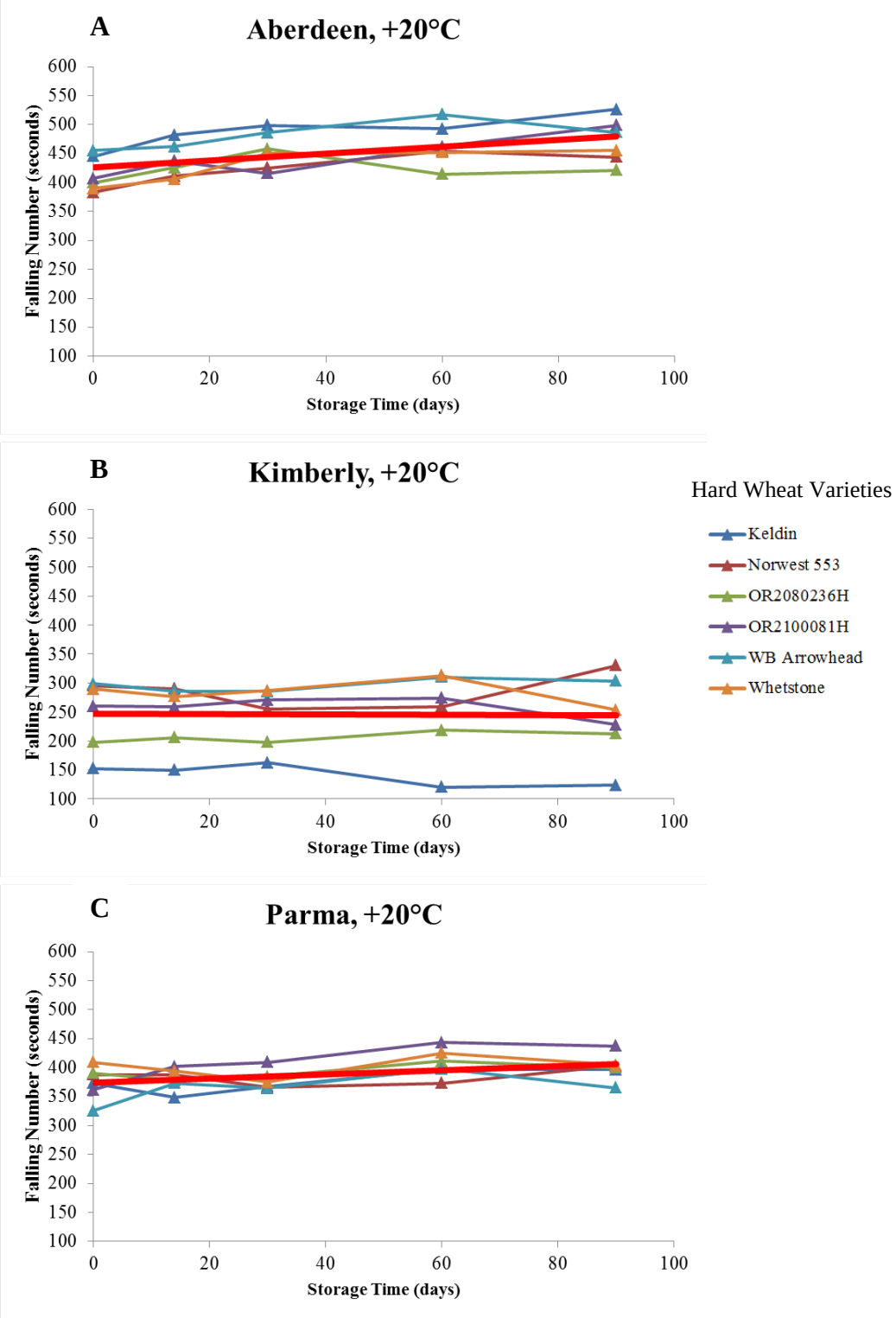

Figure 3.6 - Mean Falling Number (FN) values (s) of hard wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at +20°C. Linear regression of overall FN activities (averaged across varieties) are presented in red.

3.4.2 Storage at +20°C

Two-way ANOVA for the location*temperature treatment, Aberdeen at +20°C, is shown in Table 3.9. There were significant differences between both storage times and wheat varieties. The F-ratios indicated that storage time and wheat variety were about equally influential. The storage time*variety interaction term was not significant. Regression analyses (Table 3.11) showed that most varieties had a significant increase in FN across the 90 days of storage. The individual variety rates of increase ranged from 0 (NS) for OR2080236H and WB Arrowhead to 285 s per 30 days for OR2100081H. The correlation of overall FN for each day (across varieties) with storage time was also significant. The overall rate of increase across varieties was 18 s per 30 days. The results show that for this location*temperature treatment, storage of grain was associated with overall systematic increases in FN.

Two-way ANOVA for the location*temperature treatment Kimberly at +20°C is shown in Table 3.9. There were significant differences between both storage times and varieties. The F-ratios indicated the major influence was variety. The storage time*variety interaction term was also significant. Regression analyses (Table 3.11) showed that only one variety (Keldin) had a significant change in FN across the 90 days of storage. In contrast to all other significant correlations observed so far in this study, this change was a ***decrease*** in FN. The rate of decrease for Keldin was 12.3 s per 30 days. The correlation of overall FN for each day (across varieties) with storage time was not significant. The results show that for this location*temperature treatment, storage of grain was not associated with overall systematic increases in FN and with a decrease in one of the tested varieties.

Two-way ANOVA for the location*temperature treatment, Parma at +20°C, is shown in Table 3.9. There were significant differences between both storage times and wheat varieties. The F-ratios indicated that storage time and wheat variety were each about equally influential. The storage time*variety interaction term was not significant. Regression analyses (Table 3.11) showed that only one variety (OR2100081H) had significant increases in FN across the 90 days of storage. The rate of increase for OR2100081H was 23.7 s per 30 days. The correlation of overall FN for each day (across varieties) with storage time was weak but significant, although this may be due primarily to the significant increases in the FN of OR2100081H. The results show that for this location*temperature treatment, storage of grain was associated with some overall systematic increases in FN.

In summary, at +20°C there were systematic changes in FN values associated with grain storage. However, this was primarily in wheats from Aberdeen and Parma where initial FN values were all > 300 s. All varieties from Aberdeen (except OR2080236H and WB Arrowhead) had significant systematic increases in FN values over storage time. The overall mean FN change over storage time was significant in wheats from Parma as well. However, only one variety (OR2100081H) from Parma had a significant increase in FN values over time. When a linear model was applied to this treatment without OR2100081H the mean change in FN over time was still significant. In observing Figure 3.5C it is evident that there was a general trend towards higher FN values over storage time at +20°C for the Parma samples, particularly in contrast to the flat trend for the location Kimberly (Figure 3.6B). However, the upward trend observed from Parma was not as large as the trend observed in wheats from Aberdeen (Figure 3.6A). The significant positive correlations seen when looking across all varieties from Parma may have

been a function of the higher degrees of freedom in the analysis afforded by not dividing the analyses by variety. Notably, the variety Keldin from Kimberly had significant decreased in FN values over storage time at +20°C.

Table 3.11 - Linear regression of Falling Number (FN) values (s) of hard wheats from Aberdeen, Kimberly, and Parma stored at +20°C for 90 days.

+20°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Keldin	461	0.72	0.77	<0.001	158	-0.41	-0.80	<0.001	359	0.45	0.62	ns
Norwest 553	397	0.68	0.78	<0.001	275	0.29	0.34	ns	379	0.12	0.24	ns
OR2080236H	422	0.05	0.08	ns	199	0.19	0.71	ns	384	0.24	0.64	ns
OR2100081H	407	0.95	0.86	<0.001	269	-0.27	-0.52	ns	380	0.79	0.77	<0.001
WB Arrowhead	463	0.47	0.63	ns	290	0.17	0.51	ns	350	0.39	0.51	ns
Whetstone	403	0.72	0.81	<0.001	291	-0.19	-0.31	ns	396	0.15	0.25	ns
Overall FN	**426**	**0.60**	**0.50**	**<0.0001**	**247**	**-0.04**	**-0.02**	**ns**	**375**	**0.36**	**0.42**	**<0.001**

3.4.3 Storage at +40°C

Two-way ANOVA for the location*temperature treatment, Aberdeen at +40°C, is shown in Table 3.9. There were significant differences between both storage times and varieties. The F-ratios indicated that the major influence was storage time. This contrasts with all but one other treatments where variety was the major influence, with the exception of soft wheats from Parma at +40°C where storage time was also the major influence (Table 3.3). The storage time*variety interaction term was not significant. Regression analyses (Table 3.12) showed that all varieties had significant increases in FN across the 90 days of storage. Increases in FN ranged from a low of 90.3 s per 30 days for Norwest 553 to a high of 120 s per 30 days for WB Arrowhead. The correlation of overall FN for each day (across varieties) with storage time was also significant. The overall rate of increase across varieties was 107 s per 30 days. This was the highest rate of increases observed in soft or hard wheats in any location*storage temperature treatment (Figure

3.7A). The results show that for this location*temperature treatment, storage of grain was associated with overall systematic increases in FN and that the magnitude of increases were greater than seen with grain from this location at either of the lower temperatures (Tables 3.11 and 3.12). A preliminary analysis of a potential cause for the anomalously high rate of increase in FN values is presented in Section 3.5 below.

Two-way ANOVA for the location*temperature treatment, Kimberly at +40°C, is shown in Table 3.9. There were significant differences between storage times and varieties. The F-ratios indicated the major influence was variety. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 3.12) showed that most varieties had significant increases in FN over the 90 days of storage. Increases in FN ranged from a low of 0 s per 30 days for Keldin (NS) to a high of 35.7 s per 30 days for Norwest 553. The correlation of overall FN for each day (across varieties) with storage time was also significant. The overall rate of increase across varieties was 24.9 s per 30 days. The results show that for this location*temperature treatment, storage of grain was associated with overall systematic increases in FN. The variety Keldin grown at Kimberly stands out again. It was the most affected by PHS of the hard wheats and showed either no increase in FN, or even a decrease over the duration of storage (Figures 3.5B, 3.6B, 3.7B).

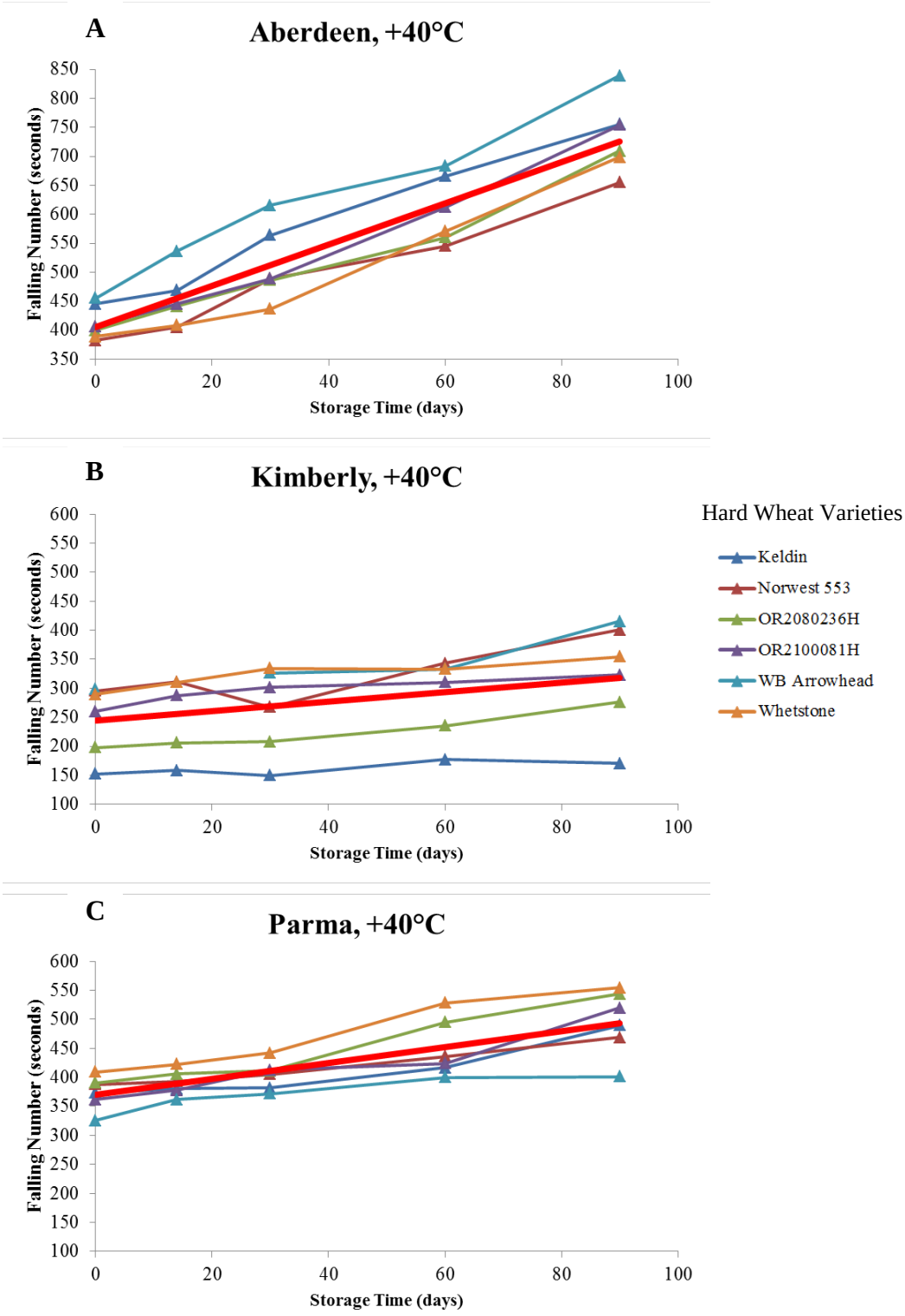

Figure 3.7 - Mean Falling Number (FN) values (s) of hard wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at +40°C. Linear regression of overall FN activities (averaged across varieties) are presented in red.

Two-way ANOVA for the location*temperature treatment, Parma at +40°C, is shown in Table 3.9. There were significant differences between storage times and varieties. The F-ratios indicated that the major influence was storage time, as was the case in hard wheats from Aberdeen stored at +40°C. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 3.12) showed that all varieties had significant increases in FN over the 90 days of storage. Changes in FN ranged from a low of 23.4 s per 30 days for WB Arrowhead to a high of 54.0 s per 30 days for OR2100236H. The correlation of overall FN for each day (across varieties) with storage time was also significant. The overall rate of increase across varieties was 40.8 s per 30 days. The results show that for this location*temperature treatment, storage of grain was associated with overall systematic increases in FN.

In summary, at +40°C there were systematic changes in FN values associated with grain storage in this collection of hard wheat varieties. The only exception was the variety Keldin from Kimberly. Overall FN values showed significant systematic increases across all varieties from all three locations when stored at +40°C. The varieties Norwest 553 and Whetstone from Aberdeen showed significant increases in FN values at all three storage temperatures. The variety Norwest 553 was not sprouted at either the Aberdeen or Parma locations (Day 0 FN of 383 s and 388 s respectively) but the magnitude of increases in FN values between the two locations was different. This suggests that even in unsprouted wheat, growing environment may influence rate of increases in FN values over storage time. The variety OR2100081H from Parma showed significant increases in FN values at all three storage temperatures (Tables 3.10, 3.11, and 3.12). The varieties Whetstone, OR2100086H, and Norwest 553 from Kimberly had significant

changes in initial FN values from < 300s to > 300 s over the duration of storage. The variety Keldin from Kimberly had no significant increases in FN values at any of the three storage temperatures. Keldin was the only variety to show a significant decrease in FN over storage time. The influence of the main effect "storage time" increased as storage temperature increased in this collection of hard wheats. This corresponds with the behaviors observed for soft wheats, suggesting that there is no categorical difference in the FN response to storage between hard and soft wheats (Tables 3.3 and 3.9). This is further evidence that higher temperature aging was successful in accelerating changes that occur in grain storage over time in this collection of wheats. Storage time was the most influential effect on hard wheat varieties from Aberdeen and Parma stored at +40°C (Table 3.9). The storage time*variety interaction term was not significant in hard wheat varieties from Aberdeen at any of the three storage temperatures. This shows that rate of change in this collection of hard wheats from Aberdeen was not different between wheat varieties. This assertion is supported by Figures 3.5A, 3.6A, and 3.7A.

Table 3.12 - Linear regression of Falling Number (FN) values (s) of hard wheats from Aberdeen, Kimberly, and Parma stored at +40°C for 90 days.

+40°C	**Aberdeen**				**Kimberly**				**Parma**			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Keldin	441	3.59	0.99	<0.0001	152	0.25	0.76	ns	359	1.26	0.93	<0.0001
Norwest 553	379	3.01	0.99	<0.0001	278	1.19	0.84	<0.001	382	0.92	0.90	<0.001
OR2080236H	391	3.31	0.96	<0.0001	191	0.86	0.96	<0.0001	380	1.80	0.96	<0.0001
OR2100081H	391	3.88	0.98	<0.0001	273	0.61	0.87	<0.001	356	1.62	0.92	<0.001
WB Arrowhead	471	3.99	0.98	<0.0001	290	1.18	0.91	<0.001	342	0.78	0.87	<0.001
Whetstone	363	3.56	0.97	<0.0001	300	0.63	0.89	<0.001	403	1.77	0.97	<0.0001
Overall FN	**406**	**3.56**	**0.91**	**<0.0001**	**244**	**0.83**	**0.38**	**<0.001**	**370**	**1.36**	**0.76**	**<0.0001**

3.5 Polymeric protein analyses

Hard wheat varieties were assessed for polymeric protein content as a first attempt at understanding the large increases in FN at Aberdeen at +40°C, which were not associated with decreased amylase activity (see Chapter 4). One suggestion had been that the high temperature storage had cross-linked or heat-set the gluten proteins *in situ* and that this was a possible cause. Increases in UPP are indicative of heat-setting of gluten (Zhang 2015). There was no significant difference in %LUPP or in %TUPP of hard wheat varieties from Aberdeen stored at either -20°C or +40°C. This suggests that the increased rate of change in FN observed in +40°C storage condition at Aberdeen (Figures 3.7A) was not due to a differential change in the polymeric proteins during storage with respect to temperature.

There was a significant difference in %TUPP between hard wheat varieties from Aberdeen or Kimberly when stored at -20°C (F-ratio = 15.23, $p < 0.001$). While protein concentration has been shown to vary between growing environment, there is evidence that the relative abundance of protein subunits expressed in a given cultivar may remain constant (Gupta et al 1993). Hard wheats from Kimberly had significantly lower %TUPP than hard wheats from Aberdeen. This shows that polymeric protein content decreased between growing environments and, by inference, degree of PHS damage. However, there was no significant difference in %LUPP between hard wheat varieties from either Aberdeen or Kimberly at -20°C. This suggests that the postulated degradation of polymeric proteins occurred relatively equally across molecular weight ranges of the proteins.

3.6 Conclusions

Changes in FN were observed over grain storage time. Changes in FN occurred differentially between growing environments, wheat varieties, storage temperatures, and between soft and hard wheat classes. Changes in FN values over storage time were less prevalent in low FN samples. Highest rates of increase in FN were observed in ungerminated hard wheats. Varieties showed different propensities to sprout. Soft wheats were generally more susceptible to PHS in this collection of wheat varieties. Rates of systematic increases in FN over storage time occurred at a higher magnitude as storage temperature increased, particularly in unsprouted hard wheats. Storage of grain at -20°C was not sufficient in all cases to stop significant systematic increases in FN values, particularly in unsprouted hard wheats. Storage at +40°C accelerated the rate of change in FN values over storage time, particularly in hard wheats from Aberdeen. Grain storage was successful as a way to raise FN values to > 300 s in very few cases. Polymeric protein content was not altered by storage temperature in hard wheats. However, there was evidence that polymeric protein content was degraded in PHS-damaged hard wheats. Changes in FN will be assessed against the results of tracking changes in αA activity over storage time and temperature in the following study (see Chapter 4).

References

See Bibliography

Chapter 4: The influence of storage time and temperature on Alpha-amylase activity in sprouted and unsprouted wheats

Mike R. Adams and Andrew S. Ross

Abstract

Pre-harvest sprouting (PHS) increases alpha amylase (αA) activity in wheat, the result of which reduces wheat end-product quality. Falling Number (FN) is the primary test used by industry to gauge PHS damage in wheats, but direct measurement of αA activity is the fundamental reference test for assessing PHS. The objective of this study was to determine if αA activity of wheat samples changed during storage and if changes were a function of storage time, storage temperature, and degree of PHS damage. Sixteen wheat varieties (ten soft, six hard) from three Idaho locations were used. Samples captured a wide range of αA activities. Samples were stored at -20°C, +20°C, and +40°C and assessed for FN and αA activity after 0, 14, 30, 60, and 90 days of storage. Across all samples FN and αA activity had the expected curvilinear relationship. High αA activities were primarily observed in soft wheats from locations that had rain events prior to harvest. Storage temperature did not influence changes in αA activity as much as it influenced changes in FN over storage time. Changes in αA activity over storage time were most prevalent in soft wheats with initial αA activities > 0.1 Ceralpha Units (CU). There were small decreases in αA activity in hard wheats but the distinction between high and low αA activity samples was not as evident as in the soft wheats because the vast majority of hard wheat samples tested had αA activities < 0.1 CU. Decreases in αA activity were often not associated with a corresponding increase in FN values over grain storage time. Increases in FN over storage time for the hard wheats significantly differed between locations. However, decreases in αA activities over storage time for the hard wheats were not significantly different between locations. Storage for 90 days

at these temperatures may not be effective in decreasing αA activity from > 0.1 CU to < 0.1 CU. Significant increases in FN to > 300 s did not have corresponding decreases in αA activity from > 0.1 CU to < 0.1 CU.

4.1 Introduction

Pre-harvest sprouting (PHS) appears to be an increasingly frequent problem in the U.S. Pacific Northwest (PNW). Rain prior to harvest germinates wheat in the field, which leads to increased alpha amylase (αA) activity in the intact caryopsis, as well as the de novo synthesis of other hydrolases: e.g. proteases (Simsek et al 2014). PHS can reduce both the functionality of processing intermediates and the quality of end-products. Wheat is visually inspected for signs of PHS upon delivery. Wheat is graded as unacceptable for milling purposes if more than 4% of the seeds are visibly sprouted (Simsek et al 2014). However, PHS can be present in the absence of visible cues (Ross and Bettge 2009). Falling Number (FN: AACCI Approved Method 56-81.03) is the standard analytical method performed at grain elevators when farmers deliver their wheat. A FN of > 300 s is presumptive evidence of low αA activity in the grain. Wheat with a FN < 300 s is presumptive evidence of excessive αA activity in the grain and is considered poor quality by processors. In consequence, grain testing with FN < 300 s leads to discounted prices paid to farmers (Hareland 2003). This is a serious problem for farmers and exporters in the PNW. While FN is a useful tool for grading, it strictly measures the enzyme's effect on the starch endogenous to the grain as opposed αA activity directly. FN values > 300 s have been observed in wheat in the absence of high αA activity. It has been shown that low grain protein content (GPC) can artificially lower FN values in ungerminated soft white winter wheat from the U.S. Pacific Northwest (Ross et al 2012).

Direct measurement of αA activity is the reference method for framing all other measures of PHS. At αA activities < 0.1 Ceralpha units (CU: AACCI Approved Method 22-02.01) αA activity in the grain is sufficiently low that it does not cause processing problems. As αA is the primary enzyme related to reduced end-product quality, effort has been spent to either reduce αA activity in PHS-affected wheat or mitigate its effects. For reduction, Hareland (2003) found that removing the bran via pearling wheat increased FN and decreased αA activity, a likely result of removing the enzyme-containing aleurone layer. However, pearling had no effect on dough characteristics (as indexed by Farinograph stability), bread loaf volume, or crumb characteristics as compared to non-pearled controls (Hareland 2003). This suggests pearling may not be a viable option to improve PHS-affected wheat processing characteristics and other methods must be explored.

Post-harvest grain storage has been suggested as another way to reduce the effects of PHS damage. Lunn et al (2000) cited anecdotal evidence that suggested that increased FN during storage was a result of inactivation pericarp-derived αA. The effect of grain storage on FN has been explored before. A number of studies have observed increased FN during grain storage. Harvest year, storage temperature, growing environment, and grain moisture content were reported to have significant effects (Lukow et al 1995, Hruskova et al 2004, Karaoglu et al 2010). Brandolini et al (2010) also observed an increase in FN with increasing storage time and temperature. However this was in stored wheat flour and not intact grain. On the contrary, Abid et al (2009) observed decreases in FN to < 300 s in six wheat varieties over 12 months of storage, but this was also done with flour as opposed to intact grain.

The common thread missing in many of the studies cited above are observations of changes in intact grains, with varying levels of sprout damage, across different relevant storage temperatures, over practical storage durations. The goals of this study are to observe if FN and αA activity of intact grain are altered by storage, if changes in FN or αA activity are affected by storage temperature, if changes during grain storage are effective in increasing wheat from poor to acceptable FN values and αA activity levels, and if potential changes in FN and αA activity are affected by the degree of PHS. We hypothesize that the extent of mitigation will be a function of wheat variety, growing conditions, storage time and temperature, and the extent to which the grain has been damaged.

4.2 Materials and Methods

4.2.1 Materials

Wheat samples

Ten soft and 6 hard wheat varieties from were selected three Idaho locations (Aberdeen, Parma, and Kimberly) were screened for FN and αA activity. Samples from Aberdeen and Kimberly were obtained from research plots planted, maintained, and harvested by the University of Idaho Agronomy Program. Samples from Parma were obtained from research plots planted, maintained, and harvested by the Oregon State University Wheat Breeding Program. Varieties and locations were chosen to capture a range of FN values and αA activities from poor to acceptable. The location Aberdeen was harvested on August 11 2014 and received on October 6 2014. Parma was harvested on August 3 2014 and received on September 25 2014. Kimberly

was harvested on August 20 2014 and received October 3 2014. After harvest, samples were stored indoors at ambient temperature and humidity in plastic tubs (Parma) or paper bags (Aberdeen and Kimberly). Upon receiving, samples were screened for FN and αA activity within 5 days. Samples were then subdivided and stored at -20°C, +20°C, and +40°C in zip-closure plastic bags in a temperature controlled cabinet. Temperature and relative humidity were continuously monitored over storage time.

Milling

For each day of testing 25 g of each wheat sample was pulverized in a Perten Laboratory Mill (LM 3100, Perten Instruments, Inc., Springfield, IL) with a 0.8 mm screen. All samples were milled 24 hours before testing. Frozen subsamples were allowed 12 hours to equilibrate to room temperature before milling.

Reagents

Reagents for αA activity measurement were prepared using contents from the Ceralpha Method kit according to procedures outlined by Megazyme International (Bray, Co. Wicklow, Ireland). Reagent materials not provided in kit were purchased in concentrated form from Sigma-Aldrich (St. Louis, MO). Preparation of reagents for αA activity assay are described in-depth in the methods section.

4.2.2 Methods

Falling Number Analysis

FN analyses were performed in duplicate after 0, 14, 30, 60, and 90 days of storage for each of the three storage temperatures. FN was performed according to the AACCI approved method 56-

81.03 using a FN apparatus (FN 1700, Perten Instruments, Inc., Springfield, IL) with one modification. Samples of 25 g, rather than the standard 300 g, were milled as a result of a limited supply of sample. Samples of wheat meal (7 g) were added to Perten Falling Number tubes followed by 25 ml deionized water. Tubes were stoppered and placed into a Perten Shakematic (SM 1095, Perten Instruments, Inc., Springfield, IL) to shake (3 s) and to hydrate wheat meal; tubes were inverted once after mixing to ensure all wheat meal was suspended. FN plungers were used to scrape down residual slurry on stoppers and upper part of tubes and then placed into tubes. Tubes were placed into boiling water bath of the FN apparatus. Within 5 s the apparatus engaged the plungers and began mixing the wheat meal suspension using an up and down oscillating motion. After 60 s of mixing the plungers were automatically lifted to the top of the tubes and released. The plungers were allowed to fall freely through the samples and upon reaching the bottom of the tubes the timers were automatically stopped. The numbers on the timers are the FN values and include the initial 60 s mixing step. FN values were reported as is (without moisture correction).

Alpha Amylase Activity

αA activity analyses were performed in duplicate after 0, 14, 30, 60, and 90 days of storage for each of the three storage temperatures. αA assays were performed using the Megazyme Ceralpha Method assay kit (K-CERA 09/11, Megazyme International, Bray, Co. Wicklow, Ireland), AACCI Approved Method 22-02.01 with some modifications. Samples of 25 g, rather than the standard 50 g, were milled as a result of a limited supply of sample. The mass of wheat meal used was reduced from the standard 3.0 g to 1.5 g and appropriate solvent volume was subsequently reduced by half in the assay.

The extraction buffer was prepared from a concentrated sodium malate buffer. The concentrated sodium malate buffer (1 M) was prepared by dissolving 134.1 g malic acid, 70 g NaOH, and 58.4 g NaCl in 800 ml deionized water. After solution cooled to room temperature 5.9 g $CaCl_2 \cdot 2H_2O$ was added. The pH was adjusted to 5.4 by dropwise addition of 4 M NaOH or 4 M HCl. Concentrated sodium malate buffer then received 1 g sodium azide and volume was adjusted to 1 liter. The sodium malate buffer was stored at room temperature. The extraction buffer was prepared to 50 mM and pH 5.4 by diluting 50 ml concentrated sodium malate buffer to 1 liter with deionized water. The extraction buffer was stored at refrigeration temperature for up to three months.

A substrate solution was prepared by dissolving contents of one vial (54.5 mg blocked *p*-nitrophenyl maltoheptaoside (BPNPG7) and 125 U thermostable α-glucosidase) of Amylase HR reagent from Megazyme International in 10 ml of room temperature, previously-boiled water. Prepared substrate was divided into 2 ml aliquots and stored frozen.

The assay stopping reagent was prepared by dissolving 10 g anhydrous tri-sodium phosphate in 1 liter deionized water and adjusting pH to 11. Stopping reagent was stored at room temperature for up to 3 months.

αA assay was performed by weighing 1.5 g samples into 50 ml centrifuge tubes with lids. Samples were suspended in extraction buffer (10 ml) via vortex and placed in 40°C water bath for 20 min. Tubes were agitated every 5 min. After 20 min of extraction, samples were

centrifuged for 10 min at 1,000 RCF. The resulting supernatant was assayed within 2 hours of centrifugation. Substrate reagent (0.1 ml) was added to glass test tubes and equilibrated to 40°C for 5 min; wheat meal extract was also equilibrated to 40°C for 5 min. Each tube of temperature-equilibrated substrate received 0.1 ml temperature-equilibrated wheat meal extract, was vortexed, and then incubated in 40°C water bath for exactly 20 min (from time of addition). After 20 min, assay was terminated by the addition of stopping reagent (1.5 ml) and then vortexed. After addition of stopping reagent 0.2 ml of the resulting solution was transferred to 96-well spectrophotometer plate (in duplicate). Absorbance of samples and reaction blank (0.1 ml extraction buffer, 0.1 ml substrate, and 1.5 ml stopping reagent) were measured against deionized water at λ = 400 nm in a Molecular Devices VersaMax Tunable Microplate Reader . Calculation of αA activity was performed using equation: $\frac{\Delta E_{400}}{T_I} \times \frac{V_T}{V_A} \times \frac{1}{E_{mM}} \times \frac{V_{Ex}}{M_S} = Units\ \frac{CU}{g\ flour}$ where ΔE_{400} = [(sample absorbance – deionized water absorbance) – (blank absorbance – deionized water absorbance)], T_I = incubation time (20 min), V_T = volume in test tube (1.7 ml), V_A = volume of aliquot assayed (0.1 ml), E_{mM} = absorbance of *p*-nitrophenyl at λ = 400 nm in 1% tri-sodium phosphate (18.1, provided by Megazyme International), V_{Ex} = Extraction volume (10 ml), and M_S = weight of sample (1.5 g). This equation yields the Ceralpha Units (CU) per gram of flour which is equal to the amount of enzyme required to release 1 µmol of *p*-nitrophenyl from BPNPG7 in one minute under assay conditions in the presence of thermostable α-glucosidase.

Statistical Analysis

Multifactor analyses of variance and correlation analyses were carried out using JMP 11 (SAS Institute Inc., Cary, NC). Statistical significance was set at $p < 0.01$.

4.3 Results and Discussion: Falling Number

Figure 4.1 shows the curvilinear relationship between αA activity and FN. A power law function was applied to this data set with an equation of $y = 23.98*x^{-1.004}$ ($r = 0.87$, $p < 0.0001$). This curvilinear relationship is consistent with observations from all studies about αA activity and FN since the FN method was applied to wheat (Hagberg 1961). This shows that the relationship between αA activity and FN observed in this collection of wheat varieties is not anomalous. It is evident from figure 4.1 that there is an asymptote at αA activity = 0.05 CU. This is likely due to the detection limit of the αA assay.

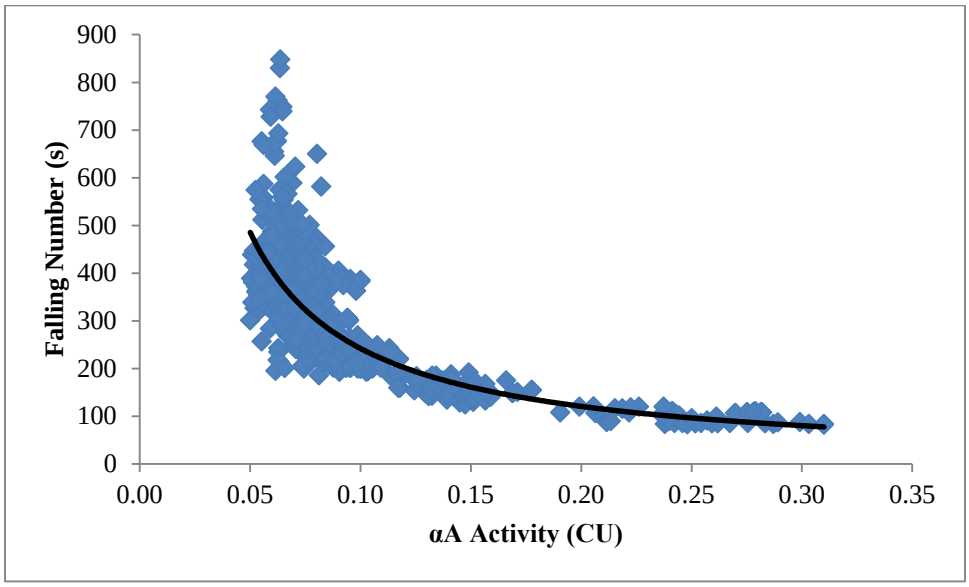

Figure 4.1 - Scatterplot of Falling Number (s) by alpha amylase (αA) activity (Ceralpha Units, CU). A power law fit is presented in black.

4.4 Results and Discussion: Soft wheat

Summary statistics for soft wheat αA activity are shown in table 4.1. The full-factorial four-way ANOVA showed that αA activities varied significantly between growing locations, wheat varieties, and days of storage (Table 4.2). F-ratios from the ANOVA indicated that environment

had the largest influence on αA activity, followed by variety and storage time. Environment was also the most influential main effect in both soft and hard wheat FN (Tables 3.2 and 3.8). Storage temperature was not significant. Most of the two-way interaction terms were significant. The largest F-ratios were for the location*variety and then the location*storage time interactions.

Table 4.1 shows that the location Kimberly had both the highest absolute αA activity and the highest mean αA activity of the 3 locations. This indicated that Kimberly was likely to have been affected by PHS. This was expected as our collaborators at the University of Idaho indicated a noteworthy rain event after grain reached physiological maturity. Aberdeen and Parma both had maximum αA activities of 0.1, which is diagnostic of PHS damage. However, the FN values associated with these two measurements were 383 s (Aberdeen) and 386 s (Parma) suggesting otherwise. Both of these observations occurred in the wheat variety Skiles.

All wheat varieties had minimum αA activities of < 0.1 CU. This indicated either no evidence of PHS at one or more locations, or a decrease in αA activity over storage time. Most wheat varieties had maximum αA activities of > 0.1 CU suggesting that most were affected by PHS at one or more locations. LCS Artdeco and LCS Biancor had the highest mean αA activities. This suggests that both varieties either had high susceptibility to PHS, or that they may be earlier maturing varieties. In this case seed dormancy may have already been eroding while the harvest-ripe seeds waited in the field to be harvested. The logistics of harvesting breeding nurseries means that in practice all plots are harvested on the same day, and only after the latest maturing varieties reach harvest maturity.

Table 4.1 - Summary Statistics of soft wheat alpha amylase activities (Ceralpha Units).

Location	**Min**	**Max**	**Mean**	**SD**	**SE**
Aberdeen	0.051	0.100	0.072	0.01	<0.01
Kimberly	0.063	0.310	0.131	0.07	<0.01
Parma	0.050	0.100	0.067	0.01	<0.01
Variety					
LCS Artdeco	0.062	0.310	0.141	0.09	0.01
LCS Biancor	0.050	0.282	0.123	0.09	0.01
WB 1070 CL	0.061	0.178	0.093	0.04	0.01
Kaseberg	0.056	0.159	0.091	0.04	0.01
Rosalyn	0.062	0.117	0.081	0.02	0.01
Skiles	0.055	0.100	0.080	0.01	0.01
OR2090473	0.060	0.113	0.077	0.01	0.01
SY Ovation	0.058	0.091	0.073	0.01	0.01
Bobtail	0.059	0.093	0.072	0.01	0.01
Mary	0.051	0.104	0.069	0.02	0.01
Temperature					
-20°	0.052	0.310	0.090	0.05	<0.01
+20°	0.050	0.310	0.090	0.05	<0.01
+40°	0.050	0.310	0.090	0.05	<0.01
Storage Time (Days)					
0	0.053	0.310	0.097	0.06	<0.01
14	0.051	0.303	0.094	0.05	<0.01
30	0.053	0.282	0.087	0.05	<0.01
60	0.051	0.257	0.086	0.04	<0.01
90	0.050	0.275	0.087	0.04	<0.01

Table 4.2 - F-statistics from ANOVA of alpha amylase (αA) activities (Ceralpha Units) of all factors and interactions in soft wheats.

Soft Wheats	F-statistic
Location	7408.7*
Variety	973.5*
Temperature	ns
Storage Time	258.6*
Location*Variety	941.1*
Location*Temperature	7.4*
Location*Storage Time	76.7*
Variety*Temperature	ns
Variety*Storage Time	16.6*
Temperature*Storage Time	ns
Location*Variety*Temperature	ns
Location *Variety*Storage Time	10.0*
Location*Temperature *Storage Time	ns
Variety*Temperature*Storage Time	2.4*
Location* Variety*Temperature*Storage Time	2.3*

*significant at $P < 0.01$, ns not significant at $P < 0.01$

F-statistics from 4-way ANOVA (Table 4.2) indicated significant influences on αA activity for all main effects except storage temperature. The majority of interaction terms were also significant. Given the number of significant interactions it was deemed prudent to analyze each location*temperature treatment separately. Figures 4.2, 4.3, and 4.4 show changes in αA activities over time of storage for the 10 soft varieties for each location*temperature treatment. It is evident that αA activity changed in some varieties at some storage temperatures.

4.4.1 Storage at -20°C

The two-way ANOVA for the location*temperature treatment, Aberdeen at -20°C, is shown in Table 4.3. There were significant differences between both storage times and varieties. The F-ratios indicated the major influence was variety, as was the case for FN in this treatment. The

storage time*variety interaction term was also significant. Regression analyses (Table 4.4) showed that two varieties, Kaseberg and LCS Biancor, had significant decreases in αA activity across the 90 days of storage. Neither of these two varieties in this treatment had significant increases in FN over storage time (Section 3.3.1). The rates of decrease for both Kaseberg and LCS Biancor were barely detectable at < 0.01 CU per 90 days. The correlation of the overall αA activity for each day (across varieties) with storage time was not significant. The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity across all varieties.

Table 4.3 - F-statistics (F-stat) from two-way ANOVA of soft wheat alpha amylase (αA) activity (Ceralpha Units) by storage time, variety and the storage time*variety interaction for each location*temperature treatment.

Aberdeen, -20°	**F-stat**	**Kimberly, -20°**	**F-stat**	**Parma, -20°**	**F-stat**
Storage Time	15.6*	Storage Time	109.1*	Storage Time	7.7*
Variety	122.6*	Variety	1285.3*	Variety	104.4*
Storage Time*Variety	7.0*	Storage Time*Variety	12.9*	Storage Time*Variety	6.5*
Aberdeen, +20°	**F-stat**	**Kimberly, +20°**	**F-stat**	**Parma, +20°**	**F-stat**
Storage Time	52.7*	Storage Time	92.2*	Storage Time	114.6*
Variety	116.4*	Variety	2174.4*	Variety	104.8*
Storage Time*Variety	10.3*	Storage Time*Variety	11.7*	Storage Time*Variety	28.6
Aberdeen,+ 40°	**F-stat**	**Kimberly, +40°**	**F-stat**	**Parma, +40°**	**F-stat**
Storage Time	62.5*	Storage Time	100.4*	Storage Time	19.4*
Variety	102.1*	Variety	2370.5*	Variety	102.0*
Storage Time*Variety	6.6*	Storage Time*Variety	16.8*	Storage Time*Variety	10.5*

*significant at P < 0.01, ns not significant at P < 0.01

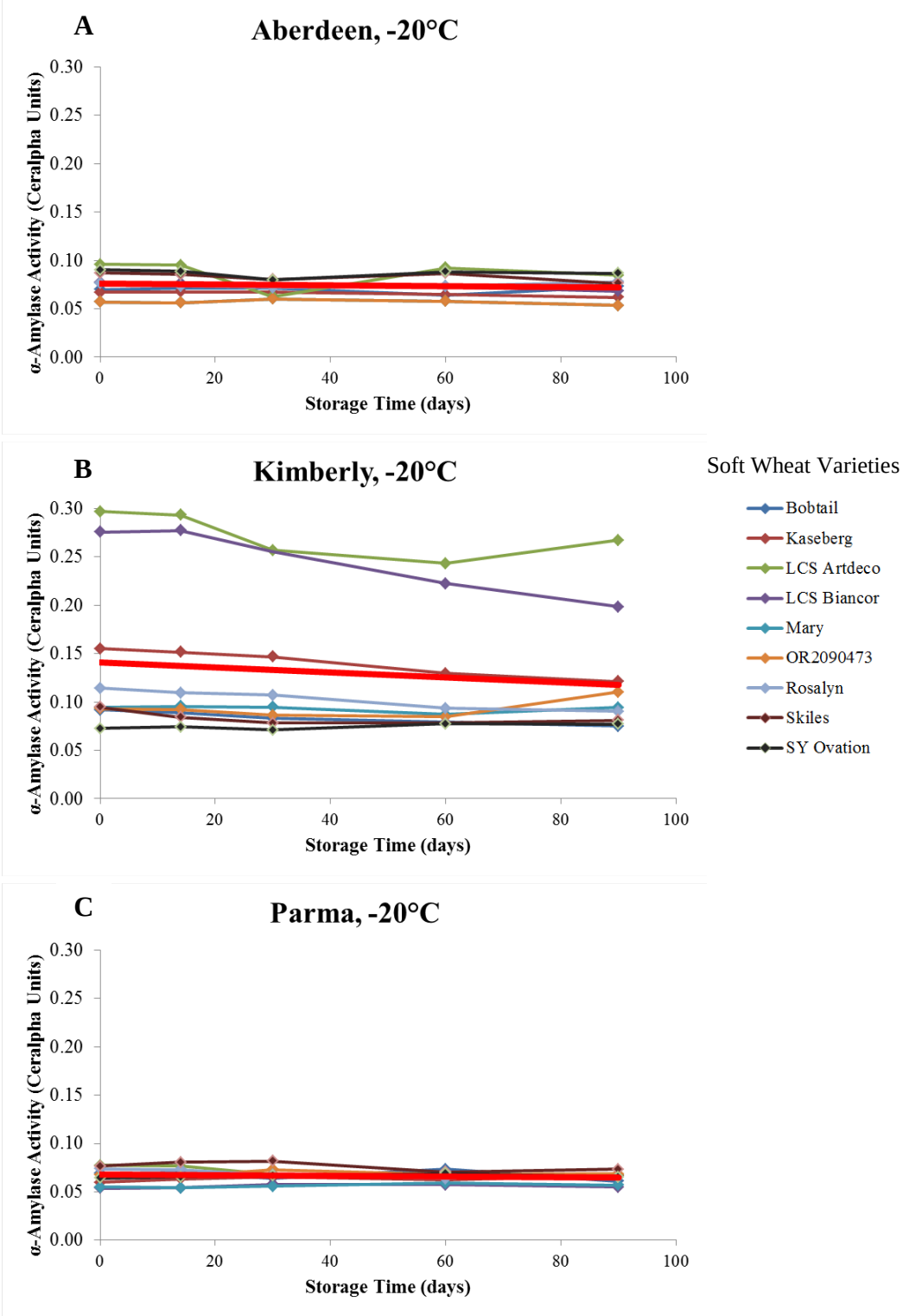

Figure 4.2 - Mean alpha amylase (αA) activities (Ceralpha Units) of soft wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at -20°C. Linear regression of overall αA activities (averaged across varieties) is presented in red.

The two-way ANOVA for the location*temperature treatment, Kimberly at -20°C, is shown in Table 4.3. There were significant differences between both days of storage and wheat varieties. The F-ratios indicated the major influence was variety, followed by storage time. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 4.4) showed that half of the wheat varieties had a significant decrease in αA activity across the 90 days of storage. However, the correlation of the mean αA activity for each day (across varieties) with storage time was not significant. Significant decreases in αA activity ranged from a low of 0.016 CU per 90 days from Bobtail to a high of 0.084 CU per 90 days from LCS Biancor. Only the variety Kaseberg from this treatment had a significant increase in FN values over storage time (Section 3.3.1). The variety Rosalyn in this treatment had a decrease in αA activity over time of storage from 0.11 CU to 0.09 CU, but it did not have a significant increase in FN over storage time. Rosalyn was the only variety to have a significant decrease in αA activity from > 0.1 CU to < 0.1 CU at this storage temperature. Figure 4.2B shows that wheat varieties with initial (day 0) αA activities > 0.1 CU behaved differently over storage time than wheats with initial αA activities < 0.1 CU. This conjecture is supported by the significant storage time*variety term (Table 4.3). Separate linear regression models were applied to wheat varieties from Kimberly with initial αA activities > 0.1 CU and < 0.1 CU. There was no significant decrease over time in wheat varieties with initial αA activities > 0.1 CU ($r = -0.08$, $p = 0.1$). There was also no significant decrease over time in wheat varieties with initial αA activities < 0.1 CU($r = -0.09$, $p = 0.53$). The results show that for this location storage of grain for 90 days at -20°C was associated with some variety specific systematic decreases in αA activity. The trend of decreases in αA activities over storage time occurred, primarily, in wheat varieties with higher day 0 αA activities. This is in direct contrast to the results from the FN

study. Increases in FN over storage time primarily occurred in wheat varieties with day 0 FN > 300 s.

The two-way ANOVA for the location*temperature treatment, Parma at -20°C, is shown in Table 4.3. There were significant differences between storage times and varieties. The F-ratios indicated the major influence was variety. The storage time*variety interaction term was also significant. Regression analyses (Table 4.4) showed that most varieties did not have a significant decrease in αA activity across the 90 days of storage. Significant decreases in αA activity ranged from a low of 0.01 CU per 90 days from LCS Artdeco and Rosalyn to a high of 0.13 CU per 90 days from WB 1070 CL. The variety Rosalyn from this treatment also had a significant increase in FN over storage time, but LCS Artdeco and WB 1070 CL did not (Section 3.3.1). The other variety from this treatment that had significant increases in FN over storage time was SY Ovation, which did not have significant decreases in αA activity when stored at -20°C. The correlation of the overall αA activity for each day (across varieties) with storage time was not significant. The results show that for this location*temperature treatment, storage of grain was associated with some variety specific systematic decreases in αA activity.

In summary, at -20°C there were very few systematic changes in αA activity associated with grain storage in this collection of soft wheat varieties. The largest decreases were seen in presumptively sprouted wheats from Kimberly, which directly contrasts results from the FN study where significant increases in FN at -20°C primarily occurred in sound wheat varieties. The varieties Rosalyn and WB 1070 CL showed systematic decreases in αA activities at both Kimberly and Parma, but with no accompanying increase in FN over time (except Rosalyn). The

varieties Kaseberg and LCS Biancor showed systematic decreases in αA activities at both Aberdeen and Parma, but neither variety from these treatments showed significant increases in FN over storage time. Decreases in overall αA activities across all varieties over 90 days of storage was not significant in wheats from Aberdeen, Kimberly, or Parma at -20°C. This contrasts with the significant increases in overall FN values observed in soft wheats from Parma at -20°C.

Table 4.4 - Linear regression of alpha amylase (αA) activities (Ceralpha Units) of soft wheats from Aberdeen, Kimberly, and Parma stored at -20°C for 90 days.

-20°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Bobtail	0.070	-2.2E-06	-0.02	ns	0.090	-1.8E-04	-0.96	<0.0001	0.069	-4.7E-05	-0.36	ns
Kaseberg	0.068	-6.6E-05	-0.86	<0.001	0.157	-4.1E-04	-0.98	<0.0001	0.062	5.2E-05	0.65	ns
LCS Artdeco	0.089	-6.1E-05	-0.16	ns	0.287	-4.2E-04	-0.60	ns	0.076	-1.1E-04	-0.67	<0.01
LCS Biancor	0.077	-9.3E-05	-0.92	<0.001	0.282	-9.3E-04	-0.98	<0.0001	0.055	1.6E-05	0.28	ns
Mary	0.058	-2.7E-05	-0.35	ns	0.095	-4.0E-05	-0.26	ns	0.055	3.7E-05	0.59	ns
OR2090473	0.074	3.7E-05	0.51	ns	0.087	1.5E-04	0.53	ns	0.068	1.2E-05	0.15	ns
Rosalyn	0.074	-2.5E-05	-0.27	ns	0.114	-2.8E-04	-0.96	<0.0001	0.073	-1.1E-04	-0.84	<0.001
Skiles	0.086	-8.5E-05	-0.53	ns	0.088	-1.2E-04	-0.62	ns	0.080	-8.3E-05	-0.58	ns
SY Ovation	0.088	-2.2E-05	-0.18	ns	0.072	5.7E-05	0.63	ns	0.065	2.3E-05	0.43	ns
WB 1070 CL	0.073	-8.3E-05	-0.67	ns	0.167	-6.2E-04	-0.85	<0.001	0.077	-1.4E-04	-0.81	<0.001
Overall αA	**0.076**	**-3.8E-05**	**-0.12**	**ns**	**0.141**	**-2.6E-04**	**-0.40**	**ns**	**0.068**	**-3.5E-05**	**-0.15**	**ns**

4.4.2 Storage at +20°C

The two-way ANOVA for the location*temperature treatment, Aberdeen at +20°C, is shown in Table 4.3. There were significant differences between both storage times and varieties, as was the case for FN in soft wheats from this treatment. The F-ratios indicated the major influence was variety. The storage time*variety interaction term was also significant. Regression analyses (Table 4.5) showed that two varieties, OR2090473 and WB 1070 CL, had significant decreases in αA activity across the 90 days of storage. However, neither of these two varieties from this treatment had significant increases in FN over storage time (Section 3.3.2). The only soft wheat

variety from this treatment with a significant increase in FN over storage time was Mary, which had no corresponding significant decrease in αA activity. Significant decreases in αA activity ranged from a low of 0.011 CU per 90 days from WB 1070 CL to a high of 0.014 CU per 90 days from OR2090473. The correlation of overall αA activity for each day (across varieties) with storage time was not significant, as was the case with overall increases in FN for this treatment. The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in in αA activity. However, there were variety specific decreases.

The two-way ANOVA for the location*temperature treatment, Kimberly at +20°C, is shown in Table 4.3. There were significant differences between both storage times and varieties. The F-ratios indicated the major influence was variety, as was the case for FN in this treatment. The storage time*variety interaction term was also significant. Regression analyses (Table 4.5) showed that some varieties had significant decreases in in αA activity across the 90 days of storage. Significant decreases in αA activity ranged from a low of 0.014 CU per 90 days from Skiles to a high of 0.068 CU per 90 days from LCS Artdeco. LCS Artdeco was the only soft wheat variety from this treatment to have a significant increase in FN values over storage time (Section 3.3.2). The variety Rosalyn from Kimberly, as it was when stored at -20°C, was the only variety that had a significant decrease in αA activity from > 0.1 CU to < 0.1 CU when stored at +20°C, however there was no corresponding significant increase in FN. The correlation of overall αA activities for each day (across varieties) with storage time was not significant. The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity, however, there were variety specific decreases.

The two-way ANOVA for the location*temperature treatment, Parma at +20°C, is shown in Table 4.3. There were significant differences between both storage times and varieties. The F-ratios indicated that storage time and wheat variety were about equally influential, as was the case for FN in this treatment. The storage time*variety interaction term was also significant. Regression analyses (Table 4.5) showed that only two varieties, LCS Artdeco and WB 1070 CL, had significant decreases in αA activity across the 90 days of storage. Decrease in αA activity was 0.014 CU per 90 days from LCS Artdeco and 0.002 CU per 90 days from WB 1070 CL. Neither of these two varieties from this treatment had corresponding significant increases in FN values over storage time (Section 3.3.2).The only soft wheat variety from this treatment with a significant increase in FN over storage time was SY Ovation, a variety that had no evidence of PHS damage on day 0 (αA activity = 0.065 CU, FN = 302 s). The correlation of overall αA activity for each day (across varieties) with storage time was not significant, which is in direct contrast with the overall significant increase in FN across all varieties observed in this treatment (Section 3.3.2). The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity overall. However, there were variety specific decreases.

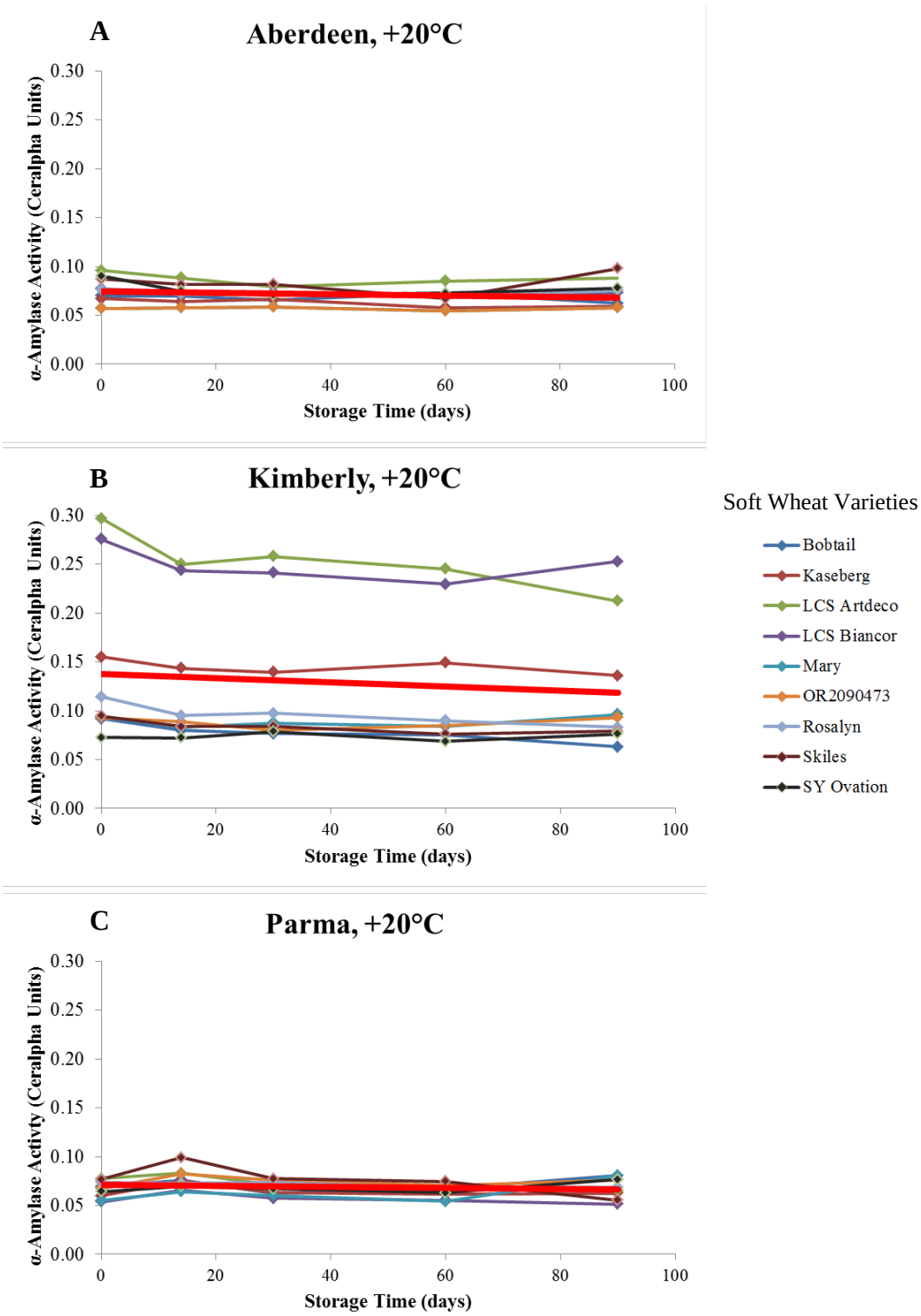

Figure 4.3 - Mean alpha amylase (αA) activities (Ceralpha Units) of soft wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at +20°C. Linear regression of overall αA activities (averaged across varieties) is presented in red.

In summary, at +20°C there were very few systematic changes in αA activity associated with grain storage in this collection of soft wheat varieties. These changes were, as it was for -20°C, primarily in presumptively sprouted wheats from Kimberly. The variety WB 1070 CL showed systematic decreases in αA activities at both Aberdeen and Parma with no corresponding significant increases in FN. The variety LCS Artdeco showed systematic decreases in αA activities at both Kimberly and Parma with only a corresponding significant increase in FN from Kimberly. Overall αA activities across all varieties over 90 days of storage at +20°C did not significantly decrease in wheats from Aberdeen, Kimberly, or Parma. This is corroborated with the observed flat trend lines in Figure 4.3. Notably, the varieties Bobtail and Rosalyn from Kimberly and the varieties LCS Artdeco and WB 1070 CL from Parma all had significant decreases in αA activity over storage time at both -20°C and +20°C. This may suggest that αA from these varieties at these locations is susceptible to degradation in storage. However, none of these four varieties from those two locations had corresponding increases in FN over storage times at either -20°C or +20°C. This is consistent with the observation from soft wheats stored at -20°C that significant systematic decreases in αA activity over storage time primarily occur in wheats with high initial levels of αA while significant systematic increases in FN over storage time primarily occur in wheats with initial FN values > 300 s.

Table 4.5 - Linear regression of alpha amylase (αA) activities (Ceralpha Units) of soft wheats from Aberdeen, Kimberly, and Parma stored at +20°C for 90 days.

+20°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Bobtail	0.070	-5.0E-05	-0.41	ns	0.087	-2.6E-04	-0.92	<0.001	0.068	7.9E-05	0.39	ns
Kaseberg	0.066	-9.3E-05	-0.67	ns	0.150	-1.4E-04	-0.58	ns	0.066	-4.4E-05	-0.26	ns
LCS Artdeco	0.092	-1.8E-04	-0.62	ns	0.281	-7.5E-04	-0.87	<0.001	0.080	-1.6E-04	-0.84	<0.001
LCS Biancor	0.076	-4.8E-05	-0.56	ns	0.256	-2.0E-04	-0.40	ns	0.060	-7.6E-05	-0.16	ns
Mary	0.057	-1.5E-06	-0.03	ns	0.087	4.3E-05	0.23	ns	0.055	2.0E-04	0.21	ns
OR2090473	0.076	-1.5E-04	-0.90	<0.001	0.087	1.1E-05	0.07	ns	0.074	1.5E-05	0.09	ns
Rosalyn	0.073	-6.0E-06	-0.06	ns	0.107	-2.8E-04	-0.87	<0.001	0.074	-7.0E-05	-0.75	ns
Skiles	0.081	5.6E-05	0.18	ns	0.090	-1.6E-04	-0.81	<0.001	0.089	-3.3E-04	-0.76	ns
SY Ovation	0.081	-8.6E-05	-0.40	ns	0.073	1.4E-05	0.12	ns	0.065	8.7E-05	0.56	ns
WB 1070 CL	0.074	-1.2E-04	-0.88	<0.001	0.155	-4.1E-04	-0.68	ns	0.079	-1.9E-05	-0.28	<0.001
Overall αA	**0.075**	**-7.6E-05**	**-0.24**	**ns**	**0.137**	**-2.1E-04**	**-0.10**	**ns**	**0.071**	**-4.9E-05**	**-0.17**	**ns**

4.4.3 Storage at +40°C

The two-way ANOVA the location*temperature treatment, Aberdeen at +40°C, is shown in Table 4.3. There were significant differences between storage times and varieties, as was the case in FN values for this treatment. The F-ratios indicated the major influence was from variety. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 4.5) showed that some varieties had significant decreases in αA activity across the 90 days of storage. Significant decreases in αA activity ranged from a low of 0.01 CU per 90 days from Bobtail and WB 1070 CL to a high of 0.023 CU per 90 days from SY Ovation. Both of these varieties, as all soft wheat varieties form this treatment, exhibited significant systematic increases in FN values over storage time (Section 3.3.3). The correlation of overall αA activity for each day (across varieties) with storage time was also significant. The change overall change in αA activity across all varieties was barely detectable at 0.008 CU per 90 days. The results show that for this location*temperature treatment, storage of grain was associated with systematic decreases in αA activity.

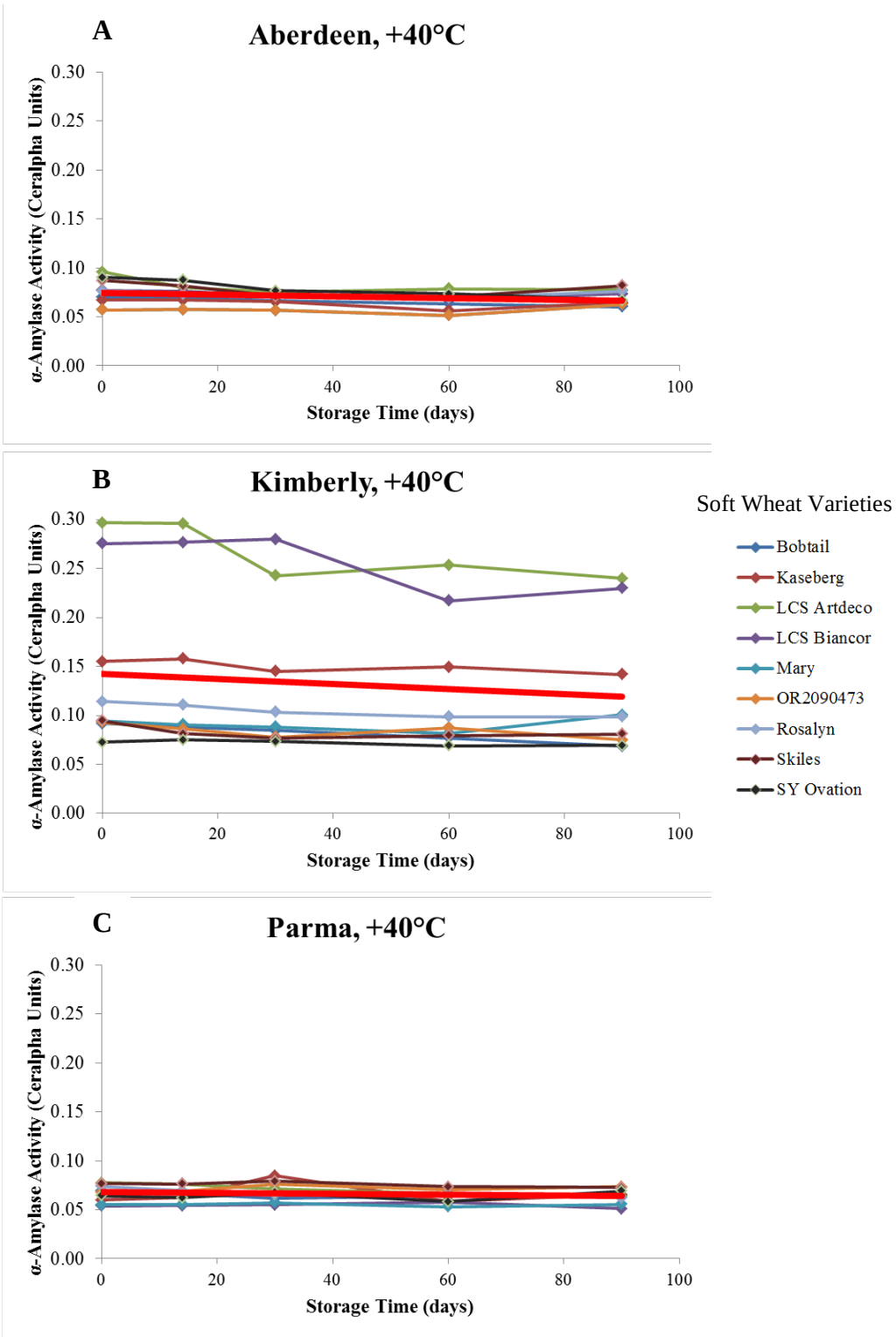

Figure 4.4 - Mean alpha amylase (αA) activities (Ceralpha Units) of soft wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at +40°C. Linear regression of overall αA activities (averaged across varieties) is presented in red.

The two-way ANOVA for the location*temperature treatment, Kimberly at +40°C, is shown in Table 4.3 There were significant differences between both storage times and varieties. The F-ratios indicated the major influence was variety, followed by storage time. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 4.6) showed that some varieties had significant decreases in αA activity across the 90 days of storage. Significant decreases in αA activity ranged from a low of 0.016 CU per 90 days from Rosalyn to a high of 0.063 CU per 90 days from LCS Biancor. The variety Rosalyn from Kimberly, as it was when stored at -20°C and +20°C, was the only variety that had a significant decrease in αA activity from > 0.1 CU to < 0.1 CU when stored at +40°C, however this decrease was only to 0.099 CU which was higher than its day 90 αA activity when stored at either 20°C or +20°C. The decrease in αA activity over storage time at +40°C for LCS Biancor from Kimberly was higher than the decrease seen at -20°C (0 CU per 90 days, NS) but lower than the decrease seen at +20°C (-0.068 CU per 90 day). All of the soft wheat varieties from this treatment that had significant systematic decreases in αA activity over storage time also had corresponding significant systematic increases in FN over storage time. The correlation of overall αA activity for each day (across varieties) with storage time was not significant, which was also the case for overall changes in FN values over storage time for this treatment (Section 3.3.3). Figure 4.4B shows that varieties with an initial (day 0) αA activity > 0.1 CU behaved differently than those with initial αA activity < 0.1 CU. Separate linear regression models were applied to varieties with initial > 0.1 CU and < 0.1 CU. There were significant systematic decreases in αA over time in soft wheats with initial αA < 0.1 CU ($r = -0.38$, $p = 0.007$). However, there was no significant decrease in αA activity over storage time in soft wheat varieties with initial αA activity > 0.01 CU ($r = -0.19$, $p = 0.19$). This is observation is in contrast

with previous storage temperatures where significant decreases in αA activities primarily occurred in soft wheats with high initial αA activities. The overall rate of decrease in αA activity for soft wheats with initial αA activity < 0.01 CU was 0.013 CU per 90 days. The results show that for this location*temperature treatment, storage of grain was associated with significant systematic decreases in αA activity in soft wheats with initial αA activities < 0.01 CU but not those with initial αA activities > 0.01 CU. All of the varieties from this treatment that had significant systematic decreases in αA activity over storage time also had significant increases in FN.

The two-way ANOVA for the location*temperature treatment, Parma at +40°C, is shown in Table 4.3. There were significant differences between both storage times and varieties. The F-ratios indicated that the major influence was variety. The storage time*variety interaction term was also significant. Regression analyses (Table 4.6) showed most varieties did not have significant decreases in αA activities across the 90 days of storage. Significant decreases in αA activity ranged from a low of 0.009 CU per 90 days from Rosalyn to a high of 0.014 CU per 90 days from LCS Artdeco and WB 1070 CL. All three of these varieties from this treatment that had significant decreases in αA activity had significant increases in FN over storage time as well (Section 3.3.3). The correlation of overall αA activity for each day (across varieties) with storage time was not significant, this is in contrast to the overall significant increase in FN over storage time observed from this treatment. The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity. However, there were variety specific decreases.

In summary, at +40°C there were some systematic changes in αA activity associated with grain storage in this collection of soft wheat varieties. However, this was primarily observed in wheats from Aberdeen and, as it was for storage at -20°C and +20°C, from Kimberly. Notably, the varieties Bobtail and Rosalyn from Kimberly and the variety LCS Artdeco from Parma showed systematic decreases in αA activities at all storage temperatures. This suggests that αA from these varieties may be susceptible to degradation of αA activity when stored. The variety WB 1070 CL showed systematic decreases in αA activities at Parma at all storage temperatures and at Aberdeen at both +20°C and +40°C. This suggests that αA from this variety, when unsprouted, may be susceptible to degradation of activity during storage when stored at ambient or higher temperatures. Variety was the most influential effect as assessed by F-ratio for all location*temperature treatments (except Parma at +20°C where variety and storage time were equally influential) (Table 4.3). The variety Rosalyn from Kimberly was the only variety to have a decrease in αA activity from > 0.01 CU to < 0.01 CU, however this occurred at a higher degree when stored at -20°C and +20°C than when stored at +40°C. This suggests that the variety Rosalyn may be susceptible to degradation of αA activity over storage time, particularly when sprouted. The pattern of increasing influence by storage time with increasing storage temperature was not as clear in αA activity as it was in FN. This suggests that differences in αA activity in this collection of soft wheats were primarily variety dependent and that time of storage was less influential. This is corroborated by Figures 4.2, 4.3, and 4.4 where the majority of trends in linear regressions were more or less flat.

Table 4.6 - Linear regression of alpha amylase (αA) activities (Ceralpha Units) of soft wheats from Aberdeen, Kimberly, and Parma stored at +40°C for 90 days.

+40°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Bobtail	0.070	-1.1E-04	-0.97	<0.001	0.092	-2.6E-04	-0.99	<0.0001	0.067	-3.9E-05	-0.43	ns
Kaseberg	0.067	-6.2E-05	-0.48	ns	0.156	-1.5E-04	-0.76	ns	0.066	-5.3E-06	-0.02	ns
LCS Artdeco	0.087	-1.4E-04	-0.59	ns	0.290	-6.3E-04	-0.78	<0.001	0.077	-1.6E-04	-0.92	<0.001
LCS Biancor	0.076	-5.9E-05	-0.56	ns	0.283	-7.0E-04	-0.83	<0.001	0.006	-2.2E-05	-0.27	ns
Mary	0.056	3.3E-05	0.29	ns	0.090	3.8E-05	0.18	ns	0.055	-6.5E-06	-0.14	ns
OR2090473	0.076	-1.5E-04	-0.91	<0.001	0.089	-1.4E-04	-0.66	ns	0.069	4.6E-05	0.43	ns
Rosalyn	0.074	-8.5E-06	-0.07	ns	0.112	-1.8E-04	-0.88	<0.001	0.071	-1.0E-04	-0.80	<0.001
Skiles	0.081	-6.2E-05	-0.29	ns	0.086	-1.0E-04	-0.53	ns	0.078	-5.2E-05	-0.56	ns
SY Ovation	0.089	-2.6E-04	-0.95	<0.0001	0.074	-6.2E-05	-0.63	ns	0.063	2.9E-05	0.24	ns
WB 1070 CL	0.072	-1.1E-04	-0.86	<0.001	0.149	-3.5E-04	-0.47	ns	0.077	-1.6E-04	-0.81	<0.001
Overall αA	**0.075**	**-9.3E-05**	**-0.33**	**<0.001**	**0.142**	**-2.5E-04**	**-0.12**	**ns***	**0.068**	**-4.7E-05**	**-0.19**	**ns**

*Overall αA was significant in varieties with initial (day 0) αA activities < 0.01 CU.

4.5 Results and Discussion: Hard Wheats

Summary statistics for hard wheat αA activity are shown in Table 4.7. The full-factorial four-way ANOVA showed that αA activity varied significantly between growing locations, wheat varieties, days and temperature of storage (Table 4.8). F-ratios from the ANOVA indicated that environment had the largest influence on αA activity, followed by variety. This was also the case in both soft and hard wheat FN as well soft wheat αA activity (Tables 3.2, 3.8, and 4.2). Storage time and temperature were less influential. There were many significant interaction terms in the 4-way ANOVA. The largest F-ratio for an interaction term was for location*variety, which was also one of the main significant interactions in soft wheat αA activity (Table 4.1).

Table 4.7 shows that the location Kimberly had both the highest absolute αA activity and the highest mean αA activity of the 3 locations. This indicated that Kimberly was likely to have been affected by PHS. This was expected as our collaborators at the University of Idaho indicated a noteworthy rain event after grain reached physiological maturity. Aberdeen and Parma had

maximum αA activities < 0.1 CU, which indicates no PHS damage for hard wheats at these two locations.

Table 4.7 - Summary Statistics of hard wheat alpha amylase activity (Ceralpha Units).

Location	Min	Max	Mean	SD	SE
Kimberly	0.055	0.206	0.091	0.03	<0.01
Aberdeen	0.054	0.082	0.068	0.01	<0.01
Parma	0.052	0.092	0.065	0.01	<0.01
Variety					
Keldin	0.059	0.206	0.093	0.04	<0.01
OR2080236H	0.055	0.109	0.080	0.01	<0.01
OR2100081H	0.055	0.101	0.072	0.01	<0.01
WB Arrowhead	0.055	0.086	0.068	0.01	<0.01
Whetstone	0.052	0.084	0.068	0.01	<0.01
Norwest 553	0.053	0.083	0.065	0.01	<0.01
Temperature					
-20°	0.053	0.206	0.075	0.02	<0.01
+20°	0.055	0.158	0.075	0.02	<0.01
+40°	0.052	0.158	0.074	0.02	<0.01
Storage Time (Days)					
0	0.058	0.158	0.079	0.02	<0.01
14	0.057	0.155	0.076	0.02	<0.01
30	0.055	0.157	0.074	0.02	<0.01
60	0.053	0.206	0.073	0.02	<0.01
90	0.052	0.157	0.071	0.02	<0.01

All wheat varieties had minimum αA activities of < 0.1 CU (Table 4.7). This indicated either no evidence of PHS at one or more locations, or an increase in αA activity over storage time. Three of the six hard wheat varieties had maximum αA activities < 0.1 CU indicating no presence of PHS damage at any of the three locations. This suggests that these varieties either had low

susceptibility to PHS or that they may be later maturing varieties. The variety Keldin had the highest absolute αA activity and mean αA activity. This suggests that Keldin either had high susceptibility to PHS, or that it may be an earlier maturing variety. In the case of Keldin seed dormancy may have already been eroding while the harvest-ripe seeds waited in the field to be harvested. The logistics of harvesting breeding nurseries means that in practice all plots are harvested on the same day, and only after the latest maturing varieties reach harvest maturity.

Mean αA activities were significantly different between the 3 storage temperatures when assessed by F-ratio from the four-way ANOVA (Table 4.8). The -20°C storage temperature had the highest absolute αA activity and mean αA activity. This was higher than maximum αA activity at either +20°C or +40°C (Table 4.6). Mean αA activities were significantly different between days of storage when assessed by F-ratio from the 4-way ANOVA (Table 4.8).

F-statistics from 4-way ANOVA (Table 4.8) indicated significant influences on αA activity for all main effects. The majority of interaction terms were also significant. Given the number of significant interactions it was deemed prudent to analyze each location*temperature treatment separately. Figures 4.5, 4.6, and 4.7 show changes in αA activities over time of storage for the six hard varieties for each location*storage temperature treatment. Changes in αA activities over time of storage in hard wheats was not as evident as changes in αA activities over time of storage in soft wheats. Changes in αA activities over time of storage in general were also not as evident as changes in FN.

Table 4.8 - F-statistics from ANOVA of alpha amylase (αA) activity (Ceralpha Units) of all factors and interactions in hard wheats.

Hard Wheats	**F-statistic**
Location	985.1*
Variety	282.3*
Temperature	8.3*
Storage Time	50.0*
Location*Variety	216.6*
Location*Temperature	ns
Location*Storage Time	ns
Variety*Temperature	3.1
Variety*Storage Time	4.6*
Temperature*Storage Time	9.1*
Location*Variety*Temperature	3.7*
Location *Variety*Storage Time	5.0*
Location*Temperature *Storage Time	12.0*
Variety*Temperature*Storage Time	4.6*
Location* Variety*Temperature*Storage Time	4.3*

*significant at $P < 0.01$, ns not significant at $P < 0.01$

Table 4.9 - F-statistics (F-stat) from two-way ANOVA of soft wheat alpha amylase (αA) activities (Ceralpha Units) by storage time, variety, and storage time*variety interaction for each storage location*temperature treatment.

Aberdeen, -20°	**F-stat**	**Kimberly, -20°**	**F-stat**	**Parma, -20°**	**F-stat**
Storage Time	11.2*	Storage Time	37.2*	Storage Time	ns
Variety	18.6*	Variety	643.7*	Variety	52.5*
Storage Time*Variety	5.8*	Storage Time*Variety	9.8*	Storage Time*Variety	6.7*
Aberdeen, +20°	**F-stat**	**Kimberly, +20°**	**F-stat**	**Parma, +20°**	**F-stat**
Storage Time	15.9*	Storage Time	71.4*	Storage Time	33.8*
Variety	28.9*	Variety	81.8*	Variety	38.6*
Storage Time*Variety	3.4*	Storage Time*Variety	50.4*	Storage Time*Variety	12.3*
Aberdeen, +40°	**F-stat**	**Kimberly, +40°**	**F-stat**	**Parma, +40°**	**F-stat**
Storage Time	34.3*	Storage Time	35.4*	Storage Time	11.1*
Variety	5.9*	Variety	980.7*	Variety	54.8*
Storage Time*Variety	6.5*	Storage Time*Variety	12.3*	Storage Time*Variety	5.3*

*significant at $P < 0.01$, ns not significant at $P < 0.01$

4.5.1 Storage at -20°C

The two-way ANOVA for the location*temperature treatment, Aberdeen at -20°C, is shown in Table 4.9. There were significant differences between both storage times and varieties, as was the case in FN for this treatment as well. The F-ratios indicated that storage time and variety were about equally influential. The storage time*variety interaction term was also significant. Regression analyses (Table 4.10) showed that only one variety, Whetstone, had a significant decrease in αA activity over the 90 days of storage. Whetstone also had a significant increase in FN over storage time as well (Section 3.4.1). The rate of decrease for Whetstone was 0.018 CU per 90 days. The correlation of overall αA activity for each day (across varieties) with storage time was not significant, which directly contrasts the significant increase in FN values observed

in hard wheats from this treatment. The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity.

The two-way ANOVA for the location*temperature treatment, Kimberly at -20°C, is shown in Table 4.9. There were significant differences between both days of storage and wheat varieties. The F-ratios indicated the major influence was variety, which was also the case in FN for both hard and soft wheats in this treatment as well as αA activity for soft wheats. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 4.10) showed that two of the wheat varieties, WB Arrowhead and Whetstone, had significant decreases in αA activities over the 90 days of storage. Whetstone from Aberdeen also had a significant decrease in αA activity when stored at -20°C. The rate of decrease for WB Arrowhead was 0.014 CU per 90 days. The rate of decrease for Whetstone was 0.02 CU per 90 days. The correlation of overall αA activity for each day (across varieties) with storage time was not significant. The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity.

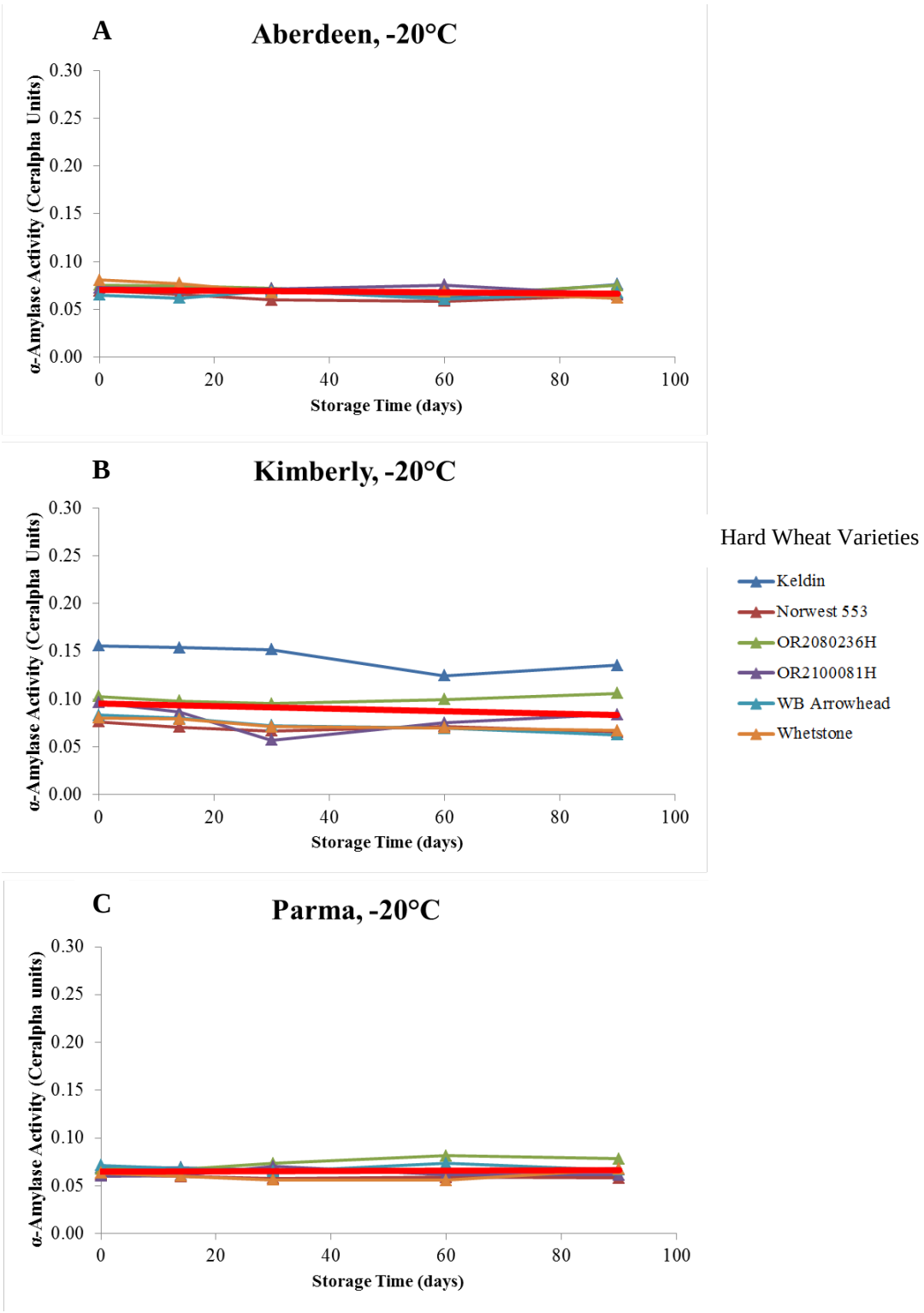

Figure 4.5 - Mean alpha amylase (αA) values (Ceralpha Units) of hard wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at -20°C. Linear regression of overall αA activities (averaged across varieties) is presented in red.

The two-way ANOVA for the location*temperature treatment, Parma at -20°C, is shown in Table 4.9. There were significant differences between wheat varieties but not storage time. The storage time*variety interaction term was also significant, but to a lesser degree than wheat varieties. Regression analyses (Table 4.10) showed that only one variety, OR2080236H, had a significant decrease in αA activity over the 90 days of storage, however OR2080236H from this treatment did not have a corresponding increase in FN over time. The rate of decrease for OR2080236H was barely detectable at 0.004 CU per 90 days. The correlation of overall αA activity for each day (across varieties) with storage time was not significant, as was also the case for the change in FN over time at this treatment (Section 3.4.1). The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity.

In summary, at -20°C there were no systematic changes in αA activity associated with grain storage in this collection of hard wheat varieties. This is consistent with the very few instances of change in FN values over storage time observed in this collection of hard wheat varieties at this temperature. However, this is in contrast to the higher number of observations of systematic decreases in αA activity over storage time observed in soft wheats at this temperature. There were some varietal exceptions. Kimberly had the most varieties that showed significant decreases in αA activities over storage time. The variety Whetstone showed systematic decreases in αA activities at both Aberdeen and Kimberly. The varieties that had significant decreases in αA activity over time at this temperature were all varieties with initial αA activity < 0.01 CU. This contrasts with the observation that decreases in soft wheat αA activity over time primarily occurred in varieties with initial αA > 0.01 CU. Overall αA activities across all varieties over 90

days of storage did not significantly decrease in wheats from Aberdeen, Kimberly, or Parma when stored at -20°C.

Table 4.10 - Linear regression of alpha amylase (αA) activities (Ceralpha Units) of hard wheats from Aberdeen, Kimberly, and Parma stored at -20°C for 90 days.

-20°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Keldin	0.687	3.1E-05	0.67	ns	0.156	-3.1E-04	-0.76	ns	0.069	-3.14E-05	-0.45	ns
Norwest 553	0.066	-5.0E-05	-0.34	ns	0.073	-8.3E-04	-0.68	ns	0.060	-2.63E-05	-0.38	ns
OR2080236H	0.074	-4.0E-05	-0.32	ns	0.098	5.6E-05	0.42	ns	0.068	-4.09E-05	-0.80	<0.001
OR2100081H	0.074	-4.9E-05	-0.46	ns	0.083	-9.5E-05	-0.23	ns	0.063	-5.31E-06	-0.04	ns
WB Arrowhead	0.064	1.6E-05	0.12	ns	0.082	-2.2E-04	-0.94	<0.0001	0.070	-1.4E-05	-0.14	ns
Whetstone	0.079	-2.0E-04	-0.91	<0.001	0.079	-1.5E-04	-0.90	<0.001	0.059	3.69E-05	0.27	ns
Overall αA	**0.071**	**-4.9E-05**	**-0.27**	**ns**	**0.095**	**-1.3E-04**	**-0.16**	**ns**	**0.065**	**1.9E-05**	**0.10**	**ns**

4.5.2 Storage at +20°C

The two-way ANOVA for the location*temperature treatment, Aberdeen at +20°C, is shown in Table 4.9. There were significant differences between both storage times and varieties, as was the case in both hard wheat FN and soft wheat αA activity in this treatment. The F-ratios indicated the major influence was variety. The storage time*variety interaction term was also significant, but to a lesser degree than the main effects. Regression analyses (Table 4.11) showed that half of the varieties had significant decreases in αA activity across the 90 days of storage. Significant decreases in αA activity ranged from a low of 0.006 CU per 90 days for Keldin and OR2100081H to a high of 0.01 CU per 90 days for Norwest 553. All of the varieties with significant decreases in αA activity (except Whetstone) also had corresponding increases in FN values over storage time in this treatment (Section 3.4.2). The correlation of the overall αA activity for each day (across varieties) with storage time was also significant, as was the case for FN values in this treatment as well. Overall change in αA activity was 0.006 CU per 90 days of storage. The results show that for this location*temperature treatment, storage of grain was

associated with both systematic decreases in in αA activity and systematic increases in FN in hard wheats.

The two-way ANOVA for the location*temperature treatment, Kimberly at +20°C, is shown in Table 4.9. There were significant differences between both storage times and varieties, as was the case for hard what FN in this treatment and soft wheat αA activity. The storage time*variety interaction term was also significant. The F-ratios for the two main effects and their interaction were in the same order of magnitude. Regression analyses (Table 4.11) showed that only one variety, WB Arrowhead, had a significant decrease in in αA activity across the 90 days of storage. The rate of decrease for WB Arrowhead was 0.018 CU per 90 days. There was no corresponding significant increase in FN over storage time for this variety in this location*temperature treatment. The correlation of overall αA activity for each day (across varieties) with storage time was not significant, as was the case for both hard wheat FN and soft wheat αA activity for this treatment as well. The results show that for this location*temperature treatment, storage of grain was not associated with either systematic decreases in αA activity or systematic increases in FN in both hard and soft wheats.

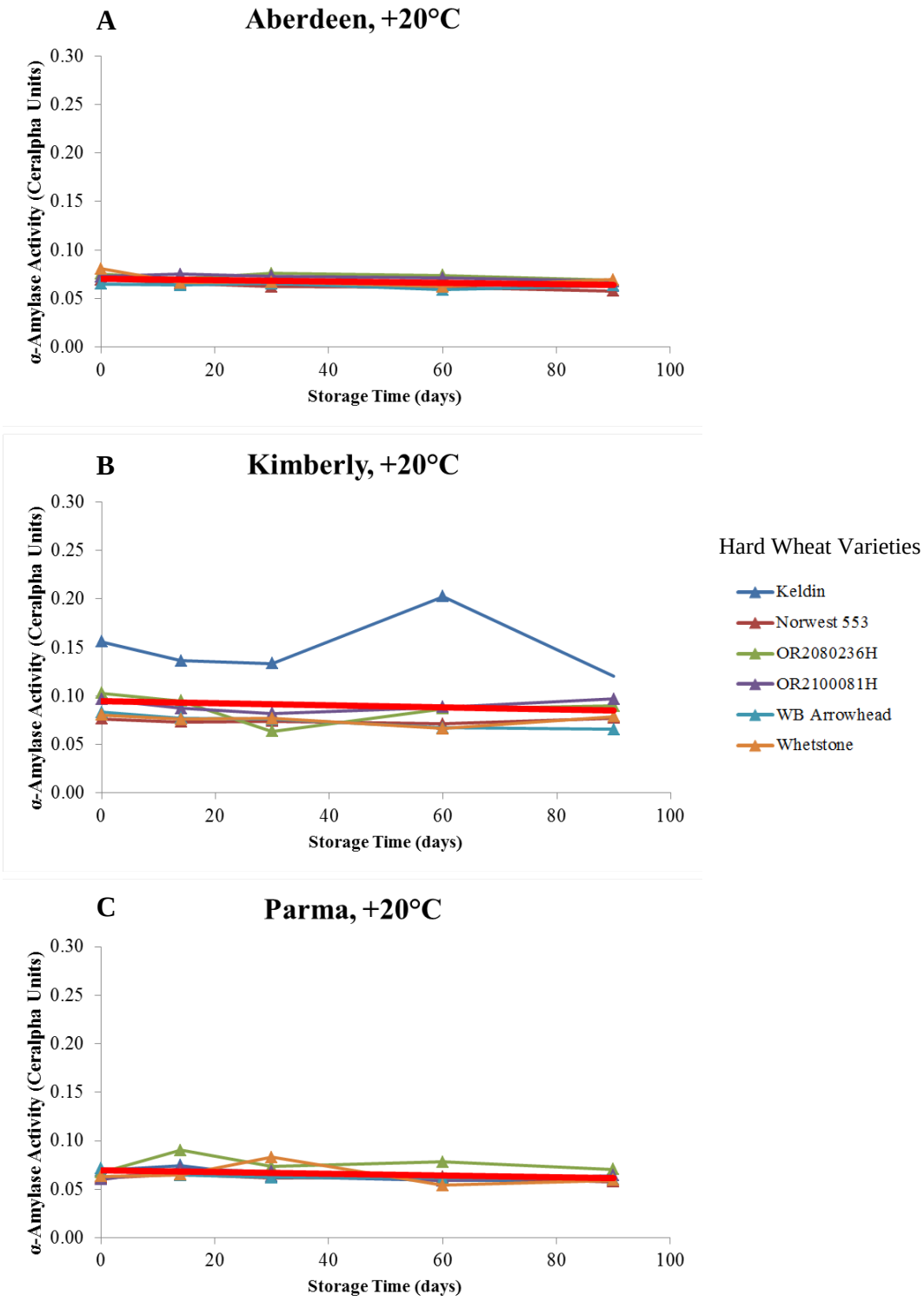

Figure 4.6 - Mean alpha amylase (αA) values (Ceralpha Units) of hard wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at +20°C. Linear regression of overall αA activities (averaged across varieties) is presented in red.

The two-way ANOVA for the location*temperature treatment, Parma at +20°C is shown in Table 4.9. There were significant differences between both storage times and varieties. The F-ratios indicated that storage time and wheat variety were about equally influential, as was also the case in FN for hard wheats as well as αA activity for soft wheats in this treatment. The storage time*variety interaction term was also significant. Regression analyses (Table 4.11) showed that only one variety, Keldin, had a significant decrease in αA activity across the 90 days of storage. The rate of decrease for Keldin was 0.014 CU per 90 days. However, Keldin did not have a significant increase in FN values over storage time at this treatment. The correlation of overall αA activity for each day (across varieties) with storage time was weakly significant. The overall change in αA activity across all varieties was barely detectable at 0.007 CU per 90 days of storage. The weak but significant correlation of the mean αA activity may be due to the significant change in αA activity of the variety Keldin. This also occurred in both hard and soft wheat FN for this treatment but with OR2100081H and Skiles (respectively) as the only varieties that had significant changes over time. This contrasts with soft wheats for this treatment where there were no significant decreases in αA activity over time. The results show that for this location*temperature treatment, storage of grain was associated with some variety specific systematic decreases in αA activity for hard wheats as well as variety specific increases in FN for both soft and hard wheats.

In summary, at +20°C there were some variety specific systematic changes in αA activity associated with grain storage in this collection of hard wheat varieties. However, this occurred primarily in unsprouted wheats from Aberdeen. This is consistent with the observation that increases in FN over time primarily occurred in presumptively unsprouted hard wheats.

However, this is in contrast to the observation in soft wheats where significant decreases in αA activity primarily occurred in samples with initial αA activities > 0.1 CU. Keldin was the only variety to show systematic decrease in αA activity over storage time from Parma. When a linear model excluding Keldin was applied to Parma overall αA activities did not show a significant decrease over storage time (r = -0.27, p = .06). This suggests that overall significant systematic decreases in αA activity observed in wheats from Parma was likely influenced by the significant change observed in Keldin. The variety WB Arrowhead from Kimberly showed systematic decrease in αA activities over storage time at both -20°C and +20°C. This suggests that this variety may exhibit degradation of αA activity over storage time.

Table 4.11 - Linear regression of alpha amylase (αA) activities (Ceralpha Units) of hard wheats from Aberdeen, Kimberly, and Parma stored at +20°C for 90 days.

+20°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Keldin	0.070	-6.9E-05	-0.83	<0.001	0.134	8.8E-04	0.70	ns	0.071	-1.5E-04	-0.79	<0.001
Norwest 553	0.068	-1.1E-04	-0.78	<0.001	0.074	9.1E-06	0.11	ns	0.064	-4.5E-05	-0.50	ns
OR2080236H	0.075	-5.0E-05	-0.49	ns	0.091	-9.0E-05	-0.22	ns	0.078	-4.7E-05	-0.18	ns
OR2100081H	0.075	-7.0E-05	-0.82	<0.001	0.089	3.7E-05	0.19	ns	0.066	-3.8E-05	-0.24	ns
WB Arrowhead	0.065	-4.0E-05	-0.38	ns	0.081	-2.0E-04	-0.92	<0.001	0.068	-9.6E-05	-0.75	ns
Whetstone	0.072	-8.4E-05	-0.44	ns	0.773	-4.5E-05	-0.29	ns	0.070	-1.2E-04	-0.38	ns
Overall αA	**0.071**	**-7.1E-05**	**-0.40**	**<0.001**	**0.095**	**-1.1E-04**	**-0.11**	**ns**	**0.069**	**-8.3E-05**	**-0.34**	**<0.001**

4.5.3 Storage at +40°C

The two-way ANOVA for the location*temperature treatment, Aberdeen at +40°C, is shown in Table 4.9. There were significant differences between both storage times and varieties. The F-ratios indicated the major influence was from storage time, rather than variety as seen in previous location*temperature treatments. The most influential main effect for FN values in hard wheats in this treatment was also storage time. The storage time*variety interaction term was also significant. Regression analyses (Table 4.12) showed that all varieties, except Norwest 553 and

WB Arrowhead, had significant decreases in αA activities across the 90 days of storage. These varieties were are presumptively unsprouted which is in contrast to the observation in soft wheats where changes in αA activity over storage time occurred primarily in wheats with initial αA activities > 0.1 CU. Significant decreases in αA activity ranged from a low of 0.007 CU per 90 days for to a high of 0.026 CU per 90 days for Whetstone. All varieties from this treatment that had significant decreases in αA activity over time also had significant increases in FN over time (Section 3.4.3). The correlation of overall αA activity for each day (across varieties) with storage time was also significant, as was also the case for the change in αA activity in soft wheats and the change in FN for both soft and hard wheats from this treatment. Overall change in αA activity across all varieties was 0.013 CU per 90 days of storage. The results show that for this location*temperature treatment, storage of grain was associated with systematic decreases in αA activity in soft and hard wheats as well as systematic increases in FN in soft and hard wheats.

The two-way ANOVA for the location*temperature treatment, Kimberly at +40°C is shown in Table 4.9. There were significant differences between both storage times and varieties. The F-ratios indicated the major influence was variety, as was also the case for the change in FN for hard wheats from this treatment. The storage time*variety interaction term was also significant. Regression analyses (Table 4.12) showed that two varieties, OR2080236H and WB Arrowhead, had significant decreases in αA activities across the 90 days of storage. The change in αA activity by OR2080236H was 0.025 CU per 90 days. The change in αA activity by WB Arrowhead was 0.014 CU per 90 days. Both of these varieties from this treatment also had corresponding increases in FN over storage time as well. The correlation of overall αA activity for each day (across varieties) with storage time was not significant. This contrasts with the

observation that FN significantly increased over time for hard wheats in this treatment. The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity. However, there were variety specific decreases in αA activity.

The two-way ANOVA for the location*temperature treatment, Parma at +40°C, is shown in Table 4.9. There were significant differences between both storage times and varieties. The F-ratios indicated that the major influence was variety, followed by storage time. This contrasts the observation that the main influence on changes in FN was storage time for hard wheats in this treatment. The storage time*variety interaction term was also significant. Regression analyses (Table 4.12) showed that two varieties, Keldin and Whetstone, had significant decreases in αA activities over the 90 days of storage. The change in αA activity by Keldin was barely detectable at 0.007 CU per 90 days. The change in αA activity by WB Arrowhead barely detectable at 0.008 CU per 90 days. Both of these varieties from this treatment also had significant increases in FN over storage time. The correlation of overall αA activity for each day (across varieties) with storage time was not significant. This was also the case for the change in soft wheat αA activity over time for this treatment as well. The results show that for this location*temperature treatment, storage of grain was not associated with systematic decreases in αA activity. However, there were variety specific decreases in αA activity.

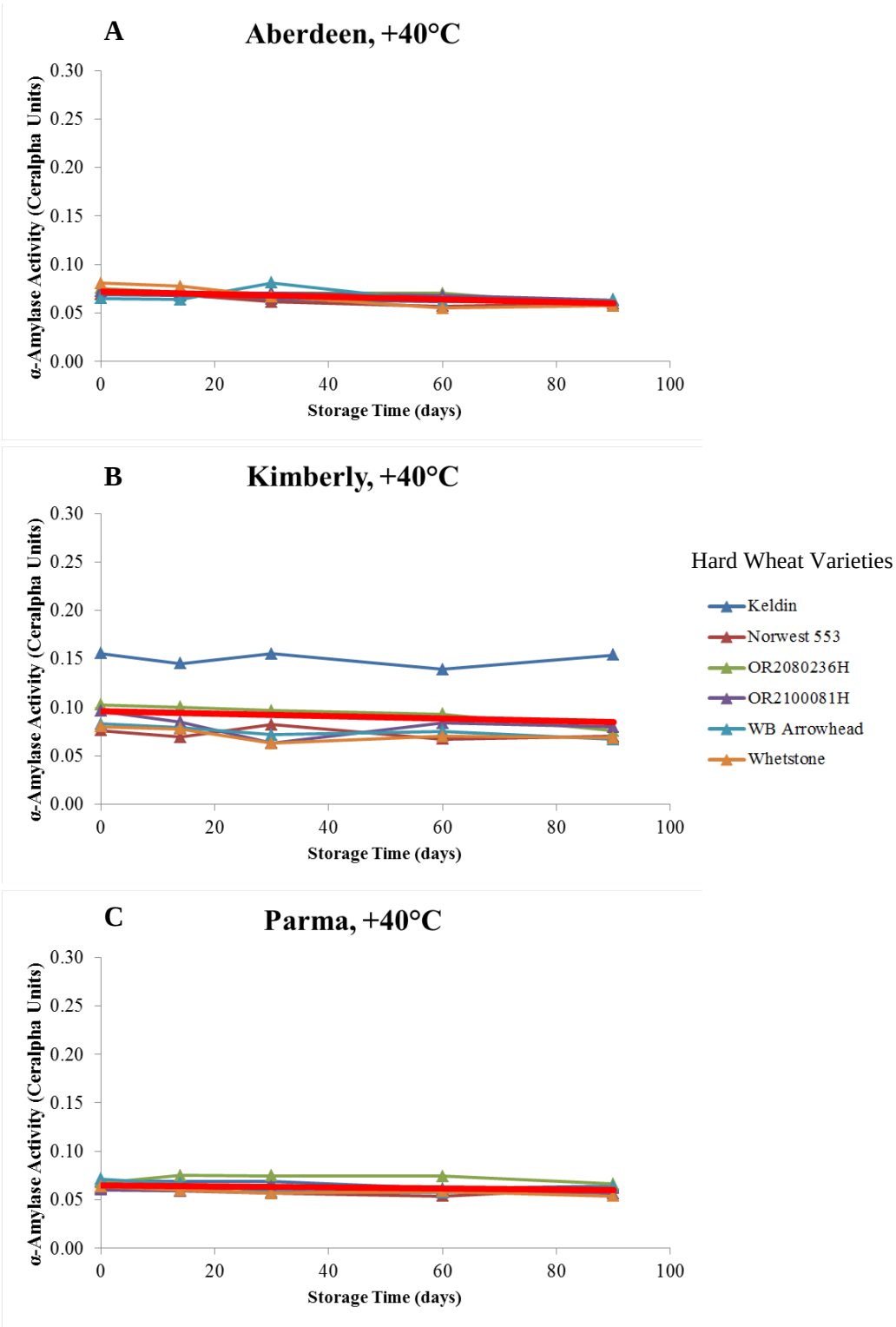

Figure 4.7 - Mean alpha amylase (αA) values (Ceralpha Units) of hard wheats over storage time at (A) Aberdeen, (B) Kimberly, and (C) Parma stored at +40°C. Linear regression of overall αA activities (averaged across varieties) is presented in red.

In summary, at +40°C there were some variety specific systematic changes in αA activity associated with grain storage in this collection of hard wheats. This occurred, as it was in +20°C, primarily in presumptively unsprouted wheats from Aberdeen. This observation is in direct contrast with what was seen in soft wheats. Decreases in αA activity in soft wheats primarily occurred in varieties with initial αA activities > 0.1 CU. The variety Keldin from Kimberly had significant decreases in αA activity from > 0.1 CU to < 0.1 CU when stored at -20°C and +40°C as well. This was the only variety, hard or soft, that had this decrease. However, this decrease did not have a corresponding increase in FN values to > 300 s. Notably, the variety Keldin from both Aberdeen and Parma showed significant systematic decreases in αA activity at both +20°C and +40°C with corresponding increases in FN over storage time. This suggests that this variety, when presumptively unsprouted, may be susceptible to the degradation of αA activity over storage time at ambient and higher temperatures and that this degradation may be responsible for increasing FN values. However, while the decline in αA activity was similar for hard wheats from Aberdeen and Parma stored at 40°C, FN values increases at a higher rate in hard wheats from Aberdeen (Figures 3.7A and B, 4.7A and B). This suggests that the changes modulating FN over storage time at high temperatures are not solely influenced by decline in αA activity. As was the case in soft wheats, variety was the dominant effect influencing αA activity in hard wheats (except in the cases of Aberdeen stored at +40°C and Parma stored at +20°C). Also, the trend of the main effect storage time increasing in significance as storage temperature increases that was observed in FN was not evident for changes in αA activity for both soft and hard wheats. This suggests that the changes in αA activity were not as influenced by higher storage temperature as compared to changes in FN in this collection of wheat varieties. The storage time*variety interaction term was influential in for all treatments in both soft and hard wheat

varieties. This suggests that, within each treatment, changes in αA activity occurred similarly between wheat varieties.

Table 4.12 - Linear regression of alpha amylase (αA) activities (Ceralpha Units) of hard wheats from Aberdeen, Kimberly, and Parma stored at +40°C for 90 days.

+40°C	Aberdeen				Kimberly				Parma			
Variety	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**	**Intercept**	**Slope**	**r**	**p-value**
Keldin	0.068	-7.4E-05	-0.77	<0.001	0.151	-2.6E-05	-0.12	ns	0.070	-8.3E-05	-0.79	<0.001
Norwest 553	0.068	-1.3E-04	-0.64	ns	0.076	-7.6E-05	-0.44	ns	0.059	-8.4E-06	-0.08	ns
OR2080236H	0.076	-1.8E-04	-0.87	<0.001	0.104	-2.8E-04	-0.94	<0.0001	0.073	-2.9E-05	-0.22	ns
OR2100081H	0.073	-1.1E-04	-0.85	<0.001	0.086	-1.0E-04	-0.29	ns	0.061	-4.0E-05	-0.47	ns
WB Arrowhead	0.069	-4.9E-05	-0.21	ns	0.081	-1.5E-04	-0.79	<0.001	0.067	-9.4E-05	-0.55	ns
Whetstone	0.079	-2.9E-04	-0.91	<0.001	0.077	-1.2E-04	-0.57	ns	0.062	-8.5E-05	-0.79	<0.001
Overall αA	**0.072**	**-1.4E-04**	**-0.07**	**<0.0001**	**0.096**	**-1.3E-04**	**-0.17**	**ns**	**0.065**	**-5.7E-05**	**-0.30**	**ns**

4.6 Conclusions

αA activity and FN values had a curvilinear relationship as previously observed in the literature (Hagberg 1961, Perten 1964, d'Appolonia et al 1982).

Changes in αA activity were observed over grain storage time. Changes in αA activity occurred differently across growing environments, wheat varieties, and between soft and hard wheat classes. Different wheat varieties had different propensities to sprout as measured by αA activity. High αA activities were primarily observed in soft wheats from locations that had rain events prior to harvest. Storage temperature did not have as much influence on changes in αA activity as it did on changes in FN over storage time.

Changes in αA activity over storage time were most prevalent in soft wheats with initial αA activities > 0.1 CU. There were small decreases in αA activity in hard wheats but the distinction

between high and low αA activity samples was not as evident as in the soft wheats because the vast majority of hard wheat samples tested had αA activities < 0.1 CU.

Decreases in αA activity were often not associated with corresponding increases in FN values over grain storage time. The changes that occur in stored wheat that modulate FN over time were seemingly not wholly influenced by αA activity. Hard wheats from the location Aberdeen as compared to Parma had higher rates of increase in FN over time. However, the decrease in αA activity between the two locations may not have been different enough to be the sole reason for the changes in FN.

Storage for 90 days at these temperatures may not be effective in decreasing αA activity from > 0.1 CU to < 0.1 CU. Changes in αA activity from > 0.1 CU to < 0.1 CU only occurred in one variety, Keldin from Kimberly. This significant decrease in αA activity did not have a corresponding increase in FN to > 300 s.

References

See Bibliography

Chapter 5: Lactic acid solvent retention capacity for dough strength prediction at early generations in wheat breeding programs

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Abstract

It is the goal of wheat breeders to produce distinct cultivars that meet the demands of farmers, millers, and processors. Early generation screening improves breeding program efficiency. The Mixograph is used to measure dough mixing properties in breeding programs but requires time and over 50 g of grain. Total polymeric protein (TPP) can also be used as a proxy for dough properties, uses less sample, but requires time and sophisticated equipment. Lactic acid Solvent Retention Capacity (LASRC) test has been proposed as an alternative screening tool for hard wheat. The objectives of this research were to provide preliminary information on the ability of LASRC, on its own, to predict dough mixing properties and to assess the correlation between LASRC and TPP. Ninety-eight hard winter wheats from the 2014 harvest year were used. Samples were F5 lines from yield trials in Pendleton, OR. Wheat samples were categorized by flour protein concentration (FPC). Mixographs were analyzed both by eye and by the proprietary Mixsmart software. TPP content was assessed as % large unextractable polymeric proteins (%LUPP) using size exclusion high performance liquid chromatography In this collection of hard wheats, dough mixing parameters were slightly better correlated with LASRC than they were with %LUPP. Correlations between LASRC, %LUPP, and dough mixing parameters were different between FPC categories, particularly in low FPC samples. A strict cutoff of 115% LASRC effectively screened out the bottom 10% of low quality hard wheats but retained a nearly equal amount of low quality hard wheats that would have been screened out by mixograph analysis. LASRC and %LUPP are not likely to be effective predictors of dough properties, but

may have some value as screening methods for hard wheat quality in the early generations of a wheat breeding cycle.

5.1 Introduction

It is the goal of wheat breeders to produce distinct cultivars that meet the demands of the market, processors, and consumers. Wheat breeding programs begin with the crossing of divergent genotypes. The result is thousands of progeny with a range of traits (Baenziger and DePauw 2009) some of which are quality related. Samples are cultivated in greenhouses for several generations before enough seed is produced to begin field trials. Many lines are dropped from the program based on agronomic characteristics such as straw height, lodging (falling over in the field), color, and disease susceptibility (Baenziger and DePauw 2009). Those that are retained are harvested and grain is analyzed for a range of quality parameters. Larger amounts of grain are produced at each subsequent generation. This allows either a larger range of quality tests to be applied or the application of tests requiring more sample. But, the longer a line is kept in the breeding program before being screened out, the less efficient the program is. Therefore, it is very desirable to predict wheat quality at early generations.

Hard wheat is used to make bread products. Characteristics associated with improved bread quality have been extensively studied, wheat kernel proteins in particular (Delcour 2012). The gluten forming proteins in wheat are glutenin and gliadin. These proteins give dough its viscoelastic qualities: glutenin providing elasticity and gliadin providing viscosity. Viscoelasticity gives dough the unique quality of being able to trap gasses produced during microbial fermentation, permanently deform during shaping and rising, and yield a leavened

product. The glutenins are the most studied proteins with regard to dough properties and have the most leverage over genotypic differences in dough behavior (Delcour 2012). Glutenin subunits can be classified as high molecular weight (HMW-GS) and low molecular weight (LMW-GS). Glutenins, primarily the HMW-GS, form large, ramifying networks called glutenin macropolymer (GMP) (Don et al 2003). High levels of HMW-GS, and by inference GMP, are associated with increased dough strength and superior bread quality (Weegels et al 1996). Dough strength can be measured using recording dough mixers (RDMs) (Delcour and Hoseney 2010). In breeding programs the Mixograph method is the most common RDM as a result of smaller sample size (10 g) and high speed, relative to other RDMs. However, compared to other, non-RDM screening methods the Mixograph method requires a relatively large amount of refined flour.

GMP can be measured as total polymeric protein (TPP) content (Gupta et al 1993, Johansson et al 2001, Kuktaite et al 2004). TPP analysis via HPSEC distinguishes protein fractions by retention time. Large polymeric proteins (LPP) elute first, small polymeric proteins (SPP) elute second, large monomeric proteins (LMP, i.e. gliadins) elute third, and small monomeric proteins (SMP, i.e. albumins and globulins) elute last. TPP is all polymeric glutenins: i.e. both HMW- and LMW-GS (LPP and SPP, respectively) (Zhang 2015). Unextractable polymeric protein (UPP) are those HMW-GS that cannot be extracted from the flour by the solvent/buffer alone but require sonication for extraction. Total unextractable polymeric protein (TUPP) is the proportion of UPP:TPP. Large unextractable polymeric protein (LUPP) is the proportion of large unextractable polymeric protein to all large polymeric protein. These are normally reported as percentages. High LUPP content has been associated with increased dough strength and bread

quality (Johansson et al 2001). However, direct measurement of TPP is time, labor, and equipment intensive. Therefore, for early generation screening methods that are both high throughput and use less sample than RDM analysis might be appropriate, unless only mg quantities of flour are available.

The Solvent Retention Capacity (SRC) test is proposed as an early generation screening tool for wheat breeding programs (Kweon et al 2011). The SRC test examines the individual contributions of the major polymeric components of wheat flour and predicts their influence on processing-intermediate functionality and end-product quality (Kweon et al 2011). While initially designed to be a tool for predicting soft wheat quality, SRC has also been applied to predicting hard wheat quality (Ram and Singh 2004, Ram et al 2005, Xiao et al 2006, Kweon et al 2011, Jayaram et al 2014). Xiao et al (2006) showed LASRC was not significantly correlated with Mixograph peak time, but LASRC was significantly correlated with bread loaf volume ($r = 0.83$, $p < 0.0001$). In 2005, Ram et al showed that LASRC was able to explain 14% of the variability in Mixograph peak time ($r = 0.38$, $p < 0.001$). In SRC methodology, the four solvents used are deionized water (WSRC), 5% w/w aqueous lactic acid (LASRC), 5% w/w aqueous sodium carbonate (NaSRC), and 50% w/w aqueous sucrose (SucSRC). Lactic acid preferentially swells glutenin subunits, sodium carbonate swells damaged starch, and sucrose swells arabinoxylan (AX) and gliadins (Slade and Levine 1994). Water is used as the reference solvent as it hydrates all of the polymeric components of wheat flour, but to a lesser degree than the other solvents (Ross and Bettge 2009). The solvent of interest for hard wheat quality is lactic acid, as lactic acid favors the swelling of glutenin subunits. LASRC has been shown to correlate

with loaf volume, SDS-sedimentation, Farinograph absorption, and Mixograph parameters (Ram et al 2005, Xiao et al 2006).

This study explored the correlations between LASRC and TPP on mixing properties measured by Mixograph. The objectives of this research were to provide preliminary information on the usefulness of using LASRC, on its own, to predict dough mixing properties aimed at application to predictions in early generations in a wheat breeding program. We also examined inter-relationships between TPP, LASRC, and Mixograph parameters both from the perspective of reassessing the utility of the TPP parameters as predictors of dough properties and to use TPP as an independent frame of reference for examining the utility of the LASRC test.

5.2 Materials and methods

5.2.1 Materials

Wheat samples

Ninety-eight F5 hard winter wheat samples were obtained from the Oregon State University Wheat Breeding Program's Preliminary Yield Trials. Samples were harvested in 2014. Samples were grown in one m^2 research plots in Pendleton, OR. Hard wheat samples were genetic lines of the F5 generation used in elite yield trials.

Reagents

Reagents were purchased in concentrated form from Sigma-Aldrich (St. Louis, MO).

5.2.2 Methods

Grain Characteristics

Grain samples were analyzed for protein concentration (GPC, %) using near infra-red spectroscopy (NIR, Infratec 1241, FOSS NIT Systems Inc., Denmark) according to AACCI approved method 39-10.01. NIR apparatus was also fitted with a test weight (TWT) module that was used for TWT measurement, reported in lbs/bu.

Grain hardness was analyzed using the Single Kernel Characterization System (SKCS 4100, Perten Instruments, Inc., Springfield, IL: AACCI approved method 55-31.01) using 200 seeds. Kernels were visibly inspected to remove broken seeds and foreign material. Kernel hardness, diameter, and moisture data were collected. Kernel hardness was reported in SKCS hardness units (HU).

Milling

Samples were tempered to 15% moisture content with deionized water 12-14 hours before milling using based on moisture obtained from SKCS. Water addition for tempering was calculated using the equation: $weight\ of\ water\ to\ add = \left(\frac{100-original\ sample\ moisture\ (\%)}{100-desired\ sample\ moisture\ (\%)}\right) \times weight\ of\ sample$ (AACCI Approved Method 26-95.01). Right before milling samples were weighed, this weight was used to calculate flour yields. Samples were milled to break flour in a modified Brabender Quadramat Senior mill (Brabender GmbH & Co., Germany). After break milling, sample was sieved at 500 µm (no. 35 sieve) and 150 µm (no. 100). After 1 min of sieving, sieve was stopped and contents of topmost sieve (500 µm) was emptied, weighed, and discarded as bran. Bran yield was calculated via equation: $Bran\ Yield\ (\%) =$

$\left(\frac{mass\ of\ bran}{initial\ grain\ mass}\right) \times 100$. Flour that passed through both sieves after 3 min was considered break flour. Break flour yield (BFY, %) was calculated via equation: $BFY\ (\%) = \left(\frac{mass\ of\ break\ flour}{initial\ grain\ mass}\right) \times 100$. Flour that did not pass through the 150 µm sieve (middlings) after 3 min was retained.

Flour Characteristics

Flour moisture contents (FMC, %) and flour protein concentration (FPC, %) were determined on duplicate samples using NIR spectroscopy (Infratec 1241, FOSS NIT Systems Inc., Denmark). Flour samples were categorized into high (>11%), medium (9-11%), and low (<9%) protein categories (Figure 5.1).

Mixograph analysis

Mixograph (National Manufacturing Co., Lincoln, NE) analyses were performed in duplicate according to the AACCI approved method 54-40.02. Flour (10 g, 14% moisture basis) was added to a Mixograph bowl. A well was made in the flour and deionized water was added. Water rate of addition was initially determined by the equation: $water\ addition\ (ml)\ =\ 1.5\ \times (FPC) + 43.6$, to calculate optimum hydration and mixed for 10 min. Dough hydration was corrected if necessary based on visual inspection of the resulting mixogram (AACCI Approved Method 54-40.02). Mixograms were analyzed both by eye and by using the proprietary software Mixsmart obtained from the Mixograph manufacturer (National Manufacturing Co., Lincoln, NE). Mixograms were visually inspected and graded numerically using a Mixogram Reference Chart similar to the one reported by Baenziger et al (2001), and also used by our collaborating USDA Wheat Quality Lab in Pullman WA, and authored but not formally published by Stephen

Baenziger. The Mixograph Reference Chart assigned a numerical score of 1-8 (Mixograph Score) based on mixogram appearance and protein category (high, medium, or low as described above: Figure 5.1). In industry, processors prefer wheat flour with peak dough development times from three to seven minutes (USDA 2006).

Manual peak time was determined as the crossover point of straight lines created by the slopes of the left and right sides of the peak (Figure 5.1). Mixogram band widths were measured using a ruler (cm), ignoring "wild swings" (National Manufacturing Co. 2013). Breakdown, or tolerance to mixing, was determined by dividing the bandwidth at peak time by the bandwidth two min after peak time and reporting the ratio.

The Mixsmart software outputs 60 measures of dough strength. Many are redundant and many are of no value. Therefore it was necessary to select a manageable number for statistical analysis (Chung et al 2001). One measure of dough strength and one measure of dough resistance to mixing were selected from both the envelope and midline analysis of the mixograms. The parameters selected were envelope peak time, envelope right integral, midline peak time, and midline right integral. Integrals were measured as percent torque times minutes of mixing (%TQ*min).

Solvent Retention Capacity

SRC analyses were performed in duplicate according to AACCI approved method 56-11.02 with one modification. For this experiment only water and 5% (w/w) lactic acid SRC were used. Flour (5 +/- 0.01 g) was weighed into 50 ml pre-weighed centrifuge tubes with caps, flour weight was

recorded. Solvent (25 g) was added to each tube and then mixed in a modified Perten Shakematic (SM 1095, Perten Instruments, Inc., Springfield, IL) and shaken for 3 s. Tubes were inverted once to ensure all sample was suspended and then placed on a rotational agitator for exactly 20 min. After 20 min, samples were centrifuged for 15 min at 1,000 RCF. After centrifugation tubes were drained and inverted to 90° for exactly 10 min. After draining, tubes were re-capped and weighed. SRC value was determined on a 14% moisture basis by equation: $SRC\ value\ (\%) = \left\{\left[\left(\frac{tube,cap,gel\ weight - tube,cap\ weight}{flour\ weight}\right) - 1\right] \times \left(\frac{86}{100 - flour\ moisture}\right) \times 100\right\}$.

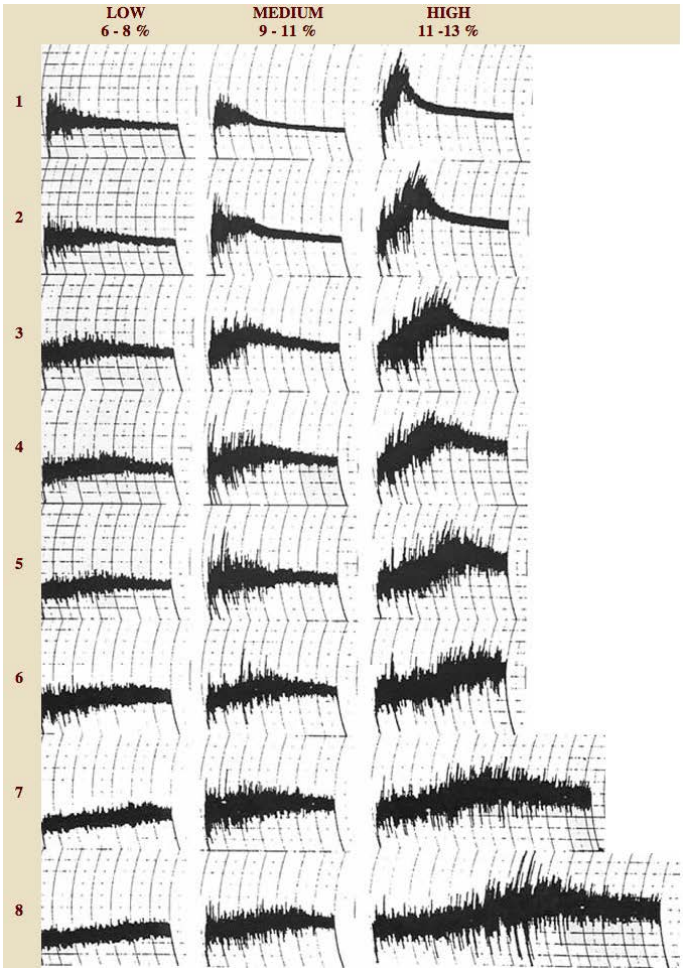


Figure 5.1- Mixogram Reference Chart used for visually inspecting mixograms by flour protein content and Mixograph performance (USDA Western Wheat Quality Laboratory 2010) Numerals in the left hand column indicate "Mixograph Score".

Total Polymeric Proteins

TPP analysis was performed using a two-stage sodium dodecylsulphate (SDS) extraction adapted from Gupta et al 1993. The first extraction yields SDS-extractable proteins and the second extraction yields the SDS-unextracatable proteins (Kuktaite et al 2004, Batey et al 1990, Singh et al 1990a, Singh et al 1990b). Wheat meal (0.01 g) was suspended in 1 mL 0.5% SDS at pH 6.9, vortexed for 5 s, and then centrifuged (16,000 RCF) for 20 min. The resulting supernatant was filtered (0.45 µm) into HPLC vials. The remaining pellet received 1 mL of the SDS buffer and was resuspended. The resuspended samples were then sonicated (30 s, amplitude 5) to extract the insoluble polymeric proteins, centrifuged for 20 min at 16,000 RCF, and the resulting supernatant was filtered (0.45 µm) into HPLC vials (Gupta et al 1993). Wheat protein was fractionated via size-exclusion high performance liquid chromatography (SE-HPLC) in a Waters 2695 Separations Module using a Phenomenex BioSep-SEC-s4000 column (5 µm particle size, 500 Å pore size) with a KJO-4282 guard column and AJ0-4489 cartridge. The detector was a Waters 2996 Photodiode Array detector set at $\lambda = 214$ nm (Kuktaite et al 2004). Separation was achieved in 15 minutes by loading 10 µl of sample in elution conditions of a 1:1 ratio 50% (v/v) acetonitrile and deionized water containing 0.1% (v/v) trifluoroacetic acid (TFA) with a flow rate of 0.45 ml/min (Johansson et al 2001). The chromatograms were integrated at 4.85, 5.55, and 7.80 min (Zhang 2015: Figure 5.2) which divided the chromatogram into 4 main sections of decreasing molecular size: HMW-glutenin (A), LMW-Glutenin (B), gliadins (C), and albumins + globulins (D) in the first (a) and second (b) extractions. The percentage of large unextractable polymeric protein (%LUPP) was calculated as [A2 / (A2 + A1)] x 100. The percentage of total unextractable polymeric protein (%TUPP) was calculated as [(A2 + B2) / (A1 + B1 + A2+ B2] x 100. Both %LUPP and %TUPP were calculated, these measurements were significantly

correlated ($r = 0.98$, $p < 0.0001$) and reporting both parameters was deemed redundant. %LUPP was chosen as the measure of polymeric proteins for this study.

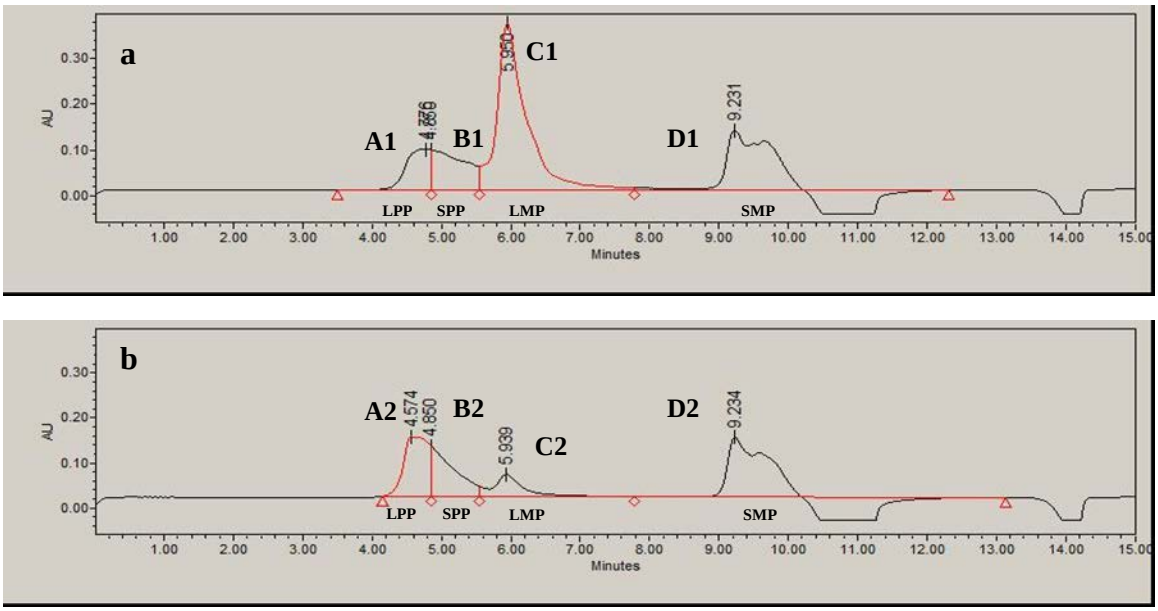


Figure 5.2 - Representative SE-HPLC chromatograms (a) First extraction: SDS-extractable proteins and (b) Second extraction SDS-unextractable proteins. Chromatograms were separated into four parts including large polymeric protein (LPP), small polymeric protein (SPP), large monomeric protein (LMP), and small monomeric protein (SMP). A1 and A2 represent the LPP fractions, B1 and B2 represent the SPP fractions, C1 and C2 represent the LMP fractions, and D1 and D2 represent the SMP fractions (Zhang 2015).

Statistical Analysis

Correlation analyses were carried out using JMP 11 (SAS Institute Inc., Cary, NC). Statistical significance was set at $p < 0.01$. Kendall's τ test was used for nonparametric analyses of correlations. Kendall's τ test was chosen over Spearman's ρ test because this dataset violates the assumption that both variables in the correlation are ratio, interval, or ordinal data necessary to apply Spearman's ρ test. Also, Kendall's τ test is a more conservative correlation analysis method than Pearson's ρ test (Newson 2002).

5.3 Results and Discussion

Table 5.1 shows the summary statistics for the complete sample set. TWT indicated all samples had TWT greater than 58 lbs/bu. This indicates they would grade U.S. #2 or better with respect to TWT (GIPSA 2014). SKH values indicated that the samples all fell within commonly accepted targets for SKH values for commercial hard wheat varieties (USDA 2006). There was a useful range of FPC from what would be commonly accepted as high protein to low protein (Figure 5.1). This data set also captured a range of dough mixing quality parameters as measured by Mixograph score, peak times, and resistance to mixing (Table 5.1, Figure 5.1). Mixograph peak time, for example, had a range of 0.5 – 6.8 min, depending on method of analysis. This range of peak times nearly covers the full range of mix times from lowest to highest preferred by industry, and across Mixograph scores (M-score) from 1 to 8 (Table 5.1, Figure 5.1, USDA 2006). N values show that there were several samples where insufficient flour was available to perform Mixograph analysis in duplicate. This further highlights the importance of quality tests that can be performed with smaller amounts of flour. In addition there were four Mixograph tests done where the trace was so flat that a peak time could not be manually assigned. Similarly, in the electronic Mixograph data missing values were related to the inability of the software to assign a value to a specific parameter.

Table 5.1 - Summary statistics of grain, flour, and dough quality parameters.

	n	Mean	SD	Minimum	Maximum
TWT (lbs/bu)	98	61.9	1.15	59.6	64.2
SKH (HU)	98	69.6	3.82	64.0	78.9
FPC (%)	98	9.8	1.14	6.9	13.9
LASRC (%)	98	135.8	14.72	101.7	171.1
%LUPP	98	65.3	5.35	51.9	77.3
M-Score	94	4.3	1.76	1	8
MPT (min)	94	3.9	1.10	0.5	6.1
MBD	94	0.70	0.16	0.38	1.5
EPT (min)	94	3.2	1.17	1.1	5.7
ERInt (%TQ*min)	94	96.0	24.63	39.3	148.8
MLPT (min)	94	4.1	1.15	1.5	6.8
ML RInt (%TQ*min)	94	358.8	59.73	195.8	473.6

Abbreviations: TWT: Test weight; SKH: Single kernel hardness; FPC: Flour protein concentrations; LASRC: Lactic acid Solvent Retention Capacity; LUPP: Large unextractabale polymeric proteins; M-Score: Mixograph score; MPT: Manual peak time; MBD: Manual breakdown; EPT: Envelope peak time; ERInt: Envelope right integral; MLPT: Midline peak time; MLRInt: Midline right integral

Table 5.2 shows the correlation coefficients between LASRC and %LUPP and other measures of grain, flour, and dough quality for the entire sample set. LASRC and %LUPP were significantly correlated with all measures of dough quality except for MBD (the manual calculation of resistance to mixing). There was a significant positive correlation between LASRC and FPC as would be expected. There was no significant correlation between %LUPP and FPC, this observation is consistent with the literature (Gupta et al 1993, Lemelin et al 2005). The nonparametric correlations between M-Score and both LASRC and %LUPP were also significant. This suggests that LASRC and %LUPP can explain some of the variability between grades given to mixograms by visual inspection.

The correlations between LASRC and all other measures of grain, flour, and dough mixing quality had slightly higher "r" values than for %LUPP. This suggests that the LASRC test may be more appropriate for predicting hard wheat quality on its own than would %LUPP. However, across the full data set LASRC on its own was only able to account for a maximum 35% of the variability of any dough mixing parameter. This suggests that its predictive capability is limited.

Table 5.2 - Correlation coefficients of lactic acid Solvent Retention Capacity (LASRC) and % large unextractable polymeric proteins (%LUPP) on grain, flour, and dough quality parameters.

	LASRC	**%LUPP**
TWT (lbs/bu)	ns	ns
SKH (HU)	ns	ns
FPC (%)	0.33	ns
LASRC (%)	--	0.60
MPT (min)	0.56	0.50
MBD	ns	ns
EPT (min)	0.54	0.41
ERInt (%TQ*min)	0.44	ns
MLPT (min)	0.51	0.48
MLRInt (%TQ*min)	0.59	0.50
M-Score*	0.44	0.32

Abbreviations: TWT: Test weight; SKH: Single kernel hardness; FPC: Flour protein concentrations; LASRC: Lactic acid Solvent Retention Capacity; LUPP: Large unextractabale polymeric proteins; MPT: Manual peak time; MBD: Manual breakdown; EPT: Envelope peak time; ERInt: Envelope right integral; MLPT: Midline peak time; MLRInt: Midline right integral; M-Score: Mixograph score.
*Correlation calculated via Kendall's τ test

One of the key issues of breeding and selecting hard wheats in the Pacific Northwest (PNW) growing environment is the need to make decisions on hard wheat dough properties on samples that have lower than optimum FPC for dough testing. To investigate this we divided the sample set into three categories: high, medium, and low FPC. The cut offs (Section 5.2.2, Figure 5.1)

were chosen to align with other work (Figure 5.1) and correspond to market feedback that shows processors use similar cut off points of FPC to guide buying decisions (Ross 2015, personal communication).

Table 5.3 shows the summary statistics of grain, flour, and dough quality parameters in the high, medium, and low flour protein concentration categories. N values show that the majority of samples were of medium FPC. In the high FPC category the salient details are that there was still a useful range of LASRC, %LUPP, and dough properties to observe correlations, despite the deliberately reduced range of FPC. Distributions of TWT and SKH were similar to the full data set.

In the medium FPC category there was also a useful range of LASRC, %LUPP, and dough properties to observe correlations (Table 5.3). Distributions of TWT, SKH, and measures of dough quality were all similar to the full dataset as well. This was expected as the majority of samples were in this FPC category (Tables 5.1 and 5.3). Notably, the medium FPC category contained the highest values for MPT, MLPT, and M-score in the entire dataset. This supports the assertion that protein quality is of greater importance than protein quantity in determining dough mixing characteristics and, by inference, end-product quality.

Table 5.3 - Summary statistics of grain, flour, and dough quality parameters in high protein concentration wheats.

High Protein	n	Mean	SD	Minimum	Maximum
TWT (lb/bu)	12	61.0	1.08	59.7	62.9
SKH (HU)	12	71.1	3.35	66.1	78.9
FPC (%)	12	12.0	0.79	11.1	13.9
LASRC (%)	12	145.5	17.41	113.9	171.1
%LUPP	12	67.3	4.12	59.3	75.0
M-Score	11	4.4	1.29	2	7
MPT (min)	12	3.6	0.93	2.1	4.8
MBD	12	0.64	0.09	0.47	0.81
EPT (min)	12	3.2	0.86	1.9	4.7
ERInt (%TQ*min)	12	87.6	15.54	61.9	113.7
MLPT (min)	12	4.0	0.95	2.6	5.4
MLRInt (%TQ*min)	12	370.5	53.68	294.2	457.6
Medium Protein	**n**	**Mean**	**SD**	**Minimum**	**Maximum**
TWT (lb/bu)	60	61.8	1.16	59.6	64.2
SKH (HU)	60	69.6	3.96	64.0	78.5
FPC (%)	60	9.9	0.53	9.0	10.8
LASRC (%)	60	136.4	14.26	101.7	163.8
%LUPP	60	65.3	5.73	51.9	77.3
M-Score	60	4.8	1.79	2	8
MPT (min)	58	4.0	1.04	1.9	6.1
MBD	58	0.69	0.18	0.38	1.5
EPT (min)	58	3.4	1.12	1.4	5.7
ERInt (%TQ*min)	58	100.3	25.22	39.3	148.8
MLPT (min)	58	4.2	1.16	1.5	6.8
MLRInt (%TQ*min)	57	363.3	59.22	195.8	473.6
Low Protein	**n**	**Mean**	**SD**	**Minimum**	**Maximum**
TWT (lb/bu)	26	62.4	0.84	61.0	64.0
SKH (HU)	26	69.2	3.66	64.4	75.0
FPC (%)	26	8.6	0.45	6.9	150.3
LASRC (%)	26	129.9	12.04	105.1	76.2
%LUPP	26	64.4	4.84	52.4	5.4
M-Score	26	3.1	1.23	1	6
MPT (min)	24	3.7	1.29	0.50	1.0
MBD	24	0.74	0.13	0.48	5.6
EPT (min)	26	2.7	1.29	1.1	130.8
ERInt (%TQ*min)	26	89.9	25.07	42.0	6.4
MLPT (min)	26	4.0	1.22	2.0	461.0
MLRInt (%TQ*min)	26	343.2	62.69	211.1	8.9

Abbreviations: TWT: Test weight; SKH: Single kernel hardness; FPC: Flour protein concentrations; LASRC: Lactic acid Solvent Retention Capacity; LUPP: Large unextractabale polymeric proteins; M-Score: Mixograph score; MPT: Manual peak time; MBD: Manual breakdown; EPT: Envelope peak time; ERInt: Envelope right integral; MLPT: Midline peak time; MLRInt: Midline right integral

The low FPC category had a similar distribution of TWT and SKH as observed in the complete dataset, and in the high and medium FPC categories (Table 5.3). However, the low FPC category captured a narrower range of LASRC values. The low FPC category had a slightly lower mean M-score than the either the high or medium FPC categories. As can be observed in Figure 5.1, low FPC mixograms may be flat and can be difficult to interpret meaningfully.

Table 5.4 shows the correlation coefficients of LASRC and %LUPP on measures of grain, flour, and dough quality for the high, medium, and low FPC categories. For the high FPC category LASRC and %LUPP were both positively correlated with MPT. LASRC was also significantly and positively correlated with EPT and M-score (nonparametric). %LUPP was significantly and positively correlated with different dough mixing parameters, MLPT and MLRInt. Only LASRC correlated significantly with FPC. Despite the relative ease of interpreting the high FPC mix curves (Figure 5.1) the numerical values of the correlation coefficients were similar to those observed for the full data set (Table 5.2). It is of value to recall that for the full data set n = 94 for MPT and for the high FPC set n = between 11 and 12 depending on the parameter. Even in this subset of samples where mixograms were easier to interpret both visually and by machine, LASRC or %LUPP predicted at best only 48% of the variability in any dough mixing parameter.

For the medium FPC category, LASRC and %LUPP were correlated with the same dough mixing parameters (Table 5.4). LASRC consistently explained more variability in dough mixing parameters than %LUPP. The numerical values of the parametric correlation coefficients for LASRC, %LUPP and all dough quality parameters were higher in the medium FPC category than in the entire dataset (Figures 5.2 and 5.4). However, the nonparametric correlations of LASRC and %LUPP with M-score were not higher in the medium FPC category as compared to

the entire dataset. The correlations of %LUPP on MLPT and MLRInt were higher in the high FPC category than the medium FPC category. LASRC and %LUPP predicted at best only 46% of the variability in any dough mixing parameter in the medium FPC category.

Table 5.4 - Correlation coefficients of lactic acid Solvent Retention Capacity (LASRC) and % large unextractable polymeric proteins (%LUPP) on grain, flour, and dough quality parameters in high, medium, and low protein concentration wheats.

	High		Med		Low	
	LASRC	**%LUPP**	**LASRC**	**%LUPP**	**LASRC**	**%LUPP**
TWT (lbs/bu)	ns	ns	ns	ns	ns	ns
SKH (HU)	ns	ns	ns	ns	ns	ns
FPC (%)	0.69	ns	ns	ns	ns	ns
LASRC (%)	--	ns	--	0.67	--	ns
MPT (min)	0.59	0.69	0.68	0.61	0.48	ns
MBD	ns	ns	ns	ns	ns	ns
EPT (min)	0.67	ns	0.65	0.56	ns	ns
ERInt (%TQ*min)	ns	ns	0.60	0.38	ns	ns
MLPT (min)	ns	0.66	0.65	0.55	ns	ns
MLRInt (%TQ*min)	ns	0.66	0.68	0.56	ns	ns
M-Score*	0.55	ns	0.41	0.38	0.32	ns

Abbreviations: TWT: Test weight; SKH: Single kernel hardness; FPC: Flour protein concentrations; LASRC: Lactic acid Solvent Retention Capacity; LUPP: Large unextractabale polymeric proteins; MPT: Manual peak time; MBD: Manual breakdown; EPT: Envelope peak time; ERInt: Envelope right integral; MLPT: Midline peak time; MLRInt: Midline right integral; M-Score: Mixograph score.
*Correlation calculated via Kendall's τ test

For the low FPC category, Table 5.4 shows that only two dough mixing parameters were significantly correlated with LASRC. None of the correlations with %LUPP were significant. None of the correlations were higher in the low FPC category than in the entire dataset or the higher FPC categories. A lower number of significant correlations were observed in the low FPC category, compared to the high FPC category, despite the smaller sample size of the low FPC

category. The dearth of significant correlations in the low FPC subset further highlights the particular difficulty associated with interpreting the mixograms of low protein wheats. As low FPC wheats had very few significant correlations between LASRC, %LUPP, and dough mixing parameters it was deemed prudent to assess the dataset with only the high FPC and medium FPC samples.

Table 5.5 - Correlation coefficients of lactic acid Solvent Retention Capacity (LASRC) and % large unextractable polymeric proteins (%LUPP) on grain, flour, and dough quality parameters in high and medium flour protein concentration wheats.

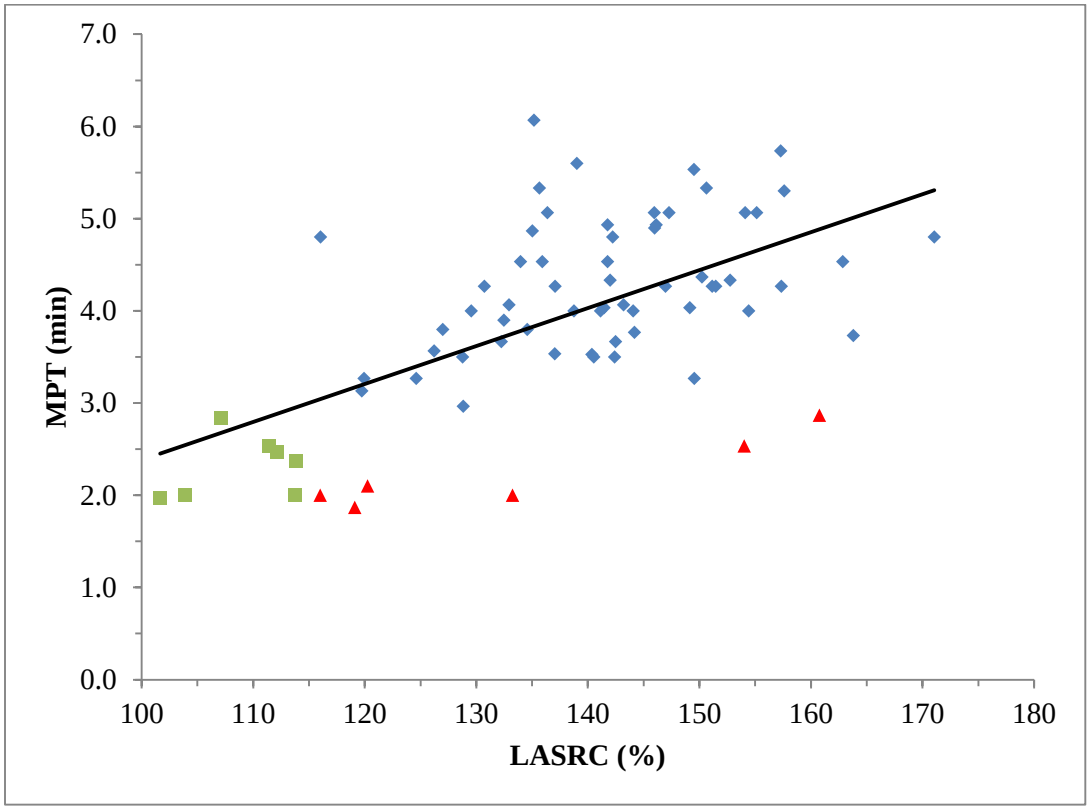
	LASRC	%LUPP
TWT (lbs/bu)	ns	ns
SKH (HU)	ns	ns
FPC (%)	ns	ns
LASRC (%)	--	0.65
MPT (min)	0.60	0.58
M BD	ns	ns
EPT (min)	0.61	0.55
ERInt (%TQ*min)	0.49	0.34
MLPT (min)	0.57	0.55
MLRInt (%TQ*min)	0.63	0.57
M-Score*	0.40	0.38

Abbreviations: TWT: Test weight; SKH: Single kernel hardness; FPC: Flour protein concentrations; LASRC: Lactic acid Solvent Retention Capacity; LUPP: Large unextractabale polymeric proteins; MPT: Manual peak time; MBD: Manual breakdown; EPT: Envelope peak time; ERInt: Envelope right integral; MLPT: Midline peak time; MLRInt: Midline right integral; M-Score: Mixograph score.
*Correlation calculated via Kendall's τ test

Table 5.5 shows the correlation coefficients of LASRC and %LUPP on measures of grain, flour, and dough quality for the subset of high and medium FPC samples. The correlation coefficients were higher for every parametric correlation between LASRC, %LUPP, and dough mixing parameters (except for FPC) for the high and medium FPC subset as compared to the full dataset.

The non-parametric correlation coefficient between LASRC and M-score was slightly lower than in the complete dataset, and also not as high as for the medium FPC subset alone.

Figure 5.3 – Scatterplot of mean Manual Peak Time (MPT) by Lactic Acid Solvent Retention Capacity (LASRC). Linear regression is shown as solid black line. Green squares represent data with LASRC < 115% and MPT < 3.0 min. Red triangles represent data with LASRC > 115% and MPT < 3.0 min. Blue diamonds represent data with with LASRC > 115% and MPT > 3.0 min.



The focus on the numerical magnitude of "r" values is related to the practical need to make predictions in a breeding program for selection purposes. Therefore the magnitude of "r" and hence "r^2", the coefficient of determination, are salient to the practical outcomes. In general, for a predictive test to be of value the OSU Wheat Quality Program only applies tests broadly if r^2 is > 0.75% (i.e. explains more than 75% of the variability in the predicted value). Based on this criterion LASRC and %LUPP would not be sufficient as predictors.

If the LASRC test were to be implemented in a wheat-breeding program it could only be used a rough screening technique, and not a predictor. In rough screening, a cutoff point would need to be determined and applied. A reasonable cutoff for MPT based on industry demands is 3.0 min (USDA 2006). Figure 5.3 shows the linear regression of *mean* LASRC and MPT values for the high and medium FPC samples ($r = 0.60$, $p < 0.0001$, $n = 70$). According to the equation from the linear regression of this subsample of the dataset, a MPT of 3.0 min corresponds to a LASRC value of 115%. If a strict 115% LASRC cutoff was applied to this dataset then 10% (7 samples) of these samples would be correctly categorized as having MPT too short to be acceptable (Figure 5.3, green squares). 8.6% (6 samples) would be incorrectly categorized. These samples have LASRC > than the cut-off but MPT < 3.0 min (Figure 5.3, red triangles). Fortunately, in this subset of the data no samples would be incorrectly categorized where LASRC was < the cutoff, but where MPT was > 3.0 min. However, if the low FPC category samples were included then two samples would be incorrectly screened out of the breeding program. By implementing a selection pressure of 10%, and LASRC specification could be set that would exclude the 10% of samples with the worst/lowest MPT. The associated cost would be including, in this sample set, 8.6% of samples that had unacceptable MPT. In this case LASRC correctly categorized 81.4% of the samples (Figure 5.3, blue diamonds). Discussions with the breeder have indicated that a 10% selection pressure is sufficient to improve the overall quality of the germplasm pool if applied consistently over a period of years. It needs to be reiterated that while successful in screening out the bottom 10% of low quality samples, there was an opportunity cost associated with retaining a similar proportion of low quality samples. Overall, LASRC may not be effective on its own as predictor of dough characteristics, but may still have value as an early generation screening tool

for hard wheat quality. In general %LUPP was similar to or slightly worse than LASRC in predictive capabilities for dough characteristics. As a result all of the considerations about LASRC as a predictor or screening tool for dough characteristics are applicable to %LUPP.

5.4 Conclusions

The effectiveness of LASRC and %LUPP as early generation screening methods for hard wheat quality was explored. Mixograph analysis was used to assess hard wheat dough characteristics. In this collection of hard wheats, dough mixing parameters were slightly better correlated with LASRC than they were with %LUPP. LASRC was significantly correlated with three measures of Mixograph peak time. The correlation coefficients between LASRC and Mixograph peak time measurements were higher in this collection of hard wheats than previously observed in the literature (Ram et al 2005, Xiao et al 2006). Correlations between LASRC, %LUPP, and dough mixing parameters were different between FPC categories, particularly in low FPC samples. A strict cutoff of 115% LASRC effectively screened out the bottom 10% of low quality hard wheats but retained a nearly equal amount of low quality hard wheats that would have been screened out by mixograph analysis (MPT < 3.0 min). LASRC and %LUPP are not likely to be effective predictors of dough properties, but may have some value as screening methods for hard wheat quality in the early generations of a wheat breeding cycle.

5.5 References

See Bibliography.

Chapter 6: General Conclusions

PHS Studies (Chapters 3 and 4)

Changes in Falling Number (FN) values and alpha amylase (αA) activity were observed over grain storage time. Changes in FN occurred differentially between growing environments, wheat varieties, storage temperatures, and between soft and hard wheat classes. Changes in αA activity occurred differently across growing environments, wheat varieties, and between soft and hard wheat classes but not storage temperature. αA activity and FN values had a curvilinear relationship as previously observed in the literature (Hagberg 1961, Perten 1964, d'Appolonia 1982). Varieties showed different propensities to sprout as measured by FN and αA activity. Soft wheats were generally more susceptible to PHS in this collection of wheat varieties. Low FN values and high αA activities were primarily observed in soft wheats from locations that had rain events prior to harvest.

Changes in FN values over storage time were less prevalent in low FN samples. Highest rates of increase in FN were observed in ungerminated hard wheats. This directly contrasted with changes in αA activity. Decreases in αA activity over storage time were most prevalent in *soft* wheats, particularly sprouted soft wheats (i.e. those with initial αA activities > 0.1 CU). Decreases in hard wheat αA activities occurred primarily in wheats with initial αA activities < 0.1 CU, but at a lower magnitude than soft wheats. These results were counterintuitive as one would expect increases in FN to correspond with decreases in αA activity.

Rates of systematic increases in FN over storage time occurred at a higher magnitude as storage temperature increased, particularly in unsprouted hard wheats. Storage temperature did not have as much influence on changes in αA activity as it did on changes in FN over storage time. Storage of grain at -20°C was not sufficient in all cases to stop significant systematic increases in FN values, particularly in unsprouted hard wheats. Storage at +40°C accelerated the rate of change in FN values over storage time, particularly in hard wheats from Aberdeen.

Decreases in αA activity were often not associated with a corresponding increase in FN values over grain storage time. The changes that occur in stored wheat that modulate FN over time were seemingly not wholly influenced by αA activity. Hard wheats from the location Aberdeen as compared to Parma had higher rates of increase in FN over time. However, the decrease in αA activity between the two locations may not have been different enough to be the sole reason for the changes in FN. Temperature-induced gluten crosslinking was explored as possible explanation for drastic increases in hard wheat FN. Polymeric protein content was not altered by storage temperature in hard wheats. However, there was evidence that polymeric protein content was degraded in PHS-damaged hard wheats.

Grain storage was successful as a way to raise FN values to > 300 s in very few cases. However, storage for 90 days was not effective in decreasing αA activity from > 0.1 CU to < 0.1 CU. Changes in αA activity from > 0.1 CU to < 0.1 CU only occurred in one variety, Keldin from Kimberly. This significant decrease in αA activity did not have a corresponding increase in FN to > 300 s.

Dough strength study (Chapter 5)

The effectiveness of LASRC and %LUPP as early generation screening methods for hard wheat quality was explored. Mixograph analysis was used to assess hard wheat dough characteristics. In this collection of hard wheats, dough mixing parameters were slightly better correlated with LASRC than %LUPP. LASRC was significantly correlated with three measures of Mixograph peak time. The correlation coefficients between LASRC and Mixograph peak time measurements were higher in this collection of hard wheats than previously observed in the literature (Ram et al 2005, Xiao et al 2006). Correlations between LASRC, %LUPP, and dough mixing parameters were different between FPC categories, particularly in low FPC samples. A strict cutoff of 115% LASRC effectively screened out the bottom 10% of low quality hard wheats but retained a nearly equal amount of low quality hard wheats that would have been screened out by mixograph analysis (MPT < 3.0 min). LASRC and %LUPP are not likely to be effective predictors of dough properties, but may have some value as screening methods for hard wheat quality in the early generations of a wheat breeding cycle.

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